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REVIEW

Nuclear Receptors: Nomenclature, Ligands, Mechanisms of Their Effects on Gene Expression

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Abstract—Nuclear receptors are DNA binding transcription factors possessing conservative domain organization. Their activity is regulated by lipophilic ligands, phosphorylation, and by interactions with other proteins. This review highlights the nomenclature of 1999 for human nuclear receptors and the ligand structure and domain organization of these receptors. The review also summarizes recent data on the structure of hormone response elements of specific genes, the structure–functional organization of receptor co-regulators (coactivators and corepressors). They mediate their effects on transcription via two main mechanisms: chromatin remodeling and the effect on the main transcriptional factors. Some attention is focused on specific features of signal transduction at negative hormone-response elements, regulation of receptor activity via phosphorylation, mechanisms of receptor cycle termination, and on physiological and biochemical properties of certain groups of receptors and processes that they regulate.

Key words: nuclear receptors, hormone response elements, co-regulators, gene expression, chromatin remodeling

Nuclear receptors provide direct control of gene expression via various extracellular and intracellular signals. Several mechanisms are involved in the regulation of these receptors: 1) the interaction with small lipophilic ligands; 2) covalent modification (usually phosphorylation), regulated by extra- and intracellular signals; 3) protein–protein interaction with other transcription factors.

1. NOMENCLATURE OF NUCLEAR RECEPTORS. RECEPTOR LIGANDS

The nuclear receptor superfamily includes several hundred members (*Drosophila* and *Nematoda* genomes contain 21 and 270 genes encoding nuclear receptors, respectively, and the human genome contains 48 or 49 genes encoding nuclear receptors) [1, 2]. These proteins are subdivided into several subfamilies according to similarities of amino acid sequences and some other properties. The table shows the modern nomenclature of human nuclear receptors [3] (see also http://www.ens-lyon.fr/LBMC/Laudet/NucRec/nomenclature-table.html). Protein products of paralogic genes are designated by Greek letters α, β, γ, δ. Many receptors exist as several variants, appearing due to employment of several alternative promoters, alternative splicing of transcripts, or alternative starts of translation. As a rule such variants are designated by Arab numbers 1, 2, etc. Expression of various genes encoding specific receptors (and their variants) is tissue specific; it is also characterized by individual regulatory features, e.g., in ontogenesis [4, 5].

Orphan receptors are a large group representing members of various subfamilies. Ligands for these orphan receptors remain unknown or they have been found after the discovery of a corresponding protein. Ligands for nuclear receptors may be hormones or non-hormonal compounds. The latter group includes dietary products or their metabolites or metabolites of endogenous compounds such as cholesterol and polyunsaturated fatty acids. Many drugs and pollutants may also act as ligands for nuclear receptors (table) [6-10].

2. DOMAINS OF NUCLEAR RECEPTORS

Nuclear receptors share a similar modular (domain) structure that usually includes from four to five independent but functionally related domains [11, 12] (Fig. 1).

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Nomenclature and ligands of nuclear receptors

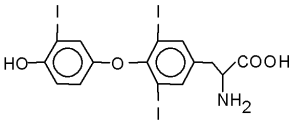
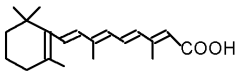
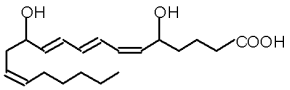
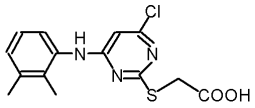
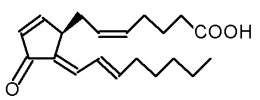
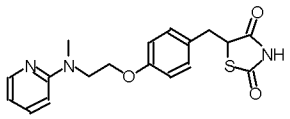
Subfamily and the group	Gene	Trivial name	Full name	Ligand structure	Ligand name
1	2	3	4	5	6
1A	NR1A1	TRα	thyroid hormone receptor α		triiodothyronine
	NR1A2	TRβ	thyroid hormone receptor β	same	same
1B	NR1B1	RARα	retinoic acid receptor α		*trans*-retinoic acid
	NR1B2	RARβ	retinoic acid receptor β	same	
	NR1B3	RARγ	retinoic acid receptor γ	same	
1C	NR1C1	PPARα	peroxisome proliferator-activated receptor α		leukotriene B_4
					WY 14,643
	NR1C2	PPARβ	peroxisome proliferator-activated receptor β	same	WY 14,643
	NR1C3	PPARγ	peroxisome proliferator-activated receptor γ		15-deoxy-$\Delta^{12,14}$-prostaglandin J_2
					BRL 49653 (Rozylglitiazone)
1D	NR1D1	REVERBα	receptor α, encoded by DNA strand complementary to coding sequence of *cERBα* gene (i.e., NR1A1)		
	NR1D1	REVERBβ	receptor β, encoded by DNA strand complementary to coding sequence of *cERBβ* gene (i.e., NR1A2)		

Table (Contd.)

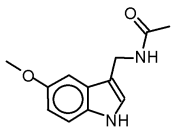
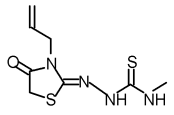
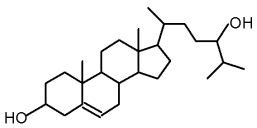
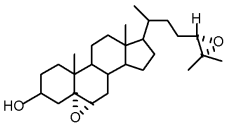
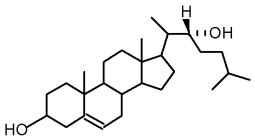
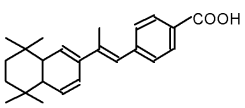
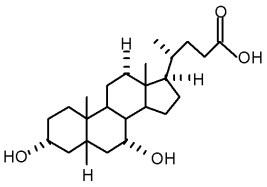
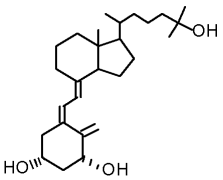
1	2	3	4	5	6
1F	NR1F1	RORα	retinoid orphan receptor α		melatonin (N-acetyl-5-methoxy-tryptamine)
					CGP 52608 (1-(3-allyl-4-oxothiazo-lidin-2-yliden)-4-methylthiosemi-carbazone
	NR1F2	RORβ	retinoid orphan receptor β	same	same
	NR1F3	RORγ	retinoid orphan receptor γ	same	same
1H	NR1H3	LXRα	liver X receptor α		24-hydroxy-cholesterol
					5,6-24(S),25-diepoxycholesterol
	NR1H2	LXRβ	liver X receptor β		22(R)-hydroxy-cholesterol
	NR1H4	FXR	farnesoid X receptor		TTNPB
					xenodeoxycholic acid
1I	NR1I1	VDR	vitamin D receptor		1α,25-dihydroxy-vitamin D_3

Table (Contd.)

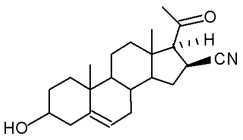
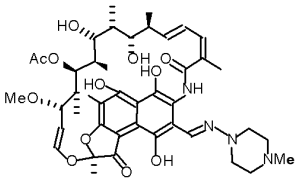
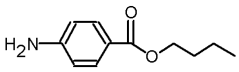
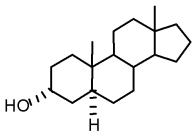
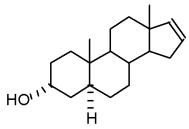
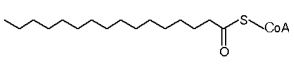
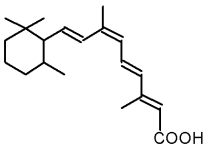
1	2	3	4	5	6
	NR1I2	PXR	pregnane X receptor		pregnenolone-16-carbonitrile
					rifampicin
		BXR	benzoate X receptor (clawed frog, *Xenopus*)		4-aminobutyl-benzoate
	NR1I3	CARα	constitutively active receptor α		5α-androstan-3α-ol
	NR1I4	CARβ	constitutively active receptor β		5α-androst-16-en-3α-ol
2A	NR2A1	HNF4α	hepatocyte nuclear factor 4 α		palmitoyl-CoA
	NR2A1	HNF4γ	hepatocyte nuclear factor 4 γ		
	NR2A1	HNF4β	hepatocyte nuclear factor 4 β		
2B	NR2B1	RXRα	retinoid X receptor α		9-*cis*-retinoic acid
	NR2B2	RXRβ	retinoid X receptor β	same	same
	NR2B3	RXRγ	retinoid X receptor γ	same	same
2C	NR2C1	TR2	testicular receptor 2		
	NR2C2	TR4	testicular receptor 4		
2E	NR2E1	TLX	homolog of gene encoding tailless in *Drosophila* flies (tailless X)		

Table (Contd.)

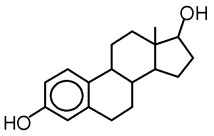
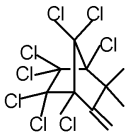
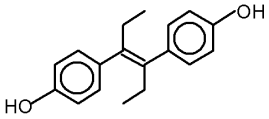
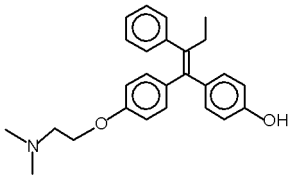
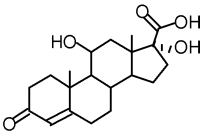
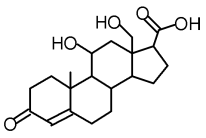
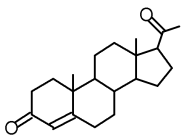
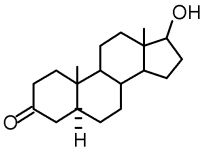
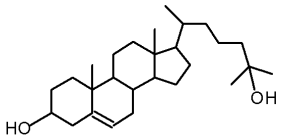
1	2	3	4	5	6
2F	NR2F1	COUP-TFI	chicken ovalbumin upstream promoter transcription factor I		
	NR2F2	COUP-TFII	chicken ovalbumin upstream promoter transcription factor II		
3A	NR3A1	ERα	estrogen receptor α		estradiol
	NR3A2	ERβ	estrogen receptor β	same	same
3B	NR3B1	ERRα	estrogen-related receptor α		toxafen
	NR3B2	ERRβ	estrogen-related receptor β		diethylstilbestrol
	NR3B3	ERRγ	estrogen-related receptor γ		4-hydroxy-tamoxifen
3C	NR3C1	GR	glucocorticoid receptor		hydrocortisone
	NR3C2	MR	mineralocorticoid receptor		aldosterone
	NR3C3	PR	progesterone receptor		progesterone

Table (Contd.)

1	2	3	4	5	6
	NR3C4	AR	androgen receptor		5α-dihydro-testosterone
4A	NR4A1	NGFIB	nerve growth factor inducible-B		
	NR4A2	NURR1	Nur-related factor 1		
	NR4A3	MINOR	mitogen-inducible nuclear orphan receptor		
5A	NR5A1	SF1	steroidogenic factor 1		25-hydroxy-cholesterol (?)
	NR5A2	LRH1	liver receptor homologous protein 1		
6A	NR6A1	GCNF1	germ cell nuclear factor 1		
0B	NR0B1	DAX1	dosage-sensitive sex reversal, AHC critical region on the X chromosome, gene 1		
	NR0B2	SHP	small heterodimeric partner		

Note: Empty cells in the table correspond to receptors whose ligands have not been identified yet.

The modulator A/B domain is the most variable in terms of length and amino acid sequence. Most receptor isoforms appear due to modification of this domain. The A/B domain contains a transcription activation autonomic function-1 (AF-1) that synergistically acts with ligand dependent AF-2 located opposite the receptor molecule. AF-1 is responsible for specificity of receptor activity in accordance with promoter context and a given cell type. This suggests that this domain interacts with tissue-specific cofactors. Differences in the structure of the A/B-domain may provide differences in responses to the ligand acting at related receptors. The A/B domain is the target for receptor regulating protein kinases (see Section 9). Various group-specific (SRCs, p300, CBP) and selective (MMS19, DEAD-domain containing proteins p72/p68) coactivators of nuclear receptors directly interact with this domain and this results in an increase in receptor activity [13-18].

DNA-binding domain and hormone-response elements. DNA-binding domain (DBD) or C-domain is the most conservative domain. DBD recognizes enhancer-like DNA sites called the hormone-response elements (HREs). They contain one or two consensus half-sites recognized by mono- or dimeric receptors, respectively. Two half-sites may be arranged as palindromes and repeats which represent either inverted or everted structures. Steroid receptors (excluding estrogen receptors) recognize the consensus half-site AGAACA; estrogen receptors (and other nuclear receptors) recognize the AGGTCA half-site. Monomeric receptor HREs contain a 5′-terminal extension including from three to six bases enriched with A/T. Natural HREs do not entirely correspond to these consensus sequences. A gene may contain one or several HREs for a given hormone and these HREs are usually located at 5′-flanking region (and rarely in intron and exon sequences or at 3′-flanking region) [19]. The existence of multiple HREs provides cooperative augmentation of the ligand effect.

X-Ray analysis of isolated DBDs and their dimers of various receptors [19, 20] allowed characterizing the receptor–nucleic acid interaction in more detail. DBD contains three modules: two zinc fingers (including 66-70 amino acid residues in total), and a C-terminal extension (CTE) of 25 residues. Amino acid residues of the zinc finger (P-box region) are directly involved in recognition of HRE half-site. The second zinc finger nonspecifically interacts with DNA sugar-phosphate backbone; it acts as one of the surfaces for receptor dimerization (regions of D- and DR-boxes). CTE has several functions. It interacts with the HRE half-site flanking bases. This is especially important for specific and tight binding of HRE with monomeric receptors and for right orientation of heterodimer receptors interacting with asymmetric HREs. CTE also forms a surface for the interaction of the receptor with other proteins; CTE operates as a "ruler" for discrimination of HREs by size of spacer separating two half-sites [6, 19, 21] (Fig. 2). The size of spacer separating two HRE half-sites may vary from 0 to 5-6 bp. In the case of HREs organized as direct repeats, the size of spacer is important for selectivity of HRE interaction with various receptors.

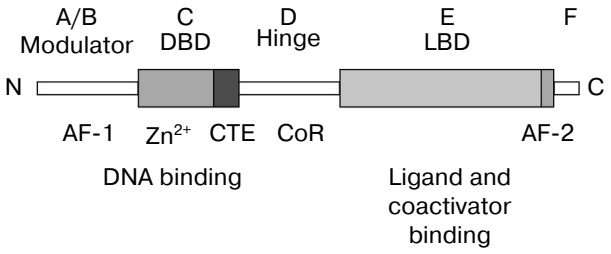
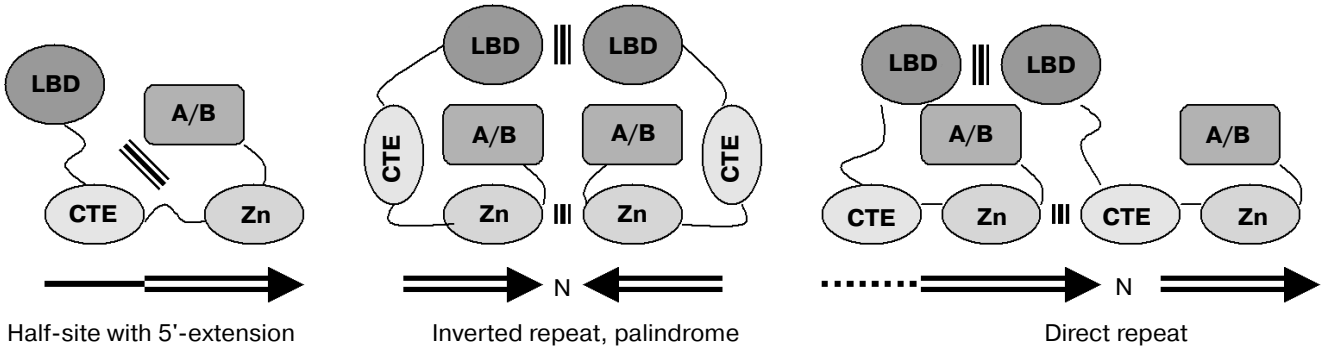


Fig. 1. Domain organization of nuclear receptors. Designations: DBD) DNA-binding domain; LBD) ligand binding domain; AF-1, AF-2) transactivation function 1 and 2, respectively; Zn^{2+}) zinc finger; CTE) C-terminal extension of DNA-binding domain; CoR) the region for corepressor interaction.

A hinge region D of receptors is variable by length and amino acid sequence. Its flexibility can provide DBD rotation along LBD by 180°; this is important for the interaction of receptor dimers with asymmetric HREs representing direct repeats and HREs representing inverted repeats. This region also forms a surface for receptor interaction with some co-regulators [22] and it may contain nuclear localization signal [23].

Ligand binding domain (LBD) or E region is moderately conservative. In the region of the fourth helix, it contains a motif (signature) that is common for nuclear receptors. A highly conservative sequence located at the C-terminus (in many receptors the twelfth helix) corresponds to ligand dependent activation function AF-2. X-Ray analysis of free (apo) LBD of many receptors and their complexes with ligands [12, 24-31] revealed the conservative arrangement of this domain: 11-13 α-helices are packed into three layers and a hydrophobic ligand-binding pocket is located deep inside the LBD. Besides ligand binding, this domain is involved in receptor dimerization and interaction with heat shock proteins. It also contains nuclear localization signal, and is involved in the transactivation effect of the receptor. The latter requires AF-2 and coactivator recruitment. Ligand binding induces conformational changes in the receptor molecule resulting in altered orientation of the AF-2 motif. This "locks" ligand and forms a surface for the interaction with coactivators at the "entrance" to the ligand binding pocket. (This surface includes AF-2 and at least three other helices.) Receptor antagonists cannot induce such conformational changes in the receptor molecule and consequently they cannot promote the receptor interaction with coactivators. Selective agonists/antagonists also called selective receptor modulators induce "wrong" ori-



Monomers	Homodimers	RXR-heterodimers	Homodimers
RVR	ERR	PPAR	Rev-Erb
ROR	ER	LXR	HNF4
TLX	AR	FXR	RXR
ERR	PR	PXR	TR2
NGFI-B	GR	CAR	TLX
FTZ-F1	MR	NGFI-B	COUP-TF
			GCNF

Fig. 2. Interaction of nuclear receptor with distinctly organized hormone responsive elements. N designates a spacer between HRE half-sites. Other designations are the same as in Fig. 1.

entation of H12, which provides receptor activity only in certain gene and cellular context. Such direction in modern pharmacology is very promising and knowledge of nuclear receptor LBD structure may help to develop ligands with predetermined biological properties [32-34].

The F domain was not found in all receptors. This domain may be involved in additional discrimination between receptor agonists and antagonists. For example, removal of this domain from progesterone or glucocorticoid receptors "converted" antagonists into agonists. It is possible that this domain can bind some corepressor [35].

Domains are functionally interlinked. After ligand or DNA binding, the interaction of distant receptor domains with other molecules may be changed due to conformational receptor plasticity [36, 37].

3. RECEPTOR EXPRESSION

Expression of nuclear receptors is under multiple controls by numerous factors, including the receptor ligands. Genes encoding nuclear receptors contain hormone-response elements responsible for positive or negative feedback. Expression of receptors may be tissue-specific (i.e., limited to one or a few tissues as in the case of LXR or SF-1) or it may occur in almost all tissues (e.g., RXR). Some receptors are expressed at the earliest stages of embryonal development (e.g., SF-1 determines the development of gonads) or at the later stages (e.g., AR and ER mediate the effects of products of gonad functioning, androgens and estrogens). As a rule, nuclear receptor expression is preserved during further ontogenesis, but it may decline during aging. The level of nuclear receptor expression determines cell sensitivity to a certain hormone, and this feature is employed in clinical practice for diagnostics of effectiveness of hormone therapy [33, 38].

4. INTRACELLULAR LOCALIZATION

Most nuclear receptors are constitutively (i.e., ligand-independently) localized in the nucleus. In the absence of a ligand, the major proportion of steroid receptors may be located in cytoplasm. Nuclear localization of receptors is mainly determined by protein–protein interactions (e.g., a dimerization partner such as RXR or corepressor as NCoR) rather than DNA binding [39]. Irrespectively to the receptor type, a corresponding ligand causes redistribution of the receptor between nucleoplasm and chromatin and hetero- and euchromatin. In cytoplasm steroid receptors are bound to heat

shock proteins (Hsp) preventing receptor transportation through the nuclear membrane (which normally occurs due to the presence of nuclear localization signal in the receptor LBD) and receptor binding to DNA due to the shielding of receptor DBD [40]. The mature form of the steroid receptor exists as the receptor complex with dimeric Hsp90 and possibly immunophilin. Processing of newly synthesized steroid receptor may include ATP-dependent (and independent) association of the receptor molecule with eight chaperone proteins, which are probably responsible for the rendering to the receptor a required conformation [41].

5. CO-REGULATORS

In the absence of ligand, nuclear receptors may be transcriptionally inactive (e.g., steroid receptors) or may exert repressor (as thyroid hormone receptors) or stimulatory (as constitutively active CAR receptors) effects on transcription. Ligand binding causes changes in transcriptional activity of receptors by increasing or decreasing initial receptor activity. These changes are realized by receptor interaction with co-regulator proteins (see reviews [42-45]). In dependence on the receptor state, these co-regulators form a transcriptionally permissible or impermissible environment in the promoter region; they may also bind the receptor with general transcriptional factors and RNA-polymerase II. Direct, ligand-dependent binding of some nuclear receptors with the components of the transcriptional complex (e.g., TBP, TFIIF, TFIIH, and TFIIB) is also established. Such interaction is suggested to influence an assembly of transcriptional complexes. However, selectivity of nuclear receptor effects (the dependence on a cell type and controlled promoter) is mainly achieved by ligand-regulated interaction with corepressors, coactivators and CBP cointegrators (CREB-binding proteins)/p300.

Coactivators. Several types of coactivators of nuclear receptors are known. Figure 3 shows structure–functional organization of steroid hormone receptor coactivator 1 (SRC-1), which is typical for the whole family of these coactivators [42]. They preferentially interact with receptor AF-2, thus providing functional synergism between AF-2 and AF-1. These proteins may also interact with the main transcriptional factors, such as TFIIB and TBP. Members of this family (SRC-1, -2, -3) are encoded by three genes, and each of them exists as several splicing variants. Structural differences mainly observed in the C-terminal region particularly include the size of polyglutamine fragments. Consensus motifs LXXLL (where L and X are leucine and any amino acid, respectively) provide coactivator binding to hydrophobic groove of the receptor due to formation of a hydrophobic surface including leucine residues of amphipatic α-helix. (The receptor groove is formed in the presence of agonist by several fragments of the receptor molecule with obligate involvement of the 12th α-helix). In the presence of antagonists, this helix becomes a steric obstacle for the receptor interaction with LXXLL coactivator motif. The existence of several such motifs in the coactivator molecule allows the coactivator to contact with both receptor molecules of the dimer. Similar motifs have been also recognized in other coregulators of nuclear receptors. Variations of these motifs may determine selectivity of coregulator binding to certain type of receptors.

Besides members of the SRC family, other proteins function as coactivators of nuclear receptors. These include E3 ubiquitin protein ligase E6-AP and RPF-1 [46] (see Section 8). The SUG-1 coactivator lacks LXXLL motifs. Apparently, it interacts with receptor fragments that differ from AF-2 [47]. The L7/SPA coac-

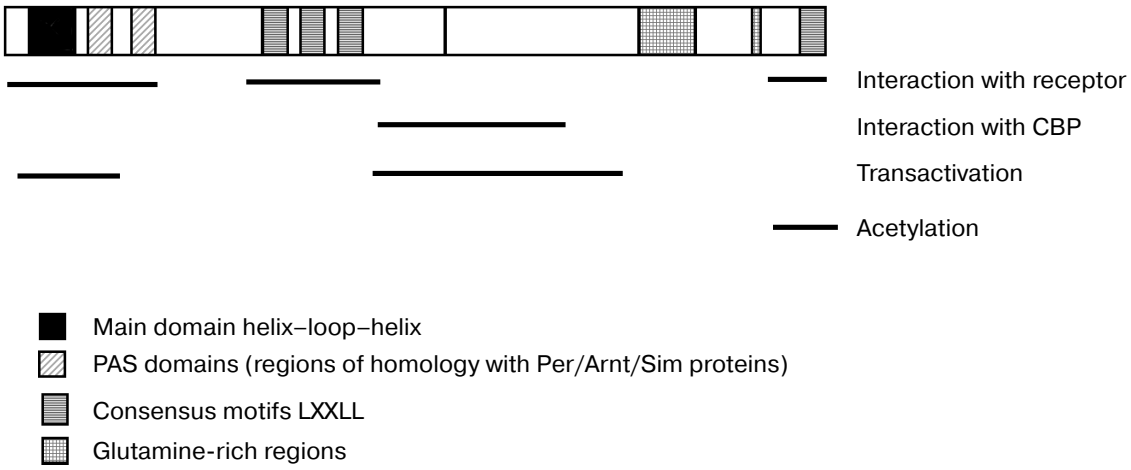


Fig. 3. Structure–functional organization of steroid receptor coactivator 1 (SRC-1); CBP is CREB-binding protein.

tivator potentiates the activity of partial hormone agonists; however, it does not influence the activity of full agonists or antagonists [48]. It is possible that this (or similar) coactivator is involved in effects of selective hormone analogs. Multicomponent protein coactivator complex TRAPs/DRIPs interacts with receptors via TRAP220/PBP [49]. This complex is also involved in the action of other transcription factors, including members of the GATA family (this is supported by data on embryonal lethality in *TRAP220* knockout mice [50]). Other factors such as positive cofactors (PCs) may also be involved in realization of nuclear receptor effects [51]. In contrast to most coactivators, PGC-1 (PPARγ coactivator-1) interacts not only with AF-2 but also with a hinge region of the receptor [52]. Expression of this coactivator (as well as PPARγ) predominates in brown adipose tissue and skeletal muscles and depends on the ambient temperature; the latter suggests a key role of this coactivator in thermogenesis [53]. Expression of hepatic PGC-1 induced by starvation and insulin insufficiency is required for induction of gluconeogenesis [54]. This coactivator can also interact with splicing factors. The group of TAFII proteins associated with TATA-binding protein (interacting with TATA-sequence in the promoter of the controlled gene) may also contribute to selectivity of nuclear receptor effects. It is suggested that various nuclear receptors promote formation of different variants of transcription factor TFIID complex by selective interaction with various TAFII proteins [55].

More selective activators include SRA. The latter is a non-protein (RNA) coactivator which selectively potentiates AF-1 activity of steroid receptors [56]. Apparently, SRA functions as a bridge between receptor AF-1 and coactivators (SRC) associated with AF-2. (SRA interacts with coactivator proteins p72/p68, containing SRA and SRC binding sites [15].) Members of ARA (androgen receptor activator) coactivator group selectively interact with AF-1 or AF-2 of the androgen receptor; they may modulate agonist activity of partial agonist/antagonists [57] (i.e., they exhibit L7/SPA coactivator-like effect, see above). The TIF-1/SL1 complexes may realize the effect of nuclear receptors on rDNA promoters and this effect depends on TIF-1 acetylation [58]. Proteins of highly mobile group (HMG-1 and HMG-2) selectively increase DNA-binding activity of steroid hormone receptors due to formation of stable ternary complexes receptor–HMG–DNA. It is suggested that HMG functionally substitutes for the C-terminal extension of DBD of other receptors which is absent in steroid receptors and which is required for receptor interaction with the minor groove of DNA [59].

The cointegrators CBP and p300. CBP (CREB binding protein) and p300 act as coactivators not only for nuclear receptors but also for other transcription regulatory proteins. CBP may simultaneously interact with nuclear receptors and several coactivators including members of SRC family and histone acetylases. According to the "fire and reload" hypothesis, p300 and the receptor cooperatively increase transcription only during the first round and repeated assembly of the preinitiation complex requires only the receptor [60].

Some evidence exists that *in vivo* several coactivators present as preformed relatively stable oligomeric complexes, which are recruited by the active receptor; these complexes may also recruit additional coregulators by forming loose bonds with them [61].

Thus, agonist binding stimulates receptor complex formation with one or a few coactivators such as steroid receptor coactivator (SRA), p300/CBP associated factor (p/CAF), and CPB/p300. Coactivators SRC-1, p/CAF, and CBP possess their own histone acetylase activity. These and other coactivators recruit histone acetylases with distinct substrate specificity. In both cases histone acetylation results in relaxation and depression of chromatin due to reduced interaction of histones with negatively charged groups of DNA (Fig. 4) and bond loosening between nucleosomes. Besides histones, nuclear receptor regulated acetyl transferases catalyze modification of some transcription factors (e.g., protein p53), which is accompanied by change in their functional activity. Protein complexes NURD and Mi-2 exhibiting ATPase activity act in a similar manner (but with the opposite effect). These proteins are suggested to influence chromatin structure, which becomes susceptible to corepressors associated with histone deacetylases, which fix the inactive form of chromatin [62]. Protein NSD-1 interacting with nuclear receptors contains SET domain, and activator and repressor domains. This protein can probably stabilize and relax chromatin structure in dependence on the stage of development [63].

A nucleosome may not only prevent gene transcription activity, but also increase this activity (the latter requires corresponding positioning). DNA winding around the histone core may bring together distant regulatory sites (including hormone-response elements) of the gene and its proximal sites interacting with basic transcription factors. Consequently, nuclear receptors associated with hormone-response elements may directly (or indirectly) interact with the basic transcription factors and stabilize preinitiation transcription complex. Nucleosome positioning is one of possible modes of tissue specificity of hormone action. For example, estrogen-response element of the prolactin gene is localized out of the nucleosome only in cells expressing this hormone; estrogen-response element in nucleosome is inaccessible for the estrogen receptor [64].

Corepressors. In the absence of the hormonal ligand, type II nuclear receptors and some others may decrease the basal activity of gene promoters. This effect is determined by receptor interaction with corepressor proteins. Figure 5 shows structural–functional organization of NCoR (nuclear receptor corepressor) [65]. The corepres-

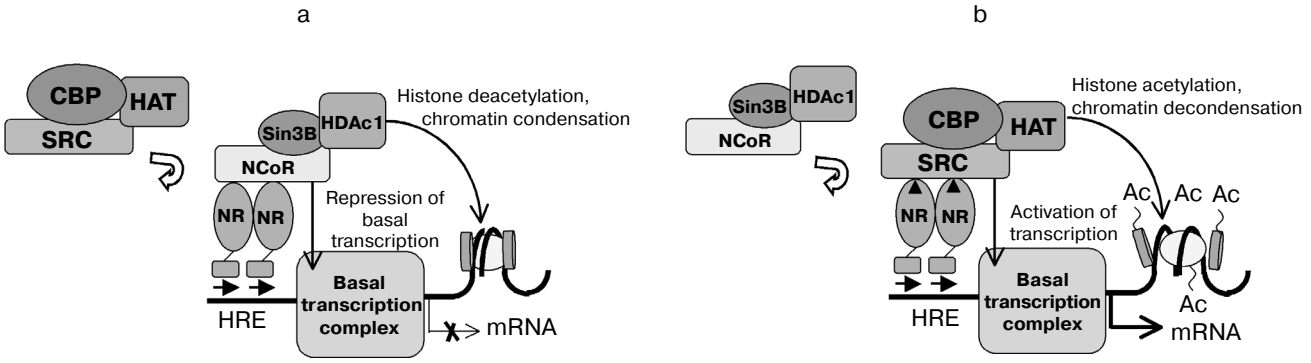


Fig. 4. Mechanism of repression (a) and activation (b) of transcription by nuclear receptors. Designations: NR) nuclear receptor; HRE) hormone-responsive element; NCoR) nuclear receptor corepressor; Sin3B) corepressor Sin3B; HDAc-1) histone deacetylase 1; SRC) steroid receptor coactivator; CBP) CREB-binding protein; HAT) histone acetyl transferase. The dark triangle designates receptor ligand.

sor SMRT (silence messenger of retinoid receptors and thyroid hormones) shares similar organization and significant homology with NcoR; however, the former lacks amino acid stretches corresponding to RDI and RDII [66, 67].

In the absence of the ligand, NcoR may simultaneously interact with two receptor molecules forming the dimer on the hormone-response element; this is achieved by the interaction of two domains involved in interaction with nuclear receptors (ID-N and ID-C) which are located at the C-terminus of the NcoR molecule and include α-helical elements containing motif LXXI/HIXXXI/L [65, 68, 69]. Later the other ID with the core-sequence IDVII was identified near ID-N. Replacement of leucine residues in coactivator IDs for isoleucine in corepressor IDs resulted in preferential corepressor binding to aporeceptors but not to ligand-bound receptors [70]. It remains unclear why an additional site of receptor binding exists on the NcoR molecule. It is possible that its presence is determined by known selectivity of some IDs with respect to various receptors. It is also possible that additional ID provides binding of trimer receptor; the latter is suggested to be formed on some negative HREs (see Section 6).

Regions involved in the interaction with corepressor are located on N- and C-termini of receptor LBD (and in some receptors at a hinge domain [71]). They contain a hydrophobic groove formed by several α-helices (H3-H4 and H11-H12), which is partially overlapped with the surface of the interaction of receptors with coactivators [72]. In the case of ligand regulated receptors, the 12th α-helix plays a special role: changes of its spatial orientation upon ligand binding form a steric obstacle for receptor interaction with corepressor and the receptor can interact with the coactivator. The H12 of RXR, the partner for dimerization of other nuclear receptors, plays an addi-

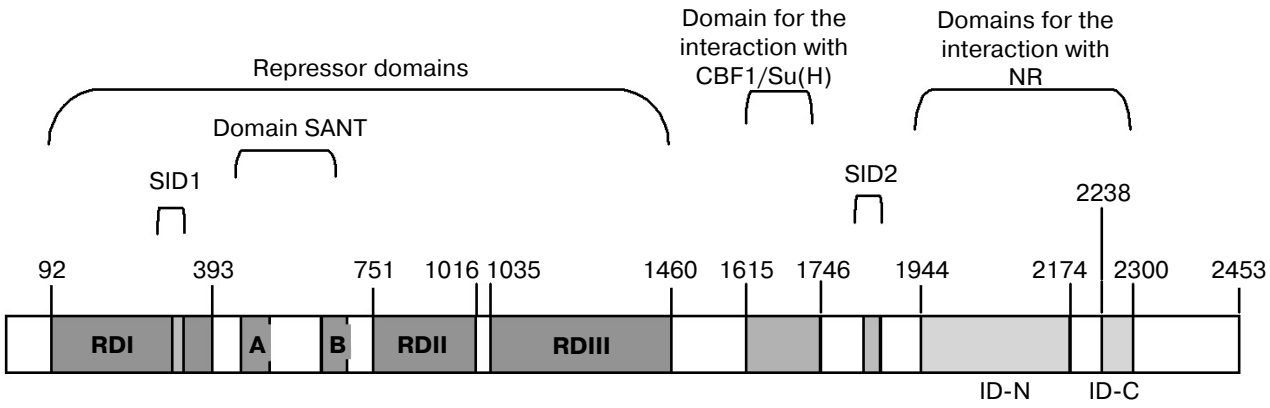


Fig. 5. Structure-functional organization of nuclear receptor corepressor (NCoR): RDI-III) repressor domains I-III; NR) nuclear receptor; ID-N, ID-C) domains for the interaction with nuclear receptors; SID1, SID2) domains for the interaction with corepressors Sin3A/B; CBF/Su(H)) corepressor; SANT) domain typical for the transcription regulators (SW13/ADA2/NcoR/TFIIIB) interacting with histone acetylases. Numbers indicate positions of amino acid residues at the domain borders.

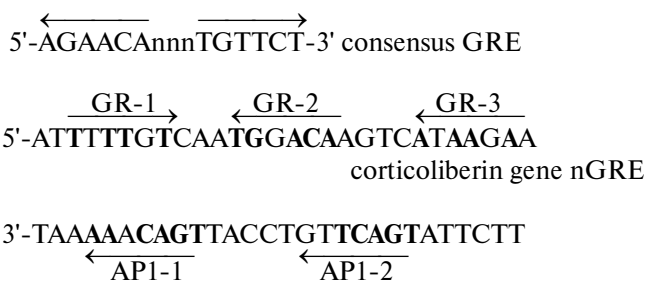
tional role. In apo-RXR the H12 is the steric obstacle for corepressor binding. In the ligand-lacking heterodimers (TR/RXR), orientation of this helix involved into inter-receptor interactions promotes corepressor binding to the dimer [73].

Members of the nuclear receptor family may preferentially interact with a certain corepressor. Besides SMRT and NCoR, there are other corepressors possessing distinct functions. For example, protein TRUP reduces interactions of the dimers TR-RXR and RAR-RXR with corresponding HREs [74]. The protein SUN-CoR (small unique corepressor of nuclear receptors) can interact with nuclear receptors and with corepressors SMRT and NCoR and this interaction involves its C-terminal domain. The SUN-CoR N-terminal domain includes autonomic repressor function with unknown action mechanism [75]. Recently, a new type of corepressor, PSF (polypyrimidine tract binding protein-associated splicing factor) has been recognized. It interacts with receptor DBD but not with LBD. In contrast to SMRT and NCoR, agonists do not abolish PSF binding to receptors [76]. The interaction of central nervous system-specific corepressor NIX1 (neuronal interacting factor X1) with nuclear receptors is also unique: this interaction requiring the presence of AF-2 in the receptor molecule is observed only in the presence of receptor agonists but not antagonists (i.e., this interaction corresponds to the interaction with receptor coactivators) [77]. Another action mechanism of this corepressor involves a simple competition with coactivators for the receptor molecule. The repressor SHARP (SMRT/HDAC1 associated repressor protein) exhibits binary effect. In the absence of a ligand it acts as a bridge between SMRT and histone acetylases and inhibits transcription activity of the receptors, but in the presence of the ligand SHARP interacts with coactivator SRA and inhibits its activity [78].

Effects of coactivators and corepressors on transcription involve at least two mechanisms: chromatin remodeling and change of transcription complex activity. Chromatin remodeling occurs due to the corepressor-induced recruitment of histone acetylases responsible for maintenance the repressed state of chromatin. This recruitment may occur by direct corepressor effect [79] or it may involve additional secondary corepressors. The latter include Sin3A/B proteins, which bind to receptors indirectly via corresponding domains of corepressors NCoR, SMRT, or other corepressors [76]. The resulting complex of four types of proteins suppresses local chromatin relaxation and inhibits pre-initiation transcription complex functioning (Fig. 4). Inhibition of basic transcription factors may occur via their direct interaction with corepressors. For example, NCoR may bind to TAFII32, TFIIB, and TAFII70 by its repressor domains and receptor interacting domains. These interactions are non-competitive because they involve different surfaces of the NCoR molecule. Moreover, TFIIB binding to the NCoR–receptor complex may occur simultaneously during binding of NCoR with second corepressor Sin3B and Sin3B-associated histone deacetylase. Binding to NCoR impairs the functional interaction between TAFII32 and TFIIB, and this fixes the pre-initiation complex in the inactive state [80]. (TAFII32 recruits TFIIB into the pre-initiation complex whereas TAFII70 recruits TAFII250 histone acetyl transferase). It is suggested that as in the case of coactivators various corepressor oligomeric complexes including also histone deacetylases are formed in the cell before interaction with receptors [81].

6. NEGATIVE HORMONE RESPONSE ELEMENTS

Hormones can induce and repress gene expression, and the ratio of genes induced and repressed by a given hormone may be close to unity. Mechanisms underlying gene repression are poorly understood. The repression effects of hormones involve four DNA regulatory elements denominated as negative hormone-response DNA elements (nHREs). They are usually located at the 5′-flanking region of the controlled gene. These elements may differ from canonic positive HREs by nucleotide sequence in half-sites, by amount and mutual orientation of these half-sites, and also by the size of the separating spacers [82-84]:



(bases corresponding to the consensus ones are shown in bold, nGRE is the negative glucocorticoid-response element).

In many cases nuclear receptors directly interact with similar sequences and oligomerization state of receptors on nHRE may be higher or lower than the receptor oligomerization on positive HREs [85, 86]. Very frequently, nHREs neighbor or overlap with elements interacting with other transcription factors (in the example shown above, there is the overlapping with the activation factor AP1 binding sites) [87]. This implies physical or functional interaction of the receptor with such (or similar) factor or their competition for the overlapping DNA elements as in the case of prolactin gene nGRE [88]. In some cases direct receptor binding to nHRE was not detected. Such binding is suggested to be mediated via

non-receptor protein factors, which directly bind to nHRE; in the case of distal nGRE of luliberin gene, octameric protein Oct-1 plays a role of such factor [89].

In the absence of ligand, direct or indirect interaction of nuclear receptors with nHREs may increase the basal transcription activity of the gene, and ligand abolishes this effect and may cause additional decrease of the gene expression. Both effects involve corepressors, and corepressor functioning is quite opposite than on positive HREs. For example, in the absence of hormone TR activating effect on the expression of genes encoding α- and β-subunits of thyrotropin and thyroliberin requires TR contact with corepressor (SMRT or NCoR). However, in the absence of contact between receptor and corepressor inhibitory effect of triiodothyronine is not abolished [90]. The phenomenon of such change of corepressor functioning in dependence of type of HRE (positive or negative) still does not have a plausible explanation which would be confirmed in direct experiments. Structure of negative nHREs significantly differs from that of positive HREs. Taking into consideration high conformational plasticity of receptors, we can assume that the conformation of receptors bound to two types of HREs is different and these differences may be transduced to the corepressor structure and this is accompanied by reversal of its functioning. There is some indirect evidence which seems to support this hypothesis: glucocorticoid and progesterone antagonist RU486 may acquire full or partial agonist activity of nGRE. Besides ligand and corepressor, the transcription activity of receptors on nHRE may be regulated by a heterodimerization partner, RXR and its ligand, which in the case of TRb suppress its basal activity of TRβ [86]. Unusual receptor heterodimerization was found on nGRE of the gene encoding 5-HT1A serotonin receptor; this nGRE is a direct repeat with spacer of 6 bp. Here heterodimers of glucocorticoid and mineralocorticoid receptors are formed, and they are more effective in suppression of transcription than homodimers of these receptors [84].

7. OTHER POSSIBLE MECHANISMS OF ACTION OF NUCLEAR RECEPTORS

Thymine-DNA glycosidase involved in excision of non-complementary DNA base pairs may physically and functionally interact with retinoid receptors RAR and RXR. It is possible that nuclear receptors may be somehow involved in DNA reparation and *vice versa* (reparation factors may influence nuclear receptor functioning) [91].

Atypical short-term non-genomic effects of some hormones usually acting via nuclear receptors are documented. It is possible that some of these effects can be realized via nuclear receptors anchored in the plasma membrane by covalent modification of the receptor (due to introduction in its molecule of a lipophilic fragment or interaction of the receptor molecule with membrane proteins). In fact, plasma membranes of hypophysis, hippocampal neurons, mammary glands, spermatozoids etc. contain an antigen recognized by a few antibodies against ERα. The amount of this antigen can be reduced by suppression of ER expression caused by administration of the antisense oligonucleotide [92]. Antagonists of nuclear ER block rapid stimulating effect of estradiol on NO synthase in endothelial cells. The transfection of COS-7 cells with ERα expression vector provides rapid activation of NO synthase by estrogen. This effect depends on Gα and such second messengers as Ca^{2+}, cGMP, and also tyrosine kinase and MAPKs [93, 94]. However, it should be noted that in most cases plasma membrane hormone binding activity of some cells differs from the nuclear receptors by specificity and antigenic determinants and short-term non-genomic hormone effects persist after blockade of their nuclear receptors [94-96].

8. TERMINATION OF THE RECEPTOR CYCLE

Mechanisms of termination of hormonal effects via nuclear receptors are quite different and may vary for certain types of receptors. In the case of ERs and some other receptors the hormonal ligand significantly accelerates degradation of its own receptor (e.g., estradiol reduces the half-life for ER from ~5 days to 3-4 h). However, the same parameter of GR is ligand-independent (20-25 h). Ligand-dependent degradation of human ER and possibly hen PR involves a ubiquitin-proteasome pathway operating for selective degradation of short-living regulatory proteins [97, 98].

Ubiquitin is a small protein which covalently labels proteins for subsequent degradation by conjugation with their lysine residues. The resultant chains of polyubiquitin-degraded protein are recognized and degraded by the 26S-proteasome. Besides labeling for subsequent degradation, ubiquitin conjugation may also have other regulatory functions (e.g., change of intracellular protein localization) [99, 100]. This conjugation is mediated by a ubiquitin activating enzyme (UBA, E1), a family of ubiquitin carrier proteins (UBCs, E2), and a ubiquitin-protein ligase E3 with different substrate specificities. Some ubiquitin-protein ligases can act as coactivators of nuclear receptors. In these proteins, ligase and coactivator functions are independent and spatially separated. For example, Anhelman syndrome associated mutations of the protein E6-AP are accompanied by a loss of its ubiquitin-protein ligase activity but not coactivator function with respect to the nuclear receptors [46] (Fig. 6). (This syndrome is an inherited neurological disorder characterized by mental retardation, seizures, slowed speech, etc.)

Degradation of VDR also involves the proteasome mechanism. Direct ligand-dependent interaction of one

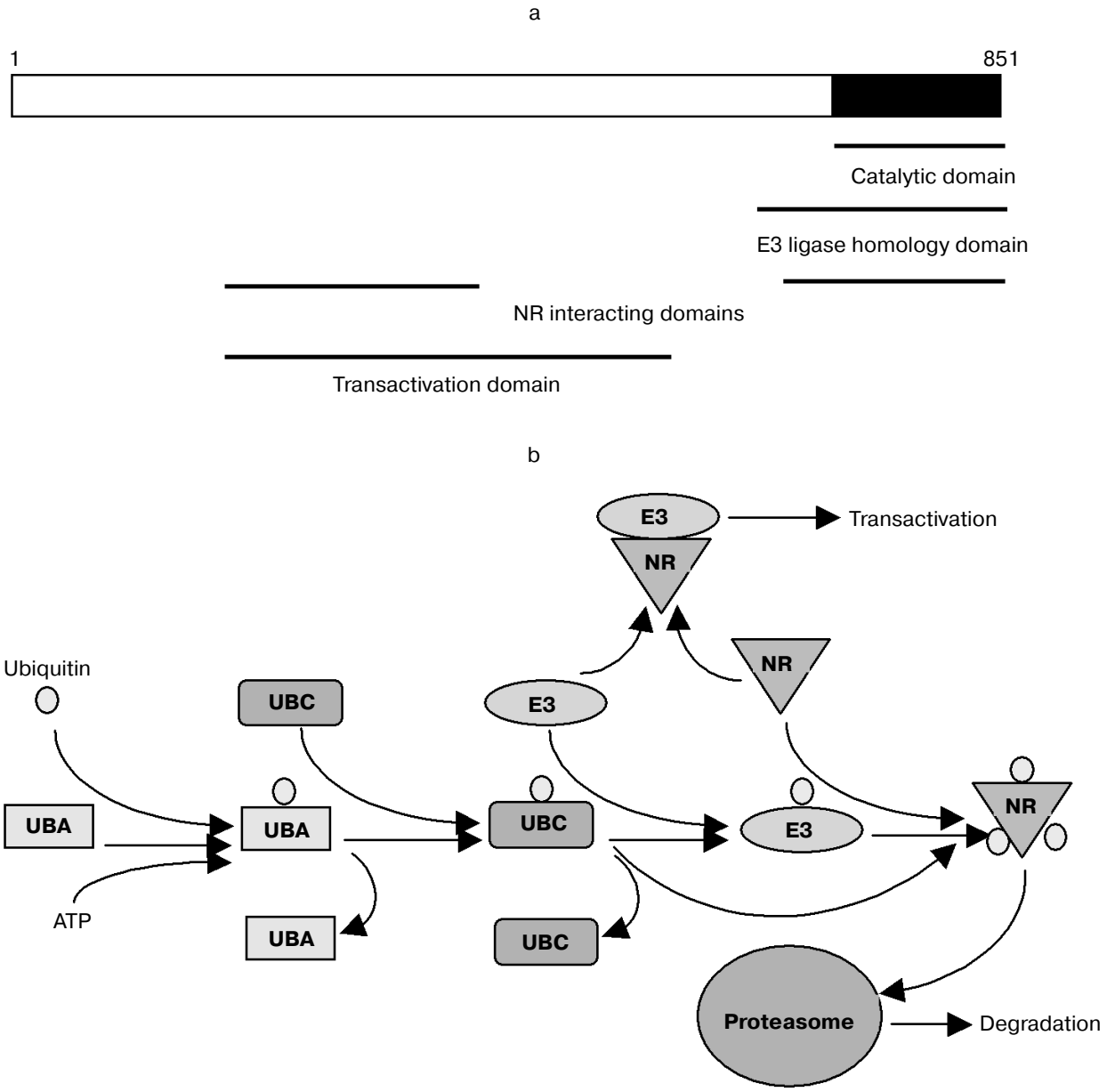


Fig. 6. Ubiquitin-protein ligase in nuclear receptor functioning: a) structure functional organization of E6-AP ubiquitin-protein ligase; b) coupling between signal transduction and receptor degradation involving ubiquitin-protein ligase. UBA) ubiquitin activating enzyme (E1); UBC) ubiquitin conjugating enzyme (E2); E3) ubiquitin-protein ligase; NR) nuclear receptor.

of the integral proteasome proteins, gal1 suppressor (SUG1), with AF-2 VDR region is the ultimate precondition for the receptor degradation [101, 102]. It should be noted that SUG1 (also known as Trip1) may also function as coactivator. The necessity of AF-2 for proteasome degradation was demonstrated for ER [103].

The other pathway of desensitization may include blockade of receptor interaction with coactivators due to their acetylation by the same cofactors (p300/CBP) involved into signal transduction [104]. Thus, desensitization and signal transduction by nuclear receptor may be interrelated. This interrelationship is typical also for membrane receptors. For example, β-arrestins stimulate internalization of receptors coupled to G-proteins and formation of complexes involved in the MAPK cascade [105].

9. REGULATION OF NUCLEAR RECEPTOR ACTIVITY BY PHOSPHORYLATION

Nuclear receptors can be substrates for various protein kinases. This provides control of the receptor activi-

ty by other regulatory factors including auto-, para-, and endocrine factors and also factors regulating the cell cycle (see for review [106-108]). Phosphorylated residues are preferentially localized in A/B domain of receptors including ligand-independent transactivation function AF-1. Some of these residues are phosphorylated constitutively while the phosphorylation of others depends on ligand. In the case of GR only agonists (but not antagonists) exhibit phosphorylation stimulating activity. The population of the receptor is highly heterogenic in the phosphorylation pattern. The phosphorylated amino acid residues of the receptors are located within consensus motifs recognized by various protein kinases including cyclin-dependent kinases (CDK), mitogen activated protein kinases (MAPKs), casein kinase II (CK II), calmodulin-dependent protein kinase II (CaMK II), glycogen synthase kinase 3 (GSK3), DNA-dependent protein kinase (DNA-PK), and others. In fact, some protein kinases (MAPK, cyclin E/CDK2, cyclin A/CDK2) can phosphorylate receptors *in vitro*. The phosphorylation of various sites causes different (even opposite) changes of the functional activity of the receptors. For example, GR phosphorylation by CDK *in vitro* increases the transcription activity of GR whereas MAPK phosphorylation causes the opposite effect. (Various hormones, e.g., glucagon, inhibit MAPK activity via cAMP and protein kinase A (PKA), whereas tyrosine kinase activating factors such as insulin activate it. Growth factors belonging to transforming growth factor β (TGFβ) inhibit the activity of CDKs, whereas activators of CDK-activating kinases (CAKs) stimulate it.) Increase of GR phosphorylation in G2/M phase of the cell cycle is accompanied by significant decrease in transactivation activity of the receptor, but it does not influence its repressor activity on negative HREs. In contrast to GR phosphorylation of ER by MAPK (as well as PKA) resulted in increase rather than the decrease in receptor transcription activity and this activity occurs in the absence of the hormonal ligand. This fact explains estrogen-like effect of epidermal growth factor (EGF) activating MAPK in uterus and mammary gland tumors. The receptor phosphorylating activity may exert different effects on its activity in various cells and at different promoters. The latter apparently reflects diversity of coregulators interacting with the receptor.

10. PECULIARITIES OF SOME NUCLEAR RECEPTORS

Receptors of steroid and thyroid hormones, α-retinoic acid, and dihydroxyvitamin D_3 were cloned in 1986-1987. Since they have been studied and analyzed in detail (see for review [109]) we leave them out of consideration, because this section is devoted to less known "orphan" receptors. Biochemistry and physiology of these orphan receptors were highlighted in several reviews [5, 6, 10, 21, 110]. So we have included references on more recent publications.

Receptors of peroxisome proliferation activators (PPARs) are the best studied orphan receptors (see review [5]). These proteins play the key role in the regulation of energy and lipid metabolisms. The particular interest in these receptors is explained by the fact that some antidiabetic, hypolipemic, and antiinflammatory drugs act via PPARs. PPARα is intensively expressed in the heart, liver, kidneys, intestine, and brown adipose tissue, where the rate of β-oxidation of fatty acids is especially high. Various factors (e.g., stress, glucocorticoids, insulin) control PPARα expression. PPARβ is expressed in many cell types and tissues, including brain, kidneys, intestine, and Sertoli cells. PPARγ isoforms are products of alternative splicing. They exhibit distinct tissue specific expression. The PPARγ1 isoform is abundant in spleen, intestine, and white adipose tissue, whereas PPARγ2 predominates in white and brown adipose tissues. PPAR-response elements (PPREs) are direct repeats containing spacer of 1 bp and 5′-extension (consensus 5′-AACT AGGNCA A AGGTCA). They have been recognized in genes regulating carbohydrate and lipid metabolism. PPARs function as heterodimers with RXR and the effects of ligands for both dimer partners are additive. For example, the thiazolidine effect on hyperglycemia and hypertriglyceridemia during starvation was potentiated by simultaneous administration of 9-*cis*-retinoic acid. Besides RXR, TRβ and LXRα can be heterodimerization partners of PPAR. Many ligands activate PPARα. These include fatty acids, eicosanoids, carbaprostacyclin, non-steroid antiinflammatory drugs, and leukotriene β_4 (LTB_4). PPARβ and PPARγ are also activated by common PPAR ligands (docosahexenic acid and some prostaglandins). PPARγ is specifically activated by thiazolidines (antidiabetic medicines), prostaglandin metabolite, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (PGJ_2), polyunsaturated fatty acids, and by non-steroid antiinflammatory medicines (e.g., ibuprofen). Relatively low selectivity of PPARs with respect to various ligands and relatively low affinity are determined by following properties of the ligand-binding pocket of these receptors. 1) The volume of this pocket significantly exceeds the ligand volume. 2) T- or Y-like shape of this pocket provides reasonable accommodation for various ligands. 3) The pocket contains an additional entry. Besides low molecular weight ligands, protein kinases (e.g., MAPK) regulate PPARs activity. Common nuclear receptor factors (SRC-1, CBP/p300, SMRT) and more selective coactivators of PPARγ (PGC-1 and PGC-2) act as coregulators of PPARs transcription activity.

Physiologically PPARα and PPARγ act as antagonists, because the former stimulates lipid catabolism, whereas the latter stimulates lipid anabolism. PPARα stimulates fatty acid utilization by increasing expression of acyl-CoA synthetase, acyl-CoA oxidase, and ketoacyl-

CoA thiolase. After several cycles of β-oxidation of fatty acids in peroxisomes, shortened products are transported into mitochondria for subsequent degradation. PPARα also stimulates the mitochondrial stage of β-oxidation by induction of carnitine-palmitoyl transferase and acyl-CoA dehydrogenase specific for moderate aliphatic chains. PPARα-dependent stimulation of fatty acid-utilization also involves induction of mitochondrial 3-hydroxy-3-methyl-glutaryl-CoA synthase responsible for synthesis of ketone bodies. PPARα attenuates expression of hepatic apolipoproteins involved in triglyceride export from the liver, and this also promotes fatty acid degradation in the liver. Starvation and stress exhibit similar effects on metabolism due to PPARα involvement in realization of these effects. Starvation is accompanied by release of free fatty acids acting as PPARα activators. PPARα-knockout mice are characterized by decreased peroxisomal β-oxidation, ketogenesis, reduced glycogen content (due to inhibition of gluconeogenesis); the latter is accompanied by severe hypoglycemia in starvation, and impairment of bile acid formation due to decreased expression of cholesterol 12α-hydroxylase [111]. PPARα also stimulates ω-oxidation of lipids due to induction of mixed type oxygenases of the CYP4A subfamily.

PPARγ is one of the factors determining adipocyte differentiation, particularly appearance of insulin-dependent glucose transport. In differentiated adipocytes PPARγ increases expression of lipoprotein lipase, fatty acid transporters, acyl-CoA synthase, malic enzyme, phosphoenol pyruvate carboxy-kinase, GLUT4 glucose transporter, insulin signal transducing elements, and factors responsible for triglyceride accumulation. The antidiabetic effect of thiazolidines restoring insulin sensitivity in type II diabetes mellitus may be (at least partially) attributed to the involvement of PPARγ. Stimulated free fatty acid influx to adipocytes is suggested to restore muscle glucose utilization inhibited by fatty acid. The other possible mechanism of antidiabetic drugs may involve PPARγ-dependent inhibition of adipocyte production of TNFα and leptin, which possess potent lipolytic effects. This is also accompanied by reduction (or disappearance) of inhibitory effect of TNFα on production of GLUT4 and insulin receptor phosphorylation and inhibitory effect of leptin on pancreatic insulin secretion. PPARγ-knockout mice are characterized by embryonal death on the 10th day due to impairments in final differentiation of trophoblast, placental vascularization, and myocardial thinning; blockade of these effects did not abolish the fatal end and the fetuses died at the end of the prenatal period due to lipodystrophy and multiple hemorrhages [112].

PPARs are involved in the inflammatory response in two ways. Being PPARα ligands, eicosanoids accelerate their own degradation. PPARγ inhibits the effects of transcription factors (AP-1, STATs, NF-κB), stimulating production of proinflammatory cytokines. Some evidence exists for the involvement of PPARs in atherogenesis and carcinogenesis. It is suggested that PPARγ ligands promote monocyte differentiation into macrophages and their subsequent conversion into foam cells. Oxidized derivatives of linoleic acid, the components of low density lipoproteins, may cause activation of PPARγ of resident macrophages accompanied by expression scavenger receptor CD36. (The latter increases lipid accumulation in atherosclerotic plaque.)

PPARβ plays an important role in implantation and decidualization of the uterus. Knockout of cyclooxygenase-2 (COX2) gene that produces natural PPARβ ligand, PGI_2, causes impairment of these processes, whereas administration of PPARβ ligands (PGI_2, carbaprostacyclin, L 165,041) to these animals restores decidualization and implantation.

The members of the Rev-Erb group are encoded by genes located on the DNA strand which is opposite to the strand carrying genes encoding TRα and TRβ. These proteins interact with DNA as monomers or homodimers. These proteins lack AF-2 function and so they are constitutive repressors of transcription. Their repressor action requires the presence of corepressors, NCoR, SMRT, SUN-CoR.

Members of the ROR group act in RXR-independent manner; they function as monomers (see for review [21]). The expression of RORβ occurs almost exclusively in the neuronal tissue related to sensoric, neuroendocrine, and limbic systems. RORγ is preferentially expressed in skeletal muscles, liver, kidneys, hepatocytes, whereas expression of its splicing variant RORγ-t was found in thymus. RORα is widely expressed in brain structures, hypophysis, adipocytes, liver, cartilage, skin, and testicles. Melatonin is a natural high affinity ROR ligand. Antiinflammatory thiazolidines (distinct from the antidiabetic medicines of this group acting at PPAR) are highly effective ligands of ROR. RORγ-t and RORα are involved into the immune response; RORα ligands stimulate formation of interleukins 2 and 6 in peripheral blood mononuclear cells. ROR ligands regulate expression of 5-lipoxygenase, one of the key enzymes in biosynthesis of antiinflammatory leukotrienes. Mutations or knockout of *RORα* gene were accompanied by some neurological abnormalities (tremor, imbalance, small body size, impairments in the development of cerebellar Purkinje cells and olfactory bulbs). Such animals are also characterized by significantly reduced expression of intestinal apolipoprotein A-1 involved in lipid transport in high density anti-atherogenic lipoproteins. This increases manifestations of atherosclerosis. Knockout of RORβ causes retinal degeneration and blindness.

LXRα is preferentially expressed in liver, and also in intestine, kidneys, and spleen. LXRβ expression is ubiquitous. Oxysterols act as selective activators of LXR and 22(R)- and 24(S)-hydroxycholesterol, 24(S),25-epoxycholesterol, and 7α-hydroxycholesterol are the most

active among them. Oxysterols are intermediates in synthesis of steroids and bile acids. LXRα-knockout mice are characterized by blockade of cholesterol metabolism, its accumulation in the liver and impaired regulation of the key enzyme of bile acid biosynthesis CYP7A (7α-hydroxylase). In contrast to normal animals, in these mice the increase of cholesterol level does not result in compensatory decrease in cholesterol intake and the increase of bile acid synthesis. Low cholesterol diet did not cause significant changes. LXRα knockout is accompanied by reduced fatty acid biosynthesis [113]. Adipocytes and peritoneal macrophages express both forms of LXR (α and β) which are involved in control of lipid metabolism. Knockout of either form results in partial blockade of oxysterol effect on the expression of apo-E, whereas double knockout totally abolishes this regulatory effect [114].

FXR is activated by high concentrations of farnesol, the isoprenoid intermediate of cholesterol biosynthesis. Bile acids are potent inducers of FXR [115]. FXR is apparently involved in concerted regulation of bile acid reutilization and *de novo* synthesis. FXR stimulates gene expression of intestine bile acid binding protein (IBABP) and inhibits promoter activity of the *CYP7A* gene [116].

PXR is preferentially expressed in the liver and intestine. Antibiotics rifampicin, synthetic pregnanes, glucocorticoid and other steroid agonists and antagonists (e.g., dexamethasone, pregnenolone-16α-carbonitrile, antimineralocorticoid spironolactone, antiandrogen ciproterone acetate, antiestrogen tamoxifen, phytoestrogens) act as its activators. These ligands in PXR-dependent manner stimulate gene expression of cytochrome P450 3A family involved in steroid hydroxylation and metabolism of drugs and other xenobiotics. Natural high affinity PXR ligands remain unknown. It is possible that PXR mediates many known side effects of rifampicin. For example, long-term treatment with this antibiotic increases the rate of metabolic clearance of steroids. Rifampicin also causes rapid reduction of administered steroids (e.g., in patients with Addison's disease treated with corticosteroids or in women using oral contraceptive preparations). BXR (an RXR ortholog found in clawed frog embryos) binds benzoate metabolites (alkyl esters of amino- and hydroxybenzoic acid) which are possibly involved in the regulation of morphogenesis. These ligands share structural similarity with *p*-aminobenzoic acid, the nutritional precursor of folic acid.

In contrast to the majority of receptors, CAR ligands (androstanol, androstenol) decrease rather than increase transcription activity of the receptor; they cause dissociation of the receptor–coactivator complex. (This situation is similar to nuclear receptor on negative HREs.) Phenobarbital and its analogs restore CAR activity (with respect to induction of genes encoding proteins of cytochrome P450 2B family) by competing with androstanes.

PXR, CAR, PPAR, and some other nuclear receptors may be involved in nonspecific adaptation of organisms due to signal transduction from endogenous and exogenous ligands, inducing induction of cytochromes P450, which metabolize toxic xenobiotics [117] and stimulate subsequent biliar excretion [118].

HNF4α is intensively expressed in the liver and some other organs, whereas HNF4γ is not expressed in the liver (in spite of its name). Homodimers of the members of this group bind HREs, which exist as direct repeats with spacer of 1 bp. Such HREs were found in many liver-specific genes and genes encoding proteins involved in regulation of carbohydrate, lipid, and amino acid metabolism. HNF4α binds various ligands including long chain acyl-CoA thioesters; poly- and monounsaturated acyl-CoA thioesters inhibit constitutive receptor activity, whereas saturated ones increase (e.g., palmitoyl-CoA) or decrease (e.g., stearoyl-CoA) it [119]. Mutations of the *HNF4α* gene are associated with maturity-onset diabetes of the young [120]. Knockout of this gene in mice causes ectodermal apoptosis, impairments of mesodermal differentiation, and embryonal death [121]. Later damage of this gene is accompanied by decreased expression of glycolytic enzymes and glucose and fatty acid transporters.

Specific binding of 9-*cis*-retinoic acid and noncyclic terpenoids (methoprene and phytanic acid) activates RXR (α, β, γ). Phytanic acid, the product of chlorophyll decay, is a nutrient, and methoprene is an industrial pollutant. RXR may act as the homodimer (RXR/RXR) or a heterodimer with other nuclear receptors (Fig. 2). Activation of non-permissive heterodimers requires obligatory presence of RXR partner ligand (for dimers RAR/RXR, TR/RXR, VDR/RXR). Activation of permissive homodimers requires the presence of RXR ligands or its partner ligand. Knockout of *RXRα* gene results in impairments of morphogenesis of placenta, heart, and eyes, accompanied by embryonal death. Damage of this gene in hepatocytes of adult animals is accompanied by multiple impairments of those metabolic processes (e.g., cholesterol metabolism, cytochrome P450 expression) which are regulated by RXR heterodimers with other nuclear receptors (PPARα, CARβ, PXR, LXR, FXR). This is also accompanied by shortening of hepatocyte life-time especially during regeneration and increase of cell ploidy [122, 123]. Damage of the gene encoding RXRβ is accompanied by disturbances in spermatogenesis and premature morphogenesis of lung alveoles [124]. Knockout of *RXRγ* gene causes impairments in the development or activation of cholinergic neurons of brain extrapyramidal system [125], hippocampal functions related to orientation and memory [126].

Receptors of the TR2 group are highly expressed in testicles, where they preferentially function as transcription repressors. These receptors interact with DNA as homo- or TR2/TR4 heterodimers. Their HREs are direct

repeats, where the size of the spacer varies from 1 to 5 bp. TR4 may also act as the monomer [127]. The repressor effect includes direct interaction with histone deacetylases.

TLX preferentially interacts with modified HRE, in which the second consensus G is replaced for A. TLX is preferentially expressed in the embryonal forebrain. TLX-knockout mice are characterized by hypoplasia of forebrain structures (particularly, olfactory bulbs, amygdala, and dentate gyrus). Such animals become very aggressive, and the females refuse to care for the offspring [128].

Members of the COUP-TF group act as transcription repressors inhibiting stimulatory effects of numerous nuclear receptors. In homodimeric form (as well as heterodimers with RXR) they can interact with many types of HRE. Knockout of *COUP-TFI* gene in mice results in impaired development of the peripheral nervous system; the latter is accompanied by disturbances of sucking and swallowing in neonates followed by their subsequent death from starvation and dehydration [129]. Knockout of the *COUP-TFII* gene results in embryonal death on the 10th day of development due to defects in angiogenesis and heart development [130].

Estrogen receptors share some properties of both steroid receptors and other nuclear receptors. Like other steroid receptors, ERs interact with heat shock proteins and their HREs are organized as palindromes. However, nucleotide sequences of these elements are close to HREs for nuclear receptors distinct from the steroid receptors.

ERR receptors are widely expressed in the organism. They can interact with DNA in both monomeric and dimeric forms [131]. In the dimer form they recognize estrogen-response elements. Transcription activity of ERR is regulated by synthetic estrogen analogs and pollutants [7-9, 132]. Endogenous ligands of ERRs remain unknown. Knockout of *ERRβ* gene results in impaired formation of placenta and embryonal death at the 10.5th day of development [133]. Pilot experiments revealed that knockout of *ERRα* gene causes disturbances in lipid metabolism; this correlates with the preferential expression of this receptor in tissues where β-oxidation of fatty acids is especially intensive [6].

Steroid hormone receptors GR, MR, PR, and AR possess some characteristic features that make them different from other nuclear receptors. They interact with the heat shock protein Hsp90, blocking DNA-binding domain of these receptors. The hormonal ligand binding causes dissociation of Hsp90. The second characteristic feature of this receptor sub-group consists of the type of recognized HREs-palindrome structures representing 6-membered half-sites, which are separated by a spacer of 3 bp.

Receptors of the NGFI-B subfamily are preferentially expressed in the cells of nervous system and represent a part of the immediate response to such stimuli as growth factors and depolarization. These receptors are also expressed in hypophysis, adrenals, liver, and some other organs. They interact with DNA in the monomeric form and as homo- and heterodimers (with RXR). Nuclear localization, binding to DNA, and transcription activity of NGFI-B are sensitive to regulation by phosphorylation. Damage to the *NGFI-B* gene causes insignificant effect on the mouse genotype [134]; however, knockout of the *NURR1* gene is accompanied by the death of animals soon after birth. These mice are characterized by underdeveloped midbrain dopaminergic neurons [135].

SF1 is preferentially expressed in steroidogenic tissues and also in hypothalamus and hypophysis. This monomeric receptor interacts with HREs of several genes encoding enzymes involved in biosynthesis of steroid hormones, anti-Müllerian hormone, luteinizing hormone, and ACTH receptor. Its transcription activity is under control by cAMP-dependent phosphorylation stimulated by ACTH and luteinizing hormone [136]. Phosphorylation of AF-1 domain causes recruitment of SRC-2 coactivator. Pilot data that 25-, 26-, and 27-hydroxycholesterols are activating ligands of SF1 [137] were not confirmed in subsequent studies [138, 139]. Knockout of the *SF1* gene in mice results in agenesis of gonads and adrenals, impairments of sexual differentiation, and death of animals soon after birth [140].

GCNF is preferentially expressed in the germ cells; it plays an important role in embryogenesis. Knockout of the *GCNF* gene in mice is accompanied by embryonal death on the 10.5th day of the development possibly due to cardiovascular insufficiency and defects of organogenesis (the neurotubule remains opened, somite amount is reduced, ectopic tailbuds appear under conditions of impaired connections between chorion and allantois [141]).

DAX-1 and SHP receptors differ from other nuclear receptors by the absence of typical DBD. DAX-1 recognizes DNA hairpin structures, whereas SHP does not interact with DNA. DAX-1 is expressed in hypothalamus, hypophysis, adrenals, and gonads. Together with SF1 and product of the *SRY* gene it determines sexual differentiation and the development of gonads and adrenal cortex. Forming heterodimers with SF1 DAX-1 inhibits transcription activity of SF1 [136]. Male DAX-1-knockout mice are infertile due to impairments in differentiation and proliferation of Leydig's and Sertoli's cells in testes. One third of one year old animals (and older) suffer from reproductive tract tumors [142]. SHP forms heterodimers with many nuclear receptors, and this results in their reduced transcription activity. This effect is realized by two mechanisms: inhibition of binding to DNA and activity of own repressor function located in the N-terminal region of SHP [143]. Mutations of the *SHP* gene are associated with obesity [144].

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