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Review

NR4A nuclear receptors are orphans but not lonesome

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ABSTRACT

The NR4A subfamily of nuclear receptors consists of three mammalian members: Nur77, Nurr1, and NOR-1. The NR4A receptors are involved in essential physiological processes such as adaptive and innate immune cell differentiation, metabolism and brain function. They act as transcription factors that directly modulate gene expression, but can also form trans-repressive complexes with other transcription factors. In contrast to steroid hormone nuclear receptors such as the estrogen receptor or the glucocorticoid receptor, no ligands have been described for the NR4A receptors. This lack of known ligands might be explained by the structure of the ligand-binding domain of NR4A receptors, which shows an active conformation and a ligand-binding pocket that is filled with bulky amino acid side-chains. Other mechanisms, such as transcriptional control, post-translational modifications and protein–protein interactions therefore seem to be more important in regulating the activity of the NR4A receptors. For Nur77, over 80 interacting proteins (the interactome) have been identified so far, and roughly half of these interactions has been studied in more detail. Although the NR4As show some overlap in interacting proteins, less information is available on the interactome of Nurr1 and NOR-1. Therefore, the present review will describe the current knowledge on the interactomes of all three NR4A nuclear receptors with emphasis on Nur77.

1. Introduction

The nuclear receptor family of 48 proteins comprises steroid hormone receptors, nuclear receptors that heterodimerize with retinoid X receptors (RXRs), and a number of so called orphan receptors. A typical nuclear receptor contains a central double zinc finger DNA-binding domain (DBD), a carboxyl-terminal ligand-binding domain (LBD) composed of 12 α-helices, and an unstructured amino-terminal domain (N-term) (Fig. 1) [1]. The ligands of nuclear receptors are usually small, non-protein compounds such as steroid hormones, retinoids, fatty acids or cholesterol derivatives. In this review we describe the NR4A-subfamily of orphan receptors comprised of Nur77 (also known as NR4A1, TR3, NGFI-B), Nurr1 (NR4A2) and NOR-1 (NR4A3), for which no ligand has been identified yet. The amino acid sequences of the different NR4A DBDs are almost identical, whereas the LBDs show a sequence similarity of 58–65%. Meanwhile, the N-terminal domains are most divergent, with only 26–28% amino acid sequence similarity between the NR4As.This domain is therefore also the most likely to exhibit diversity in protein–protein interactions (Fig. 1). NR4A receptors are involved in a plethora of cellular processes and their activity is mainly regulated through alterations in gene expression, post-translational modifications and interactions with coregulatory proteins. In this review we put together the wealth of information that is available on the interactome of the NR4A receptors with a focus on Nur77, for which most protein–protein interactions have been described. We categorized the Nur77-binding proteins into three groups: transcription factors, transcriptional coregulators and kinases. The proteins interacting with Nurr1 and NOR-1 are described in a separate part of the review. The protein–protein interactions described in this review are summarized in Tables 1 through 5, while the protein–protein interactions of Nur77 that have a known binding site are also shown schematically in Fig. 2.

2. Interactions between Nur77 and other transcription factors

Nur77 acts as a transcription factor with its two zinc fingers in the DBD mediating direct binding to DNA. Nur77 binds as a monomeric factor on the NGFI-B response element (NBRE; AAAGGTCA) or as a homodimer to Nur-response elements (NurREs; TGATATTTn$_6$AAATGCCA) in

Abbreviations: 6-MP, 6-mercaptopurine; 9-cis-RA, 9-cis-retinoic acid; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; Cam-kinase, calcium/calmodulin-dependent protein kinase; CREB, cAMP response element binding protein; DBD, DNA-binding domain; DSB, DNA double-strand break; E2, 17-β-estradiol; HAT, histone acetyltransferase; HDAC, histone deacetylase; HIF-1α, hypoxia inducible factor-1α; HPA, hypothalamo-pituitary-adrenal; LBD, ligand-binding domain; LXRs, liver X receptors; MDM2, mouse double minute 2; NBRE, NGFI-B response element; NES, nuclear export sequence; N-term, amino-terminal domain; NurRE, Nur-response elements; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol(3,4,5)phosphate; PKC, protein kinase C; POMC, pro-opiomelanocortin; PPARs, peroxisome proliferator-activated receptors; βRARE, RA-response element of the RARβ promoter; RXRs, retinoid X receptors; StAR, steroidogenic acute regulatory protein; Treg, regulatory T cells

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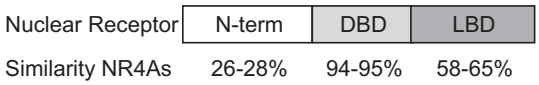

Fig. 1. Schematic representation of the domain structure of nuclear receptors. Nuclear receptors are composed of an N-terminal domain (N-term), a central DNA-binding domain (DBD) and a ligand-binding domain (LBD). The amino acid similarity between the individual domains of Nur77 with Nurr1 and NOR-1 is given in percentages below the domains.

promoter sequences of its downstream target genes. The interaction of Nur77 with other transcription factors modulates its transcriptional activity. Vice versa, such protein–protein interactions can also result in enhancement or inhibition of the activity of the interacting transcription factor itself (see Table 1). The following paragraphs will focus on those interactions between Nur77 and other transcription factors for which the outcome on transcriptional activity has been determined in a physiological context.

2.1. Nur77 in the regulation of endocrine signals and steroid hormone synthesis

Nur77 is expressed in endocrine tissues and in organs that are crucial for steroid hormone synthesis such as the adrenal glands, the pituitary gland and the testes. The first functional NurRE was identified in the promoter of the pro-opiomelanocortin (POMC) gene of pituitary-derived AtT-20 cells [2]. Nur77 can bind this NurRE either as a homodimer or as a heterodimer with either one of the other two NR4A receptors Nurr1 and NOR-1. Interestingly, it was shown that these heterodimers enhance POMC gene transcription more potently than homodimers of Nur77 do, suggesting that there is interdependency between the NR4A receptors in activating their target genes [3].

The NurRE sequence in the POMC promoter also partially overlaps with a STAT1-3 response element. Philips et al. showed that Nur77 and STAT1-3 bind simultaneously to this so called NurRE-STAT composite site and synergistically enhance transcription of the POMC gene. However, Nur77 and STAT1-3 do not interact directly, which suggests that one or more facilitating factors are involved in NurRE-STAT driven transcription. Mynard et al. showed that this third factor is cAMP response element binding protein (CREB), which binds both STAT1-3 and Nur77 and indirectly enhances transcription of the POMC gene by facilitating the synergistic activation of the NurRE-STAT composite site by STAT1-3 and Nur77 [4].

Nur77 also plays an important role in the steroidogenic acute regulatory protein (StAR)-mediated testosterone production by Leydig cells. StAR is required for the transport of cholesterol through the mitochondrial membrane to initiate steroid hormone synthesis. Nur77 binds to an NBRE in the StAR promoter, which is in close proximity to an AP-1 response element. In response to cAMP stimulation c-Jun and Nur77 synergistically increase StAR gene expression [5], presumably through a direct interaction between c-Jun and the LBD of Nur77 [6]. On the other hand, c-Jun has also been shown to suppress expression of the hydroxylase P450c17 gene by blocking the DNA-binding activity of Nur77 in response to stimulation of Leydig cells with reactive oxygen species [7]. The effect of c-Jun on the transcriptional activity of Nur77 therefore seems to depend on other factors as well. One of these factors could be the atypical nuclear receptor DAX1 (NR0B1), which lacks a DBD and associates with multiple coregulatory proteins. DAX1 binds Nur77 directly and represses its ability to enhance transcription of the previously mentioned P450c17 gene. This inhibition is mainly the result of competition

Table 1
Transcription factors interacting with Nur77.

Transcription factor	Also known as	Interacting domain of Nur77	References
Coactivators of Nur77			
CREB	CREB1	N-term	[4]
NOR-1	NR4A3; MINOR		[3]
Nur77	NR4A1; TR3; NGFI-B		[3]
Nurr1	NR4A2; NOT		[3]
RXRγ	NR2B3; RXRC; RXRgamma	N-term; LBD	[15]
Coactivated by Nurt77			
c-Jun	AP-1; AP1; c-Jun; C-JUN; cJun	LBD	[7]
RXRα	NR2B1; RXR alpha	LBD	[16,17]
SP1			[34,35]
SP4			[34,35]
p53	TP53; TRP53	DBD–LBD	[67]
Corepressed by Nur77			
COUP-TFI	NR2F1; EAR3		[26]
COUP-TFII	NR2F2; COUPTFB		[26]
GR	NR3C1; GCR; GRL; GCCR	DBD	[11]
NF-κB (p65)	EBP-1; KBF1; NF kappa B; NFκB		[42]
p53	TP53; TRP53	DBD–LBD	[38]
RXRα	NR2B1; RXR alpha	LBD	[21]
Corepressor of Nur77			
AR	NR3C4	N-term	[12]
DAX1	NR0B1; DAX-1; DSS	LBD	[8]
ERα	NR3A1; ER; ESR1; Era; ESRA;	DBD–LBD	[13]
GR	NR3C1; GCR; GRL; GCCR	DBD	[9,10]
c-JUN	AP-1; AP1; c-Jun; C-JUN; cJun	LBD	[6]
NF-κB (p65)	EBP-1; KBF1; NF kappa B; NFκB	LBD	[14]
Notch-1	hN1; TAN1	DBD–LBD	[37]
PML	MYL; TRIM19	DBD	[36]
RARα	NR1B1; RAR		[22]
RXRα	NR2B1; RXR alpha	DBD	[22]
SHP	NR0B2; SHP-1	N-term	[28]
Other function			
ß-catenin	CTNNB1; armadillo	Nur77 inhibits β-catenin signaling by inducing its degradation	[33]
RXRα	NR2B1; RXR alpha	Translocation of hetero-dimers to the cytoplasm	[17,20,21,24]
VHL	pVHL; VHL1	Nur77 inhibits VHL-associated E3-ligase activity	[40]

for the interaction with Nur77 between DAX1 and steroid receptor coactivator-1 (SRC-1), which is a transcriptional coactivator of Nur77 that will be described in more detail later on in this review [8].

Recruitment of Nur77 to the StAR promoter is inhibited by the synthetic glucocorticoid receptor (GR) ligand dexamethasone [5]. GR and Nur77 interact directly through their DBDs and subsequent transrepression of Nur77 involves the formation of a transcriptionally inactive complex comprised of GR, Brg1, Brm and HDAC2 on the POMC promoter [9–11]. Interestingly, dexamethasone stimulation increases DAX1 expression levels, suggesting that DAX1 also plays a role in GR-mediated transrepression of Nur77 [11]. Additionally, Nur77 was shown to reciprocally inhibit the activity of GR in CV-1 cells, presumably through the formation of the previously mentioned transcriptionally inactive complexes [11].

Steroid hormone synthesis is tightly regulated, and both the androgens and 17-β-estradiol (E2) activate negative feedback loops involving Nur77. Upon ligand binding, the androgen receptor (AR) can inhibit Nur77 activity through interaction of the N-terminal domain of Nur77 with the DBD of AR. Competition for SRC-1 binding was identified as the underlying mechanism for this inhibition of Nur77 activity, analogous to DAX1 inhibition [12]. The estrogen receptor alpha (ERα) binds with its DBD-hinge region the DBD–LBD domains of Nur77, thereby blocking Nur77 DNA-binding and thus activity [13]. Finally, TNFα was shown to inhibit the expression of steroidogenic enzymes through induction of NF-κB and subsequent inhibition of Nur77 transcriptional activity [14]. The interaction between Nur77 and NF-κB will be described in more detail below.

2.2. Interaction between Nur77, RXRs and RARs modulates apoptosis

The three retinoid X receptors (RXRα, β and γ) can form heterodimers with multiple nuclear receptors and bind 9-*cis*-retinoic acid (9-*cis*-RA) in their ligand-binding pocket. RXRs are silenced when dimerized with the retinoic acid receptors (RARs). In contrast, both Nur77 and Nurr1, but not NOR-1, can form activating heterodimers with RXRs on DR5 response elements ($GGTTCAn_5AGTTCA$). So far, Nur77 has been shown to interact with both RXRα and RXRγ. RXRγ was found to enhance Nur77 transcriptional activity, which was further increased upon addition of the RXR ligand 9-*cis*-RA. This interaction can therefore make Nur77 indirectly responsive to retinoic acids [15]. Similarly, Nur77 can form heterodimers with RXRα that are also activated by 9-*cis*-RA with Nur77 acting as an activator of RXRα-dependent transcription [16]. In the absence of RXRα activating ligands, Nur77 and RXRα heterodimerize via their DBDs. Ligand binding by RXRα induces a conformational change, which alters the dimerization interface and allows for dimerization of Nurr77 and RXRα through their LBDs. This interaction masks the leucine-rich nuclear export signal (NES) of RXRα, thereby preventing nuclear export and increasing transcription of RA-response genes [17]. 9-*cis*-RA was also shown to arrest Nur77-RXRα heterodimers in the nucleus of neuronal cells, thereby protecting these cells from glutamate-induced apoptosis [18] and in pluripotent embryonic stem cells [19]. In contrast to the above findings, 9-*cis*-RA was shown to induce rather than inhibit nuclear export and mitochondrial localization of Nur77-RXRα dimers in gastric cancer cells [20,21]. Futhermore, Nur77 was shown to decrease RXRα transcriptional activity by competing with the coactivator CBP/p300 for RXRα binding in these cells [21]. Finally, in Jurkat cells RA-dependent repression of Nur77 activity by both RAR and RXR has been described [22]. Together these data illustrate that the outcome of Nur77-RXR interaction is highly dependent on the cell type, the stimulus and the presence of retinoic acids. Furthermore, retinoic acids are unstable and are metabolized in different ways in distinct cell types so one may speculate that a specific metabolite of 9-*cis*-RA is responsible for nuclear export and regulation of activity of Nur77-RXRα dimers. This hypothesis remains to be tested however [23].

Other stimuli also play a role in modulating the interaction between Nur77 and RXRα: in response to either apoptotic stimuli or nerve growth factor (NGF) treatment, Nur77 translocates from the nucleus to the cytoplasm as part of a Nur77-RXRα heterodimer. Although these two stimuli have similar downstream effects (nuclear export of Nur77-RXRα), the exact mechanism through which they achieve this effect seems to be either fundamentally different or very dependent on cell type [17,24]. In PC12 cells NGF-induced translocation requires the presence of a NES in the LBD of Nur77 thereby regulating the subcellular distribution of RXRα [24]. On the other hand, RXRα is required for shuttling of Nur77 from the nucleus to the cytoplasm in response to apoptotic stimuli in prostate cancer cells, as the export of Nur77-RXRα heterodimers in these cells is dependent on a NES present in the LBD of RXRα [17]. It could be hypothesized that cell-type specific tertiary proteins that modulate the interactions between Nur77 and RXRα described above cause these differences in the nuclear export mechanism. For example, in smooth muscle cells, interferon stimulated gene 12 (ISG12) interacts with and inhibits the transcriptional activity of Nur77 by facilitating Crm1-dependent nuclear export of Nur77 [25]. After nuclear export, Nur77-RXRα heterodimers are targeted to the mitochondria where Nur77 induces apoptosis via interaction with Bcl-2, a process that will be described in more detail further on in this review.

Related to the interactions between Nur77 and the RXRs are COUP-TFI and COUP-TFII. These nuclear receptors bind and repress RARs through competition for DNA binding. Interactions between Nur77 and COUP-TFI/II may therefore result in enhanced expression of RAR-responsive genes. At the same time, binding of the COUP-TFs to Nur77 also inhibits Nur77-RXR heterodimer formation, thereby inhibiting the transcriptional activity of these complexes [26]. In short, COUP-TFs add an additional layer of regulation to the interactions between Nur77 and the RXRs.

2.3. Interaction of Nur77 with other transcription factors in cell proliferation and apoptosis

Nur77 has been implicated in the regulation of cellular survival, proliferation and apoptosis in different tissues and cell types involving various mechanisms, as reviewed by Moll et al. [27]. In this section we focus on the interaction of Nur77 with transcription factors that are actively involved in these processes.

In cultured hepatocytes the atypical nuclear receptor SHP interacts with a variety of nuclear receptors, including Nur77. The SHP protein only consists of a putative LBD and binds either Nur77 or the coactivator CBP/p300. Through this binding it can sequester this activator away from Nur77 resulting in inhibition of Nur77 activity [28]. In addition to this direct interaction, Yeo et al. also described SHP as a modulator of Nur77-mediated apoptosis in hepatocytes. The exact role of Nur77 in hepatocyte apoptosis is still unclear however, as adenovirus-mediated overexpression of Nur77 in mouse liver does not result in extensive apoptosis in this organ [29,30].

β-catenin is a dual function protein: it can either regulate cell–cell adhesion as a transmembrane protein or alter gene expression as a transcription factor after proteolytic release from the membrane. Notably, Nur77 gene expression is indirectly increased through activation of the AP-1 transcription factor complex by β-catenin [31]. In addition to regulation of Nur77 gene expression, β-catenin and Nur77 also interact directly and this interaction plays an important role in ubiquitin-mediated proteasomal degradation of β-catenin. More specifically, Nur77 binds β-catenin through its LBD and subsequently becomes ubiquitinated on its N-terminal domain. The proteasome subsequently degrades this entire complex of ubiquitinated Nur77 and β-catenin. Inhibition of nuclear export of Nur77 through mutagenesis of the NES in the LBD of Nur77 abolishes this degradation-inducing interaction with β-catenin. Additionally, Nur77 mutants lacking a DBD and associated nuclear localization signals (NLS) show increased binding and degradation of β-catenin. These two observations strongly suggest that this interaction takes place exclusively in the cytoplasm [31–33].

In pancreatic cancer cells, Nur77 was shown to bind Sp1 and Sp4 transcription factors, which are recruited to GC-rich sites in the promoter

of the cyclin-dependent kinase inhibitor p21. The effect on the survival and growth of these cells remains unclear although the enhanced p21 expression would potentially inhibit cell proliferation [34]. However, in another study of the same pancreatic cancer cells it was demonstrated that the Nur77/Sp1-complex induces expression of Survivin, a member of the inhibitors of the apoptosis family, resulting in cell survival and proliferation [35].

The promyelocytic leukemia protein (PML) is a tumor suppressor involved in apoptosis and regulation of cell cycle progression. PML interacts with the DBD of Nur77 via its coiled-coil domain thereby inhibiting Nur77 binding to DNA [36]. In the human osteosarcoma U2OS cell line Nur77-dependent apoptosis is enhanced by PML. Finally, the intracellular region of the transmembrane protein Notch-1 is released upon proteolytic cleavage and translocates to the nucleus where it can bind Nur77 and inhibit Nur77-dependent cell death of T-cells [37].

Taken together, these data illustrate that Nur77 interacts with a plethora of transcription factors that are active in cellular survival, growth and apoptosis and that the final outcome of these interactions on both Nur77 activity and cellular behavior is variable. One should realize that not all proteins mentioned are expressed to the same extent and under the same conditions in the different cell systems, which most likely explains the diverse outcome on Nur77 function.

2.4. Nur77 modulates tumor progression and angiogenesis through interactions with p53 and HIF-1α

The anti-cancer function of the tumor suppressor p53 involves cell cycle arrest, maintenance of genomic stability, apoptosis and inhibition of angiogenesis. Acetylation of p53 is blocked upon complex formation between p53 and Nur77, which suppresses the transcriptional activity of p53 [38]. The p53 target gene mouse double minute 2 (MDM2) is an E3 ligase that binds and ubiquitinates p53, but not Nur77. Nur77 can bind to p53 with a higher affinity than MDM2 and through this competition for binding Nur77 inhibits MDM2-mediated ubiquitination and degradation of p53. As a consequence, self-ubiquitination and subsequent degradation of MDM2 is promoted [38]. Taken together, the interaction of Nur77 with p53 downregulates both MDM2 mRNA (through a decrease in p53 transcriptional activity) and protein levels (through an increase in self-ubiquitination). Nur77 also stabilizes hypoxia inducible factor (HIF)-1α in a similar fashion: HIF-1α protein is continuously degraded due to interaction with MDM2, but is stabilized under hypoxic conditions to induce the expression of genes crucial in angiogenesis. Nur77 expression is also increased in response to hypoxia, leading to reduced MDM2 expression, decreased ubiquitination and degradation of HIF-1α and enhanced expression of HIF-1α target genes [39]. An additional mechanism by which Nur77 inhibits degradation of HIF-1α involves interaction with the von Hippel–Lindau protein (pVHL). In the presence of oxygen pVHL forms a complex that exhibits E3 ubiquitin ligase activity, leading to HIF-1α ubiquitination and subsequent degradation. Under hypoxic conditions Nur77 binds to the α-domain of pVHL, thereby blocking pVHL-mediated HIF-1α ubiquitination and thus degradation [40].

2.5. Nur77 modulates inflammatory responses through interaction with NF-κB

In mice, Nur77 is involved in negative selection of thymocytes, regulatory T cell differentiation, development of Ly6C-low monocytes and attenuation of the chronic inflammatory response of macrophages in atherosclerosis [1,41]. In Jurkat cells the anti-inflammatory function of Nur77 is mediated at least partly through its direct interaction with the p65 (RelA) subunit of NF-κB [14]. The NF-κB binding sites in IL2- and IL8-promoters have been shown to preferentially bind p65- or c-Rel homodimers or p65-c-Rel heterodimers. Nur77 respresses the transcriptional activity of p65 and c-Rel on these low affinity binding sites, but not on the high affinity NF-κB response element derived from the HIV-LTR. Reciprocally, p65 was shown to inhibit the transcriptional activity of Nur77 on NurREs [42].

3. Interactions between Nur77 and transcriptional coregulatory proteins

Target gene transcription by Nur77 is modulated through interaction with coregulatory factors that either enhance or repress interactions between Nur77 and the transcriptional machinery [43]. Both the N-terminal domain and LBD of Nur77 play a crucial role in transcriptional activation, coregulator recruitment and intra- and intermolecular interactions [15]. Some coregulators of Nur77 display specificity for either the NBRE or the NurRE promoter element and most coregulators have been characterized in a cell type- and stimulus-specific manner (Table 2). Coregulators of Nur77 have diverse enzyme functions

Table 2
Transcriptional coregulators interacting with Nur77.

Name	Also known as	Effect on Nur77 activity	Interacting domain of Nur77	References
ARR19	CKLFSF2; CMTM2	−	LBD	[51]
AXIN2	AXIL			[54]
BCL2	Bcl-2; PPP1R50		LBD	[56,57]
BRG1	SMARCA4; BAF190; SNF2; SWI2; hSNF2b	−		[9]
BRM	SMARCA2; BAF190; NCBRS; SNF2; SNF2L2; SWI2; Sth1p; hSNF2a	−		[9]
CBP/p300	CBP; CREBBP; KAT3A; RSTS; p300; EP300; KAT3B	+	N-term; LBD	[15,28,46,47]
CRIF1	CKBBP2; GADD45GIP1	−	N-term	[50]
FHL2	DRAL; SLIM3	−	N-term; DBD	[59]
HDAC1	GON-10; HD1; RPD3; RPD3L1	−	LBD	[46]
ISG12	IFI27; ISG12A; P27	−		[25]
PCAF	KAT2B	+	N-term	[15]
PIN1	DOD; UBL5	+	N-term; DBD	[63,64]
PRMT1	HRMT1L2; IR1B4	+	DBD–LBD	[49]
RB1	p105-Rb; pp110; pRb	+	DBD	[45]
SMAD7	CRCS3; MADH7; MADH8			[54]
SMRT	NCOR2; SMAP270; SMRTE; TRAC1	−	LBD	[43,52]
SRC-1	NCOA1; F-SRC-1; RIP160	+	N-term; LBD	[15,43,44]
SRC-2	NCOA2; GRIP1; NCoA-2; TIF2	+	N-term	[15]
TIM-1	HAVCR1; KIM-1	−	LBD	[60]
TIM-3	HAVCR2; KIM-3	−	LBD	[60]
TIM-4	SMUCKLER	−	LBD	[60]
TIF1β	TRIM28; KAP1; TIF1B	+	N-term	[48]
TRAP220	MED1; CRSP1; DRIP205; PBP; PPARBP; RB18A; TRIP2		N-term	[105]

N-term, indicates N-terminal domain; −, binding partner represses Nur77 activity; +, activation of Nur77 transcriptional activity.

among which are histone acetyltransferase (HAT), histone deacetylase (HDAC), methyltransferase and isomerase activities.

3.1. Interactions with steroid receptor coactivators (SRCs) increase the transcriptional activity of Nur77 and are extensively modulated by other protein–protein interactions

Muscat et al. generated a model for the structure of the LBD of Nur77 and predicted that the coregulator-binding groove that is formed in the LBDs of most nuclear receptors upon ligand binding is absent in Nur77 [15]. The lack of this binding groove, which can interact with LxxLL motifs of coregulatory proteins, may explain why most coregulators interact with the N-terminal domain of Nur77 instead.

The steroid receptor coactivators (SRCs)1-3 are three members of the p160 family of histone acetyl transferases (HATs) that are critical for transactivation of many nuclear receptors. Both SRC-1 and SRC-2 can directly interact with the N-terminal domain of Nur77 and enhance its transcriptional activity. Interestingly, SRC-1 can also interact with the LBD of Nur77 even though this domain does not contain the previously mentioned coregulator-binding groove [15]. Additionally, SRC-1 only increases the transcriptional activity of Nur77 on NurREs (which are bound by NR4A dimers) but not NBREs (which are bound by NR4A monomers) [15,44]. The latter two facts suggest a possible role for SRC-1 in facilitating NR4A dimerization through interaction with the LBD of Nur77. At present, both the role of SRC-1 in NR4A dimerization and the importance of the innate acetyltransferase activity of SRC-1 or SRC-2 in the transcriptional activation of Nur77 remain unknown. Furthermore, many other coregulatory proteins have been found to either increase or decrease the activation of Nur77 by SRC-1 and SRC-2. This finding suggests that cells regulate Nur77 activation by SRC-1 and SRC-2 through protein–protein interactions instead of ligand binding, which fits with the fact that Nur77 does not contain a classical nuclear receptor LBD that recruits coregulators like SRC-1 in a ligand-dependent fashion.

Multiple proteins have been shown to increase transactivation of Nur77 by SRC-1 or SRC-2. First, retinoblastoma tumor suppressor protein (Rb) is a repressor of genes involved in cell cycle progression. Rb and its related proteins p107 and p130 increase the activity of Nur77 through direct interaction with the N-terminal domain and the DBD of Nur77. Although SRCs can bind Nur77 in the absence of Rb, Rb acts as a synergistic potentiator of SRC-2 coactivator function, thereby promoting Nur77 activity [45].

Second, p300/CBP-associated factor (PCAF) is a HAT that binds directly to the N-terminal domain of Nur77 and mediates recruitment of additional coactivators. Together with SRC-1, SRC-2 and CBP/p300, PCAF works with the mediator complex to remodel local chromatin structure, thereby enhancing transcription [15]. In addition to interactions with PCAF, Nur77 is also a target for acetylation by CBP/p300 itself. This acetylation increases protein stability of Nur77 and is directly antagonized by histone deacetylase 1 (HDAC1) [46]. Interestingly, Nur77 induces expression of both the acetylase p300 and the deacetylase HDAC1, pointing to the existence of a negative feedback loop that regulates Nur77 activity and is itself regulated at the post-transcriptional level [46,47].

Third, transcription intermediary factor 1β (TIF1β) is an intrinsic component of two chromatin remodeling and histone deacetylase complexes: the NCoR1 complex and the nucleosome remodeling and deacetylation (Mi-2/NuRD) complex. TIF1β increases the transcriptional activity of Nur77 upon corticotropin-releasing hormone (CRH) stimulation of AtT-20 cells by forming a transcriptional activation complex together with SRC-2 on the N-terminal domain of Nur77. It should be noted that TIF1β acts only on Nur77/NR4A dimers but not on monomers. Additionally, TIF1β also interacts with the other two members of the NR4A family, which will be discussed in a separate section of this review [48].

Finally, protein arginine methyltransferase 1 (PRMT1) physically interacts with Nur77 and synergizes with SRC-2 to increase Nur77 transcriptional activity. However, Nur77 is not methylated by PRMT1 [49]. Instead, PRMT1 increases the protein stability of Nur77 through direct interaction independent of its methyltransferase activity. Interestingly, this interaction also blocks the methyltransferase activity of PRMT1 by masking its catalytic domain, resulting in decreased methylation of PRMT1 substrates such as STAT3 and Sam68, indirectly leading to either a decrease in their transcriptional activity (for STAT3) or a decrease in their nuclear localization (for Sam68) [49].

On the other hand, two proteins have been shown to decrease the activation of Nur77 by direct competition with either SRC-1 or SRC-2. First, CR6-interacting factor 1 (CRIF1), a putative regulator of cell cycle progression and cell growth, has been shown to interact directly with the N-terminal domain of Nur77 via its mid-region and decrease transactivation of Nur77 by SRC-2. In thyroid cells this binding resulted in a decrease of Nur77-dependent induction of E2F1 promoter activity and Nur77-mediated G1/S progression of the cell cycle [50].

The second, androgen receptor corepressor-19 (ARR19), is a member of the chemokine-like factor superfamily and reduces the expression of steroidogenic enzymes. ARR19 can inhibit the transcriptional activity of Nur77 by directly competing with SRC-1 for binding of the LBD of Nur77 [51]. As was previously stated, SRC-1 could hypothetically affect dimerization of Nur77. Unfortunately, Qamar et al. did not look at the effect of ARR19 or SRC-1 on NR4A dimerization in this study.

Similarly, although not directly related to modulation of SRC-1 and SRC-2 activity, the silencing mediator for retinoid and thyroid hormones (SMRT) was shown to bind the LBD of Nur77 and repress its transcriptional activity. SMRT is a known corepressor of multiple nuclear receptors and associates with HDACs to form repressive complexes. The HDAC inhibitor Trichostatin A (TSA) does not block the repressive effects of SMRT on Nur77 however, suggesting that HDACs do not play a role in this process. Instead, Sohn et al. hypothesize that SMRT competes with other coregulatory proteins (such as the SRCs) for binding of Nur77 [52]. Furthermore, it should be noted that the inhibitory effect of SMRT on Nur77 is repressed by Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIVc), which facilitates the translocation of SMRT from the nucleus to the cytoplasm [52]. Additionally, increases in cytosolic calcium enhance expression of Nur77 [53], while increases in intracellular cAMP levels enhance expression of both Nur77 and SMRT [43]. When taking these three facts together, it could be hypothesized that the effect of a stimulus that sequentially increases cytosolic Ca^{2+} and cAMP levels (such as those relayed by the β-adrenergic receptors) on Nur77 is a two-stage affair that is regulated by SMRT: first, when cytosolic calcium and cAMP levels are high, expression of both SMRT and Nur77 is increased while SMRT protein is sequestered in the cytosol by CaMKIVc, leading to an increase in Nur77 transcriptional activity. Then, when the said stimulus has passed and cytosolic calcium levels return to normal, SMRT is no longer exported from the nucleus and starts inhibiting Nur77, thereby ending the Nur77-dependent response of the cell to such a stimulus.

Finally, Sohn et al. also describe the coactivator ASC-2 (NCOA6), which increases the transcriptional activity of Nur77 but does not bind it directly. The interaction between ASC-2 and Nur77 is said to be mediated by an intermediary protein that has not been identified yet [52]. Since it has been shown that ASC-2 interacts with both RXRα and RARα [54], it could be hypothesized that the previously mentioned dimerization of Nur77 with these two receptors plays a role in the activation of Nur77 by ASC-2.

3.2. Nur77 promotes thymocyte apoptosis through interaction with Bcl-2

Nur77 has been shown to induce cell death through a transcription-dependent pathway in thymocytes [55], while Bcl-2 family proteins are evolutionarily conserved regulators of apoptosis [56]. Upon apoptotic

stimulation of thymocytes Nur77 translocates from the nucleus to mitochondria, a process which is tightly controlled by post-translational modifications such as phosphorylation (more on this later). When Nur77 binds to Bcl-2 in mitochondria cytochrome C is released into the cytosol, which subsequently triggers apoptosis. The interaction of Nur77 with Bcl-2 induces a conformational change resulting in the conversion of Bcl-2 from an anti-apoptotic to a pro-apoptotic protein [56]. The translocation of Nur77 from the nucleus to the mitochondria is also observed in cancer cells derived from lung, ovary, prostate, stomach and breast [27]. Nur77 and NOR-1 associate with the proapoptotic BH3 domain of Bcl-2 in stimulated thymocytes, which was confirmed in cells from T-cell receptor-transgenic mouse models [57]. Recently, Kolluri et al. developed a short Nur77-derived peptide, NuBCP-9, which potentiates the pro-apoptotic function of Bcl-2 to induce apoptosis of cancer cells in vitro and in animal models [58].

3.3. Regulation of Nur77 transcriptional activity by FHL2, TIMs and TGFβ signaling

Four and a half LIM domain protein-2 (FHL2) is a typical adaptor protein that facilitates interactions between different proteins and interacts directly with all three NR4As [59]. All four LIM domains of FHL2 can bind Nur77 and both the N-terminal domain and the DBD of Nur77 are involved in this interaction. FHL2 inhibits Nur77 transcriptional activity, presumably through recruitment of additional, repressive coregulators of Nur77 [59].

T-cell immunoglobulin and mucin domain (TIM) proteins such as TIM-1, TIM-3 and TIM-4 function as cell-surface signaling receptors in T-cells and scavenger receptors in antigen-presenting cells. All three TIM proteins interact with Nur77 and repress its transcriptional activity [60]. It was also shown that TIM-1 is constitutively endocytosed and that this dynamic cycling of TIM-1 is important for simultaneously targeting Nur77 to the lysosome for degradation. As such, the association of TIM-1 with Nur77 in renal tubular epithelial cells may confer protection against apoptosis [60].

More recently, it was shown that Nur77 strongly potentiates oncogenic TGFβ signaling in breast tumor cells by interacting with both Axin2 and Smad7. These interactions lead to increased Axin2-dependent degradation of Smad7, a negative regulator of TGFβ signaling [61]. So far it is unknown whether or not these interactions also have a reciprocal effect on the activity of Nur77, although it has been shown that Axin2, like Nur77, enhances degradation of β-catenin, suggesting that Axin2 plays a role in this Nur77-mediated degradation process as well [62].

4. Interactions with kinases and phosphorylation of Nur77

Phosphorylation is a post-translational protein modification that can change the conformation of an entire protein or, alternatively, alter the accessibility of only a single region of it. These conformational changes can in turn lead to an increase or decrease in the protein's activity, stability or ability to interact with other proteins. A number of kinases have been shown to phosphorylate or interact with Nur77 directly, and these interactions and modifications occur in all three of the domains of Nur77 (Table 3).

4.1. Phosphorylation inhibits proteasomal degradation of Nur77

The peptidyl-prolyl isomerase Pin1 plays a role in post-translational regulation of protein function by recognizing phosphorylated serine or threonine residues and isomerizing the adjacent proline residues (so called pSer/pThr-Pro motifs). Pin1 interacts with all three NR4A nuclear receptors and enhances their transcriptional activity [63,64]. However, the exact regions of Nur77 that Pin1 binds or isomerizes are subjects of debate as Nur77 contains 17 putative pSer/pThr-Pro motifs for isomerization by Pin1 and two papers have identified different motifs as being essential for Pin1–Nur77 interactions. The first paper, by Chen et al., reported that Pin1 recognizes both JNK1-phosphorylated Ser95 and ERK2-phosphorylated Ser431 and subsequently isomerizes their adjacent proline residues, which ultimately leads to increased protein stability and transcriptional activity of Nur77, respectively [63,65]. In contrast, van Tiel et al. reported that Pin1 enhances Nur77 transcriptional activity independent of its isomerase activity and that the isomerase activity of Pin1 is only required to increase the protein stability of Nur77. Additionally, van Tiel et al. reported that phosphorylation of Nur77 at the N-terminal domain Ser152 residue by casein kinase 2 (CK2) is required for isomerization to take place, which then leads to increased protein stability by blocking ubiquitination of Nur77 [64]. Both studies used Pin1 and Nur77 mutants to verify binding and isomerization sites and all experiments were performed in HEK293 cells. So far there is no explanation for the discrepancies between these two papers.

4.2. Phosphorylation by DNA-PK promotes the p53 transactivation and DNA double-strand break repair activity of Nur77

DNA-PK is a serine/threonine protein kinase complex that is expressed in the nucleus, where it plays an essential role in the non-homologous end joining (NHEJ) pathway of DNA double-strand break (DSB) repair [66]. DNA-PK has been shown to phosphorylate Nur77 at two different sites with distinct downstream effects: first, phosphorylation of Nur77 at the Ser164 residue promotes the Nur77-mediated interaction between DNA-PK and p53, resulting in phosphorylation of p53 and subsequent enhancement of its transcriptional activity [67].Second, Nur77 can also be phosphorylated by DNA-PK at residue Ser337 in response to DNA damage, and this phosphorylation is crucial for the proper progression of DSB repair [68]. However, the enhancement of DSB repair does not depend on the transcriptional activity of Nur77, excluding the

Table 3
Kinases that interact directly with Nur77.

Kinase	Also known as	Effect of phosphorylation	Phosphorylation site	Interaction site	References
AKT1	AKT; PKB	Decreases transcriptional activity, blocks nuclear export of Nur77	Ser351	DBD	[71,75]
CHEK2	CDS1; CHK2; RAD53	Increases DNA-binding ability of Nur77	Thr88	N-term	[84]
CK2	CSNK2A1/A2/B; CKII	Mediates stabilization of Nur77 by Pin1	Ser152	N-term	[64]
DNA-PK	PRKDC	Promotes DSB-repair activity of Nur77	Ser164 Ser337	N-term DBD	[67,68]
ERK2	p38; MAPK1; MAPK2	Mediates transcriptional activation of Nur77 by Pin1	Thr143 Ser431	N-term DBD	[63,76,81,82]
ERK5	ERK4; BMK1; MAPK7	Increases transcriptional activity of Nur77	n.d. (not Thr143)	n.d.	[83]
JNK	JNK1; MAPK8	Reduces DNA-binding ability, promotes nuclear export of Nur77	Ser95	N-term	[63,65,71,77]
LKB1	hLKB1; STK11	No direct phosphorylation. LKB1 stays in the nucleus by binding Nur77, thereby inhibiting AMPK signaling	n.d.	LBD	[87]
PKA	PKAC; PRKAC	Decreases DNA-binding ability of Nur77, but increases DNA-binding ability of Nur77 homodimers	Ser341 Ser351	DBD	[44,74]
PKC	PRKC	Induces mitochondrial translocation of Nur77. Inhibition of the catalytic activity of PKCΘ	Ser351 n.d.	DBD	[74,79,80]
RSK1/2	S6K; p90-RSK; RPS6KA1/3	Mitochondrial translocation of Nur77 and apoptosis in T cells	Ser351	DBD	[73,77,78]

Phosphorylation sites are given for human NR4A1, isoform 1 (GI:21361342); n.d., not determined.

possibility of Nur77 enhancing transcription of genes involved in DNA repair. Additionally, phosphorylation by DNA-PK is not required for recruitment of Nur77 to sites of DNA repair, as DNA-PK only phosphorylates Nur77 after it is already localized to DNA repair sites. Instead, the enzymatic activity of poly-ADP-ribose polymerase-1 (PARP-1) was shown to be essential for recruitment of Nur77 to DNA repair sites [68]. Increased DSB repair by Nur77 may therefore be seen as a two-step process: first, ribosylation by PARP-1 of either Nur77 itself (unconfirmed, but direct interactions between PARP-1 and Nur77 do occur [69]) or the chromatin surrounding the DSB (as has been previously shown to occur [70]) paves the way for a stable association of Nur77s with DSBs. Such an association then allows for recruitment of DNA-PK, which phosphorylates Nur77 and thereby paves the way for recruitment of additional DNA repair factors.

4.3. Subcellular localization of Nur77 is regulated by competing kinase signaling pathways

Two major signaling pathways play an important role in mediating the intracellular relay of cell proliferation and apoptosis signals: the phosphatidylinositol 3-kinase to Akt/protein kinase B (PI3K-Akt) pathway and the Ras to mitogen-activated protein kinase (MAPK) pathway. These two pathways competitively alter the subcellular localization of Nur77, with PI3K-Akt signaling promoting nuclear sequestration and transcriptional inactivation of Nur77, and Ras-MAPK signaling enhancing mitochondrial translocation and transcriptional activation of Nur77 (Fig. 3). As described earlier in this review, translocation of Nur77 to the mitochondria is a potent inducer of apoptosis. Therefore, the choice in subcellular localization of Nur77 as decided by these two signaling pathways can also be seen as a choice for either apoptosis or survival of the cell. The effects of the PI3K-Akt signaling pathway on Nur77 are a direct result of phosphorylation of Nur77 by Akt at residue Ser351. Phosphorylation of this residue, which is located in the DBD, has two effects on Nur77: first, it blocks export of Nur77 from the nucleus, presumably through phosphorylation-dependent interactions between Nur77 and the 14-3-3 family of inactivating proteins (which have been shown to interact with Nur77, albeit in vitro only) [71–73]. Second, phosphorylation of Ser351 inhibits the ability of Nur77 to bind DNA and thereby decreases its transcriptional activity [74,75].

On the other hand, activation of the MAPK pathway has less straightforward effects on Nur77, as it can induce both nuclear sequestration and non-apoptotic cell death through ERK2 [76], as well as mitochondrial translocation and apoptosis through RSK/JNK [65,71,73,77,78]. More specifically, 90-kDa ribosomal S6 kinase (RSK2; also known as p90-RSK) can phosphorylate the same Ser351 residue in the DBD of Nur77 that was previously shown to be phosphorylated by Akt [73,77,78]. Since phosphorylation of Ser351 can have seemingly opposite effects depending on the kinase involved, it seems logical that a third factor determines the final effect phosphorylation of this residue has on localization of Nur77. This third factor could be phosphorylation by JNK, as it was shown that when the Ser95 residue in the N-terminal domain of Nur77 is phosphorylated by JNK, nuclear export and translocation of Nur77 to the mitochondria is increased and Bcl-2 mediated T-cell apoptosis is promoted [65,71]. Another possibility is involvement of protein kinase C (PKC), which also induces mitochondrial translocation and is known to phosphorylate Nur77 in vitro [74,79]. Whether the kinases described above synergistically promote translocation of Nur77, or whether phosphorylation of Nur77 by these two kinases only occurs separately from each other depending on cell type remains to be determined. It should also be noted that in addition to the phosphorylation by PKC described above, interaction between the LBD of Nur77 and a highly conserved glycine-rich loop of PKC was also shown to inhibit its kinase activity [80].

ERK2 can phosphorylate Nur77 at residue Thr143, which also sequesters Nur77 in the nucleus and thereby induces an alternative, non-apoptotic form of programmed cell death that requires transcriptional activation of Nur77 [76,81,82]. Additionally, ERK5 phosphorylates Nur77 as well (albeit at a different site than ERK2) and this phosphorylation was shown to also increase apoptosis through induction of its transcriptional activity [83]. The idea of transcription-dependent induction of cell death by Nur77 is made more plausible by the fact that phosphorylation of residue Thr88 in Nur77 by checkpoint kinase 2 (CHEK2) has already been described to have a similar cell death-inducing effect by enhancing Nur77-dependent down-regulation of expression of the anti-apoptotic genes BRE and RNF-7 [84]. In summary, phosphorylation by Akt inactivates and sequesters Nur77 in the nucleus and thereby inhibits apoptosis, whereas the MAPK pathway and/or PKC (possibly depending on the cell type) can either enhance mitochondrial translocation of Nur77 and apoptosis through JNK and RSK, or sequester Nur77 in the nucleus and thereby promote non-apoptotic programmed cell death through ERK2.

Finally, it should be noted that the Ser351 residue of Nur77, whose phosphorylation by kinases of the MAPK pathway and Akt was discussed in the preceding paragraphs, is also phosphorylated by PKA [74]. The exact effect of this phosphorylation by PKA is unclear however, as activation of PKA has been shown to both increase and decrease transcriptional activity of Nur77 [44,74].

4.4. Nur77 indirectly regulates AMPK activity through interactions with LKB1

During times of energy starvation cellular levels of adenosine monophosphate (AMP) rise and the appropriately named AMP-activated protein kinase (AMPK) becomes activated through phosphorylation by liver kinase B1 (LKB1). Activated AMPK is a major driver of energy conservation in the cell, because it turns on energy producing catabolic pathways such as fatty acid oxidation and glycolysis while simultaneously shutting down energy-requiring processes such as fatty acid synthesis, gluconeogenesis and cell growth [85]. Nur77 has been shown to have the opposite effect of AMPK: it promotes gluconeogenesis, glucose metabolism and sensitivity to insulin [86]. This competition for the metabolic state of the cell between AMPK and Nur77 is not just indirect however, as Nur77 can also inhibit the activity of AMPK through modulating the localization of LKB1. Nur77 directly interacts with nuclear LKB1 and this interaction prevents LKB1 from leaving the nucleus and activating cytosolic AMPK [87]. In the regulation of nuclear export of LKB1 phosphorylation at its Ser428 residue is most important, which is a target site for protein kinase C zeta (PKCζ) [88]. The LBD of Nur77 and the region surrounding the PKCζ target residue Ser428 of LKB1 interact with each other. This interaction prevents phosphorylation of LKB1 and thus its nuclear export [87].

5. Interacting proteins of Nurr1 and NOR-1

The interactome of Nurr1 is not as well described as that of Nur77, whereas for NOR-1 even less data are available on its interacting proteins. The reason why Nurr1 has been studied more elaborately than NOR-1 may be its crucial function in development of dopaminergic neurons as is illustrated by the brain phenotype of Nurr1-deficient mice [89]. In this paragraph Nurr1-interacting proteins will be presented first, followed by a description of our current knowledge on the protein–protein interactions of NOR-1 (Tables 4–5).

5.1. The interactome of Nurr1

Nurr1 is both in its monomeric and homodimeric form an active transcription factor, but it can also form heterodimers with Nur77 or RXR. Nurr1 interaction with RXR has been described in great detail demonstrating that alanine substitution of Pro560 or Leu562 completely disrupts this interaction, as well as the triple substitution of Lys554–Leu555–Leu556 [90]. RXR is an inactive receptor in other nuclear receptor dimeric complexes however, it creates a hormone-dependent

Table 4
Nurr1 interacting proteins.

Name	Also known as	Effect on Nurr1 activity	Interacting domain of Nurr1	References
CTNNB1	ß-Catenin; armadillo	Increased	LBD	[96]
CDKN1C	Cyclin-dependent kinase inhibitor 1C; p57Kip2	Decreased	N-term	[94]
COPS5	COP9 signalosome subunit 5; Jab1	nd		[102]
coREST	REST corepressor 1; RCOR1	nd	DBD	[98]
ERK2	p38; MAPK1; MAPK2	Increased	N-term	[92,93]
ERK5	MAPK7	Increased	N-term-LBD	[92]
FHL2	Four and a half LIM domain 2; DRAL; SLIM3	Decreased	LBD–DBD	[59]
GR	Glucocorticoid receptor; NR3C1	Increased	DBD or N-term	[10,91]
LEF1	Lymphoid enhancer-binding factor 1	nd	LBD	[96]
LIMK1	LIM kinase 1	Decreased	N-term-LBD	[92]
NCOR1	Nuclear receptor corepressor 1	nd		[103]
NCOR2	Nuclear receptor corepressor 2; SMRT	Decreased	LBD	[95,103]
NFKB	NFKB-p65; NF-κB	nd		[98]
NR4A1	Nur77; NAK1; TR3; NGFI-B	nd	LBD	
PIAS4	Protein inhibitor of activated STAT protein 4; PIASgamma	Decreased	N-term	[100]
Pin1	Peptidyl-prolyl isomerase 1; DOD; UBL5	Increased		[64]
PRKDC	DNA-PKcs; DNA-PK	nd	DBD	[68]
RSK1/2	S6K; p90-RSK; RPS6KA1/3	nd	DBD	[73]
RUNX1	AML1; CBFA2; EVI-1; PEBP2aB	nd		[97]
RXRA	NR2B1	nd	LBD	[16,90,102]
SFPQ	PSF; POMP100	nd		[95]
SIN3A	SIN3 transcription regulator family member A	nd		[95]
TP53	p53; TRP53	nd	DBD	[101]
TRIM28	TIF1β; KAP; TIF1B1	Increased		[48]
FXR, PPAR, STAT3, PARP-1, p85 βPIX, MxiI		nd		[92]

N-term, indicates N-terminal domain; n.d., not determined.

complex upon interaction with Nurr1 or Nur77. Of note, the transcriptional activity of Nurr1 itself is reduced upon complex formation with RXR [16]. Nurr1 also interacts with the glucocorticoid receptor (GR) although some discrepancy exists in literature on the domain of Nurr1 involved in this reaction, which has been described to be the DBD or the N-terminal domain [10,91]. Nurr1 inhibits the transcriptional activity of GR in AtT20 cells, whereas GR enhances Nurr1 activity in a dexamethasone-dependent manner in PC12 cells [10,91].

In search for proteins that interact with Nurr1 extensive pull-down experiments were performed, which revealed that the MAP kinases ERK2 and ERK5 both bind to Nurr1 and enhance its transcriptional activity in a phosphorylation-dependent manner [92]. For ERK5 it was demonstrated that the Nurr1 Thr168A- and Ser177Ala-variants were no longer responsive to ERK5 activation, whereas the exact phosphorylation sites in Nurr1 for ERK2 were later identified as Ser126 and Thr132 [93]. ERK2 interacts with the N-terminal domain of Nurr1, whereas ERK5 was shown to bind the N-terminal domain, where the target residues (Thr168, Ser177) are localized, and the LBD.

LIM kinase1 and cyclin-dependent kinase inhibitor 1C (CDKN1C or p57Kip2) are known to interact and have independently been shown to bind the N-terminal domain of Nurr1 to inhibit the activity of Nurr1 [92,94]. Therefore, it is conceivable that the p57kip2–LIM kinase1–Nurr1 complex exists. Moreover, LIM-domain containing proteins are known to recruit the Sin3A-histone deacetylase co-repressor complex and Nurr1 interacts directly with Sin3A, making the existence of an even larger protein complex feasible [95].

The peptidyl-prolyl isomerase Pin1 was identified in a yeast-two-hybrid screen and shown to interact with the N-terminal domain and DBD of Nurr1. Pin1 enhances the transcriptional activity of Nurr1 without changing its protein stability, in contrast to Nur77 protein stability that is increased by Pin1 [63,64]. In the same screen the LIM-only domain protein FHL2 was identified and, similarly as observed for Nur77, FHL2 binds the N-terminal domain and DBD of Nurr1 and inhibits its activity [59, unpublished data van Tiel et al.].

As indicated above, Nurr1 is crucial in development of dopaminergic neurons. In these cells Nurr1 is bound in corepressor complexes via lymphoid enhancer-binding factor-1 (LEF-1). β-catenin competes for this specific interaction with the Nurr1 LBD and upon binding to Nurr1 β-catenin facilitates downstream gene expression of both Nurr1 and Wnt [96].

More recently, Nurr1 has been demonstrated to be involved in expression of the Forkhead transcription factor Foxp3, which defines the differentiation of regulatory T cells (Treg) [97]. This regulation of gene expression in $CD4^+$ T cells is mediated through direct interaction of Nurr1 with Runx1. In macrophages and microglia an anti-inflammatory function has been attributed to Nurr1 involving inhibition of NF-κB p65 activity [98]. Indeed, a direct interaction between Nurr1 and NF-κB p65 has been established, which requires phosphorylation of p65 at Ser468. Subsequently, the corepressor complex with CoRest is recruited and this factor also binds Nurr1 directly. It is the Nurr1/CoREST-mediated transrepression complex that blocks NF-κB p65 activity.

In analogy with Nur77, Nurr1 is important in DNA repair through interaction with DNA-PK, which phosphorylates Nurr1 at Ser337 in the NR4A-conserved sequence 'TDSLKG'. To substantiate its involvement in dsDNA repair, overexpression of the Ser337Ala Nurr1-variant was shown to hamper DNA repair [68].

The SUMO-E3 ligase PIASγ represses Nurr1 transcriptional activity in two independent ways. One mechanism involves PIASγ-mediated SUMOylation of Lys91 in Nurr1 resulting in reduced activity in complex promoters. The other type of repression is mediated through a direct interaction between Nurr1 and PIASγ independent of its E3-ligase activity [99,100].

Proteins directly interacting with Nurr1, but for which the effect on Nurr1 function has not been studied in detail are the nuclear receptors FXR and PPAR, the transcription factors STAT3 and p53, PARP-1, which is involved in apoptosis, p85 βPIX, a guanine nucleotide exchange factor and JAB1, a component of the COP9 signalosome that acts as a positive regulator of E3 ubiquitin ligases [92,101].

JAB1 is involved in the degradation of cyclin-dependent kinase inhibitor CDKN1B/p27Kip1, but its effect on Nurr1 activity or protein stability has not been studied [69,92,102].

Among the less well-defined Nurr1 interacting proteins are also the signaling pathway constituents MxiI and NCoR-1 [92]. For NCoR-1 it has been shown that a peptide comprising its LxxLL motif interacts with the novel co-regulator interaction site that has been defined in the Nurr1 LBD domain, based on the LBD crystal structure and NMR-analyses

Table 5
NOR-1 interacting proteins.

Name	Also known as	Effect on NOR-1 activity	Interacting domain of NOR-1	References
BCL2	Bcl-2; PPP1R50	nd		[79]
CBP/p300	CBP; CREBBP; p300; EP300	nd		[46]
ERK2	p38; MAPK1; MAPK2	nd		[82]
FHL2	DRAL; FHL-2; SLIM3	Decreased		[59]
TRAP220	MED1; RSP1; DRIP205; PBP; PPARBP; RB18A; TRIP2	Increased		[104]
NCOA2	SRC-2; TIF2; GRIP1; NCoA-2	Increased	N-term	[104]
Pin1	Peptidyl-prolyl isomerase 1; DOD; UBL5	Increased	N-term	[64]
PARP1	poly-ADP-ribose polymerase-1	Decreased		[69]
PKC	PRKC	nd		[79]
PRKDC	DNA-PKcs; DNA-PK	nd	DBD	[68]
RSK1/2	S6K; p90-RSK; RPS6KA1/3	nd	DBD	[73]
SIX3	HPE2	Decreased	DBD	[108]
TRIM28	TIF1-β; KAP; TIF1B1	Increased	DBD and LBD/DBD	[48]

N-term, indicates N-terminal domain; n.d., not determined.

[103]. Similarly, the peptide containing the LxxLL motif of NCoR-2 (also known as SMRT) interacts with this novel co-regulator interaction site in the LBD. Pitx3 disturbs the interaction between Nurr1 and NCoR-2/SMRT and even though Nurr1 does not bind directly to Pitx3, these proteins both bind the corepressor PSF indicating that Pitx3 and Nurr1 are present in the same complex [95].

5.2. The interactome of NOR-1

NOR-1 is less well studied than Nur77 and Nurr1 and most of the data on interacting proteins of NOR-1 are presented in studies that are mainly focused on its homologues. As a consequence, NOR-1 protein–protein interactions are described with limited detail, for example the HAT p300/CBP acetylates NOR-1 similarly as Nur77, however, the effect on NOR-1 activity has not been described [79]. Likewise, NOR-1 interacts with the co-regulator TIF1β resulting in enhanced NOR-1 activity, but the domain involved in the interaction is unknown [48]. Similar to Nur77, PKC and RSK1/2 were shown to induce NOR-1 mitochondrial translocation [73,79] and DNA-PK binds the DBD of NOR-1. Even though Nurr1 and Nur77 are both essential for optimal DSB repair the function of NOR-1 in this process remains to be studied [68]. Both FHL2 and the peptidyl-prolyl isomerase Pin1 bind the N-terminal domain and DBD

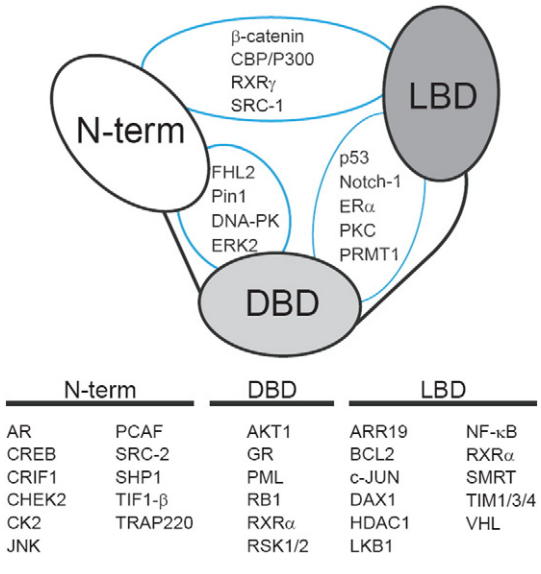

Fig. 2. Nur77 and its interacting proteins. Schematic overview of the protein–protein interactions with Nur77 for which the domains of interaction have been elucidated. Details are described in the text and in Tables 1–3, which also contain the full names of the indicated proteins. N-term, N-terminal domain; DBD, DNA-binding domain; LBD, ligand-binding domain.

of NOR-1, resulting in reduced or enhanced transcriptional activity of NOR-1, respectively [59,64].

Muscat and co-workers performed detailed studies to identify co-regulators of NOR-1 and were the first to reveal the absence of a conventional ligand-binding pocket in the LBD of NOR-1, through molecular modeling and hydrophobicity analysis of the LBD [104]. Based on these analyses, the relative importance of the N-terminal domain of NOR-1 in regulation of the transcriptional activity of NOR-1 became apparent and direct interaction of a number of crucial co-regulators to this domain was shown; SRC-2 (GRIP-1), SRC-1, SRC-3, p300, DRIP250/TRAP220 and PCAF [104]. The interaction between the N-terminal domain of NOR-1 and TRAP220 is independent of PKA- and PKC phosphorylation sites in TRAP220. Most interestingly, the purine derivative 6-mercaptopurine, which enhances the activity of NR4As without directly binding these nuclear receptors promotes the interaction between NOR-1 and TRAP220 [105].

Both Nur77 and NOR-1 are involved in T-cell receptor mediated apoptosis of developing T cells [106]. During activation of T cells the expression of NOR-1 is induced and protein kinase C (PKC) becomes active. NOR-1 is a PKC substrate that is phosphorylated and subsequently translocates from the nucleus to the mitochondria where it binds Bcl-2. Most interestingly, as already indicated above the interaction between NOR-1/Nur77 and Bcl-2 causes a conformational change in Bcl-2 allowing its BH3 domain to be exposed, resulting in the conversion of Bcl-2 from an anti-apoptotic into a pro-apoptotic protein. For Nur77 it is exactly known which amino acids are involved to provoke the functional switch in Bcl-2, which is not the case for NOR-1 [57,79].

Initially, the homeobox domain containing protein Six3 was identified in a yeast-two-hybrid study as a protein that interacts uniquely with the DBD and LBD of NOR-1 without binding or inhibiting the activity of Nur77 or Nurr1. Of interest, NOR-1 and Six3 show overlap in expression in the rat fetal forebrain on embryonic day 18 [107]. In a later study this specificity of Six3 for NOR-1 was not found, rather interaction with all three NR4As was observed [108]. NOR-1 is part of the EWS/NOR-1 fusion protein that is expressed in human extraskeletal myxoid chondrosarcoma tumors. Six3 enhances the activity of NOR-1 (and Nur77 and Nurr1), whereas the activity of EWS/NOR-1 is inhibited and the interaction only requires the DBD of NOR-1. The opposing data in these two studies may be explained by the use of different cell types for the activity assays, as well as the use of Gal4-fusion proteins in the latter study.

PARP-1 specifically and effectively interacts with the DBD of NOR-1 independent of the enzymatic activity of PARP-1 [69]. Nurr1 interacts with lower affinity, whereas EWS/NOR-1 and Nur77 do not bind PARP-1, unless the N-terminal domain of Nur77 is deleted. The latter experiment nicely illustrates that the N-terminal domains of Nur77 and EWS/NOR-1 disturb PARP-1 interaction with the DBD. This may be the underlying mechanism of differential function of NOR-1 and the EWS/NOR-1 fusion protein. In line with the binding characteristics,

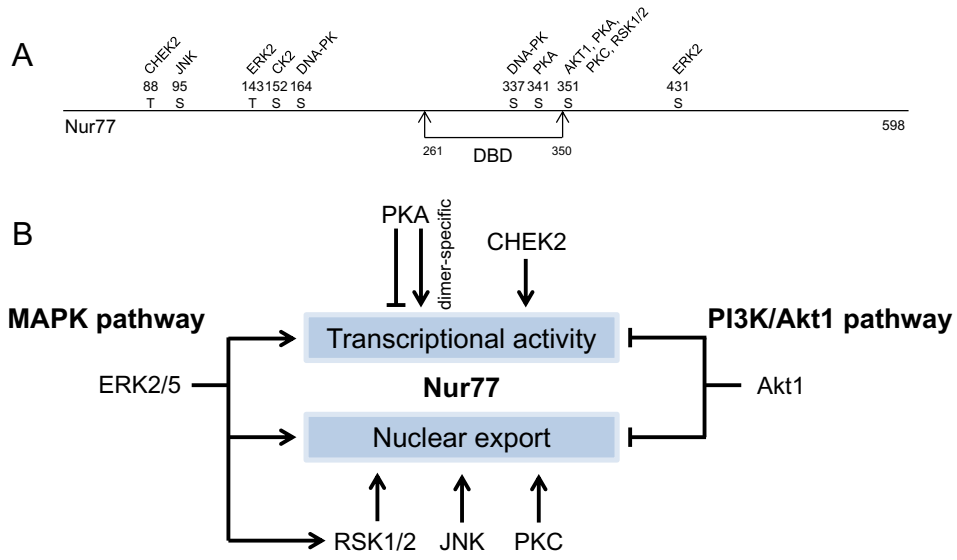

Fig. 3. Nur77 and kinases modulating its activity and localization. A, Overview of the amino-acid sequence of Nur77 with known phosphorylation sites and associated kinases indicated (T = threonine, S = serine). B, Schematic illustration of effects of different kinases on Nur77 transcriptional activity and subcellular localization. See Table 3 for definitions of the abbreviations of the kinases shown.

PARP-1 only inhibits the activity of NOR-1 effectively, again independently of the ribose polymerase activity of PARP-1.

6. Discussion and concluding remarks

This review summarizes the currently available knowledge on the protein–protein interactions of the NR4A nuclear receptor family and their downstream effects. When looking at the information gathered in this review three main observations can be made. First, there are a large number of protein–protein interactions that regulate the activity of Nur77 and there is a large variation in the effects of these interactions on the 'target' protein, be it Nur77 or the interacting protein itself. These effects include modulation of transcriptional activity, protein stability, post-translational modification and cellular localization: all processes that are tightly regulated by ligand binding in other nuclear receptors. In light of the many interactions it undergoes with other proteins, Nur77 could also be considered to be a molecular 'chameleon': a protein that selectively adopts the responsiveness of other proteins by directly interacting with them. Secondly, the protein–protein interactions with Nur77 described in this review have been studied in a wide range of cell types, such as immune cells (T-cells, thymocytes, monocytes and macrophages); somatic cells (neurons, smooth muscle cells, endothelial cells and hepatocytes) and cancer cells from diverse origins. We reason that a stimulus- and cell type-specific expression pattern of interacting proteins may be decisive in determining both the interactions of NR4As with other proteins and their activity in general. The well-studied interaction between Nur77 and RXRα, which has unique outcomes depending on both the cell type studied and the stimulus used, is one such interaction that is modulated by stimulus- or cell type-specific auxiliary proteins.

Lastly, there is a large amount of overlap in interacting proteins between the three NR4A nuclear receptors. All three domains of the NR4As are involved in interactions with other proteins (Tables 1–5, Fig. 2), and we think that the unstructured N-terminal domains are of special interest as they have the lowest overall amino acid similarity (Fig. 1). Based on this dissimilarity, it could be hypothesized that the N-terminal domain of each NR4A receptor interacts with a unique set of proteins that specifically regulates each of their activities, if it were not for the fact that this review has shown that the interacting partners of the NR4As strongly overlap. However, a closer look at the N-terminal domains of Nur77, Nurr1 and NOR-1 reveals small stretches of relatively high similarity within the amino acid sequences (Fig. 4). The possible importance of these small stretches of high similarity is most readily apparent when looking at phosphorylation sites of the NR4As. For

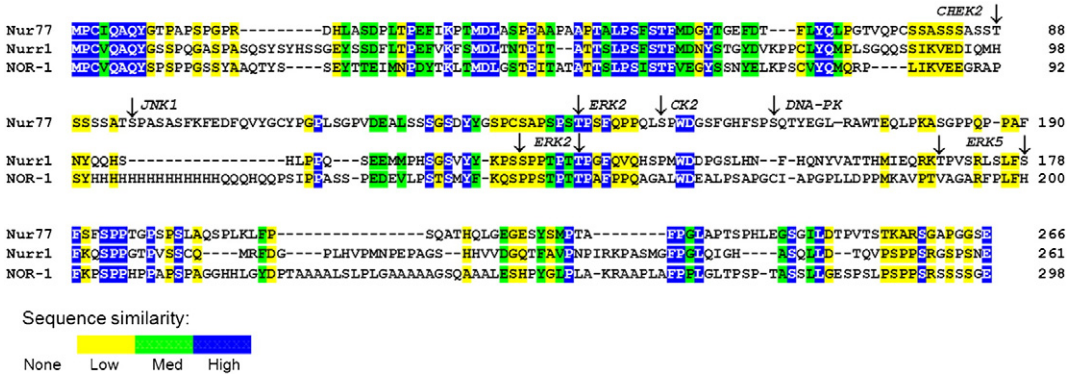

Fig. 4. Amino-acid sequence similarity between the N-terminal domains of the NR4A receptors. The amino-acid sequence of the N-terminal domains of Nur77, Nurr1 and NOR-1 was aligned and the extent of sequence similarity is indicated with colors; e.g. blue indicates the regions where the sequence of the three NR4As is identical. In the Nur77 sequence, the CHEK2 target Thr88, the JNK1 target Ser95, the ERK2 target Thr143, the CK2 target Ser152, and the DNA-PK target Ser164 are indicated with arrows. In the Nurr1 sequence, the ERK2 targets Ser126 and Thr132, and the ERK5 targets Thr168 and Ser177 are indicated with arrows.

example, ERK2 phosphorylates both Nur77 and Nurr1 at highly conserved sites in their N-terminal domains (Thr143 of Nur77 [82]; Ser126 and Thr132 of Nurr1) [93]. Additionally, NOR-1 is phosphorylated by ERK2 in vitro [82], but the exact phosphorylation site is not known. Based on our hypothesis, it might be located in the same conserved stretch of the N-terminal domain that is targeted by ERK2 in Nur77 and Nurr1. Similarly, PKC and RSK1/2 were both shown to target all three NR4As in their highly conserved DBDs.

In contrast, some kinases phosphorylate amino-acid residues that are not located in conserved stretches. In line with our hypothesis, these kinases only affect one of the NR4As. For example: protein stabilization of Nur77 by Pin1, which requires phosphorylation of the poorly conserved residues Ser95 or Ser152 by JNK1 or CK2, respectively. Pin1 does not affect Nurr1 or NOR1 protein stability, which could be caused by the lack of CK2 or JNK1 consensus sites in these two proteins.

Similarly, DNA-PK and CHEK2 have only been shown to phosphorylate Nur77 so far, and these two kinases both target poorly conserved amino acid residues (Ser164 and Thr88, respectively) as well. Finally, two ERK5 phosphorylation sites in Nurr1 (Thr168 and Ser177) are localized in a region of the N-terminal domain with low amino acid sequence similarity between the three family members, suggesting that Nur77 and NOR-1 are not phosphorylated by ERK5 in this amino-acid stretch.

In summary, this review shows that even though the NR4As have no known ligands, they are still tightly regulated through a plethora of protein–protein interactions. Our current knowledge on the NR4A interactome is most likely far from complete and the exact interplay between the interacting proteins and the regulation of such interactions will provide deeper inside in NR4A receptor function.

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