NR4A Orphan Nuclear Receptors
Transcriptional Regulators of Gene Expression in Metabolism and Vascular Biology

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Abstract—Members of the nuclear hormone receptor superfamily, including the peroxisome proliferator–activated receptor and the liver X receptor subfamilies, orchestrate transcriptional networks involved in the control of metabolism and the development of vascular disease. In addition to these well-characterized ligand-activated transcription factors, the nuclear receptor (NR) superfamily comprises many orphan receptors, whose ligands and physiological functions remain unknown. Among this group of orphan receptors is the NR4A subfamily, including Nur77 (NR4A1), Nurr1 (NR4A2), and NOR1 (NR4A3). These orphan NRs constitute an evolutionary ancient and highly conserved group of transcription factors. In contrast to other members of the superfamily, NR4A receptors function as ligand-independent transcription factors and immediate- or early-response genes, which are rapidly induced by a pleiotropy of environmental cues. Early functional studies have pointed to a critical role of NR4A receptors in regulating differentiation, proliferation, and apoptosis. More recent research has characterized NR4A receptors as key transcriptional regulators of glucose and lipid homeostasis, adipogenesis, inflammation, and vascular remodeling. In this review, we will summarize recent advances in understanding the molecular biology and physiological functions of NR4A receptors and discuss their role in the transcriptional control of metabolism and vascular remodeling. (Arterioscler Thromb Vasc Biol. 2010;30:1535-1541.)

Key Words: gene expression • metabolism • vascular biology • nuclear receptors

Members of the nuclear hormone receptor superfamily have emerged as a potentially large class of therapeutic targets for the treatment of obesity, diabetes mellitus, and atherosclerotic disease. Most signaling pathways in these complex diseases ultimately converge to control networks of gene expression through signal-regulated transcription factors, including nuclear receptors (NRs). The understanding of their ability to sense environmental cues and translate endocrine and metabolic signals into specific gene expression programs in metabolism and vascular biology has considerably expanded our knowledge on the pathophysiology of these prevalent diseases. For example, the adopted orphan NRs of the peroxisome proliferator–activated receptors and liver X receptors subfamilies have been characterized to orchestrate gene expression programs involved in the control of glucose homeostasis, lipid metabolism, inflammation, and proliferation in the vascular wall. Pharmacological ligands for these 2 NRs have been developed, and their ligand-induced activation improves glucose metabolism and prevents atherosclerosis in murine models, attesting to the importance of these receptors and the approach to develop pharmacological ligands. The human genome contains 48 members of the NR superfamily. In addition to the classic endocrine receptors and the adopted orphan receptors, the NR superfamily comprises an even larger group of orphan receptors. Although the ligands for these orphan receptors remain unknown, considerable progress has been made in identifying their regulated target genes and characterizing their physiological functions. Among these orphan receptors is the NR4A subfamily, including Nur77 (NR4A1), Nurr1 (NR4A2), and NOR1 (NR4A3). Members of this subfamily function as ligand-independent NR and early-response genes regulating key cellular processes, including inflammation, proliferation, differentiation, and survival. In this review, we will summarize recent progress in understanding the physiological function of NR4A receptors and discuss their role as transcriptional regulators of gene expression in metabolism and vascular biology.

Molecular Biology of NR4A Orphan NRs
NRs share a common structure consisting of a ligand-independent AF-1 transactivation domain in the N-terminal region, a highly conserved DNA-binding domain composed of 2 zinc fingers recognizing specific DNA sequences, and a ligand-binding domain (LBD) that contains a ligand-dependent AF-2 transactivation domain in its C-terminal...
NR4A receptors share this common NR structure, and the 3 members reveal a high degree of homology in their genomic structure and conservation of their DNA-binding domain (degree of conservation >90%). However, several lines of evidence indicate that NR4A receptors may represent a distinct group of transcription factors that do not function in a classic manner. Mutation analysis indicated that NR4A receptors function as constitutively active receptors whose transcriptional activity is independent of the LBD. Instead, their transcriptional activity and coactivator recruitment appear to be dependent on the N-terminal AF-1 domain, which constitutes a common distinction of ligand-independent transcriptional activation by NR. This initial observation was supported by the finding that the LBD of NR4A receptors contains hydrophilic surfaces instead of the classic hydrophobic cleft that mediates coactivator recruitment of other NRs. Finally, this unusual structure of the NR4A LBD has recently been confirmed by X-ray crystallography, demonstrating that the Nur1 LBD contains no cavity as a result of hydrophobic residues in the region normally occupied by ligands. Considering these observations, NR4A receptors are thought to function as constitutively active and ligand-independent receptors, whose transcriptional activity is primarily dependent on the expression of the receptor and its posttranslational modification.

NR4A receptors are early immediate-response genes, which are induced by a pleiotropy of stimuli, including growth factors, inflammatory stimuli, cytokines, peptide hormones, and cellular stress. Once their expression is induced, NR4A receptors activate transcription by binding as monomers or homodimers to canonical DNA target sites, the nerve growth factor–induced protein B–responsive element (NBRE) consisting of an octanucleotide (AAAGGTCA) motif. NR4A homodimers preferentially bind to the Nur-responsive element, which constitutes an everted repeat of the NBRE-related sequence (AAAT(G/A)(C/T)CA) found in the pro-opiomelanocortin gene promoter. In addition, Nur77 and Nur1 (but not NOR1) heterodimerize with retinoid X receptor (RXR) and activate transcription through a DR-5 element in a 9-cis-retinoic acid–dependent manner. This heterodimerization of Nur1 with RXR is isotype specific because Nur1 interacts only with RXRα and RXRγ but not with RXRB. Furthermore, different NR4A receptors can form heterodimers to synergistically activate transcription. Although it was initially thought that NR4A receptors only activate genes, a recent study provided the first evidence that Nur1 can also repress inflammatory gene promoters by recruiting corepressor complexes.

In addition to the rapid expression as early-response genes, the transcriptional activity of NR4A receptors is regulated by posttranslational modification. All 3 NR4A receptors are phosphorylated at serine residues in response to growth factor–dependent activation of various kinases, including mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB/Akt), Jun-N-terminal kinase (JNK), and ribosomal S6 kinase (RSK). For example, Nur77 is phosphorylated at serine 350 and serine 354 within the DNA-binding domain, which inhibits the transactivation activity. Furthermore, phosphorylation of Nur77 at serine 105 induces nuclear export of the Nur77/RXR heterodimer complex, providing an additional mechanism by which phosphorylation may inhibit the transcriptional activity of Nur77. In addition to phosphorylation, all NR4A receptors contain sumoylation consensus sites, and sumoylation of Nur1 induces or inhibits the transcriptional activity in a sumoylation site-specific manner. Although still in their infancy, these posttranslational modifications regulate the transcriptional activity and may represent a major mode of the control of gene expression by NR4A receptors.

**NR4A Receptors in Metabolism and Energy Balance**

**Carbohydrate Metabolism**

All 3 NR4A receptors are potently induced in the liver in response to physiological stimuli, including fasting and glucagon stimulation (Figure 1). Furthermore, hepatic NR4A receptor expression is increased in diabetic mice as a model of pathological gluconeogenesis. Functional studies further
demonstrated that adenoviral overexpression of Nur77 increases the expression of genes involved in gluconeogenesis and stimulates hepatic glucose production in mice. Interestingly, Nur77 overexpression induces several gluconeogenic genes, including glucose-6-phosphatase (G6pc), fructose bisphosphatase 1 (Fbp1), Fbp2, and enolase 3 (Eno3), which all contain NBRE consensus sites in their promoters. Therefore, this study provides the first experimental evidence that NR4A receptors regulate gluconeogenesis and may serve to link hormonal stimulation to downstream metabolic gene expression.

In skeletal muscle, NR4A receptors are induced by growth factors, β-adrenergic signaling, and endurance exercise. Maxwell et al first demonstrated that knockdown of Nur77 in muscle cells results in decreased lipolysis and the expression of genes regulating energy expenditure and lipid homeostasis, including AMP-activated protein kinase, uncoupling protein (UCP) 3, glucose transporter-4 (GLUT4), CD36, adiponectin receptor 2, and cavelin 3. Conversely, Chao et al reported that overexpression of Nur77 in C2C12 muscle cells increases the expression of genes involved in glucose and glycogen metabolism, whereas Nur77 deficiency in mice reduces the expression of genes involved in skeletal muscle glucose use in vivo. Consistent with this role of Nur77 in promoting glucose utilization was the observation that Nur77-deficient mice develop skeletal muscle insulin resistance when fed a high-fat diet as the result of altered insulin signaling and reduced Glut4 expression. Although glucose metabolism has not been studied in NOR1-deficient mice, knockdown of NOR1 in skeletal muscle cells attenuates the expression of genes that control fatty acid oxidation and pyruvate use (ie, peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, PGC-1β, lipin-1α, pyruvate dehydrogenase phosphatase (PDP)-1r, and PDP1c), indicating that NOR1 may be necessary for oxidative metabolism. Finally, NOR1 has recently been demonstrated to also promote insulin-stimulated glucose uptake in adipocytes by augmenting insulin signaling and Glut4 translocation. These intriguing observations point to a key role of NR4A receptors in the transcriptional control of glucose homeostasis and oxidative metabolism.

**Lipid Metabolism**

Accumulating evidence indicates that NR4A receptors regulate various aspects of lipid metabolism. Initial experiments by Maxwell et al demonstrated that Nur77 promotes lipolysis in muscle. Subsequently, Pols et al revealed that Nur77 modulates plasma lipoprotein profiles and hepatic lipid metabolism in mice. In this study, adenoviral-mediated overexpression of Nur77 increased plasma LDL cholesterol and decreased HDL cholesterol while reducing hepatic triglyceride levels, which was thought to be the result of repression of the lipoprotein transcription factor SREBP1c. Consistent with these data, Chao et al noted hepatic steatosis and increased SREBP1c expression in Nur77-deficient mice fed a high-fat diet. However, because Nur77 did not directly affect SREBP1c activity in reporter assays, it was concluded that the hepatic steatosis in Nur77-deficient mice was likely secondary to the lipogenic effect of hyperinsulinemia.

In 3T3-L1 preadipocytes, NR4A receptor expression is induced during adipogenesis and initiation of the differentiation program. Initial studies using small interfering RNA (siRNA) approaches and overexpression of an Nur77 mutant lacking the N-terminal AF-1 transactivation domain indicated that Nur77 is not required for adipocyte differentiation. However, a functional role for NR4A receptors in adipogenesis was suggested by 2 recent in vitro studies, which demonstrated that constitutive NR4A receptor expression in 3T3-L1 preadipocytes inhibits adipocyte differentiation. One of the mechanisms proposed for this negative regulation of adipogenesis by NR4A receptors has been the inhibition of the mitotic clonal expansion of preadipocytes. However, considering that the initial mitotic expansion step is primarily a prerequisite for 3T3-L1 preadipocyte differentiation, further studies are required and there are likely additional mechanisms involved by which NR4A receptors inhibit adipogenesis. These may include direct regulation of target genes affecting adipogenesis, including extracellular matrix genes. In addition, NR4A receptors may cross talk with adipogenic signaling and transcriptional programs, particularly because Nur1 and Nur77 have been reported to interact with Wnt signaling pathways or the glucocorticoid receptor, which both play important roles in adipogenesis.

**Energy Homeostasis**

Brown adipose tissue plays a key role in energy balance and is the primary organ involved in thermogenesis through uncoupling of mitochondrial respiration by the action of UCPs. Early studies have demonstrated that Nur77 expression is highly induced in response to β-adrenergic stimulation of brown adipocytes, whereas transcript levels of all 3 NR4A receptors are induced during cold exposure. Kanzleiter et al demonstrated a repressive effect of Nur77 on the UCP-1 promoter in brown adipocytes, which was likely indirect because Nur77 did not directly interact with the UCP-1 promoter. Despite this repression of UCP-1, nonshivering thermogenesis was not affected by Nur77 deficiency in mice. In contrast, Kumar et al observed that NOR1 transcriptionally upregulates UCP-1 expression by binding to an NBRE site on the UCP-1 promoter. Furthermore, overexpression of an Nur77 mutant lacking the N-terminal AF-1 transactivation domain prevented UCP-1 transcription induced by β-adrenergic signaling. The reasons underlying these seemingly conflicting 2 studies remain unclear but are likely the result of differential regulation of UCP-1 by Nur77 and NOR1. Moreover, NR4A receptors may affect the central regulation of energy homeostasis because injection of NOR1 siRNA into the third cerebral ventricle significantly suppresses food intake and body weight in mice. In concert, these intriguing studies characterize NR4A receptors as important regulators of energy balance and food intake, although the underlying mechanisms remain elusive and warrant further studies in gene-targeted mice.

**NR4A Receptors in Vascular Biology**

An increasing number of studies have demonstrated that all 3 members of the NR4A subfamily are expressed in the developing neointima and in advanced atherosclerotic le-
sions. Moreover, accumulating evidence indicates that NR4A receptors constitute important transcription factors in the control of vascular gene expression and play critical roles in essentially all aspects of vascular remodeling, including cell viability, proliferation, and inflammation (Figure 2). In the following section, we will briefly summarize these studies, pointing to a previously unrecognized function of NR4A receptors in vascular biology.

Cell Viability and Proliferation
Endothelial cell injury, followed by the expression of adhesion molecules and the subsequent recruitment of circulating monocytes, constitutes a critical event for the initiation of atherosclerosis. All 3 NR4A receptors are potently induced by a variety of proatherogenic stimuli in endothelial cells, including atherogenic lipoproteins, inflammatory cytokines, growth factors, and hypoxia (Figure 2). The transcriptional mechanisms governing this inducible expression have been primarily studied in the context of growth factor – and hypoxia-induced NOR1 expression. Although the former mechanisms involve cAMP response element binding (CREB) protein–dependent activation of the NOR1 promoter, NOR1 expression in response to hypoxia is dependent on hypoxia-inducible factor 1 binding to a hypoxia response element in the promoter. Arkenbout et al performed the first functional experiments in endothelial cells and demonstrated that adenoviral overexpression of Nur77 inhibits proliferation of this cell type by upregulating p27Kip1 and downregulating cyclin A. However, the role of Nur77 for endothelial cell proliferation remains controversial because Zeng et al reported that Nur77 induces proliferation and cell cycle gene expression. Moreover, this report noted that angiogenesis is induced by overexpression of Nur77 and is decreased in Nur77−/− mice. With respect to the sibling NOR1, Rius et al identified a mitogenic role for this receptor by demonstrating that antisense oligonucleotides against NOR1 inhibit endothelial cell growth and wound repair after injury. Consistent with these observations, NOR1 has recently been characterized as a prosurvival gene in endothelial cells exposed to hypoxia by inducing the expression of cellular inhibitor of apoptosis protein 2. Collectively, these studies establish a role for Nur77 and NOR1 in regulating endothelial cell survival and proliferation; however, little is known about the transcriptional target genes and molecular mechanisms. At present, only 2 direct NR4A target genes have been identified in endothelial cells. Gruber et al characterized plasminogen activator inhibitor 1 as a Nur77 target gene, which is activated by the receptor through direct binding to an NBRE site in the promoter. In addition, You et al demonstrated that Nur77 overexpression prevents nuclear factor (NF)κB nuclear translocation in endothelial cells by enhancing the expression of IκBα, which is mediated through direct transactivation of an NBRE site in the IκBα promoter. Interestingly, the functional relevance of Nur77-dependent IκBα expression was confirmed by the finding that Nur77 inhibited the expression of vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 in endothelial cells.

Similar to endothelial cells, NR4A receptor expression is rapidly induced in response to atherogenic stimulation of smooth muscle cells (SMCs), including lipoproteins, cyclic stretch, and mitogenic stimuli. The transcriptional induction of NOR1 in SMCs is mediated through mitogen-induced CREB, which binds to CRE sites in the NOR1 promoter and can be pharmacologically inhibited by simvastatin. Consistent with the previously described growth-inhibitory function of Nur77 in endothelial cells, Nur77 overexpression inhibits SMC proliferation in vitro by stabilizing p27Kip1 and reduces neointima formation in vivo. Interestingly, data from the same group have further recently suggested that SMC-specific overexpression of Nur77 inhibits pathological outward remodeling in response to carotid artery ligation, which was associated with decreased macrophage accumulation and matrix metalloproteinase expression. Although these studies clearly indicate that Nur77 prevents SMC proliferation, NOR1 has been reported to act mitogenic, suggesting a function that is distinct from that of Nur77. A proliferative role of NOR1 was first reported by Martínez-González et al using antisense NOR1 oligonucleotides. Consistent with these initial observations, our own studies have demonstrated a proliferative defect and an increased propensity for apoptosis in SMCs isolated from NOR1-deficient mice. In vivo, the proliferative response and neointima formation following endovascular femoral
artery guide wire injury was decreased in NOR1-deficient mice. At the molecular level, this mitogenic activity of NOR1 was at least in part mediated by transactivation of a canonical NBRE site in the cyclin D1 promoter, characterizing cyclin D1 as a bona fide NOR1 target gene in SMCs. Furthermore, DNA microarray profiling revealed lower expression of NOR1 in elongated SMCs, whereas NOR1 knockdown suppressed DNA synthesis, further supporting the mitogenic function of NOR1 and pointing to a potential role of NOR1 in regulating cell shape. These studies establish an important and distinct role for Nur77 and NOR1 in the control of vascular cell proliferation and remodeling. Continued investigation will be required to define the transcriptional target genes and the molecular basis underlying the differential function of NOR1 and Nur77 in SMC biology.

Inflammation

The first evidence linking NR4A expression with inflammatory signaling was reported by Woronicz et al. who noted that Nur77 is induced in apoptotic T cells and that inhibition of Nur77 function prevented apoptosis. However, mice deficient in Nur77 exhibit unimpaired T-cell apoptosis, and functional redundancy of Nur77 and NOR1 in T-cell apoptosis has been suggested. Similar to T cells, Nur77 expression is increased in apoptotic macrophages; however, in contrast to the experiments performed in T cells, peritoneal macrophages isolated from Nur77-deficient mice reveal a phenotype of reduced cell death. In response to inflammatory activation, all 3 NR4A receptors are potently induced in macrophages. This inducible expression of NR4A receptors in macrophages depends on the activation of NF-κB signaling, as exemplified by the recruitment of NF-κB to response elements in the Nur77 promoter. Functional studies have indicated that NR4A receptors both activate and repress inflammatory genes in macrophages. An initial microarray analysis by Pei et al. discovered that NR4A overexpression in macrophages induces proinflammatory gene expression. Interestingly, among the identified direct Nur77 target genes was the inducible kinase inhibitor of nuclear factor kappa-B kinase (IKK)/IKKe, which functions as an NF-κB–activating kinase, providing a potential mechanism for the activation of inflammatory gene expression by Nur77 in macrophages. In contrast to these studies, Bonta et al. revealed that lentiviral overexpression of each NR4A member reduces certain inflammatory genes (ie, interleukin [IL] 1β, IL-6, IL-8, macrophage inflammatory protein (MIP)1α, MIP1β, and monocyte chemoattractant protein (MCP)-1) and the uptake of oxidized low-density lipoprotein. Finally, a recent study by Saijo et al. identified that Nur1 transcriptionally represses the inflammatory genes tumor necrosis factor α, inducible nitric oxide synthase, and IL-1β in microglia and the murine RAW264.7 cell line. This transrepression was mediated through an Nur1-dependent recruitment of the corepressor for element-1 silencing transcription factor (CoREST) complex to the target promoter and the subsequent clearance of NF-κB. Although these studies indicate that NR4A receptors function as important transcriptional regulators of inflammatory gene expression, further in vivo evidence using animal models deficient for either of the NR4A receptors seems required, particularly with respect to the development of atherosclerosis.

Concluding Remarks

In conclusion, the ligand-independent NR4A orphan NRs are immediate early-response genes whose protein products are rapidly induced in metabolic and vascular tissues in response to a pleiotropy of stimuli. Emerging evidence indicates that NR4A receptors regulate the transcription of genes involved in glucose homeostasis, lipid metabolism, and energy balance. Moreover, the initial characterization of their function in vascular biology has implicated these transcription factors in control of inflammation, proliferation, apoptosis, thrombosis, and angiogenesis. Despite recent advances in understanding the role of NR4A receptor function in physiological and pathological processes, important questions remain for future research. For example, future effort will require further validation of NR4A receptor function in murine models and rely on various gene-targeting approaches. In particular, it seems essential to determine whether the 3 different NR4A receptors exhibit similar or distinct functions in various tissues. Furthermore, few NR4A receptor-regulated genes have been identified; it will not only be important to characterize target genes but also to define the detailed transcriptional mechanisms underlying this regulation. Finally, the possibility of modulating the expression and/or transcriptional activity of NR4A receptors may provide pharmacological applications. Considering the lack of a classic ligand-binding pocket, such an approach might involve the modulation of cofactor recruitment and/or posttranslational modifications. Continued investigation of these questions and identification of NR4A-regulated target genes will provide new insights into how these orphan NRs participate in the development of physiology and disease.

Sources of Funding

This study was supported by grant R01 HL084611 from the National Institutes of Health (Dr Bruemmer). Dr Zhao is the recipient of Predoctoral Fellowship Grant 0815514D from the American Heart Association.

Disclosures

None.

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Arterioscler Thromb Vasc Biol. 2010;30:1535-1541
doi: 10.1161/ATVBAHA.109.191163
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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