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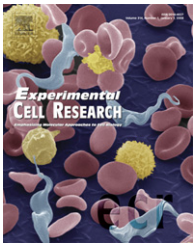

Research Article

Mutations in the AF-2 region abolish ligand-induced intranuclear immobilization of the liver X receptor α

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ABSTRACT

The liver X receptors (LXR) α and β are ligand-induced transcription factors that regulate the expression of genes important for cholesterol metabolism, lipogenesis, and other metabolic pathways. Despite their high degree of similarity, LXRs have redundant as well as nonredundant functions. The regulation of LXRs' intranuclear mobility most likely plays a major role in the regulation of their transcriptional activities. In order to elucidate how ligand binding, receptor–protein and receptor–DNA interactions affect intranuclear receptor mobility, we expressed transcriptionally active yellow fluorescent protein (YFP)-LXR α and YFP-LXR β in Cos-7 cells. We used the fluorescence recovery after photobleaching (FRAP) technique and confocal laser scanning microscopy as well as Triton X-100 permeabilization experiments and fluorescence microscopy to measure differences in the intranuclear mobility between LXR α and LXR β. The image analyses revealed that after agonist binding, LXR α exhibits slower intranuclear trafficking and greater intranuclear immobilization compared with LXR β. In addition, mutational analysis showed that the integrity of the Activation Function (AF)-2 region of LXR α is essential for its immobilization whereas the integrity of the DNA binding domain is not. These findings imply that specific protein interactions with the AF-2 region of LXR α play a role in its intranuclear immobilization.

Introduction

Nuclear receptors such as liver X receptors (LXR) are agonist-induced transcription factors. LXRs bind as heterodimers with RXR to response elements in promoter regions of target genes to regulate their transcription. Natural ligands for LXRs are oxysterols, as well as the RXR ligand 9-*cis* retinoic acid [1–3]. LXRs are found as two isotypes, LXR α and LXR β. Both LXR α and LXR β regulate the expression of gene products for cholesterol [4–6], and for lipogenesis [7,8].

LXR α and LXR β share a 78% amino acid sequence similarity, bind to similar DNA response elements, and are activated by similar ligands. Therefore, the similarity in the general mechanisms required for the activation of transcription by nuclear receptors suggests the activation of similar genes by LXR α and LXR β. In tissues such as liver, however, where both receptors are expressed, LXR α is the dominant isotype and LXR β cannot substitute for the lack of LXR α. Knockout studies have shown that while LXR α knockout mice exhibited a dramatic reduction in the ability to regulate high levels of dietary cholesterol, LXR β knockout mice resembled wild-type mice [4,6] although both LXRs can regulate lipogenic genes in the liver [4,9]. Concurrently, the LXR α knockout mice showed a decrease in the expression of certain lipogenic genes, whereas the LXR β knockout mice showed wild-type levels of those lipogenic genes [6,10]. These data indicate the presence of regulatory mechanisms that are

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selective for LXR α and LXR β. We have recently shown that nuclear localization of LXR α and LXR β is differentially regulated [11]. Regulation of nuclear localization most likely plays a role in the regulation of transcriptional activity.

The packaging of DNA in chromatin has important consequences in terms of its availability as a template for transcription, so chromatin structure is a critical aspect of gene expression. Agonist binding causes a conformational change in nuclear receptors that allows co-regulators to bind to their activation function (AF)-2 regions [12,13]. The current model of temporal and spatial events leading to transcription of genes regulated by nuclear receptors includes recruitment of a scaffold protein Transcription/TRanslation Associated Protein (TRRAP) [14]. TRRAP recruits histone acetyl transferases such as steroid receptor co-activator (SRC)-1. Histone acetylation then allows ATP-dependent chromatin remodeling SWI/SNF complexes to bind [15]. SWI/SNF complexes mediate nucleosome sliding, better accessibility to DNA with histone bound, complete dissociation of histones and DNA at specific sites [16], or histone replacement with a variant histone [17]. Then, nuclear receptors bind to DNA and attract co-regulators such as glucocorticoid receptor interacting protein (GRIP) as part of the mediator complex that ultimately attracts RNA polymerase II to the start site of target genes.

Components of SWI/SNF complexes, SRC-1, and TRRAP contain the LXXLL nuclear receptor interaction motif [17] that allows them to bind to the AF-2 regions of type I nuclear receptors such as the glucocorticoid receptor (GR), the estrogen receptor (ER), and the androgen receptor [18–20] as well as type II nuclear receptors such as thyroid hormone receptor, small heterodimerizing protein, retinoid acid receptors, peroxisome proliferator-activated receptor vitamin D receptor [21]. Conserved leucines/methionines in the AF-2 of ER and the retinoid X receptor (RXR) play a role in binding to chromatin remodeling proteins related to SWI/SNF complexes [18,22].

Nuclear receptors are highly mobile. Such mobility allows nuclear receptors to be continuously accessible to signaling pathways, as well as ligand–receptor, receptor–DNA, and protein–protein interactions including receptor–cofactor interactions. When functional fluorescent protein chimeras of nuclear receptors are expressed in living cells, fluorescence recovery after photobleaching (FRAP) experiments can visualize their intranuclear trafficking in these living cells. The fluorescence of the protein chimera is bleached using a laser beam focused on a defined subnuclear compartment and recovery of fluorescence in the bleached area over time determines the diffusion rates of the fluorescent protein chimeras within the nucleus. This technique showed that nuclear receptors moved quickly through the nucleus but were excluded from the nucleoli, and that these movements can be affected by various stimuli [23]. Longer residence times of GR at the transcription site were associated with greater transcriptional output [24].

The efficiency of nuclear receptors including LXR α and LXR β to activate gene expression likely correlates to their efficiency to recruit co-regulators. In addition, selectivity of gene expression is likely determined by the selective recruitment of co-regulators. Our previous data indicated that the regulation of LXR α and LXR β activity is mostly in the nucleus. Therefore, we compared the intranuclear mobility and subnuclear retention of LXR α and LXR β and explored the role of the DNA binding region and of the AF-2 region in the partial immobilization we found for agonist-bound LXR α.

Materials and methods

Cells and expression constructs

Transcriptionally active yellow fluorescent protein (YFP)-LXR α and YFP-LXR β were generated as described earlier [16]. YFP, YFP-LXR α and YFP-LXR β were expressed in Cos-7, HEK293, and HepG2 cells which were obtained from ATCC (Manassas, VA) and grown as described previously (25,29). Briefly, cell cultures were maintained in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Hyclone, Logan, UT), 2 mM glutamine (Invitrogen, Carlsbad, CA), and 0.1 mg/ml gentamicin (Invitrogen, Carlsbad, CA).

Microscopy

Cos-7, HEK293, or HepG2 cells were plated onto chambered cover slips (Nalge Nunc Int., Naperville, IL) and transfected with either wild-type or mutant YFP-LXR α and YFP-LXR β (0.5 μg/slide for HEK293 and Cos-7, 2 μg/slide for HepG2 cells) using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Cells were used for microscopy within 48 h of transfection. Prior to imaging experiments, cells expressing wild-type or mutant YFP-LXR α and YFP-LXR β were cultured for 18 h in media containing 5% FBS without lipoproteins (Intracel, Frederick, MD) to deplete the cells of ligands for LXR. Cells were then treated with vehicle (0.1% ethanol) or agonist T090317 (1 μM). Images were collected from a Leica TCS SP2 confocal system (Leica Microsystems, Mannheim, Germany) with the 514 nm line of a krypton–argon laser and a spectral detector of emission between 525–600 nm.

Fluorescence recovery after photobleaching (FRAP) experiments

Cos-7, HEK293, or HepG2 cells plated and transfected as described above were pretreated for 20 min with T090317 (1 μM) or vehicle on the day after transfection. The nuclei of 20 transfected cells with similar brightness were chosen for FRAP experiments. A 4.7 μm^2 region of interest (ROI) in these nuclei was photobleached with a laser beam (set to 100%, compared to 8% for imaging). Fluorescence recovery in this ROI was monitored over time using the FRAP software provided by Leica. As a control, cells transfected with YFP and cells transfected with YFP-LXR β and fixed with 4% paraformaldehyde were used for similar experiments. As controls, fluorescence intensities were monitored in three additional ROIs within and outside the tested nucleus (Fig. 2A). Recovery half-time for each 20 cells was evaluated and normalized according to Leica's instructions and the data is presented as the mean ± standard error. Significance of differences between data sets was calculated using Student's *t*-test.

Nuclear extraction experiments

Cos-7 cells were plated onto Poly-L-lysine-coated chamber slides (Nalge Nunc Int., Naperville, IL) and transfected with either wild-