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Review

Ligand-independent actions of the orphan receptors/corepressors DAX-1 and SHP in metabolism, reproduction and disease

Anna Ehrlund, Eckardt Treuter*

Center for Biosciences, Department of Biosciences and Nutrition, Karolinska Institutet, S-14183 Huddinge/Stockholm, Sweden

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ABSTRACT

DAX-1 and SHP are two closely related atypical orphan members of the nuclear receptor (NR) family that make up the NR0B subfamily. They combine properties of typical NRs and of NR-associated coregulators: both carry the characteristic NR ligand-binding domain but instead of a NR DNA-binding domain they have unique N-terminal regions that contain LxxLL-related NR-binding motifs often found in coregulators. Recent structural data indicate that DAX-1 lacks a ligand-binding pocket and thus should rely on ligand-independent mechanisms of regulation. This might be true, but remains to be proven, for SHP as well. DAX-1 and SHP have in common that they act as transcriptional corepressors of cholesterol metabolism pathways that are related on a molecular level. However, the expression patterns of the two NRs are largely different, with some notable exceptions, and so are the physiological processes they regulate. DAX-1 is mainly involved in steroidogenesis and reproductive development, while SHP plays major roles in maintaining cholesterol and glucose homeostasis. This review highlights the key similarities and differences between DAX-1 and SHP with regard to structure, function and biology and considers what can be learnt from recent research advances in the field.

This article is part of a Special Issue entitled 'Orphan Receptors'.

Contents

DAX-1 (NR0B1) and SHP (NR0B2) possess a unique place within the nuclear receptor (NR) family. Instead of binding to regulatory DNA sequences directly they appear to control transcription mainly as corepressors by associating with NRs and other transcription factors. Whether or not the activity of the orphan receptors DAX-1 and SHP can be regulated by ligands remains an intriguing open issue. Considering recent progress in understanding their physiological roles individually (reviewed in [1–4]) we attempt in this review to highlight the apparent similarities and some key differences between DAX-1 and SHP with respect to structure, function and physiology.

1. A brief history of NR0B research

NR0B1, commonly known as DAX-1 (*D*osage-sensitive sex reversal (DSS), *A*drenal Hypoplasia Congenita (AHC) critical region on chromosome *X*, gene *1*), derived its name from two syndromes caused by genetic alterations of the NR0B1 locus in humans. In DSS, a duplication of the DAX-1 gene locus causes XY-individuals to develop as females [5]. The study describing DSS initiated the discovery of the *DAX-1* gene in 1994, which was recognized to encode an unusual NR family member. It was found that mutations in the DAX-1 coding sequence caused yet another

* Corresponding author. Tel.: +46 52481060; fax: +46 7745538.
E-mail address: eckardt.treuter@ki.se (E. Treuter).

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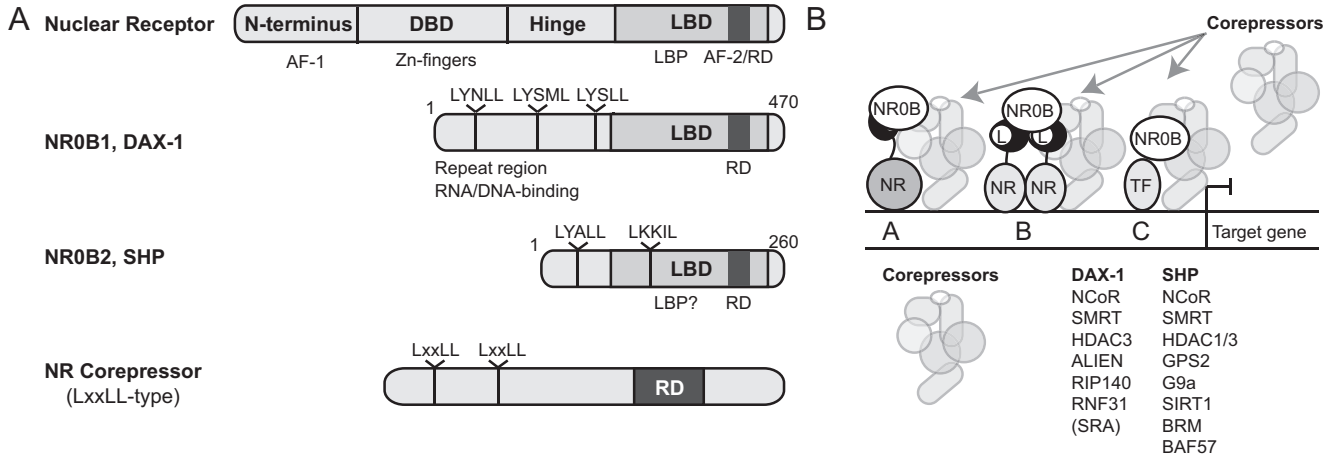

Fig. 1. Basic domain structure and corepressor function of NR0B orphan receptors. (A) Domain structure of conventional NRs (subfamilies 1–6), of NR subfamily 0 group B members, and of a prototypical LxxLL-corepressor. NR0B1/2 combine structural domains of NRs, i.e. a NR LBD including a functional repressor domain (RD), with the NR-binding domain (LxxLL-motif) of NR-associated LxxLL-corepressors such as RIP140, PROX1 and LCoR. The NR0B1 N-terminus consists of three repeats containing LxxLL-motifs and RNA/DNA-binding capability. The NR0B2 N-terminus is similar to one NR0B1 repeat and contains one LxxLL-motif, while a second LxxLL-motif is located within the LBD (see Fig. 2). All highlighted domains and key elements are conserved between mammalian NR0B members. *Abbreviations*: AF-1: activation function-1, a ligand-independent, non-conserved N-terminal activation domain of some NRs; AF-2: activation function-2, a conserved binding surface for LxxLL-type coactivators/corepressors, ligand-dependent or constitutively active; RD: repressor domain, a binding surface for distinct (non-LxxLL-type) corepressors, e.g. NCoR; DBD: zinc finger-type NR DNA-binding domain; LBD NR ligand-binding domain mediating binding of ligands, transcriptional coactivators and corepressors and NR dimerization; LBP: ligand-binding pocket consisting of LBD residues that are in direct contact with the NR ligand. (B) NR0B transcriptional corepressor mechanism. A. NR0Bs recruited to LBD of monomeric NRs e.g. LRH-1, SF-1 forming an atypical heterodimer/trimer. B. NR0Bs recruited to NR dimers (homo- or heterodimers), "ternary complex" formation of unknown stoichiometry. C. NR0Bs recruited to non-NR transcription factors e.g. EWS/FLI1, HNF1, SREBP1c or NFkB. A–C. Two-step mechanism of NR corepression by NR0B: displacement of NR-coactivators and recruitment of NR0B-associated corepressors via the RD.

developmental disorder, X-linked Adrenal Hypoplasia Congenita (AHC). In AHC the adrenal development is disrupted leading to adrenal hormone insufficiency at birth although mild cases may remain undetected for several years. This syndrome is also associated with hypogonadotropic hypogonadism [6,7]. To date, a plethora of different mutations of NR0B1 causing AHC have been described including frameshifts, premature terminations and single amino acid mutations. Interestingly, the severity of AHC symptoms varies among the patients, even among patients carrying the same mutation. Genetic background seems to be of importance making some individuals more sensitive than others [8,9].

Subsequent investigations led to the discovery that DAX-1 has a restricted expression pattern, in principle limited to the Hypothalamic–Pituitary–Adrenal/Gonadal (HPA)-axis [10]. Another NR family member, steroidogenic factor-1 (SF-1, NR5A1), has a strikingly similar expression pattern [11,12] and was discovered to work in pair with DAX-1 to regulate the transcription of enzymes active in the conversion of cholesterol to steroid hormones. These enzymes include the cholesterol transporter StAR [13] that moves cholesterol through the mitochondrial membrane, members of the cytochrome P450 enzyme family [14–16] and genes important for gonadal function [17,18]. It was noted that DAX-1 seemed to function as a transcriptional repressor rather than an activator in these early studies. A time line with key references is given in Table 1.

The DAX-1 gene is located on chromosome Xp21.3 in humans (NCBI reference sequence: NG_012143.1) and consists of two exons encoding a conserved protein of approximately 470 aa in mammalian species. Human DAX-1 has two splice variants resulting from use of an alternative second exon (exon 2 and exon 2A). The longer, 470 aa isoform, DAX-1, is the most described compared to the shorter, 400 aa long isoform DAX-1A [19]. The functional importance of the shorter form has yet to be characterized but it seems it is expressed to a lesser extent compared to DAX-1 in the steroidogenic tissues [20].

The second subfamily member NR0B2, commonly known as SHP (*S*mall *H*eterodimer *P*artner) was identified in a number of laboratories using two-hybrid protein–protein interaction screenings aimed at discovering NR-interacting proteins. Moore and colleagues were the first to report in 1996 the cloning of human SHP and its initial characterization [21]. Their studies revealed that SHP, like DAX-1, represents an unusual orphan NR that lacks a DNA-binding domain and apparently heterodimerizes with a variety of NRs, letting the authors propose the name "small heterodimer partner". As with DAX-1 an early observation was that SHP interactions generally inhibit the transcriptional activities of its target NRs, and up till now this seems the most critical function of NR0Bs in the diverse transcriptional pathways that they regulate. Subsequent studies clarified the molecular details of how SHP interacts with, and represses the activity of, NRs (see below, Fig. 1 and Table 1).

Regarding physiological functions, researchers noted that SHP is abundantly expressed in the enterohepatic system, for example in liver and intestine. Pioneering work by the groups of Kliewer, Mangelsdorf, Moore, Schwarz, including the generation of conventional SHP knockout mice, emphasized a role of SHP in regulatory NR cascades governing bile acid synthesis and cholesterol homeostasis [22–25]. Talianidis and colleagues employed liver-specific SHP transgenic mice to demonstrate a pleiotropic effect of SHP on these and additional hepatic metabolic pathways [26]. Moreover, the initial analysis of the first conditional liver-specific SHP knockout mouse reported by Wyeth in 2009 demonstrated that selective inhibition of SHP in liver protects against dyslipidemia and hepatic inflammation [27]. A time line with key references is given in Table 2.

The SHP gene, in humans located on chromosome 1p36 (NCBI reference sequence: NG_012143.1), consists of two exons and encodes a protein of approximately 260 aa. Splicing variants, which would generate different protein isoforms, are currently unknown. However, genetic polymorphisms and mutations in the human SHP gene have been identified, some of which might be linked to obesity and diabetes [28].

At both gene and protein level, SHP is most homologues to DAX-1, both forming the NR subfamily 0 group B of atypical NRs that presumably derived from a common ancestor during vertebrate