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Modulation of nuclear receptor function by cellular redox poise

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ABSTRACT

Nuclear receptors (NRs) are ligand-responsive transcription factors involved in diverse cellular processes ranging from metabolism to circadian rhythms. This review focuses on NRs that contain redox-active thiol groups, a common feature within the superfamily. We will begin by describing NRs, how they regulate various cellular processes and how binding ligands, corepressors and/or coactivators modulate their activity. We will then describe the general area of redox regulation, especially as it pertains to thiol–disulfide interconversion and the cellular systems that respond to and govern this redox equilibrium. Lastly, we will discuss specific examples of NRs whose activities are regulated by redox-active thiols. Glucocorticoid, estrogen, and the heme-responsive receptor, Rev-erb, will be described in the most detail as they exhibit archetypal redox regulatory mechanisms.

1. Introduction

Nuclear receptors (NRs) comprise a superfamily of eukaryotic ligand-responsive transcription factors that regulate genes involved in a vast array of cellular processes including metabolism, differentiation, reproduction, inflammation, and circadian rhythms. This class of proteins includes, among others, peroxisome proliferator-activated receptors (PPARs) that bind fatty acids, steroid receptors, and the heme-responsive receptor, Rev-erb. The human genome encodes 48 NRs, some of which remain orphaned as their endogenous ligands have yet to be identified. Of those NRs in which the ligand is known, several serve as pharmaceutical targets for the treatment of disease including estrogen receptors for an anti-breast cancer drug (tamoxifen), PPAR-γ for thiazolidinediones used in the treatment of type II diabetes, and the glucocorticoid receptor for dexamethasone, used in the treatment of inflammation [1,2].

NRs are modular proteins that have a conserved architecture, consisting of a highly variable N-terminal A/B domain that is involved in ligand-independent transcriptional regulation (commonly referred to as activator function-1 (AF-1)) and is a site of phosphorylation (Fig. 1, A). Adjacent to the A/B region is the highly conserved DNA-binding domain (DBD) or C region that harbors two Cys_4 zinc-finger motifs. The DBD binds to specific DNA response elements in the promoter of target genes, participates in receptor dimerization, and can contain a nuclear localization signal (NLS). Linking the DBD to the C-terminal ligand-binding domain (LBD) is a poorly conserved hinge or D region that imparts flexibility to the receptor and in many cases contains a NLS. Near the C-terminus is the LBD (E region), which also contributes to receptor dimerization, can contain a NLS, and binds signaling molecules. In addition to the LBD, some receptors also harbor an F region that does not appear to play a role in ligand-dependent transcriptional activation [3–5].

Ligand recognition by some NRs leads to dissociation from cytoplasmic heat shock protein complexes with subsequent nuclear localization. In the nucleus, the NR binds to its cognate DNA response element in the promoter of target genes and recruits transcriptional coactivators such as steroid receptor coactivator (SRC-1, 2, and 3) that ultimately govern the level of target gene activation. Conversely, other NRs are predominantly nuclear and repress gene transcription in the ligand-free state by complexing with corepressors, e.g., nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid hormone receptors (SMRT); in this case, binding of ligand leads to dissociation of the corepressor in exchange for a coactivator [4–6]. With most NRs, ligand binding is accompanied by a major conformational change of LBD α-helix 12 (a.k.a. activator function-2 helix, AF-2) from a position loosely associated with the rest of the globular domain to a compact structure in which it interacts with ligand and forms part of a hydrophobic cleft that serves as the binding interface for coactivators [7].

2. An overview of cellular redox systems and thiol–disulfide redox regulation

In addition to regulation by ligand and corepressor/coactivator binding, some NRs have been found to contain redox-sensitive thiol groups. Early studies of steroid hormone and vitamin D receptors demonstrated that thiol-attacking and modifying compounds like mercurials and iodoacetamide (IAA) adversely affected ligand and DNA binding

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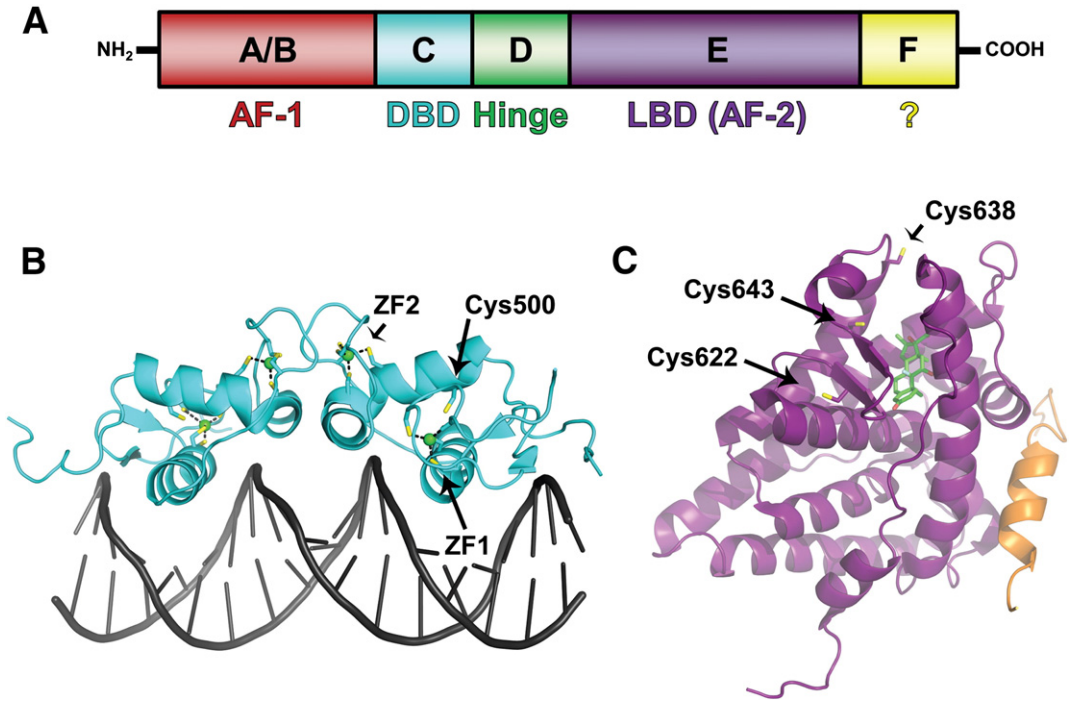

Fig. 1. Nuclear receptor domain organization and three-dimensional structure using glucocorticoid receptor as an example. (A) Modular domain organization of a typical NR. The N-terminal A/B region harbors AF-1 ligand-independent regulatory activity and is a site of phosphorylation. A/B is followed by the highly conserved C region, or DBD that is connected to the LBD (E region, containing ligand-dependent AF-2 activity) by the hinge (D region). Some receptors contain an F region with unknown function. (B) Three-dimensional structure of the rat GR DBD homodimer in complex with a palindromic GRE where the two half-sites are separated by four base pairs (PDB access code 1GLU). Cysteine sulfhydryls involved in redox modulation are highlighted in yellow. In each subunit of the dimer, eight out of nine redox-active cysteine sulfhydryls comprise the two Cys_4 zinc-finger motifs (ZF1 and ZF2). The last sulfhydryl of Cys500 (equivalent to Cys481 in the human GR sequence) resides in NLS1 and is conserved among nuclear receptors. (C) Three-dimensional structure of the human GR LBD in complex with the agonist dexamethasone (depicted as sticks with carbon atoms in green, oxygen in red, and fluorine in cyan) and a peptide derived from the coactivator TIF2 (orange) harboring a LXXLL motif (PDB access code 1M2Z). The three cysteine sulfhydryls implicated in redox modulation of ligand binding are highlighted in yellow and labeled; Cys638 and Cys643 are the pair of arsenite-reactive proximal thiols.

([8] and references therein). In many cases, reductants such as dithiothreitol (DTT) reactivated the NRs, implying that reduced sulfhydryls were required for ligand and/or DNA binding and that these activities could be regulated by the ambient cellular redox potential. It is now clear that redox poise has major functional ramifications on members of the NR superfamily and the pathways they control.

Various cellular redox systems can interface with NRs, allowing the NR to respond to redox poise and to control target pathways in response to perturbations to redox homeostasis. Cellular redox homeostasis is preserved as a delicate balance among three processes, (i) production of harmful reactive oxygen species (ROS) during metabolic and enzymatic processes, (ii) neutralization of ROS by the antioxidant defense system, and (iii) maintenance of equilibrium of the primary cellular redox couples such as glutathione:glutathione disulfide (GSH:GSSG), cysteine:cystine, NADPH:$NADP^+$, and the reduced:oxidized thioredoxin system [9,10] (Fig. 2, A). ROS are generally characterized by those intermediates that arise during the successive reduction of dioxygen to water and include superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). Endogenous ROS are produced at several distinct locations in the cell including mitochondria where complex I and III of the electron transport chain can spuriously reduce dioxygen to $O_2^{\bullet-}$ [11]. Additional ROS are generated as side reactions by cytochrome P450s [12], cyclooxygenase (COX) [13,14], lipoxygenase [14,15], and uncoupled nitric oxide synthase (NOS) [16], and as physiological products of NADPH oxidases [17] and xanthine oxidase [18]. Free H_2O_2 is a substrate for Fenton chemistry in which free ferrous iron in the cell reacts with peroxide to form hydroxyl radical (•OH), a potent oxidant that can damage DNA, lipids, and proteins non-specifically [19,20]. This reaction is potentiated via the reduction of labile ferric to ferrous iron by $O_2^{\bullet-}$ completing the Haber–Weiss reaction. In addition to ROS, reactive nitrogen species are also present in the cell and include, among others nitric oxide ($NO^{\bullet}$) produced by NOS isoforms, and the potent oxidant peroxynitrite, a combinatory product of $NO^{\bullet}$ and $O_2^{\bullet-}$ that readily oxidizes free thiols of glutathione and proteins [21] (Fig. 2, B).

Under normal conditions, $O_2^{\bullet-}$ is a short lived species that undergoes enzymatic dismutation to H_2O_2 and dioxygen in a reaction catalyzed by the metalloenzyme superoxide dismutase (SOD), which exhibits a high rate constant of $2 \times 10^9\ M^{-1}\ s^{-1}$ [22]. Although most H_2O_2 generated by $O_2^{\bullet-}$ dismutation or peroxisomal oxidases is reduced to water by antioxidant enzymes like catalases, peroxidases, and peroxiredoxins, steady-state concentrations in the nanomolar range can persist in tissues [20,23,24]. Both H_2O_2 and $O_2^{\bullet-}$ can oxidize the major redox buffer glutathione leading to a more oxidizing cellular environment, although direct thiol oxidation by H_2O_2 is not likely to be a major reaction in the cell due its slow rate constant [25], rather the more rapid metal-catalyzed thiol oxidation by H_2O_2 is more likely [26]. In order to restore homeostatic GSH:GSSG levels, glutathione disulfide is reduced by glutathione reductase, an NADPH-dependent enzyme. ROS can also oxidize protein thiols to intra- or intermolecular disulfides, or to higher thiol oxidation states, including sulfinic, sulfenic, and sulfonic acids, modifications that typically augment activity (Fig. 2, B). The ubiquitous thioredoxin (Trx) system consisting of NADPH-dependent Trx reductase and Trx, which contains a redox active pair of cysteine thiolates reduces disulfides of client proteins, thus, restoring activity [20]. Lastly, to circumvent the deleterious effects of free radicals including $^{\bullet}OH$, the cell relies on a pool of low-molecular weight (LMW) antioxidants, including glutathione, tocopherols (vitamin E), and ascorbic acid (vitamin C) [27,28]. Thus, the cell has evolved distinct antioxidant mechanisms to counteract the inevitable generation of ROS and maintain redox homeostasis.

Perturbations to cellular redox homeostasis that increase ROS levels beyond the antioxidant threshold, or deplete the antioxidant pool can lead to oxidative stress. Numerous factors contribute to elevated ROS levels. For example, the natural progression of aging is accompanied by increases in oxidative DNA and protein damage and oxidation of cellular