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IMPORTANCE OF THE REGULATION OF NUCLEAR RECEPTOR DEGRADATION

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1. ABSTRACT

Nuclear hormone receptors (NHRs) represent a superfamily of structurally related ligand-activated transcription factors, which regulate diverse biological activities like growth, development, and homeostasis. Recently, it has been demonstrated that certain members of the NHR superfamily are degraded through the ubiquitin-proteasome pathway in a ligand-dependent manner. Though the signal for the down-regulation via the ubiquitin-proteasome pathway is not yet known, phosphorylation at specific amino acid residues or coactivator binding to receptors could lead to their degradation by the 26S proteasome. Activation and degradation seems to be an engineered cyclic mechanism, which provides tight control over diverse cellular processes. The degradation process involves extensive loss of proteins and requires expenditure of cellular ATP. That seems to be inevitable for a more important aim, that is efficient and appropriate regulation of transcription. Down-regulation of receptors would lead to an attenuated transcriptional response because the number of receptor molecules available to activate transcription would decrease over time. One of the obvious reasons for down-regulating NHRs thus seems to be to prevent the cell from overstimulation by the hormones or other activating signals. Nuclear receptor turnover may also reset the transcriptional apparatus in preparation for a subsequent response. Since inhibition of the ubiquitin-proteasome degradation pathway disturbs the transcriptional activitity of some of the nuclear receptors such as estrogen (ER) and progesterone (PR) receptors, it is also possible that the degradation of NHRs may enable recycling of components of receptor-cofactor complexes and general transcriptional machinary. Understanding the mechanism of nuclear hormone receptor degradation and its relation to transcription may lead to novel insights of therapuetic intervention.

2. INTRODUCTION

Steroids, thyroid hormones, vitamin D, and retinoids regulate diverse biological processes including growth, development, and homeostasis through their cognate nuclear hormone receptors (NHRs). NHRs are a large family of structurally related, ligand-activated transcriptional regulators that include more than 50 distinct proteins (1). The general mechanism for NHR-dependent transcriptional activation initially involves an interaction between the receptor and a specific ligand (2). Ligand binding induces a conformational change within the receptor that facilitates binding of coactivator proteins which, in the end, modulates the transcriptional activity of the target gene (3). NHRs are categorized into three subclasses. Class I contains receptors for steroid hormones, including progesterone (PR), androgen (AR), mineralocorticoid (MR), glucocorticoid (GR) and estrogen (ER) receptors. These receptors are associated with molecular chaperones, such as heat shock proteins (HSPs) in the absence of ligand. Class II contains receptors for thyroid hormone (TR), vitamin D_3 (VDR), 9-*cis* (RXR) and all-*trans* retinoic acid (RAR). These receptors are present in the nucleus as heterodimers with RXR, bound to their response element in the promoter of target genes in the absence of ligand. Class III NHRs contain orphan receptors, whose cognate ligands have not yet been identified and minimal information is available concerning the mechanism of receptor transactivation (2).

In order to activate gene transcription the class I receptors undergo a series of well-defined steps. When bound to hormone, these receptors undergo a conformational change; dissociate from molecular chaperones; homodimerize; become phosphorylated; migrate into the nucleus; bind DNA at a hormone response element located in the promoter of the target gene; interact with coactivators; and recruit the basal transcription

machinery to form a stable pre-initiation complex (PIC) (2, 4, 5). These events are followed by upregulation of target gene transcription. The class II receptors are located in the nucleus bound to the response element in the promotor region of the target genes. Class II receptors heterodimerize with RXR, except RXR which homodimerizes with itself. Upon hormone binding these receptors also undergo a conformational change that results in the release of corepressors and recruitment of coactivators. This leads to the recruitment of the basal transcription machinery to form a stable PIC (2, 4, 5).

3. COACTIVATORS

NHR coactivators represent a growing class of proteins, which interact with receptors in a ligand-specific manner and enhance their transcriptional activity (4). Prior to their identification, coactivators were predicted to exist based upon experiments which showed that different receptors compete for a limiting pool of accessory factors required for optimal transcription. Stimulation of one receptor resulted in trans-represssion of another receptor, presumably from the depletion of a common coactivator pool (6). The steroid receptor coactivators (SRCs) are a family of nuclear receptor interacting proteins, which include SRC-1 (7); SRC-2, also called GRIP1 (glucocorticoid receptor interacting protein 1) and TIF2 (transcriptional intermediary factor 2) (8, 9); and SRC-3, also called AIB1 (amplified in breast cancer 1), ACTR (activator of thyroid and retinoic acid receptor), RAC3 (receptor-associated coactivator 3), TRAM-1 (thyroid hormone receptor activator molecule 1), and p/CIP (p300/CBP interacting protein) (10-14). These proteins are moderately conserved with several conserved functional domains, including an N-terminal basic helix-loop-helix (bHLH)-PAS domain, a CREB-binding protein (CBP) interacting domain (AD1), a C-terminal activation domain (AD2), a Q-rich region, and several LXXLL motifs that are involved in nuclear receptor binding (15). Many coactivators have been identified to date, reflecting the diversity within the growing coactivator family (16).

Functionally, coactivators were originally envisioned to serve as molecular bridges, linking the receptor to the basal transcription machinery (4, 17, 18). Although this may be true for some coactivators, the observation that they have been shown to possess enzymatic activities that may contribute to their ability to enhance receptor-mediated transcription seems to be an additional function. SRC-1, SRC-2, SRC-3, p300/CBP, and PCAF (p300/CBP-associated factor) possess histone acetyltransferase (HAT) activity and members of the SWI/SNF complex contain ATPase activity (19-21). HAT activity is thought to disrupt the local repressive chromatin structure by neutralizing the positive charge on histones, thus uncoupling the ionic interactions between positively charged histones and the negatively charged backbone of DNA. HAT activity was the first enzymatic function implicated in nuclear hormone receptor-dependent transcription (22). Furthermore, recent evidence suggests that coactivators themselves may be acetylated by other coactivators containing HAT activity, the purpose of which remains unclear (21). Coactivators containing ATPase activity are thought to physically open the local repressive chromatin structure, allowing for easier access of other ancillary transcription proteins (23).

Recently, ubiquitin-proteasome and ubiquitin-like pathway enzymes have been identified as coactivators of the NHR superfamily. These proteins include the E3 ubiquitin-protein ligases E6-AP (E6-associated protein) and RPF1/RSP5, UBC9 (ubiquitin-conjugating enzyme 9), and an ATPase subunit of the 26S proteasome, TRIP1/SUG1 (thyroid hormone receptor interacting protein-1/suppressor of Gal4) (24-28). Identification of components of the ubiquitin-proteasome pathway as coactivators has added a new twist to the coactivator field and suggests that the ubiquitin-proteasome pathway may play an regulatory role in NHR function and receptor-dependent transcription (16).

4. UBIQUITIN-PROTEASOME PATHWAY

The ubiquitin-proteasome pathway is the major system in eukaryotes for selective degradation of cellular proteins (29, 30). A common feature of the proteasome-mediated degradation pathway is the covalent attachment of ubiquitin, a highly conserved 8.6 kDa protein present both in the nucleus and cytoplasm, to lysine residues within the target protein. This is followed by the formation of polyubiquitin chains covalently linked to the targeted protein. Polyubiquitinated proteins are recognized and degraded by the multi-subunit protease complex, the 26S proteasome. In addition to the role it plays in protein degradation, ubiquitination may serve regulatory functions such as directing the subcellular localization of proteins (31, 32). The ubiquitin-proteasome pathway also plays an important role in various cellular processes such as cell cycle regulation, signal transduction, differentiation, and antigen processing (29, 30).

Protein ubiquitination involves three classes of enzymes, namely the E1 ubiquitin-activating enzyme (UBA1), E2 ubiquitin-conjugating enzymes (UBCs) and E3 ubiquitin-protein ligases. The E1 first activates ubiquitin in an ATP-dependent manner, forming a thioester bond between the carboxy-terminal glycine residue of ubiquitin and a cysteine residue of the UBA1. Next, ubiquitin is transferred from the E1 to one of the several E2s, preserving the high energy thioester bond. In some cases, ubiquitin is transferred directly from the E2 to the target protein through an isopeptide bond between the ε-amino group of lysine residues of the target protein and the carboxyl-terminus of ubiquitin. In other instances, the transfer of ubiquitin from E2s to target proteins proceeds through an E3 ubiquitin-protein ligase intermediate. It has been proposed that the biological specificity of the ubiquitin pathway is modulated by the selective combination of UBCs and E3 proteins. To date, more than thirty UBCs and twenty-five E3 proteins have been identified (29, 30).

Though the importance of the ubiquitin-proteasome pathway in higher eukaryotes has been well established in cell cycle regulation, signal transduction and

cell differentiation, only recently has this pathway been linked to the transcriptional machinery (33). Indeed the carboxy-terminal tail of the RNA polymerase II itself is a target of ubiquitin-proteasome pathway (34, 35). The involvement of the ubiquitin-proteasome pathway in eukaryotic transcription and steroid hormone receptor-dependent transcription is further strengthened by the observations that UBC9 and E3 ubiquitin-protein ligases, E6-AP and RPF1/RSP5, interact with steroid hormone receptors and several other transcription factors and coactivate their transactivation functions (24, 27, 28). These initial observations strongly suggest that the enzymes of the ubiquitin-proteasome pathway play an important role in the coactivation functions of steroid hormone receptors.

5. NUCLEAR HORMONE RECEPTOR DEGRADATION

Ligand dependent down regulation has been observed for several nuclear steroid receptors, including ER-alpha, PR, VDR, TR, and RAR-alpha (36-40). The importance of nuclear receptor turnover has not been elucidated in detail although a number of recent studies have led to an initial understanding of this process. The cell may expend energy to degrade activated receptors, such as ER to attenuate transcriptional responses. Alternatively, nuclear receptor turnover may provide a mechanism to reset the transcriptional apparatus at its start point after each stimulus, so that previously activated receptors can be replaced with newly synthesized, fully functional molecules.

Recently, it has been observed that not only are nuclear receptors ubiquitinated in the course of their nuclear activities. Corepressors, SRC-family coactivators, CBP, and E6-AP are also ubiquitinated (41, 42). Furthermore, a series of publications also reveal that ubiquitination is concomitant with other transcription factor activities (31, 32, 43, 44). Finally, stability of mRNA is modulated by the ubiquitin-proteasome pathway (45). This widespread ubiquitination activity suggests that the ubiquitin-proteasome pathway is critical for regulated transcription by steroid receptors. The turnover process appears to occur concomitantly with transcriptional activation induced by these same receptors. In fact, addition of 26S proteasome inhibitors, MG132 and lactacystin, inhibited ER-mediated transcription indicating that the prevention of 26S proteasome function is deleterious to transcription mediated through certain members of the NHR family. The addition of coactivators does not overcome this drug-induced inhibition (Nawaz, Z., and Lonard, D., unpublished data). It is possible that proteasome function contributes to gene transcription by disrupting the preinitiation complex, allowing the elongation to proceed. It is also possible that the proteasome pathway is necessary to exchange coactivator complexes between transcription initiation and elongation. These possibilities may be supported by the recent observation that shows accumulation of the 26S proteasome (or subunits) at sites of nuclear receptor-induced gene activity (46). This led to the consideration that proteasome activity is a necessary part of regulated gene activity. Perhaps it would be more obvious to scientists that corepressors might have to be "turned over" to initiate gene expression, but why would the receptors and coactivators require similar degradation during function? Direct and indirect evidence is accumulating that the 26S proteasome activity is indeed required for this function (42, 47).

Nuclear receptor and coactivator degradation involves an energy cost at the expense of the cell. However, this energy cost seems to be an insignificant expense when compared to the goal, transcriptional regulation, which is vital for almost all functions and development of the cell. Transcriptional regulation mediated through this receptor-coactivator degradation leads to an appropriate and timely cessation or modulation of activity in coordination with *in vivo* hormonal kinetics. There is also evidence to show that energy may be required for entry of certain receptors into the nuclear compartment and that some recycling of certain receptors (e.g. GR) occurs in the course of glucocorticoid action (48). It is possible that degradation through the ubiquitin-proteasome pathway could be the way to disassemble and reassemble the coactivator complex. There is also a consideration that certain amounts of recycling and reutilization of members of the coactivator complex takes place (49).

The above mentioned hypothesis of receptor-coactivator degradation suggests that turnover of the receptor-coactivator complex would be frequent and continuous, each new complex requiring sufficient concentrations of hormonal ligand to occupy receptor, an adequate supply of receptor (or newly synthesized receptor), and sufficient coactivator concentration to maintain adequate target gene expression in the face of the appropriate hormone levels. Down regulation or desensitization is the hallmark of the endocrine system and serves as an important mechanism to prevent cellular overstimulation by hormones.

The mechanism for NHR down regulation is not well understood and more than one may exist. However, several signals for degradation have been identified that may provide insight to possible mechanisms. It has been shown that ER-alpha, PR, VDR, RAR-alpha, and TR undergo minor ligand-independent degradation (in the absence of hormone), most likely due to unstable interactions within inactive HSP:NHR complexes (36-40). However, substantial degradation occurs once the receptor becomes ligand-bound (ligand-dependent degradation). Thus, ligand binding itself may be a signal for degradation, but evidence suggests that other activating events are involved as well. For instance, NHRs undergo multiple phosphorylation events following activation by ligand binding. It has been shown that mutation of MAP kinase phophorylation sites in PR abrogates ligand-dependent degradation, suggesting phosphorylation of NHRs may be a signal for degradation (37). Separately, it has been well documented that coactivators directly bind to NHRs through the carboxy-terminal AF-2 (50). Specific amino acids have been identified within the AF-2 of ER-alpha and RAR-alpha that are indispensable for interaction with

coactivators (40, 51, 52). Mutation of these residues prevent ligand-dependent degradation suggesting coactivator recruitment may be another signal for initiating degradation (42). As mentioned above, degradation and transcription are intimately tied and transcription poses a third possible signal for NHR degradation (40, 42). Although evidence suggest that multiple degradation signals exist, it is possible that they may be inter-related and further research needs to discriminate dependence versus independence. Additional research will also identify the bone fide mechanism(s) involved in NHR degradation.

6. PERSPECTIVE

The targeted degradation of nuclear receptors and nuclear coactivators by the ubiquitin-proteasome pathway is emerging as an important phenomenon for regulation of eukaryotic gene transcription for precise and accurate response of the cell to physiological hormonal levels. A number of observations suggest that the turnover process is essential for normal NHR function. First, several members of the NHR superfamily undergo ligand-dependent degradation that is blocked by addition of proteasome inhibitors. Second, specific events in NHR activation, such as phosphorylation and coactivator recruitment, appear to be necessary for NHR degradation. Third, degradation of receptors through the ubiquitin-proteasome pathway is intimately tied to the process of NHR-dependent transcription. Although considerable progress has been made in understanding the role of NHR degradation via the ubiquitin-proteasome pathway, knowledge about the mechanism and regulation of the process is still in its infancy. Furthermore, an important question remains unanswered, why would receptors require degradation during their function? First, down regulation may be a mechanism to prevent overstimulation by hormone. Second, turnover may be a mechanism to recycle stably-associated receptor:coactivator complexes. Third, turnover may return the promoter to its basal, unstimulated state poised for another round of transcription.

7. ACKNOWLEDGMENT

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