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DEGRADATION AND BEYOND: CONTROL OF ANDROGEN RECEPTOR ACTIVITY BY THE PROTEASOME SYSTEM

TOMASZ JAWORSKI
Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, 02-093 Warsaw, Poland

Abstract: The androgen receptor (AR) is a transcription factor belonging to the family of nuclear receptors which mediates the action of androgens in the development of urogenital structures. AR expression is regulated post-

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* Corresponding author: e-mail: tomasz_jaworski@poczta.onet.pl

Abbreviations used: AATF – apoptosis antagonizing factor; APIS complex – AAA proteins independent of 20S; ARA54 – AR-associated protein 54; ARA70 – AR-associated protein 70; ARE – androgen responsive element; ARNIP – AR N-terminal interacting protein; bFGF – basic fibroblast growth factor; CARM1 – coactivator-associated arginine methyltransferase 1; CHIP – C-terminus Hsp70 interacting protein; DBD – DNA binding domain; E6-AP – E6-associated protein; GRIP-1 – glucocorticoid receptor interacting protein 1; GSK3β – glycogen synthase kinase 3β; HDAC1 – histone deacetylase 1; HECT – homologous to the E6-AP C-terminus; HEK293 – human embryonal kidney cell line; HepG2 – human hepatoma cell line; Hsp90 – heat shock protein 90; IGF-1 – insulin-like growth factor 1; IL-6 – interleukin 6; KLK2 – kallikrein 2; KLKK – lysine (K), leucine (L); LBD – ligand binding domain; LNCaP – lymph node carcinoma of prostate cell line; MAPK – mitogen activated protein kinase; Mdm2 – Murine double minute 2; NcoR – nuclear receptor corepressor; NEDD8 – neural precursor cell-expressed developmentally down-regulated; NLS – nuclear localisation signal; p/CAF – p300/CBP-associated factor; p300/CBP – CREB-binding protein; PC3 – human prostate carcinoma cell line; PEST – proline (P), glutamic acid (E), serine (S), threonine (T); PI3K – phosphoinositide-3 kinase; PIAS1 – protein inhibitor of activated STAT; PKA – protein kinase A; PKC – protein kinase C; PR – progesterone receptor; PRMT1 – protein arginine methyltransferase 1; PROTAC – proteolysis targeting chimeric molecule; PSA – prostate specific antigen; PSMA7 – proteasome alpha subunit 7; P-TEFb – positive transcription elongation factor b; PTEN – phosphatase and tensin homolog deleted on chromosome 10; RING – really interesting new gene; RNA Pol II – RNA polymerase II; SCF ligase – Skp2/cullin1/F-box; SMRT – silencing mediator of retinoic acid and thyroid hormone receptor; SNURF – small nuclear RING finger; SRC1 – steroid receptor coactivator 1; Sug1 – suppressor of Gal; SUMO – small ubiquitin-like modifier; SWI/SNF – mating type switching/sucrose non-fermenting; TBL1 – transducin-β-like; TBLR1 – transducin-β-like-related protein; TBP – TATA binding protein; TFIIB, D, F, H – general transcription factors; TRAP – thyroid hormone receptor-associated protein; TSG101 – tumour susceptibility gene 101; UAC – ubiquitin activating enzyme; UBC – ubiquitin conjugating enzyme

translationally by the ubiquitin/proteasome system. This regulation involves more complex mechanisms than typical degradation. The ubiquitin/proteasome system may regulate AR via mechanisms that do not engage in receptor turnover. Given the critical role of AR in sexual development, this complex regulation is especially important. Deregulation of AR signalling may be a causal factor in prostate cancer development. AR is the main target in prostate cancer therapies. Due to the critical role of the ubiquitin/proteasome system in AR regulation, current research suggests that targeting AR degradation is a promising approach.

Key words: AR, Degradation, Prostate cancer, Proteasome, Transcription, Ubiquitin

INTRODUCTION

Earlier research advanced the notion that ubiquitin-dependent proteasomal degradation is solely involved in protein turnover; however, newer evidence suggests that this idea should be modified. It has been well established that proteasome activity is required for transcriptional activation by nuclear receptors. This may be due to the degradation and subsequent recovery of transcription complex components. Similarly, subunits of proteasome may act as bridging factors in the transcription complex, and ubiquitin itself may play a role in transactivation. The involvement of a ubiquitin/proteasome system in the transcription complex enables the precise regulation of the transcriptional signal in order to provide an accurate transcript level. The androgen receptor (AR) is critical for the normal growth and differentiation of the prostate and other urogenital structures [1, 2]. Although the expression of AR is tightly regulated, it sometimes gets out of control, leading to tumourigenesis [3]. Prostate cancer is the most common form of cancer, and the second leading cause of cancer death among American males [4]. Given the central role of AR in prostate function, significant attention is being paid to the understanding of its function mechanisms and regulation. Precise knowledge on AR signalling would allow for the creation of therapy that targets and facilitates AR down-regulation in prostate cancer. This review summarizes the state of knowledge on the role of proteasome and the proteasome-related system in the function of AR, and discusses recent progress in prostate cancer therapies that are based on the molecular mechanisms of AR degradation.

THE MOLECULAR BIOLOGY OF AR

AR is a ligand-dependent transcription factor which belongs to the family of nuclear receptors [5]. The human *AR* gene is located at the Xq11-12 locus, and its expression is tightly regulated, being cell- and tissue-dependent [2]. It consists of eight exons which encode four distinct domains [2, 6]: the N-terminal domain, which comprises the transactivation domain; the DNA-binding domain

(DBD) composed of two zinc finger motifs; the hinge region which contains the nuclear localisation signal (NLS); and the ligand-binding domain (LBD), responsible for high affinity androgen binding. After translation from its mRNA, the inactive cytoplasmic AR protein forms a complex with heat shock proteins that regulate receptor folding and confer ligand-binding capacity [7]. Following ligand binding, AR moves to the nucleus, where it binds to androgen responsive elements (ARE) in the promoter and/or enhancer of the target genes [8]. AR regulates the expression of almost 100 genes, including prostate specific antigen (*PSA*), *probasin*, *kallikrein*, keratinocyte growth factor (*KGF*), *p21*, the ornithine decarboxylase (*ODC*) gene in mouse kidney, and the *AR* gene itself. It should be noted that AR is able not only to up-regulate but also to down-regulate gene expression [9], depending on the availability of coactivators and corepressors.

As the mechanisms regulating AR expression have been reviewed elsewhere [2, 10] only a brief description will be presented here. AR expression is regulated at the transcriptional level by a variety of transcription factors [10], post-transcriptionally by RNA binding proteins [11], and post-translationally; the latter form of regulation includes phosphorylation, sumoylation and acetylation, depending on interaction with coregulatory proteins [12, 13].

The complex regulation of AR activity is also provided by proteasome and proteasome-related machinery. This includes the degradation of AR itself and of its coregulators, and the non-proteolytic role of the ubiquitin/26S proteasome machinery. It was shown that the treatment of HepG2 and LNCaP cells with proteasome inhibitor MG132 increased AR levels [14]. The inhibition of proteasome resulted in an increase in the number of polyubiquitylated AR forms, consistent with AR being a subject of proteasomal degradation [14].

THE MECHANISM OF UBIQUITIN/PROTEASOME-MEDIATED PROTEIN DEGRADATION

Ubiquitylation is based on the attachment of ubiquitin to the lysine residues in a target protein, and involves the action in concert of three types of enzymes: E1, E2 and E3 [15] (see Fig. 1).

The three enzymes act in a defined order to ubiquitylate the target protein, which becomes degraded in a proteasome. Ubiquitin attachment to the target protein occurs in a hierarchical, three-stage reaction: ubiquitin is first activated in an ATP-requiring reaction by the E1 activating enzyme (Uac), then activated ubiquitin is transferred onto the E2 conjugating enzyme (Ubc), and then the E3 ligase transfers ubiquitin from E2 onto the protein to be degraded [15]. The protein may either be tagged with one ubiquitin moiety, or with many by extending the length of the ubiquitin chain. As we will see later, mono- and poly-ubiquitylation processes are important events determining the fate of proteins. *In vitro* studies show that in order to be degraded, a given protein requires a minimum of four ubiquitin moieties [16].

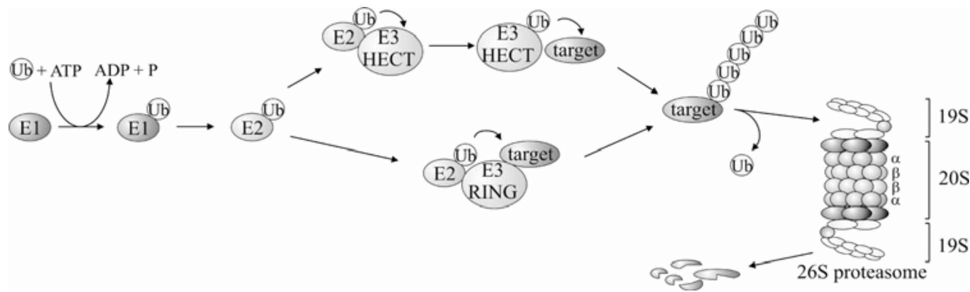


Fig. 1. A schematic diagram presenting the ubiquitin/26S proteasome pathway. In an ATP-dependent manner, ubiquitin is activated by the E1 activating enzyme and subsequently transferred from E1 onto the E2 conjugating enzyme and then from E2 onto the E3 ligase, which possesses either a HECT or RING finger domain. E3 attaches ubiquitin to the protein to be degraded in repeating steps, yielding a polyubiquitin chain that is recognised by the 19S proteasomal cap. 19S removes ubiquitin from the target protein and directs it into the 20S proteolytic core. Free ubiquitin is reutilised.

The E3 enzymes provide the specificity of substrate recognition. There are two families of E3 enzymes: Homologous to the E6-AP C-Terminus (HECT) domain E3s, and Really Interesting New Gene (RING)-finger domain E3s. Ligases belonging to both families were shown to interact with AR; a summary is given in Tab. 1.

Tab. 1. Proteins with a HECT or RING-finger domain shown to interact with AR

Protein	Domain type	Cognate E2	Function, reference
E6-AP	HECT domain	UbcH7, UbcH8	Ubiquitin E3 ligase [17-19]
ARNIP	RING finger	Ubc4-1	Ubiquitin E3 ligase [20]
ARA54	RING finger	UBE2E2, UbcH6, UBE2E3	Ubiquitin E3 ligase [21, 22]
Mdm2	RING finger	Ubc5	Ubiquitin E3 ligase [23]
PIAS	RING finger-like	Ubc9	SUMO E3 ligases [24-27]
SNURF	RING finger	UbcH5A and B, HHR6B (RAD6B), E2-25K, MmUbc7, UbcH13	Ubiquitin E3 ligase [28-30]
CHIP	U-box (modified RING finger)	Ubc4, UbcH5C	Ubiquitin E3 ligase [31-33]

The process of ubiquitylation depends on the protein-protein interaction and involves an interplay of the E3 ligase with two elements, the E2 conjugating enzyme and the recognition signal in the target [15]. There are a few examples of recognition signals for ubiquitin E3 ligases, one of which is a phosphorylated PEST sequence [34].
The 26S proteasome is a large, multisubunit complex that is localised both in the cytoplasm and in the nucleus [35]. The 26S proteasome holoenzyme is composed of a 20S core and two 19S regulatory caps, also known as PA700 [35]. The 20S proteolytic core is composed of 4 rings: two inner rings consisting of β subunits which are surrounded by two outer rings consisting of α subunits. The 20S catalytic core is located between two 19S regulatory caps. 19S regulates the activity of the proteasome by substrate recognition and its unfolding, cleaving off the polyubiquitin chain and translocating the substrate into the 20S proteolytic chamber.

LIGAND-INDEPENDENT PROTEASOMAL DEGRADATION OF AR

The AR level may be controlled by two proteolytic pathways, the first of which relies on the 26S proteasome and occurs both in the absence (ligand-independent) or presence (ligand-dependent) of the hormone. The second engages PTEN and caspase 3 activity [36] (see Fig. 2). The latter does not rely on a proteasome, and therefore will not be discussed further.

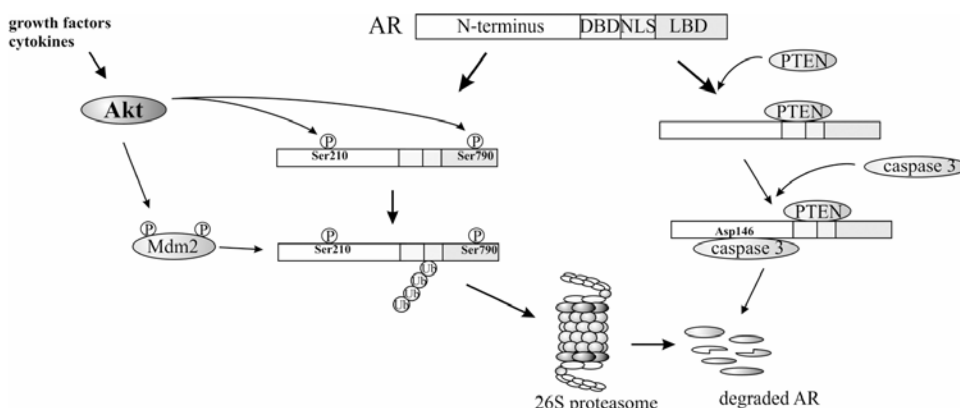


Fig. 2. The proteasome-dependent (*left*) and proteasome-independent (*right*) pathways controlling AR degradation. (*left*) Growth factor or cytokine stimulation activates Akt kinase, which phosphorylates AR at Ser210 and Ser790. Active Akt kinase also phosphorylates Mdm2, which ubiquitylates AR and targets it to degradation in the 26S proteasome. (*right*) PTEN-binding to AR facilitates the recruitment of caspase 3, which processes AR at Asp146.

Ligand-independent AR degradation occurs in response to growth factors and cytokine stimulation. AR signalling has been shown to be inhibited by IL-6 [37], basic fibroblast growth factor (bFGF) [38], and insulin-like growth factor 1 (IGF-1) [23, 39]. Growth factors and cytokines activate the PI3K/Akt kinase pathway which promotes AR phosphorylation (see Fig. 2).

The phosphorylation of AR at Ser210 and Ser790 by Akt kinase has been shown to suppress AR transactivation via the inhibition of AR-ARA70 interaction [39]. As ARA70 is an AR coactivator implicated in AR translocation to the nucleus [40], inhibiting AR-ARA70 interaction may lead to AR arrest in the cytoplasm, where it is susceptible to 26S proteasome-mediated degradation. Indeed, phosphorylation of AR by Akt promotes AR ubiquitylation and degradation via the proteasome-dependent pathway [23]. This occurs through the enhancement of AR interaction with ubiquitin E3 ligase Mdm2. Interestingly, Akt kinase exerts differential effects on AR depending on the LNCaP cell passage number [41]. Low passage cells have a low basal Akt activity, whereas high passage cells have high Akt activity. In the case of a low passage number, the PI3K/Akt pathway inhibits AR activity, and this mechanism is dependent on proteasome-mediated degradation. In the high passage, Akt actually enhances AR activity [41]. This differential regulation of AR by Akt may explain the conflicting data in the literature, as it was also reported that AR is positively regulated by Akt after activation by growth factors [42].

Glycogen synthase kinase 3β (GSK3β) is a proline-directed serine/threonine kinase that exerts its function in unstimulated cells, while growth factor-stimulation decreases its activity. GSK3β acts downstream of and is inhibited by the PI3K/Akt pathway. GSK3β is able to phosphorylate AR at Ser650 within the PEST sequence that is localised in the hinge region, and is able to suppress AR transactivation [43]. However, as in the case of Akt, other authors reported that GSK3β is required for AR gene expression [44]. The phosphorylation of Ser650 is also upregulated by stress kinase signalling and reduces AR transcription by augmentation of AR export to the cytoplasm [45].

So far, it has not been established which AR amino acid sequence is a target for Mdm2-driven ubiquitylation. It is likely that this sequence is a KLKK motif that overlaps the PEST sequence and is modified by acetylation and deacetylation, respectively driven by p300/CBP and HDAC1 [46]. Similarly, p53 contains a KSKK motif, the lysine residues of which are targets for acetylation/deacetylation. It is significant that the deacetylation of p53 by HDAC1 is required for its Mdm2-mediated degradation [47].

LIGAND-DEPENDENT PROTEASOMAL DEGRADATION OF AR

A model for ligand-dependent transcription by nuclear receptors presumes that the receptor undergoes rapid turnover in the presence of the ligand. This was established for glucocorticoid receptor GR, thyroid hormone receptor TR, retinoic acid receptor RAR, progesterone receptor PR, and estrogen receptor ER

[48-52]. There is a general view that AR is an exception in the group of nuclear receptors, as it is not degraded after ligand binding. Conversely, AR is up-regulated in the presence of the hormone. The full picture of the process is more complex. A recent report indicates that AR is ubiquitylated in response to androgen treatment [53]. Treatment of LNCaP cells with synthetic androgen R1881 resulted in the appearance of mono- and triubiquitylated AR species which remained stable for 2 hours after androgen treatment. Afterwards, there was a decline in AR stability, which was consistent with the accumulation of polyubiquitylated AR forms [53].

Ligand-dependent AR degradation could be explained by a model in which the subpopulation of receptor molecules is degraded after a short period of androgen exposure (Luke Gaughan, personal communication). This is in agreement with the observation that the same receptor molecule may be able to conduct up to four rounds of transcription. However, there is a decline in receptor transactivational competency, suggesting that each transcription cycle involves degradation of the AR subpopulation [54]. Fluctuations may also exist in the stability of the receptor over periods of androgen treatment as AR is down-regulated after a short-term androgen treatment (8 h), while its content increases after prolonged exposure of LNCaP cells to androgen (48 h) (Luke Gaughan, personal communication). Thus, the up-regulation of AR by androgens observed by many groups may result not from an increase in the stability of the receptor, but rather from the up-regulation of its own synthesis. The *AR* gene contains exonic AREs which are responsible for the up-regulation of AR mRNA [55]. This androgen-mediated increase in AR mRNA may contribute to the increase in AR protein. Therefore, it is plausible that in response to an increased proteolysis of AR, it up-regulates its own level as a result of an autoregulation mechanism. This hypothesis is supported by evidence that there is a complete loss of the receptor over 8 h of androgen treatment when AR is studied as a single population in the absence of *de novo* synthesis (in the presence of a translation inhibitor) [53].

FORMATION OF THE AR TRANSCRIPTION COMPLEX

Following ligand binding, AR translocates to the nucleus and associates with coactivators GRIP-1 (glucocorticoid receptor interacting protein 1) and histone acetylase p300/CBP (CREB-binding protein) in subnuclear foci [56]. After association with coactivators, AR migrates to target genes where GRIP-1 and p300/CBP promote the recruitment of additional components necessary for transcription [56]. These include SRC1 (steroid receptor coactivator-1) and p/CAF (p300/CBP-associated factor), which acetylate and coactivate AR [46].

To initiate transcription, the chromatin structure has to be reorganised in order to make chromatin accessible to the components of the transcriptional machinery. There are two classes of protein responsible for this. The first class utilises energy from ATP to induce changes in chromatin conformation, while the

second catalyses histone modifications to regulate transcription [57]. Histone acetyltransferases and the histone methyltransferases – coactivator-associated arginine methyltransferase 1 (CARM1) and protein arginine methyltransferase 1 (PRMT1) – have been shown to be recruited to the prostate-specific antigen (PSA) promoter after androgen treatment [58]. As methylation does not change the charge of histones, it is likely that the methylation of histones serves as a docking site for protein binding, which may facilitate subsequent acetylation by p300 [58]. Indeed, there is a crosstalk between CARM1 methylation and CBP acetylation on histone H3 [60]. The process of chromatin reorganisation is ATP dependent and driven by ATPase complexes. SWI/SNF, a chromatin remodelling complex whose two core subunits hBRM and BRG1 show ATPase activity, has been shown to stimulate AR transcription [61].

Another large protein complex that interacts with AR at the gene promoter is the TRAP-Mediator (thyroid hormone receptor-associated protein) complex. It functions to recruit RNA Pol II and general transcription factors into the transcription complex. Subunits of Mediator: TRAP220, TRAP170 and TRAP100 were shown to enhance AR transactivation [62]. The SWI/SNF and Mediator complexes can be targeted to chromatin by p300, which itself is recruited through interaction with SRC coactivators [63].

The interaction of AR with general transcriptional machinery has been reviewed [64]. Briefly, AR is able to associate with RNA Pol II, TFIIF, TFIIH and P-TEFb. Due to their kinase activity, TFIIH and P-TEFb phosphorylate the C-terminal domain (CTD) of RNA Pol II, which is a critical event for RNA Pol II to become an elongation competent [65].

After AR relocates to the target gene, it regulates the transcription of target genes by binding both enhancer and promoter sequences [8]. Occupancy of the promoter of the androgen-responsive genes PSA and kallikrein 2 (KLK2) is a dynamic, cyclic process involving the association and dissociation of AR and cofactors that correspond with the recruitment of the 19S regulatory subcomplex of the 26S proteasome [66]. Histone deacetylase 1 (HDAC1) and Mdm2 were recruited to the PSA promoter and were subject to polyubiquitylation [53]. This correlates with the observation that the ubiquitin/proteasome system is present at the promoters of nuclear receptors-regulated genes including AR in order to clean the promoter and to reinitate transcription.

INVOLVEMENT OF UBIQUITIN AND THE 26S PROTEASOME IN THE FORMATION OF THE AR TRANSCRIPTION COMPLEX

A large body of literature shows that components of the ubiquitin/proteasome system play an important role at the promoter of nuclear receptors-target genes by regulating chromatin structure and the turnover of receptors and coregulators, and by serving as bridging factors [67, 68]. Recent studies showed that proteasome activity is also important for modulating AR transcriptional activation [69]. This concurs with data indicating that both the 20S and 19S

subunits of the 26S proteasome are involved in the transcriptional process, with the latter involved in transcription initiation as well as in elongation [70-72]. Subunits of the 19S subcomplex which possess ATPase activity, the so-called APIS complex (AAA proteins independent of 20S) are recruited to the promoter upon induction of transcription independently of the 20S proteolytic core [71]. Sug1, which is one of the six proteasomal ATPases of 19S, forms a complex with the nuclear receptors [73]. However, the interaction of Sug1 with AR has not yet been studied. Evidence for the involvement of proteasome in AR-dependent transcription is provided by the fact that the S1 subunit of the 19S proteasome cap is recruited to the PSA promoter, and its occupancy by S1 correlates with AR release from the promoter [66]. Ikezoe *et al.* demonstrated that inhibition of the 26S proteasome with PS-341 decreases AR transcriptional activity without affecting AR nuclear translocation [74], indicating that the proteasome is an essential element of the AR transcriptional complex.

Activation of AR via monoubiquitylation

Recruitment of 19S to the transcriptional complex may be provided by the attachment of monoubiquitin to transcription factors. Mono- and polyubiquitylation act as important signals for the differential regulation of proteins. Monoubiquitylation is implicated in nonproteolytic processes, whereas polyubiquitylation is generally accepted to play a role in degradation [75]. For example, the attachment of a single ubiquitin moiety to VP16 transcription factor by Met30 ubiquitin ligase is required for transcriptional activation [76].

It is plausible that the linking of monoubiquitin to the target protein serves as a docking site for the 19S subcomplex, the subunits of which may promote transcription initiation. This would include formation of the complete transcriptional complex at the gene promoter, chromatin remodeling and initiation of the transcriptional process. Ubiquitin licenses transcription factors [76] by regulating their activity (monoUb) and proteolysis (polyUb). As transcription proceeds and a gene is transcribed, the ubiquitin chain arises giving a signal for the APIS complex to recruit the 20S proteolytic core [71]. 20S components are present at the promoter at a later time, after the induction of transcription. Thus, the time required for the ubiquitin chain to arise before it becomes a signal for degradation is used by the transcription factor to drive high-level transcription [71].

Androgen treatment results in the appearance of monoubiquinated AR species [53, 77]. Monoubiquitylation is critical for the efficient activation of AR, and monoubiquitylated AR is stabilised by TSG101 (tumour susceptibility gene 101) [77]. In the presence of AATF (apoptosis-antagonizing transcription factor), TSG101 binds monoubiquitylated AR and prevents its polyubiquitylation [77].

Where does the ubiquitylation signal that triggers the transcription factor activity come from? The signal for ubiquitylation for the transcription factor may be provided by components of the RNA Pol II general transcription machinery and by E3 ubiquitin ligases that are part of the RNA Pol II transcription complex

[78]. The first indication comes with the finding that the $TAF_{II}250$ subunit of TFIID, a general transcription factor, possesses both ubiquitin-activating and E2 ubiquitin-conjugating activities, and is able to monoubiquitylate histone H1 [79]. The second hypothesis is supported by the fact that p300, which is a well-characterised coactivator of nuclear receptors that possesses histone acetylase activity, was shown to be ubiquitin ligase [80]. It is also possible that the ubiquitylation of AR occurs in the cytoplasm as unliganded, cytoplasmic GR is stabilised by TSG101 [81].

AR nuclear translocation

Proteasome may act as an AR coregulator [69]. Overexpression of proteasome alpha subunit 7 (PSMA7) of the 20S core results in an increase in AR transactivation by influencing the androgen-induced translocation of AR to the nucleus [69]. 20S proteasome inhibitor MG132 is able to break the interaction of AR with coactivators ARA70 (AR-associated-70 kDa protein) and TIF2, the latter belonging to the family of SRC coactivators [69]. ARA70 increases AR-mediated transcription by confering stability to the receptor and by increasing AR nuclear localisation [40]. Moreover, ARA70 forms a platform which allows switching of the unliganded receptor into a form capable of interacting with coregulators [12]. It is therefore possible that as the nuclear localisation signal (aa: 628-669), KLKK sequence (aa: 630-633) and PEST sequence (aa: 638-658) in AR overlap, initial ubiquitylation of this motif may provide a signal for the binding of the 20S proteasome and coregulators which regulate AR translocation. The deletion of amino acids 628-646 increases the potency of AR, and that deleted hinge region induces an increased sensitivity of AR to proteasome-mediated degradation, suggesting that this fragment may be involved in controlling the transcriptional activity of AR [82]. Furthermore, mutation of lysine residues (K) in the KLKK motif delays ligand-dependent translocation, and mutated AR colocalises with proteasome, implying that proteasome is involved nuclear translocation [83]. Zhou *et al.* demonstrated that phosphorylation of Ser650 within the hinge region is important for optimal AR transactivation [84]. Ser650 in AR appears to be constitutively phosphorylated, and androgen treatment elevates its phosphorylation level [85, 86]. Given the importance of the PEST sequence in protein turnover [34], it is likely that the phosphorylation of this motif serves as a docking site for E3 ligases which promote initial AR ubiquitylation. Indeed, it was shown that the small nuclear RING finger protein SNURF, which was recently demonstrated to possess ubiquitin E3 ligase activity [30], interacts with AR in a hormone-dependent manner [28]. SNURF binds to the hinge region of AR and enhances AR-dependent transcription by serving as a bridging factor [28] and by facilitating AR import to the nucleus [29]. Importantly, the RING finger structure of SNURF may serve as an interaction interface for general transcription factors, as SNURF has been shown to interact with TBP *in vitro* [28].

Assembly of the AR transcription complex

Ubiquitin/proteasome machinery may be recruited into NR transcription complexes by transducin-β-like (TBL1) and TBL1-related protein (TBLR1) [87]. These two proteins may recruit ubiquitin conjugating/proteasome machinery to exchange nuclear receptor corepressors for coactivators after ligand binding [87]. TBL1 is recruited to the promoter of kallikrein 2 and PSA after androgen treatment, and is required for transcriptional activation by AR [87]. TBL1 and TBLR1 function as adaptor proteins that recruit the ubiquitin-conjugating enzyme UbcH5 [87]. It is likely that TBL1 is important for the recruitment of UbcH5 into the AR transcription complex, as UbcH5 is an E2 cognate of E3 ubiquitin ligase SNURF (Tab. 1). TBL1 and TBLR1 facilitate the recruitment of E6-AP to the transcriptional complex [87]. Interestingly, E6-AP and its E2 ubiquitin-conjugating enzyme UbcH7 have been shown to enhance the hormone-dependent transcriptional activity of AR [17, 19]. The coactivating function may result from the ability of E6-AP to target NcoR for degradation [88]. In the normal prostate, E6-AP is expressed at a high level where it is necessary to regulate AR levels, whereas its expression decreases in the cancerous prostate [89].

A model for ligand-dependent AR transcription has been proposed [77]: ubiquitylation of AR may occur in the cytoplasm, and TSG101 binding protects AR from premature degradation; after ligand binding, AR translocates to the nucleus and is recruited to the promoter of the androgen-responsive genes, and subsequently associates with transcriptional coactivators. Attachment of the ubiquitin moiety may also occur in the nucleus. In any case, TSG101 locks AR in the monoubiquitylated state in order to initiate transcription. Protein post-translational modifications which are important for transcription to proceed induce conformational changes that release the lock provided by TSG101, yielding polyubiquitylation, which is consistent with receptor degradation. This mechanism designates for degradation those receptors that participated in transcription, facilitating promoter clearance and a new round of transcription.

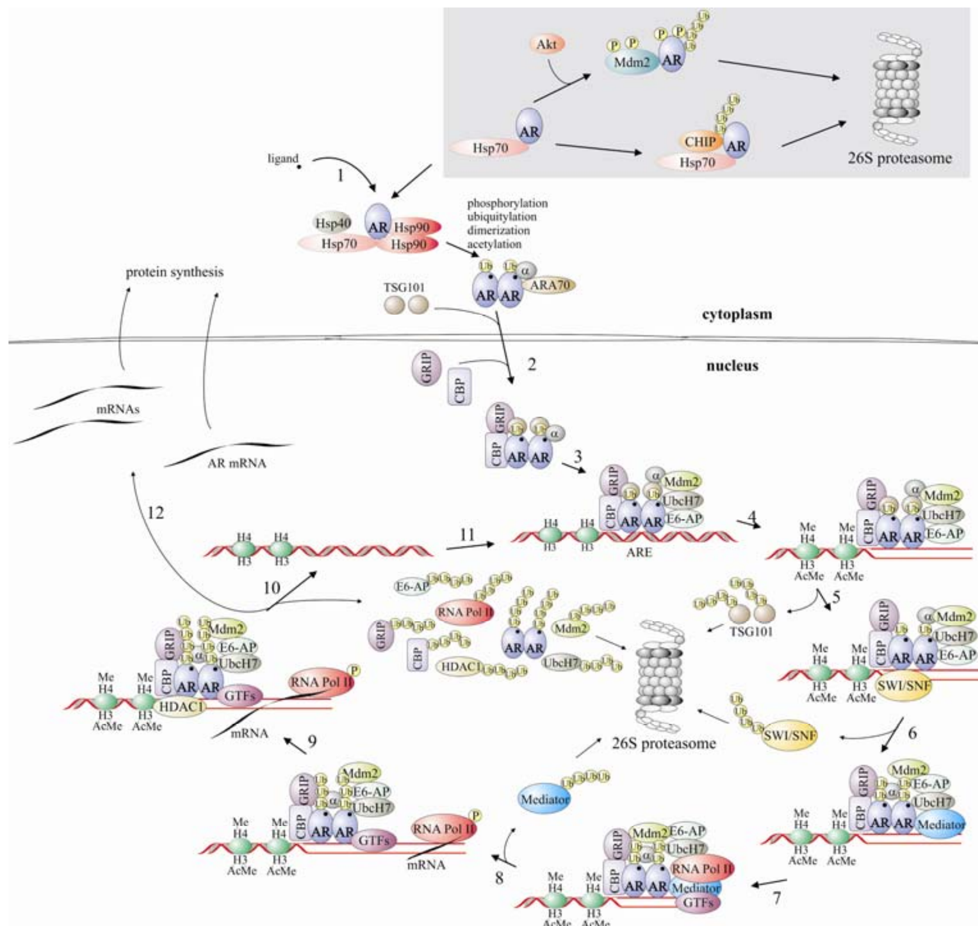
Termination of AR transcription by ubiquitin-like proteins

AR ubiquitylation is not the only condition of transcription termination. AR-dependent transcription may also be terminated through modifications by the ubiquitin-like proteins SUMO and NEDD8. Sumoylation is a general mechanism controlling the activities of the nuclear receptors [26]. The E2 SUMO-conjugating enzyme Ubc9 was shown to interact with AR [25]. PIAS1 and PIASxα, inhibitors of the STAT3 transcription factors, are SUMO-E3 ligases that sumoylate AR and repress its transactivation by facilitating its dissociation from chromatin into the nuclecytoplasmic compartment [27, 90]. It was also shown that AR may be modified by the ubiquitin-like protein NEDD8 [91]. Recent research indicates that Uba3, an activating enzyme for NEDD8, may terminate steroid receptor-mediated gene transcription and its turnover by

neddylation of the receptor or by recruitment of SCF ligases that ubiquitylate the receptor [92].

TARGETING AR DEGRADATION IN PROSTATE CANCER

Current prostate cancer therapies are based on androgen deprivation. This may be achieved either by castration or by using antiandrogens. These options are not without defects. Although they cause regression of the tumour, it usually relapses as a result of the selection of androgen-independent cells. Thus, the prostate cancer comes back and is androgen independent or androgen refractory. This is because AR may be activated by many cell signalling pathways, namely, MAPK [93, 94], PI3K/Akt [42], PKC [95] or PKA [96]; mutated AR may be activated by antagonists [97]; due to amplification, AR can be activated by small doses of remaining androgens [98]; and mutations in AR may alter its specificity



towards adrenal androgens or even towards non-androgenic steroids such as progesterone and estradiol [99, 100]. Superior therapeutic interventions are therefore needed that could take advantage of the fact that prostatic tumours are androgen-independent but remain AR-dependent [101]. Androgen-dependent and androgen-independent tumours rely on AR, so it should remain the main therapeutic target in the treatment of prostate cancers; downregulation of its level will be the advantageous approach. This may be achieved by application of hyperthermia. In LNCaP cells, heat treatment resulted in the downregulation of AR protein and in the loss of 19S proteasome activity, yet the mechanism by which heat treatment decreases AR expression remains unclear [102].

Fig. 3. Proteasome as a central regulator of the AR transcription process. The grey background depicts those activities that occur in the cytoplasm prior to ligand binding. AR may be targeted for proteasomal degradation after Akt-driven phosphorylation and Mdm2-driven ubiquitylation following growth factor stimulation. AR in its inactive state forms a complex with heat shock proteins (Hsps). Hsps form a molecular platform on which AR may be folded or targeted for proteasomal degradation after the recruitment of CHIP ligase. This mechanism provides an accurate, accessible level of receptor protein. Hsps form a multichaperone complex which confer a ligand-binding ability to the receptor, and is likely to dissociate after AR activation by the ligand, which may be testosterone or the more potent androgen dihydrotestosterone. Ligand binding induces post-translational modifications which include phosphorylation, acetylation and probably ubiquitylation; alternatively, ubiquitylation of the receptor may occur at later stages, in the nucleus at the core promoter. Ubiquitin attached to AR is protected from release by TSG101. AR translocation to the nucleus is facilitated by ARA70 coactivator and PSMA7, an α-type subunit of proteasome. 2) After nuclear import, AR associates with GRIP1 and p300/CBP coactivators; the latter may acetylate histones and AR itself. This facilitates recruitment of additional acetylases and histone modifiers like methyltransferases (omitted for clarity). These histone modifications may induce the release of TSG101. Ubiquitin attached to AR is therefore susceptible to further ubiquitylation. 6) SWI/SNF is an ATPase complex that reduces the association of histones with DNA. 7) The subsequent recruitment of Mediator facilitates the engagement of RNA Pol II and general transcription factors, and as a result, the initiation of transcription. 8) Phosphorylation of RNA Pol II by kinases associated with general transcription factors makes it elongation competent. 9) As transcription proceeds, ubiquitin ligases associated with the transcriptional complex ubiquitylate and target its components for degradation. 10) When the ubiquitin chain on AR reaches the appropriate length, AR becomes degraded, similarly to other components of the transcriptional process. It is likely that the excess of AR molecules that could not actively participate in transcription is exported to the cytoplasm retaining the ability to reenter the nucleus. 11) Promoter clearance via degradation of its transcriptional components prepares the gene for a new round of transcription. 12) AR-dependent transcription results in the production of mRNAs including AR mRNA. AR is bound co-translationally by Hsp machinery.

Another promising approach is targeting AR degradation. This is evidenced by the fact that prostate tumours lose the expression of E6-AP ubiquitin ligase [89]. Given the critical role of E6-AP in regulating AR activity, probably by targeting AR to proteasomal degradation and maintaining its proper function in normal tissue, the loss of E6-AP will be favourable in prostate cancer. Anti-cancer therapy that has gained significant interest is the application of phytochemicals, namely genistein and curcumin. Emodin seems to be a more potent phytochemical than curcumin and genistein [101]. It is a natural compound of *Rheum palmatum*, which interestingly suppresses prostate cancer growth both *in vitro* in the AR-positive LNCaP cell culture model and *in vivo* in transgenic mice bearing a PC3-AR xenograft [101]. Emodin reduces protein stability by decreasing the association of AR and Hsp90 and by increasing the association of Mdm2 with AR [101]. An interesting strategy is the application of molecules that induce degradation of selected proteins using PROteolysis TArgeting Chimeric molecules (PROTACs) [103]. PROTAC-5 is composed of DHT (a ligand for targeted AR protein), two aminocaproic acids (a linker moiety), ALAPYIP (a sequence recognised by E3 ligase), and a poly-D-arginine tag (to confer cell permeability) [103]. This molecule has been shown to down-regulate AR in HEK293 cells transfected with GFP-AR via a proteasome-dependent pathway [103]. Another approach is the application of geldanamycin in targeting AR degradation. AR in its inactive state exists as part of a multichaperone complex [104]. Geldanamycin is an inhibitor of Hsp90 chaperone, and its inhibition evokes the proteasomal degradation of several proteins, including AR [105]. Hybrids composed of testosterone and geldanamycin were shown to selectively inhibit AR in prostate cancer cells [106].

TOWARDS THE INTEGRAL MODEL

In order to provide a clear overview of the mechanism of AR action following ligand binding, the subsequent assembly of the AR transcription complex and the involvement of proteasome therein is illustrated (Fig. 3).

CONCLUSIONS

Regulation of AR transcriptional activity appears to be more complex than previously considered. This regulation involves dynamic interactions between AR and cofactors, among which proteasome plays a pivotal role. Combinatorial and sequential association and dissociation of cofactors is regulated by different cellular events which include phosphorylation, acetylation, ubiquitylation, sumoylation and others. This allows the precise regulation of each step throughout the transcriptional process. Further studies will assist in the identification of novel cofactors for AR and broaden our knowledge on the differential expression of cofactors affecting gene regulation.

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REFERENCES

1. Wilson, J.D., George, F.W. and Griffin, J.E. The hormonal control of sexual development. **Science** 211 (1981) 1278-1284.
2. Quigley, C.A., De Bellis, A., Marschke, K.B., El-Awady, M.K., Wilson, E.M. and French, F.S. Androgen receptor defects: historical, clinical and molecular perspectives. **Endocr. Rev.** 16 (1995) 271-321.
3. Grossmann, M.E., Huang, H. and Tindall, D.J. Androgen receptor signaling in androgen-refractory prostate cancer. **J. Natl. Cancer Inst.** 93 (2001) 1687-1697.
4. Jemal, A., Murray, T., Ward, E., Samuels, A., Tiwari, R.C., Ghafoor, A., Feuer, E.J. and Thun, M.J. Cancer statistics, 2005. **CA Cancer J. Clin.** 55 (2005) 10-30.
5. www.nursa.org
6. MacLean, H.E., Warne, G.L. and Zajac J.D. Localization of functional domains in the androgen receptor. **J. Steroid Biochem. Mol. Biol.** 62 (1997) 233-242.
7. Vanaja, D.K., Mitchell, S.H., Toft, D.O. and Young, C.Y. Effect of geldanamycin on androgen receptor function and stability. **Cell Stress Chaperones** 7 (2002) 55-64.
8. Shang, Y., Myers, M. and Brown, M. Formation of androgen receptor transcription complex. **Mol. Cell** 9 (2002) 601-610.
9. Asirvatham, A.J., Schmidt, M., Gao, B. and Chaudhary, J. Androgens regulate the immune/inflammatory response and cell survival pathways in rat ventral prostate epithelial cells. **Endocrinology** 147 (2006) 257-271.
10. Burnstein, K.L. Regulation of androgen receptor levels: Implications for prostate cancer progression and therapy. **J. Cell. Biochem.** 95 (2005) 657-669.
11. Yeap, B.B., Wilce, J.A. and Leedman, P.J. The androgen receptor mRNA. **BioEssays** 26 (2004) 672-682.
12. Heinlein, C.A. and Chang, C. Androgen receptor (AR) coregulators: an overview. **Endocr. Rev.** 23 (2002) 175-200.
13. Smith, C.L. and O'Malley, B.W. Coregulator function: a key to understanding tissue specificity of selective receptor modulators. **Endocr. Rev.** 25 (2004) 45-71.
14. Sheflin, L., Keegan, B., Zhang, W. and Spaulding, S.W. Inhibiting proteasomes in human HepG2 and LNCaP cells increases endogenous androgen receptor levels. **Biochem. Biophys. Res. Commun.** 276 (2000) 144-150.

15. Pickart, C.M. Mechanisms underlying ubiquitination. **Annu. Rev. Biochem.** 70 (2001) 503-533.
16. Thrower, J.S., Hoffman, L., Rechsteiner, M. and Pickart, C.M. Recognition of the polyubiquitin proteolytic signal. **EMBO J.** 19 (2000) 94-102.
17. Nawaz, Z., Lonard, D.M., Smith, C.L., Lev-Lehman, E., Tsai, S.Y., Tsai, M.J. and O'Malley B.W. The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. **Mol. Cell. Biol.** 19 (1999) 1182-1189.
18. Smith, C.L., DeVera, D.G., Lamb, D.J., Nawaz, Z., Jiang, Y.H., Beaudet, A.L. and O'Malley, B.W. Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. **Mol. Cell. Biol.** 22 (2002) 525-535.
19. Verma, S., Ismail, A., Gao, X., Fu, G., Li, X., O'Malley, B.W. and Nawaz, Z. The ubiquitin-conjugating enzyme UBCH7 acts as a coactivator for steroid hormone receptors. **Mol. Cell. Biol.** 24 (2004) 8716-8726.
20. Beitel, L.K., Elhaji, Y.A., Lumbroso, R., Wing, S.S., Panet-Raymond, V., Gottlieb, B., Pinsky, L. and Trifiro, M.A. Cloning and characterization of an androgen receptor N-terminal-interacting protein with ubiquitin-protein ligase activity. **J. Mol. Endocrinol.** 29 (2002) 41-60.
21. Kang, H.Y., Yeh, S., Fujimoto, N. and Chang, C. Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. **J. Biol. Chem.** 274 (1999) 8570-8576.
22. Ito, K., Adachi, S., Iwakami, R., Yasuda, H., Muto, Y., Seki, N. and Okano, Y. N-Terminally extended human ubiquitin-conjugating enzymes (E2s) mediate the ubiquitination of RING-finger proteins, ARA54 and RNF8. **Eur. J. Biochem.** 268 (2001) 2725-2732.
23. Lin, H.K., Wang, L., Hu, Y.C., Altuwaijri, S. and Chang, C. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. **EMBO J.** 21 (2002) 4037-4048.
24. Moilanen, A.M., Karvonen, U., Poukka, H., Yan, W., Toppari, J., Jänne, O.A. and Palvimo, J.J. A testis-specific androgen receptor coregulator that belongs to a novel family of nuclear proteins. **J. Biol. Chem.** 274 (1999) 3700-3704.
25. Poukka, H., Aarnisalo, P., Karvonen, U., Palvimo, J.J. and Jänne, O.A. Ubc9 interacts with the androgen receptor and activates receptor-dependent transcription. **J. Biol. Chem.** 274 (1999) 19441-19446.
26. Poukka, H., Karvonen, U., Jänne, O.A. and Palvimo, J.J. Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). **Proc. Natl. Acad. Sci. USA** 97 (2000) 14145-14150.
27. Nishida, T. and Yasuda, H. PIAS1 and PIASxα function as SUMO-E3 ligases toward androgen receptor and repress androgen receptor-dependent transcription. **J. Biol. Chem.** 277 (2002) 41311-41317.

28. Moilanen, A.M., Poukka, H., Karvonen, U., Häkli, M., Jänne, O.A. and Palvimo, J.J. Identification of a novel RING finger protein as a coregulator in steroid receptor-mediated gene transcription. **Mol. Cell. Biol.** 18 (1998) 5128-3519.
29. Poukka, H., Karvonen, U., Yoshikawa, N., Tanaka, H., Palvimo, J.J. and Jänne, O.A. The RING finger protein SNURF modulates nuclear trafficking of the androgen receptor. **J. Cell. Sci.** 113 (2000) 2991-3001.
30. Häkli, M., Lorick, K.L., Weissman, A.M., Jänne, O.A. and Palvimo, J.J. Transcriptional coregulator SNURF (RNF4) possesses ubiquitin E3 ligase activity. **FEBS Letters** 560 (2004) 56-62.
31. Murata, S., Minami, Y., Minami, M., Chiba, T. and Tanaka, K. CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. **EMBO Rep.** 2 (2001) 1133-1138.
32. Cardozo, C.P., Michaud, C., Ost, M.C., Fliss, A.E., Yang, E., Patterson, C., Hall, S.J. and Caplan, A.J. C-terminal Hsp-interacting protein slows androgen receptor synthesis and reduces its rate of degradation. **Arch. Biochem. Biophys.** 410 (2003) 134-140.
33. He, B., Bai, S., Hnat, A.T., Kalman, R.I., Minges, J.T., Patterson, C. and Wilson, E.M. An androgen receptor NH2-terminal conserved motif interacts with the COOH terminus of the Hsp70-interacting protein (CHIP). **J. Biol. Chem.** 279 (2004) 30643-30653.
34. Rechsteiner, M. and Rogers, S.W. PEST sequences and regulation by proteolysis. **Trends Biochem. Sci.** 21 (1996) 267-271.
35. Wolf, D.H. and Hilt, W. The proteasome: a proteolytic nanomachine of cell regulation and waste disposal. **Biochim. Biophys. Acta** 1695 (2004) 19-31.
36. Lin, H.K., Hu, Y.C., Lee, D.K. and Chang, C. Regulation of androgen receptor signaling by PTEN (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor through distinct mechanisms in prostate cancer cells. **Mol. Endocrinol.** 18 (2004) 2409-2423.
37. Yang, L., Wang, L., Lin, H.K., Kan, P.Y., Xie, S., Tsai, M.Y., Wang, P.H., Chen, Y.T. and Chang, C. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. **Biochem. Biophys. Res. Commun.** 305 (2003) 462-469.
38. Cronauer, M.V., Nessler-Menardi. C., Klocker, H., Maly, K., Hobisch, A., Bartsch, G. and Culig, Z. Androgen receptor protein is down-regulated by basic fibroblast growth factor in prostate cancer cells. **Br. J. Cancer** 82 (2000) 39-45.
39. Lin, H.K., Yeh, S., Kang, H.Y. and Chang, C. Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor. **Proc. Natl. Acad. Sci. USA** 98 (2001) 7200-7205.
40. Hu, Y.C., Yeh, S., Yeh, S.D., Sampson, E.R., Huang, J., Li, P., Hsu, C.L., Ting, H.J., Lin, H.K., Wang, L., Kim, E., Ni, J. and Chang, C. Functional domain and motif analyses of androgen receptor coregulator ARA70 and its

differential expression in prostate cancer. **J. Biol. Chem.** 279 (2004) 33438-33446.

41. Lin, H.K., Hu, Y.C., Yang, L., Altuwaijri, S., Chen, Y.T., Kang, H.Y. and Chang, C. Suppression versus induction of androgen receptor functions by the phosphatidylinositol 3-kinase/Akt pathway in prostate cancer LNCaP cells with different passage numbers. **J. Biol. Chem.** 278 (2003) 50902-50907.
42. Wen, Y., Hu, M.C., Makino, K., Spohn, B., Bartholomeusz, G., Yan, D.H. and Hung, M.C. HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. **Cancer Res.** 60 (2000) 6841-6845.
43. Salas, T.R., Kim, J., Vakar-Lopez, F., Sabichi, A.L., Troncoso, P., Jenster, G., Kikuchi, A., Chen, S.Y., Shemshedini, L., Suraokar, M., Logothetis, C.J., DiGiovanni, J., Lippman, S.M. and Menter, D.G. Glycogen synthase kinase-3 beta is involved in the phosphorylation and suppression of androgen receptor activity. **J. Biol. Chem.** 279 (2004) 19191-19200.
44. Liao, X., Thrasher, J.B., Holzbeierlein, J., Stanley, S. and Li, B. Glycogen synthase kinase-3beta activity is required for androgen-stimulated gene expression in prostate cancer. **Endocrinology** 145 (2004) 2941-2949.
45. Gioeli, D., Black, B.E., Gordon, V., Spencer, A., Kesler, C.T., Eblen, S.T., Paschal, B.M. and Weber, M.J. Stress kinase signaling regulates androgen receptor phosphorylation, transcription, and localization. **Mol. Endocrinol.** 20 (2006) 503-515.
46. Fu, M., Wang, C., Zhang, X. and Pestell, R.G. Acetylation of nuclear receptors in cellular growth and apoptosis. **Biochem. Pharmacol.** 68 (2004) 1199-1208.
47. Ito, A., Kawaguchi, Y., Lai, C.H., Kovacs, J.J., Higashimoto, Y., Appella, E. and Yao, T.P. MDM2-HDAC1-mediated deacetylation of p53 is required for its degradation. **EMBO J**. 21 (2002) 6236-6245.
48. Cidlowski, J.A. and Cidlowski, N.B. Regulation of glucocorticoid receptors by glucocorticoids in cultured HeLa S3 cells. **Endocrinology** 109 (1981) 1975-1982.
49. Dace, A., Zhao, L., Park, K.S., Furuno, T., Takamura, N., Nakanishi, M., West, B.L., Hanover, J.A. and Cheng, S. Hormone binding induces rapid proteasome-mediated degradation of thyroid hormone receptors. **Proc. Natl. Acad. Sci. USA** 97 (2000) 8985-8990.
50. Zhu, J., Gianni, M., Kopf, E., Honore, N., Chelbi-Alix, M., Koken, M., Quignon, F., Rochette-Egly, C. and de The, H. Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor alpha (RARalpha) and oncogenic RARalpha fusion proteins. **Proc. Natl. Acad. Sci. USA** 96 (1999) 14807-14812.
51. Lange, C.A., Shen, T. and Horwitz, K.B. Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase

signals their degradation by the 26S proteasome. **Proc. Natl. Acad. Sci. USA** 97 (2000) 1032-1037.

52. Nawaz, Z., Lonard, D.M., Dennis, A.P., Smith, C.L. and O'Malley, B.W. Proteasome-dependent degradation of the human estrogen receptor. **Proc. Natl. Acad. Sci. USA** 96 (1999) 1858-1862.
53. Gaughan, L., Logan, I.R., Neal, D.E. and Robson, C.N. Regulation of androgen receptor and histone deacatylase 1 by Mdm2-mediated ubiquitylation. **Nucleic Acids Res.** 33 (2005) 13-26.
54. Tyagi, R.K., Lavrovsky, Y., Ahn, S.C., Song, C.S., Chatterjee, B. and Roy, A.K. Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells. **Mol. Endocrinol.** 14 (2000) 1162-1174.
55. Dai, J.L. and Burnstein, K.L. Two androgen response elements in the androgen receptor coding region are required for cell-specific up-regulation of receptor messenger RNA. **Mol. Endocrinol.** 10 (1996) 1582-1594.
56. Black, B.E., Vitto, M.J., Gioeli, D., Spencer, A., Afshar, N., Conaway, M.R., Weber, M.J. and Paschal, B.M. Transient, ligand-dependent arrest of the androgen receptor in subnuclear foci alters phosphorylation and coactivator interactions. **Mol. Endocrinol.** 18 (2004) 834-850.
57. Kinyamu, H.K., Chen, J. and Archer, T.K. Linking the ubiquitin-proteasome pathway to chromatin remodeling/modification by nuclear receptors. **J. Mol. Endocrinol.** 34 (2005) 281-297.
58. Kang, Z., Jänne, O.A. and Palvimo, J.J. Coregulator recruitment and histone modifications in transcriptional regulation by the androgen receptor**. Mol. Endocrinol.** 18 (2004) 2633-2648.
59. Fu, M., Wang, C., Reutens, A.T., Wang, J., Angeletti, R.H., Siconolfi-Baez, L., Ogryzko, V., Avantaggiati, M.L. and Pestell, R.G. p300 and p300/cAMP response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. **J. Biol. Chem.** 275 (2000) 20853-20860.
60. Daujat, S., Bauer, U.M., Shah, V., Turner, B., Berger, S. and Kouzarides, T. Crosstalk between CARM1 methylation and CBP acetylation on histone H3. **Curr. Biol.** 12 (2002) 2090-2097.
61. Marshall, T.W., Link, K.A., Petre-Draviam, C.E. and Knudsen, K.E. Differential requirement of SWI/SNF for androgen receptor activity. **J. Biol. Chem.** 278 (2003) 30605-30613.
62. Wang, Q., Sharma, D., Ren, Y. and Fondell, J.D. A coregulatory role for the TRAP-Mediator complex in androgen receptor-mediated gene expression. **J. Biol. Chem.** 277 (2002) 42852-42858.
63. Huang, Z.Q., Li, J., Sachs, L.M., Cole, P.A. and Wong, J. A role for cofactor-cofactor and cofactor-histone interactions in targeting p300, SWI/SNF and Mediator for transcription. **EMBO J.** 22 (2003) 2146-2155.

64. Lee, D.K. and Chang, C. Molecular communication between androgen receptor and general transcription machinery. **J. Steroid Biochem. Mol. Biol.** 84 (2003) 41-49.
65. Svejstrup, J.Q. The RNA polymerase II transcription cycle: cycling through chromatin. **Biochim. Biophys. Acta** 1677 (2004) 64-73.
66. Kang, Z., Pirskanen, A., Jänne, O.A. and Palvimo, J.J. Involvement of proteasome in the dynamic assembley of the androgen receptor transcription complex. **J. Biol. Chem.** 277 (2002) 48366-48371.
67. Hager, G.L., Nagaich, A.K., Johnson, T.A., Walker, D.A and John, S. Dynamics of nuclear receptor movement and transcription. **Biochim. Biophys. Acta** 1677 (2004) 46-51.
68. Dennis, A.P. and O'Malley, B.W. Rush hour at the promoter: how the ubiquitin-proteasome pathway polices the traffic flow of nuclear receptor-dependent transcription. **J. Steroid Biochem. Mol. Biol.** 93 (2005) 139-151.
69. Lin, H.K., Altuwaijri, S., Lin, W.J., Kan, P.Y., Collins, L.L. and Chang, C. Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. **J. Biol. Chem.** 277 (2002) 36570-36576.
70. Makino, Y., Yoshida, T., Yogosawa, S., Tanaka, K., Muramatsu, M. and Tamura, T. Multiple mammalian proteasomal ATPases, but not proteasome itself, are associated with TATA-binding protein and a novel transcriptional activator, TIP120. **Genes Cells** 4 (1999) 529-539.
71. Gonzalez, F., Delahodde, A., Kodadek, T. and Johnston, S.A. Recruitment of a 19S proteasome subcomplex to an activated promoter. **Science** 296 (2002) 548-550.
72. Ferdous, A., Kodadek, T. and Johnston, S.A. A nonproteolytic function of the 19S regulatory subunit of the 26S proteasome is required for efficient activated transcription by human RNA polymerase II. **Biochemistry** 41 (2002) 12798-12805.
73. vom Baur, E., Zechel, C., Heery, D., Heine, M.J., Garnier, J.M., Vivat, V., Le Douarin, B., Gronemeyer, H., Chambon, P. and Losson, R. Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. **EMBO J.** 15 (1996) 110-124.
74. Ikezoe, T., Yang, Y., Saito, T., Koeffler, H.P. and Taguchi, H. Proteasome inhibitor PS-341 down-regulates prostate-specific antigen (PSA) and induces growth arrest and apoptosis of androgen-dependent human prostate cancer LNCaP cells. **Cancer Sci.** 95 (2004) 271-275.
75. Sun, L. and Chen, Z.J. The novel functions of ubiquitination in signaling. **Curr. Opin. Cell Biol.** 16 (2004) 119-126.

76. Salghetti, S.E., Caudy, A.A., Chenoweth, J.G. and Tansey, W.P. Regulation of transcriptional activation domain function by ubiquitin. **Science** 293 (2001) 1651-1653.
77. Burgdorf, S., Leister, P. and Scheidtmann, K.H. TSG101 interacts with apoptosis-antagonizing transcription factor and enhances androgen receptor-mediated transcription by promoting its monoubiquitination. **J. Biol. Chem.** 279 (2004) 17524-17534
78. Conaway, R.C., Brower, C.S. and Conaway, J.W. Emerging roles of ubiquitin in transcriptional regulation. **Science** 296 (2002) 1254-1258.
79. Pham, A.D. and Sauer, F. Ubiquitin-activating/conjugating activity of $TAF_{II}250$, a mediator of activation of gene expression in *Drosophila*. **Science** 289 (2000) 2357-2360.
80. Grossman, S.R., Deato, M.E., Brgnone, C., Chan, H.M., Kung, A.L., Tagami, H., Nakatani, Y. and Livingston, D.M. Polyubiquitination of p53 by a ubiquitin ligase activity of p300. **Science** 300 (2003) 342-344.
81. Ismaili, N., Blind, R. and Garabedian, M.J. Stabilisation of the unliganded glucocorticoid receptor by TSG101. **J. Biol. Chem.** 280 (2005) 11120-11126.
82. Tanner, T., Claessens, F. and Haelens, A. The hinge region of the androgen receptor plays a role in proteasome-mediated transcriptional activation. **Ann. NY Acad. Sci.** 1030 (2004) 587-592.
83. Thomas, M., Dadgar, N., Aphale, A., Harrell, J.M., Kunkel, R., Pratt, W.B. and Lieberman, A.P. Androgen receptor acetylation site mutations cause trafficking defects, misfolding and aggregation similar to expanded glutamine tracts. **J. Biol. Chem.** 279 (2004) 8389-8395.
84. Zhou, Z.X., Kemppainen, J.A. and Wilson, E.M. Identification of three proline-directed phosphorylation sites in the human androgen receptor. **Mol. Endocrinol.** 9 (1995) 605-615.
85. Wong, H.Y., Burghoorn, J.A., Van Leeuwen, M., De Ruiter, P.E., Schippers, E., Blok, L.J., Li, K.W., Dekker, H.L., De Jong, L., Trapman, J., Grootegoed, J.A. and Brinkmann, A.O. Phosphorylation of androgen receptor isoforms. **Biochem. J.** 383 (2004) 267-276.
86. Gioeli, D., Ficarro, S.B., Kwiek, J.J., Aaronson, D., Hancock, M., Catling, A.D., White, F.M., Christian, R.E., Settlage, R.E., Shabanowitz, J., Hunt, D.F. and Weber, M.J. Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites. **J. Biol. Chem.** 277 (2002) 29304-29314.
87. Perissi, V., Aggarwal, A., Glass, C.K., Rose, D.W. and Rosenfeld, M.G. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. **Cell** 116 (2004) 511-526.
88. Zhang, J., Guenther, M.G., Carthew, R.W. and Lazar, M.A. Proteasomal regulation of nuclear receptor corepressor-mediated repression. **Genes Dev**. 12 (1998) 1775-1780.

89. Gao, X., Mohsin, S.K., Gatalica, Z., Fu, G., Sharma, P. and Nawaz, Z. Decreased expression of E6-associated protein in breast and prostate carcinomas. **Endocrinology** 146 (2005) 1707-1712.
90. Jänne, O.A., Moilanen, A.M., Poukka, H., Rouleau, N., Karvonen, U., Kotaja, N., Häkli, M. and Palvimo, J.J. Androgen-receptor-interacting nuclear proteins. **Biochem. Soc. Trans.** 28 (2000) 401-405.
91. Stenoien, D.L., Cummings, C.J., Adams, H.P., Mancini, M.G., Patel, K., DeMartino, G.N., Marcelli, M., Weigel, N.L. and Mancini, M.A. Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. **Hum. Mol. Genet.** 8 (1999) 731-741.
92. Fan, M., Bigsby, R.M. and Nephew, K.P. The NEDD8 pathway is required for proteasome-mediated degradation of human estrogen receptor (ER)-alpha and essential for the antiproliferative activity of ICI 182,780 in ERalpha-positive breast cancer cells. **Mol. Endocrinol.** 17 (2003) 356-365.
93. Abreu-Martin, M.T., Chari, A., Palladino, A.A., Craft, N.A. and Sawyers, C.L. Mitogen-activated protein kinase kinase kinase 1 activates androgen receptor-dependent transcription and apoptosis in prostate cancer. **Mol. Cell. Biol.** 19 (1999) 5143-5154.
94. Yeh, S., Lin, H.K., Kang, H.Y., Thin, T.H., Lin, M.F. and Chang, C. From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. **Proc. Natl. Acad. Sci. USA** 96 (1999) 5458-5463.
95. Darne, C., Veyssiere, G. and Jean, C. Phorbol ester causes ligand-independent activation of the androgen receptor. **Eur. J. Biochem.** 256 (1998) 541-549.
96. Nazareth, L.V. and Weigel, N.L. Activation of the human androgen receptor through a protein kinase A signaling pathway. **J. Biol. Chem.** 271 (1996) 19900-19907.
97. Veldscholte, J., Ris-Stalpers, C., Kuiper, G.G., Jenster, G., Berrevoets, C., Claassen, E., van Rooij, H.C., Trapman, J., Brinkmann, A.O. and Mulder, E. A mutation in the ligand binding domain of the androgen receptor of human LNCaP cells affects steroid binding characteristics and response to anti-androgens. **Biochem. Biophys. Res. Commun.** 173 (1990) 534-540.
98. Koivisto, P., Visakorpi, T. and Kallioniemi, O.P. Androgen receptor gene amplification: a novel molecular mechanism for endocrine therapy resistance in human prostate cancer. **Scand. J. Clin. Lab. Invest. Suppl.** 226 (1996) 57-63.
99. Culig, Z., Hobisch, A., Cronauer, M.V., Cato, A.C.B., Hittmair, A., Radmayr, C., Eberle, J., Bartsch, G. and Klocker, H. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. **Mol. Endocrinol.** 7 (1993) 1541-1550.

100. Veldscholte, J., Voorhorst-Ogink, M.M., Bolt-de Vries, J., van Rooij, H.C., Trapman, J. and Mulder, E. Unusual specificity of the androgen receptor in the human prostate tumor cell line LNCaP: high affinity for progestagenic and estrogenic steroids. **Biochim. Biophys. Acta** 1052 (1990) 187-194.
101. Cha, T.L., Qiu, L., Chen, C.T., Wen, Y. and Hung, M.C. Emodin down-regulates androgen receptor and inhibits prostate cancer cell growth. **Cancer Res.** 65 (2005) 2287-2295.
102. Pajonk, F., van Ophoven, A. and McBride, W.H. Hyperthermia-induced proteasome inhibition and loss of androgen receptor expression in human prostate cancer cells. **Cancer Res.** 65 (2005) 4836-4843.
103. Schneekloth, J.S., Fonseca, F.N., Koldobskiy, M., Mandal, A., Deshaies, R., Sakamoto, K. and Crews, C.M. Chemical genetic control of protein levels: selective *in vivo* targeted degradation. **J. Am. Chem. Soc.** 126 (2004) 3748-3754.
104. Prescott, J. and Coetzee, G.A. Molecular chaperones throughout the life cycle of androgen receptor. **Cancer Lett.** 231 (2006) 12-19.
105. Vanaja, D.K., Mitchell, S.H., Toft, D.O. and Young, C.Y.F. Effect of geldanamycin on androgen receptor function and stability. **Cell Stress Chaperones** 7 (2002) 55-64.
106. Kuduk, S.D., Harris, C.R., Zheng, F.F., Sepp-Lorenzino, L., Ouerfelli, Q., Rosen, N. and Danishefsky, S.J. Synthesis and evaluation of geldanamycin-testosterone hybrids. **Bioorg. Med. Chem. Lett.** 10 (2000) 1303-1306.