Ubiquitylation of nuclear receptors: new linkages and therapeutic implications

Kyle T Helzer, Christopher Hooper, Shigeki Miyamoto and Elaine T Alarid

McArdle Laboratory for Cancer Research, Department of Oncology, 6151 Wisconsin Institutes for Medical Research, University of Wisconsin Carbone Cancer Center, University of Wisconsin–Madison, 1111 Highland Avenue, Madison, Wisconsin 53705, USA

Correspondence
should be addressed
to E T Alarid

Email
alarid@oncology.wisc.edu

Abstract

The nuclear receptor (NR) superfamily is a group of transcriptional regulators that control multiple aspects of both physiology and pathology and are broadly recognized as viable therapeutic targets. While receptor-modulating drugs have been successful in many cases, the discovery of new drug targets is still an active area of research, because resistance to NR-targeting therapies remains a significant clinical challenge. Many successful targeted therapies have harnessed the control of receptor activity by targeting events within the NR signaling pathway. In this review, we explore the role of NR ubiquitylation and discuss how the expanding roles of ubiquitin could be leveraged to identify additional entry points to control receptor function for future therapeutic development.

Key Words

- E3 ligase
- proteasome
- steroid
- NF- κ B

*Journal of Molecular
Endocrinology*
(2015) **54**, R151–R167

Brief overview of nuclear receptor signaling and drug targets

Nuclear receptors (NRs) comprise a family of transcriptional regulators that control multiple physiological processes including growth, development, reproduction and metabolism through the control of gene expression (Di Croce *et al.* 1999, Aranda & Pascual 2001). The founding member of the family, estrogen receptor alpha (ER α), was identified via its high affinity binding to radiolabeled estradiol (E₂) (Toft & Gorski 1966, Jensen *et al.* 1967). Following the cloning of the gene encoding the glucocorticoid receptor (GR, NR3C1; Miesfeld *et al.* 1984, Hollenberg *et al.* 1985), numerous other NRs were identified and combined into a superfamily composed of a total of 48 receptors in mammals, including ER α (NR3A1) and ER β (NR3A2), thyroid hormone receptor (TR, NR1A1), progesterone receptor (PR, NR3C3), androgen receptor (AR, NR3C4), retinoic acid receptor (RAR, NR1B1–3), retinoic

X receptor (RXR, NR2B1–3), vitamin D receptor (VDR, NR1I1), peroxisome proliferator-activated receptors (PPAR, NR1C1–3) and a number of orphan receptors with no known ligands (Evans & Mangelsdorf 2014). The receptors share a similar architecture consisting of an intrinsically disordered N-terminus, which in some receptors encodes a ligand-independent transactivation domain, a central DNA-binding domain containing two zinc finger motifs, and a C-terminal ligand-binding domain (LBD). The LBD mediates multiple receptor functions, including ligand binding, dimerization, co-regulator interactions and ligand-dependent transcriptional activation function. It is no surprise then that research has focused largely on the LBD and the modulation of receptor actions through both endogenous and synthetic ligands (Gronemeyer *et al.* 2004, McDonnell & Wardell 2010).

The dissection of the molecular events that regulate receptor function has greatly advanced the NR field and contributed significantly to the drug discovery toolbox. Originally, NRs were thought to participate in a relatively simple signal transduction pathway in which activated receptors directly mediated responses in the nucleus through direct DNA binding and transcriptional activation. Though fundamentally correct, the broadening knowledge of components in the NR-activation mechanism has greatly expanded the model and simultaneously expanded the opportunity to control receptor function. In the contemporary model, ligands bind to receptors in the cytoplasm or nucleus or, in some cases, to plasma-membrane-bound receptors. Ligand binding triggers a series of intracellular events, including the release of inactive receptors from heat shock protein (Hsp) complexes, changes to receptor protein conformation, mobilization, dimerization and recruitment of multi-protein transcriptional complexes. The activated NR transcriptional complexes include co-regulators (activators and repressors), chromatin modifying and remodeling complexes, and components of the basal transcriptional machinery. To date, over 300 NR co-regulators have been identified (Jung *et al.* 2005, Malovannaya *et al.* 2011; www.nursa.org). Ligand activation of membrane receptors couples receptor activation to intracellular signaling cascades (Hammes & Levin 2011). Additionally, NRs can be activated indirectly through ligand-independent mechanisms by growth factors. The complexity of NR function and regulation is further expanded by the addition of a temporal component to receptor transcriptional complexes (Métivier *et al.* 2003, Nagaich *et al.* 2004). Collectively, the elucidation of this activation cascade forms the basis for the identification of agents targeting receptors at multiple levels, including co-activator interactions (Norris *et al.* 1999, Parent *et al.* 2008, Gunther *et al.* 2009), dimerization, subcellular localization (Tran *et al.* 2009) and DNA binding (Wang *et al.* 2006, Mao *et al.* 2008, Andersen *et al.* 2010, Caboni & Lloyd 2013).

Post-translational modifications (PTMs) are another regulatory mechanism governing NR function. PTMs represent an important cross-talk mechanism by which other signaling pathways interface with NR activation. In the case of ER α , all domains of the receptor can be phosphorylated in response to ligand and/or growth factor cascades (Ali *et al.* 1993, Le Goff *et al.* 1994, Bunone *et al.* 1996, Weis *et al.* 1996, Chen *et al.* 1999, Yudt *et al.* 1999, Clark *et al.* 2001, Michalides *et al.* 2004, Held *et al.* 2012). Studies in breast cancer cell models have demonstrated that phosphorylation can impact multiple aspects of

receptor function, including protein stability, dimerization, DNA binding and co-activator preferences (Arnold *et al.* 1995, Tzeng & Klinge 1996, Chen *et al.* 1999, Henrich *et al.* 2003, Sheeler *et al.* 2003, Calligé *et al.* 2005, Valley *et al.* 2005, Likhite *et al.* 2006, Bhatt *et al.* 2012). ER α is also subject to other modifications, including acetylation (Kim *et al.* 2006), methylation (Le Romancer *et al.* 2008, Subramanian *et al.* 2008), SUMOylation (Sentis *et al.* 2005, Hilmi *et al.* 2012) and palmitoylation (Acconcia *et al.* 2005). Readers are referred to a recent comprehensive review of ER α PTMs (Le Romancer *et al.* 2011). Importantly, ER α and other NRs are targets of ubiquitylation, a PTM that couples receptor protein turnover and transcriptional function at multiple levels of the receptor signaling pathway.

NR degradation by the ubiquitin-proteasome pathway

The first studies investigating the mechanisms of NR protein turnover pointed to the role of proteasomes and, subsequently, ubiquitylation in targeting receptors to the degradation pathway. Multiple groups demonstrated that proteasome inhibitors disrupted estrogen-induced decreases in ER α protein levels (Alarid *et al.* 1999, El Khissiin & Leclercq 1999, Nawaz *et al.* 1999a). Subsequently, it was observed that RAR γ 2 and RAR α were down-regulated in response to their ligand, all-trans retinoic acid, and the down-regulation was blocked by proteasome inhibitors, MG132 and lactacystin (Zhu *et al.* 1999, Kopf *et al.* 2000). The TR, GR and mineralocorticoid receptor (MR) were also found to be down-regulated in response to ligand binding via a similar pathway (Dace *et al.* 2000, Wallace & Cidlowski 2001, Yokota *et al.* 2004). These studies established the proteasome pathway as a key regulator of NR protein stability (Alarid *et al.* 2006; Fig. 1). One caveat, however, is that proteasome inhibitor studies also disrupt NR motility and transcription (Lonard *et al.* 2000, Reid *et al.* 2003, Elbi *et al.* 2004, Stavreva *et al.* 2004). Proteasome inhibitors can also affect NR gene expression and indirectly lead to downstream changes in NR target gene regulation (Powers *et al.* 2010, Prenzel *et al.* 2011). Further, the proteasome pathway is not selective for NRs, and inhibition of such a vital cellular function can lead to both inhibition and activation of other signaling pathways, production of reactive oxygen species and induction of apoptosis (Shinohara *et al.* 1996, Emanuele *et al.* 2002, Cirit *et al.* 2012). Recent studies also implicate lysosomes in the degradation of a number of NRs, including ER α , AR and GR (He *et al.* 2011, Totta *et al.*

Figure 1

A model of conserved roles of the ubiquitin proteasome pathway in NR signaling. The primary function ascribed to ubiquitin in NR signaling is targeting receptors to the proteasome. Shown here is a simplified NR signaling pathway in which receptor (R) is activated either by ligand or by kinases from growth-factor or membrane-bound NRs. Receptors held bound by heat shock proteins can be targeted for degradation by the 26S proteasome following ubiquitylation by E3 ligases, such as CHIP. Upon binding ligand, receptors undergo dimerization (homo- and heterodimerization are not distinguished here) and can be decorated by multiple post-translational modifications (PTMs) including phosphorylation, shown as a star. Phosphorylated receptors can be directly recognized by E3 ligases, ubiquitylated and directed to the proteasome for degradation. Alternatively, PTMs can be incorporated into active transcriptional complexes of variable co-regulator components, represented by a grey multiprotein complex. The make-up of the co-regulator/receptor complexes can recruit E3 ligases that direct ubiquitylation and degradation of the transcriptional complex. In the degradative pathways, PTMs and co-regulator complexes guide E3 targeting that allows subpopulations of receptors to be selectively degraded in cells.

2014). Owing to the confounding effects of proteasome inhibitors and the complex regulation of NR protein stability, the study of NR proteolysis shifted to better understanding the role and mechanisms of ubiquitylation in targeting NRs to proteasome-mediated degradation.

Ubiquitin is a 76-residue protein that can modify target substrates by covalent attachment of its C-terminal carboxyl group to a lysine residue on the target substrate in a catalytic process involving three classes of enzymes (Hershko & Ciechanover 1998, Pickart 2001, Komander 2009). The first class of enzymes, known as E1 activating enzymes, bind ubiquitin through a catalytic cysteine

residue in an ATP-dependent mechanism, creating a high-energy thioester bond. The E1 enzyme is loaded with a second ubiquitin molecule and then recruits the second class of enzymes, E2 conjugating enzymes. The E1-Ub complex then transfers the ubiquitin to a conserved catalytic cysteine residue of the E2 enzyme, forming a thioester-linked E2-Ub complex in a process known as transthiosterification (Lee & Schindelin 2008). Lastly, the third class of enzymes, known as E3 ubiquitin ligases, facilitates the transfer of ubiquitin from the E2 conjugating enzyme, directly or indirectly, to a lysine on the substrate forming an isopeptide bond. To date, two ubiquitin-specific E1 activating enzymes (UBA1 and UBA6), ~35 E2 conjugating enzymes, and over 600 E3 ligases have been reported in humans (Bernassola *et al.* 2008, Deshaies & Joazeiro 2009, Markson *et al.* 2009, Schulman & Harper 2009, Ye & Rape 2009, van Wijk & Timmers 2010).

Substrate selectivity is primarily guided by E3 ubiquitin ligases, which belong to three subtypes: really interesting new gene (RING), homologous to E6-AP C-terminus (HECT) and RING-between-RING (RBR) (Berndsen & Wolberger 2014). These ligases are classified based on the corresponding motifs (RING, HECT and RBR) required for E3 activity as well as the distinct mechanisms involved. A RING E3 ligase can function as a monomer, dimer (homo or hetero), or multi-protein complex with the RING domain binding to specific E2s and a distinct region of the ligase (or ligase complex) binding to specific substrates. RING ligases promote the transfer of ubiquitin from ubiquitin-charged E2 without itself forming an intermediate thioester with a ubiquitin molecule. In contrast, HECT ligases accept ubiquitin from ubiquitin-charged E2s to its catalytic cysteine, which is then transferred to the substrate. RBR ligases have a combination of RING and HECT mechanisms whereby one of the RING domains binds to E2s and the other contains a catalytic cysteine that accepts a ubiquitin from ubiquitin-charged E2s and then transfers it to substrates. Table 1 lists the ubiquitin E3 ligases that participate in NR ubiquitylation that are organized by NR types and in a general chronological order of discovery in NR regulation.

In the following sections, we highlight specific ligases that appear to have generalized activities on NRs and that are implicated in distinct aspects of NR signaling pathway with the goal of highlighting processes where interference of NR ubiquitylation may be leveraged as part of on-going drug development targeting the ubiquitin system.

Table 1 E3 ligases involved in NR ubiquitylation

Nuclear receptor	E3 ligase	Class of ligase	Type of Ub	References
Estrogen receptor alpha	E6AP	HECT	Poly	Nawaz <i>et al.</i> (1999b), Li <i>et al.</i> (2006), Sun <i>et al.</i> (2012) and Rajbhandari <i>et al.</i> (2014)
	CHIP	RING (U-box)	Poly	Fan <i>et al.</i> (2005)
	Mdm2	RING	Poly	Duong <i>et al.</i> (2007)
	BRCA1/BARD1	RING	Mono	Eakin <i>et al.</i> (2007)
	EFP (TRIM25)	RING	Poly K48	Nakajima <i>et al.</i> (2007)
	SPOP	RING (Cullin)	Poly	Byun & Jung (2008)
	RBCK1	RING (RBR)	?	Gustafsson <i>et al.</i> (2010)
	CUEDC2	? ^a	?	Pan <i>et al.</i> (2011)
	Skp2	RING (F-box)	Poly	Bhatt <i>et al.</i> (2012)
	VHL	RING	Poly	Jung <i>et al.</i> (2012)
	RNF31	RING (RBR)	Mono	Zhu <i>et al.</i> (2014)
Estrogen receptor beta	CHIP	RING (U-box)	Poly K48	Tateishi <i>et al.</i> (2006)
	E6AP	HECT	Poly	Picard <i>et al.</i> (2008)
	Mdm2	RING	Poly	Sanchez <i>et al.</i> (2013)
Androgen receptor	Mdm2	RING	Poly	Lin <i>et al.</i> (2002)
	CHIP	RING (U-box)	Poly	Cardozo <i>et al.</i> (2003)
	NEDD4	HECT		Li <i>et al.</i> (2008)
	RNF6	RING	Poly K6 or K27	Xu <i>et al.</i> (2009b)
	Siah2	RING	Poly K48	Qi <i>et al.</i> (2013)
	UBR1	RING	?	Sultana <i>et al.</i> (2013)
	Skp2	RING	Poly	Li <i>et al.</i> (2014)
Glucocorticoid receptor	Hdm2	RING	Poly	Sengupta & Wasyluk (2001)
	CHIP	RING (U-box)	Poly	Connell <i>et al.</i> (2001)
	FBXW7	RING (F-box)	Poly	Malyukova <i>et al.</i> (2013)
	UBR1	RING	?	Sultana <i>et al.</i> (2013)
Progesterone receptor	CUEDC2	? ^a	?	Zhang <i>et al.</i> (2007)
	BRCA1/BARD1	RING	Poly	Calvo & Beato (2011)
Retinoic acid receptor alpha	FLRF (Rnf41)	RING	?	Jing <i>et al.</i> (2008)
Retinoic X receptor	RNF8	RING	?	Takano <i>et al.</i> (2004)
Mineralocorticoid receptor	CHIP	RING (U-box)	Poly	Faresse <i>et al.</i> (2010)
PPAR γ	Siah2	RING	Poly	Kilroy <i>et al.</i> (2012)
	MKRN1	RING	Poly	Kim <i>et al.</i> (2014)
Estrogen-related receptors	Parkin	RING (RBR)	Poly	Ren <i>et al.</i> (2011)

BARD1, BRCA1-associated RING domain protein 1; BRCA1, breast cancer type 1 susceptibility protein; CHIP, carboxyl terminus of Hsc70-interacting protein; CUEDC2, CUE domain containing 2; E6AP, E6-associated protein; EFP, estrogen-responsive finger protein; FBXW7, F-box/WVD repeat-containing protein 7; FLRF, fetal liver ring finger; Hdm2, human double minute 2; HECT, homologous to the E6-AP C-terminus; Mdm2, mouse double minute 2; MKRN1, makorin ring finger protein 1; NEDD4, neural precursor cell expressed, developmentally down-regulated 4; PPAR γ , peroxisome proliferator-activated receptor gamma; RBCK1, RanBP-type and C3HC4-type zinc finger containing 1; RING, really interesting new gene; RNF6, ring finger protein 6; RNF8, ring finger protein 8; RNF31, ring finger protein 31; Rnf41, ring finger protein 41; Siah2, seven in absentia homolog 2; Skp2, S-phase kinase-associated protein 2; SPOP, speckle-type POZ protein; Ub, ubiquitin; UBR1, ubiquitin protein ligase E3 component N-recognin 1; VHL, Von Hippel-Lindau tumor suppressor.

^aCUEDC2 is a CUE domain-containing gene that promotes the degradation of PR and ER α through ubiquitylation, see references.

Control of unliganded NR protein stability by carboxyl-terminus of Hsc70-interacting protein

The control of NRs by carboxyl-terminus of Hsc70-interacting protein (CHIP) represents specific ligase activity at early stages in NR signaling, including controlling basal NR expression and receptor availability prior to ligand binding. NRs are held stable in their unliganded state by chaperone complexes, which include Hsps, Hsp70 and Hsp90 (Smith & Toft 1993). The Hsp interaction guides appropriate folding of NR protein and stabilizes the ligand-binding pocket (Bresnick *et al.* 1989,

Smith 1993, Stancato *et al.* 1996, Pratt 1997). Disruption of the Hsp90-NR complex using a chemical inhibitor, geldanamycin, was shown to cause down-regulation of ER α , PR, GR and AR in a proteasome-dependent manner (Whitesell & Cook 1996, Pratt & Toft 1997, Bagatell *et al.* 2001, Connell *et al.* 2001, Lee *et al.* 2002, Vanaja *et al.* 2002, Fan *et al.* 2005). CHIP is a RING E3 ligase that contains a tetratricopeptide repeat, which binds and decreases the ATPase activity of Hsps. This association ultimately decreases the efficiency of the chaperone and impairs its function, leading to misfolding and subsequent degradation of its substrate by proteasomes (Ballinger *et al.*

1999, Connell *et al.* 2001). CHIP interacts with Hsp90 and incorporates itself into the NR–Hsp90 heterocomplex, causing remodeling that favors degradation of the NR. In the case of GR, CHIP can ubiquitylate GR both *in vitro* and *in vivo* and directly target it for degradation by interacting with the S5a subunit of the 26S proteasome (Connell *et al.* 2001). CHIP was also found to preferentially associate with unliganded ER α , increasing ubiquitylation and degradation of the receptor. Other NRs also appear to be ubiquitylated by CHIP, including ER β , AR, GR and MR, suggesting that ubiquitylation of NRs by CHIP is a conserved mechanism (Cardozo *et al.* 2003, Wang & DeFranco 2005, Tateishi *et al.* 2006, Faresse *et al.* 2010). Hence, CHIP ligase is a major regulator of unliganded NR protein expression (Fig. 1), which has relevance in scenarios in breast and prostate cancer where therapies such as aromatase inhibitors and abiraterone decrease hormone production yet maintain the unliganded receptor.

Regulated NR ubiquitylation by phosphorylation and coactivator complexes

Beyond control of basal NR protein levels, ligand-induced turnover of NRs revealed additional layers of complexity in E3 ligase action in NR signaling. As described above, ligand binding to NRs triggers a series of events associated with the transcriptional activation mechanism, including phosphorylation and the recruitment of multi-protein transcriptional complexes. Ubiquitylation is integrated within this activation mechanism through both phosphorylation and the protein complexes recruited to NRs (Fig. 1). For example, the RING E3 ligase mouse double minute 2 (Mdm2) is implicated in the turnover of many NRs, including AR, ER α , ER β and GR (Table 1, references therein). The targeting of NRs for ubiquitylation by Mdm2 is triggered by at least two levels of regulation, NR phosphorylation and the composition of the NR transcriptional complex. In the case of AR, phosphorylation of AR on Ser515 by cyclin-dependent kinase 7 (Cdk7), a component of the basal transcriptional machinery, is critical for recruitment of Mdm2 and the subsequent ubiquitylation and degradation of AR by the proteasome (Chymkowitch *et al.* 2011). Ubiquitylation of AR by Mdm2 can also be signaled following phosphorylation of AR by Akt on Ser210 and Ser790 (Lin *et al.* 2002). Mutation of Ser210 and Ser790, or Ser515, to alanine prevents recruitment of Mdm2 and ubiquitylation of AR. Like AR, Mdm2 is also recruited to ER α and ER β complexes when the corresponding ER is phosphorylated (Valley *et al.* 2005,

Picard *et al.* 2008, Sanchez *et al.* 2013). However, degradation of ER α upon Mdm2 over-expression provides an example in which specific NR protein complexes are also a requirement for this response – in this case, a complex with p53 (Duong *et al.* 2007). Similarly, GR degradation following dexamethasone treatment involves the formation of a GR complex containing p53 and Hdm2 (Sengupta & Wasylyk 2001). In the case of ER β , Mdm2 works in concert with a different coregulator, CREB-binding protein (CBP), to form a complex that results in ubiquitylation and ultimate degradation of ER β (Sanchez *et al.* 2013). Interestingly, unlike ER β , the Mdm2–CBP complex was unable to target ER α for degradation. These observations suggest that Mdm2 is recruited to NRs as part of larger multi-protein complexes that impart specificity of Mdm2 action in controlling ubiquitylation and degradation. Given that receptor complexes are dynamic (Métivier *et al.* 2003), this protein complex specificity, in addition to phosphorylation events, could impart temporal and context-specific regulation on the NR ubiquitylation, stability and associated functions.

Some E3 ligases control NR function through both ligase activity-dependent and -independent mechanisms. A primary example of dual-action E3 ligases is E6-associated protein (E6AP). The first studies to demonstrate ubiquitylation of endogenous NRs were done on ER α (Wijayarathne & McDonnell 2001). Subsequently, E6AP was found to be recruited to ER α in a calmodulin-dependent manner, leading to ubiquitylation and degradation of ER α (Li *et al.* 2006). In addition to calmodulin-dependent ubiquitylation, recruitment of E6AP to ER α as well as ER β requires phosphorylation of the receptor (Picard *et al.* 2008, Rajbhandari *et al.* 2014). Consistent with the ligase function of E6AP, mammary and prostate glands of E6AP-null mice show elevated levels of ER α (Gao *et al.* 2005). However, E6AP also functions as a coactivator for ER α , as well as other NRs such as PR, AR and GR (Nawaz *et al.* 1999b, Ramamoorthy & Nawaz 2008). In these cases, the disruption of ubiquitin ligase activity as well as the HECT domain of E6AP by mutagenesis had no effect on NR coactivation activity (Nawaz *et al.* 1999b). Similar ligase-independent coregulator function has been noted in NR regulation by other HECT ligases, including NEDD4-1, Rsp5 and HACE1. For example, HACE1 was identified as an NR-interacting partner in a yeast two-hybrid screen, and shown to interact with RAR α , RAR γ , ER α and TR α . Mutation of critical cysteine residues in the HECT ligase domain had no effect on its transcriptional repressor activity toward RARs (Zhao *et al.* 2009). Likewise, the ligase activity of Rsp5 is not essential for Rsp5

coactivation of PR and GR transactivation (Imhof & McDonnell 1996). Among the HECT ligases, E6AP alone thus far has been directly shown to function as a key NR regulator via ubiquitin ligase-dependent and -independent mechanisms.

Current ubiquitin-targeting therapeutics

The examples provided above indicate the potential for targeting multiple steps (protein folding, coactivator interactions and transcriptional function) in the NR signaling pathway via control of ubiquitylation. Clinical approaches in cancer therapy have thus far focused on inhibiting the 26S proteasome (Teicher *et al.* 1999). Bortezomib (Velcade, PS-341) is a general proteasome inhibitor that is FDA approved for the treatment of multiple myeloma and mantle cell lymphoma. Second-generation proteasome inhibitors have also been developed, including carfilzomib, which was approved in 2012 for multiple myeloma patients that are refractory to bortezomib therapy (Mitsiades *et al.* 2011). While the preclinical data supports the efficacy of proteasome inhibitors in other cancer types, the results outside of hematological malignancies have been disappointing (Yang *et al.* 2006). Hence, efforts are underway to more specifically target the ubiquitylation machinery and their substrates.

A glimpse into the relevance to the NR field is provided by studies of Skp1-Cullin1-F-box (SCF)–Skp2 and p27^{kip1}. Skp2 is an F-box protein and component of the SCF RING ubiquitin E3 ligase complex. Skp2 ligase ubiquitylates and degrades ER α and a high Skp2 expression in human tumors correlates with an ER α -negative status (Bhatt *et al.* 2012). Skp2 is overexpressed in human cancers, and deregulation of Skp2 is implicated in cancer progression through loss of cell-cycle control and transcription (Bloom & Pagano 2003, Kamata *et al.* 2005, Davidovich *et al.* 2008). Skp2 ligase activity was shown to be dependent on E₂-induced phosphorylation, leading to ubiquitylation of p27^{kip1} (Lecanda *et al.* 2007, Huang *et al.* 2012). The loss of nuclear p27^{kip1} has been shown to occur in E₂-induced type 1 endometrial carcinogenesis (Lecanda *et al.* 2007). Using a small molecule screen, specific agents have been identified that block Skp2-dependent ubiquitylation of p27^{kip1}, thus preventing its degradation. Treatment of E₂-induced endometrial carcinoma cell lines with these small molecules resulted in increased levels of p27^{kip1} along with decreased proliferation (Pavlidis *et al.* 2013). These experiments demonstrate that alterations of E3 ligase activity using small molecule inhibitors could be

a viable strategy for future therapeutic development. This possibility is further supported by a recent report of peptide and small molecule inhibitors of HECT ligases (Mund *et al.* 2014).

To date, three E3-targeting drugs have been approved by the FDA, and all three target the same enzyme, cereblon (CRBN). CRBN is a part of the Cul4-Rbx1-DDB1-CRBN RING ubiquitin E3 ligase complex, and the three drugs that target CRBN – thalidomide, lenalidomide and pomalidomide, commonly referred to as immunomodulatory drugs – bind to CRBN and promote the recruitment of substrates, including Ikaros (IKZF1) and Aiolos (IKZF3), which are subsequently ubiquitylated and degraded (Ito *et al.* 2010, Chamberlain *et al.* 2014, Fischer *et al.* 2014, Lu *et al.* 2014). Currently these drugs are approved for multiple myeloma therapy (Martiniani *et al.* 2012, Terpos *et al.* 2013).

Regulation of NR function by E3 ligase-mediated monoubiquitylation

While the focus in the NR field and therapeutic approaches has largely been directed to the role of ubiquitin in degradation and stability of receptors, the scope of the ubiquitin field extends well beyond degradative mechanisms associated with the proteasome pathway that requires the attachment of a ubiquitin polymer (or chain) with four or more ubiquitin moieties (Thrower *et al.* 2000). For example, attachment of a single ubiquitin molecule (monoubiquitylation) on NRs by BRCA1 has been described. BRCA1, along with its partner BARD1, form a heterodimeric RING E3 ligase implicated in numerous cellular processes including DNA repair, cell cycle control, transcriptional regulation, apoptosis and genomic stability (Deng 2006, Roy *et al.* 2012). BRCA1 ubiquitylation of both ER α and PR contributes to the their transcriptional function (Eakin *et al.* 2007, Calvo & Beato 2011). BRCA1/BARD1 monoubiquitylates ER α *in vitro* and *in vivo* (Eakin *et al.* 2007, Dizin & Irminger-Finger 2010, Ma *et al.* 2010, Zhu *et al.* 2014). This monoubiquitylation is dependent on BRCA1/BARD1 ligase activity as cancer predisposing BRCA1 mutations (C61G and C64G) affecting the ligase activity abolish the ability of BRCA1 to monoubiquitylate ER α . The site of monoubiquitylation on ER α was identified through mass spectrometry to be K302; however, the K302A ER α mutant was still monoubiquitylated *in vitro*. The adjacent lysine residue, K303, can be targeted for monoubiquitylation in lieu of K302 (Eakin *et al.* 2007). The precise function of ER α monoubiquitylation by BRCA1/BARD1 *in vivo* is still unclear, although it is hypothesized to play a role in inhibition of

ER α transcriptional activity as well as E₂-induced cell proliferation (Ma *et al.* 2010, La Rosa *et al.* 2011a). It should be noted, however, that BRCA1 mutant breast cancers are almost always ER α -negative and thus potential therapies targeting the interaction between ER α and BRCA1 would not be suitable in these cases (Karp *et al.* 1997, Loman *et al.* 1998). In the case of PR, BRCA1 induces ubiquitylation, but whether PR ubiquitylation is poly-ubiquitylation (attachment of a ubiquitin chain on a lysine) or multi-monoubiquitylation (multiple single ubiquitin attachments on different lysine sites on the substrate) is unresolved (Calvo & Beato 2011).

In addition to BRCA1, RNF31 – also known as HOIP or ZIBRA – can also monoubiquitylate ER α (Zhu *et al.* 2014). RNF31 is a RBR E3 ligase, and a component of the linear ubiquitin assembly complex (LUBAC). Studies by Zhu *et al.* (2014) show a positive correlation between RNF31 and ER α levels. Further, manipulation of RNF31 by knockdown or overexpression decreases and increases ER α -mediated transcriptional activity, respectively. Importantly, the effects on receptor transcriptional function were shown to be dependent on RNF31 E3 ligase activity, supporting the idea that monoubiquitylation of ER α can regulate receptor transcriptional activation. The control of ER α by RNF31 and BRCA1/BARD1 suggests that there may be other NRs regulated in the same manner via monoubiquitylation. Moreover, given that RNF31 can act in the context of the LUBAC E3 ligase complex (see below), additional forms of ubiquitylation may also play regulatory roles in NR function.

Non-degradative ubiquitin code

Advances in the ubiquitin field have led to an emerging concept of the ‘ubiquitin code’ (Kulathu & Komander 2012). Ubiquitin itself has seven lysine residues – K6, K11, K27, K29, K33, K48 and K63 – each of which can serve as ubiquitylation sites to assemble ubiquitin chains connected via distinct internal lysine residues (Fig. 2). The attachment of ubiquitin chains linked via lysine K48 marks the substrate for degradation by the 26S proteasome and, as mentioned above, a minimum of four ubiquitin molecules is needed for efficient recognition and degradation of substrates tagged with K48-linked polyubiquitin chains (Thrower *et al.* 2000). The aforementioned NR associated ligases form this type of ubiquitin chains. However, polyubiquitin chains linked by each of the other ubiquitin lysines has been shown to be present *in vivo* through analysis by mass spectrometry (Xu *et al.* 2009a). Moreover, the amino group of the N-terminal methionine of ubiquitin can serve to assemble ‘M1-linked’

polyubiquitin chains. Many substrates can also be multi-monoubiquitylated at multiple lysine sites as described above. Finally, different types of ubiquitin configurations could occur in a single substrate (i.e., ‘mixed’ ubiquitin linkages). These varying ubiquitin chains significantly expanded the roles that ubiquitin plays in multitudes of molecular, cellular, physiological and pathological processes. The primary decoding of the information built into distinct types of ubiquitin chains is mediated by an array of ubiquitin-binding domains (UBDs) or ubiquitin receptors (Dikic *et al.* 2009). For a more comprehensive overview of UBDs, we direct the reader to recently published reviews on UBDs (Dikic *et al.* 2009, Husnjak & Dikic 2012, Searle *et al.* 2012). The role for these less commonly studied forms of polyubiquitin linkages in NR regulation is a relatively untapped and emerging area of research. For example, ER α has recently been shown to contain a UBD in its AF-2 domain (Pesiri *et al.* 2013).

The study of RNF6 in AR biology provides a proof of principle that non-degradative ubiquitin chains can contribute to NR transcriptional function. Xu *et al.* (2009b) discovered that AR was polyubiquitylated by the E3 ubiquitin ligase, RNF6, in prostate cancer cells. Following identification as an interacting partner of AR in GST-pull down assays, the authors showed that RNF6 overexpression leads to an increase in polyubiquitylated AR without changes in AR protein levels. *In vitro* ubiquitylation assays revealed that RNF6 added K6- or K27-linked polyubiquitin chains to the AR. Overexpression of RNF6 increased the recruitment of co-factors, specifically ARA54, to androgen response elements, which suggests the possibility that specific ubiquitin linkages may contribute to recruitment and specificity of coregulator complexes on DNA. This may have implications in hormone-refractory prostate cancer where RNF6 has been shown to be overexpressed (Xu *et al.* 2009b).

To begin to elucidate the potential involvement of the ubiquitin code in NR regulation, we briefly provide an example (NF- κ B) where the roles of distinct types of ubiquitylation are better understood (Chen & Chen 2013). NF- κ B is a family of related dimeric transcription factors that are held inactive in the cytoplasm by a class of inhibitor proteins, inhibitor of κ B (I κ B). Cell signaling leads to the degradation of I κ B, liberating NF- κ B to the nucleus to initiate transcription. I κ B degradation is mediated by phosphorylation of I κ B, which creates a docking site for the β -transducing repeat containing protein (β -TrCP) RING ubiquitin E3 ligase complex that induces K48-linked polyubiquitylation of I κ B and its degradation by the 26S proteasome. Degradation of

Linkage type	Cellular role	References
K48	Proteasomal degradation	Chau <i>et al.</i> (1989) Peng <i>et al.</i> (2003) Kim <i>et al.</i> (2011)
K63	Signal transduction Translation Lysosomal degradation Endosomal trafficking DNA damage response	Xu <i>et al.</i> (2009a) Al-Hakim <i>et al.</i> (2010) Mukhopadhyay & Riezman (2007) Spence <i>et al.</i> (2000)
Met-1	Signal transduction	Kirisako <i>et al.</i> (2006) Tokunaga <i>et al.</i> (2009) Rahighi <i>et al.</i> (2009) Emmerich <i>et al.</i> (2013)
Mono	Lysosomal degradation DNA damage response DNA repair	Hoege <i>et al.</i> (2002) Freudenthal <i>et al.</i> (2010) Jackson & Durocher (2013)
K11	Cell cycle regulation Proteasomal degradation Assembled by APC/C ERAD Membrane trafficking TNF α signaling	Jin <i>et al.</i> (2008) Xu <i>et al.</i> (2009b) Dynek <i>et al.</i> (2010) Goto <i>et al.</i> (2010) Bremm & Komander (2011) Wickliffe <i>et al.</i> (2011)
K6	DNA repair DNA damage response AR transcription	Wu-Baer (2003) Nishikawa (2003) Morris & Solomon (2004) Sobhian <i>et al.</i> (2007) Wu <i>et al.</i> (2007) Xu <i>et al.</i> (2009b)
* K27	Mitochondrial maintenance Mitophagy T-cell (Treg) development Assembled by parkin AR transcription	Xu <i>et al.</i> (2009b) Geisler <i>et al.</i> (2010) Durcan <i>et al.</i> (2011) Glauser <i>et al.</i> (2011) Peng <i>et al.</i> (2011)
* K29	Lysosomal degradation Ubiquitin-fusion degradation (UFD)	Chastagner <i>et al.</i> (2006) Al-Hakim <i>et al.</i> (2008) Licchesi <i>et al.</i> (2011) Hwang <i>et al.</i> (2010)
* K33	Reduces T-cell activation	Huang <i>et al.</i> (2010) Licchesi <i>et al.</i> (2011)

Figure 2

A schematic of the cellular processes in which different polyubiquitin chain species have been implicated based on linkage type. The different types of ubiquitylated species are represented as cartoons in the left-hand column. Polyubiquitin chains can be organized into a 'closed' or 'open' conformation based solely on the type of linkage that connects them. A (*) symbolizes that structural data is currently unavailable for these

linkages; however, modeling of these structures predicts the conformation of each chain type. Cellular roles are determined based on the identification of each chain type in a specific cellular process. Currently the function of many of these chains is still unknown. This list is not meant to be comprehensive but rather to highlight the many diverse roles of ubiquitin.

I κ B is analogous to the polyubiquitylation and degradation of NRs by the proteasome. Interestingly, the mechanism of activation of I κ B kinase (IKK) complex that phosphorylates I κ B involves non-degradative polyubiquitin chains, such as K63 and M1 chains, assembled

by combinations of multiple E2s and E3s. In a simplified model, these polyubiquitin chains are recognized by UBD proteins TAK1 binding proteins 2 and 3 and NF- κ B essential modulator to induce TAK1-dependent IKK activation (Yamaoka *et al.* 1998; Fig. 3, middle panel).

Figure 3

A hypothetical model of non-degradative ubiquitin in NR signaling. Non-degradative polyubiquitin chains can serve as protein assembly scaffolds. Examples shown in the middle and right panels are assemblages described in NF- κ B signaling and DNA damage repair. Based on these models, we speculate the potential for non-degradative ubiquitin chains providing similar scaffolding in NR signaling, bringing together tertiary complexes with enzymatic activity to post-translationally modified receptor or other coregulator proteins to affect transcription. Such scaffolds could also bring other ubiquitin-binding domain (UBD) proteins into the NR complex without directly binding to NR. TAK1, TGF- β -activated kinase; TAB2/3, TGF- β activated kinase 1/MAP3K7 binding proteins 2 & 3; IKK, I- κ B kinase complex; NEMO, NF- κ B essential modulator; BRCA1, Breast cancer type 1 susceptibility protein; BARD1, BRCA1 associated RING domain 1; Rap80, Receptor-associated protein 80. Stars represent post-translational modifications such as phosphorylation, denoted as 'P'.

Thus, NF- κ B signaling highlights how non-degradative ubiquitin chains can serve as a novel scaffold to assemble multi-protein complexes. Similarly, non-degradative ubiquitin chains are used to assist in the assembly of protein complexes involved in DNA double-strand break repair (Brown & Jackson 2015; Fig. 3, right panel). It is conceivable that non-degradative ubiquitin chains could also be used to assist in assembly of NR transcriptional complexes (Fig. 3, left panel).

A new frontier in ubiquitin regulation of NR

The RNF6 study in AR mentioned above suggests that non-degradative polyubiquitin chains may interface with NR activation mechanisms at the level of co-activators (Xu *et al.* 2009b). Indeed, an inspection of NR co-activators can identify several of them to contain RING finger domains (Table 2). Whether the RING domains in these coactivators contribute to their activities in NR signaling awaits further investigation. However, intriguingly, TIF1 assembles ternary coactivator complexes as part of AR transcriptional activation (Teyssier *et al.* 2006), and MAT1 is part of the assembly of TFIID and Cdk-activating kinase complex involved in receptor phosphorylation and transcriptional synergy (Rochette-Egly *et al.* 1997, Bastien *et al.* 2000, Chen *et al.* 2000, Chymkowitch *et al.* 2011). It is tempting to speculate that the RING domains of these factors could produce ubiquitin-based assembly scaffolds similar to what is observed in NF- κ B signaling. The limited studies of different ubiquitin linkages in biological contexts, including the NR field, could be due in part to the difficulty in detecting and quantifying endogenous proteins modified by specific ubiquitin linkages. Linkage-specific antibodies are commercially available for the detection of K48, K63 or M1 linkages. These antibodies can be used in immunoprecipitation-western analyses (Haglund & Dikic 2005, Emmerich *et al.* 2013, Jackson & Durocher 2013). This approach has been used to investigate the types of ubiquitin chains formed on ER α in response to E₂ (La Rosa *et al.* 2011b). Alternatively, overexpression and knockdown or knockout of specific E2s and E3s (e.g., Ubc13 for K63 chains or LUBAC subunits for M1 chains; Kirisako *et al.* 2006, Tokunaga *et al.* 2009, 2011, Ikeda *et al.* 2011), or overexpression of ubiquitin

Table 2 NR coregulators that contain RING finger domains

Coregulator	Ring finger designation	Receptor	References
ARNIP	RNF199	AR	Beitel <i>et al.</i> (2002)
BRCA1	RNF53	ER α PR	Fan <i>et al.</i> (1999), Zheng <i>et al.</i> (2001), Kawai <i>et al.</i> (2002) and Calvo & Beato (2011)
EFP	RNF147	ER α	Inoue <i>et al.</i> (1993) and Nakajima <i>et al.</i> (2007)
MAT1	RNF66	ER α , PPAR γ	Talukder <i>et al.</i> (2003) and Helenius <i>et al.</i> (2009)
RNF8	RNF8	RXR	Takano <i>et al.</i> (2004)
RLIM	RNF12	ER α	Johnsen <i>et al.</i> (2009)
TIF1	RNF82 and RNF96	RXR, RAR ER α , VDR, AR and TR	Vom Baur <i>et al.</i> (1996), Thénot <i>et al.</i> (1997) and Teyssier <i>et al.</i> (2006)
SNURF	RNF4	AR, GR PR and ER α	Moilanen <i>et al.</i> (1998), Poukka <i>et al.</i> (2000) and Saville <i>et al.</i> (2002)

RNF, ring finger protein; ARNIP, androgen receptor N-terminal interacting protein; BRCA1, breast cancer type 1 susceptibility protein; EFP, estrogen-responsive finger protein; MAT1, menage-a-trois homologue 1; RLIM, ring finger protein, LIM domain interacting; TIF1, transcriptional intermediary factor; SNURF, SNRPN upstream reading frame.

mutants (K48R, K63R, etc.), as well as replacement of endogenous ubiquitin with ubiquitin mutants (Xu *et al.* 2009c), has been applied to interrogate the role for specific ubiquitin chains. Finally, mass spectrometry techniques have also been used to map the ubiquitylation sites that are aided by the development of anti-di-Gly antibodies to enrich ubiquitylated peptide species following trypsin digestion (Kirkpatrick *et al.* 2005, Kim *et al.* 2011). Each of these approaches has limitations in cell systems (e.g., over-expression/knockdown can have effects on multiple substrates, background of endogenous ubiquitin in mM concentrations, multiple genes encoding ubiquitin, and effects on cell viability). Despite these challenges, in combination these techniques have been instrumental in providing insight into the roles of alternative forms of ubiquitin linkages in cell signaling and regulation.

The role of non-degradative ubiquitin and the ubiquitin code in regulation of NR function is in its infancy and despite some of the current technical challenges, understanding how this protein modification regulates NR function may open new avenues of research and therapeutic design. There are many critical reagents being generated (e.g., antibodies that specifically detect different ubiquitin linkages (Newton *et al.* 2008, Matsumoto *et al.* 2012)), new techniques being developed (e.g., advanced, sensitive and quantitative MS analyses (Peng *et al.* 2003, Xu & Peng 2006, Phu *et al.* 2011)) and specific ubiquitin E2s, E3s and deubiquitinases that act on specific ubiquitin linkages are being identified (Komander *et al.* 2009, Ye & Rape 2009, Kar *et al.* 2012). These advances may accelerate the elucidation of the roles for non-degradative poly-ubiquitylation in regulation of the NR family of proteins. While capitalizing on receptor ubiquitylation has yet to be tapped for clinical application, there is much to be gained by better understanding of the expanding role of ubiquitin in NR signaling. Just as the increased complexity of receptor genomic and non-genomic activities is providing new avenues of rationale design of therapeutics for NR-associated disease, the growing roles of ubiquitin in receptor protein control and transactivation provide an alternative to existing ligand-based therapies. The marriage of NR and ubiquitin fields presents an opportunity for both fields to explore fundamental biology of these important systems with high translational potential.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work was supported by NIH grants CA159578 (to E T A), F31 CA186551 (to C H), and CA77474 and GM083681 (to S M).

Author contribution statement

K T H, C H, S M and E T A all contributed to writing and editing the manuscript.

Acknowledgements

We would like to acknowledge Dr Eric Streiter for sharing expertise in ubiquitin linkages. We would also like to acknowledge the support of McArdle Laboratories for Cancer Research and the University of Wisconsin Carbone Cancer Center.

References

- Acconcia F, Ascenzi P, Bocedi A, Spisni E, Tomasi V, Trentalance A, Visca P & Marino M 2005 Palmitoylation-dependent estrogen receptor α membrane localization: regulation by 17 β -estradiol. *Molecular Biology of the Cell* **16** 231–237. (doi:10.1091/mbc.E04-07-0547)
- Alarid ET, Bakopoulos N & Solodin N 1999 Proteasome-mediated proteolysis of estrogen receptor: a novel component in autologous down-regulation. *Molecular Endocrinology* **13** 1522–1534. (doi:10.1210/mend.13.9.0337)
- Alarid ET, Preisler-Maskek MT & Solodin NM 2006 Lives and times of nuclear receptors. *Molecular Endocrinology* **20** 1972–1981. (doi:10.1210/me.2005-0481)
- Al-Hakim AK, Zagorska A, Chapman L, Deak M, Pegg M & Alessi DR 2008 Control of AMPK-related kinases by USP9X and atypical Lys(29)/Lys(33)-linked polyubiquitin chains. *Biochemical Journal* **411** 249–260. (doi:10.1042/BJ20080067)
- Al-Hakim AK, Escribano-Diaz C, Landry M-C, O'Donnell L, Panier S, Szilard RK & Durocher D 2010 The ubiquitous role of ubiquitin in the DNA damage response. *DNA Repair* **9** 1229–1240. (doi:10.1016/j.dnarep.2010.09.011)
- Ali S, Metzger D, Bornert JM & Chambon P 1993 Modulation of transcriptional activation by ligand-dependent phosphorylation of the human oestrogen receptor A/B region. *EMBO Journal* **12** 1153–1160.
- Andersen RJ, Mawji NR, Wang J, Wang G, Haile S, Myung J-K, Watt K, Tam T, Yang YC, Bañuelos CA *et al.* 2010 Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. *Cancer Cell* **17** 535–546. (doi:10.1016/j.ccr.2010.04.027)
- Aranda A & Pascual A 2001 Nuclear hormone receptors and gene expression. *Physiological Reviews* **81** 1269–1304.
- Arnold SF, Vorojeikina DP & Notides AC 1995 Phosphorylation of tyrosine 537 on the human estrogen receptor is required for binding to an estrogen response element. *Journal of Biological Chemistry* **270** 30205–30212. (doi:10.1074/jbc.270.4.1850)
- Bagatell R, Khan O, Paine-Murrieta G, Taylor CW, Akinaga S & Whitesell L 2001 Destabilization of steroid receptors by heat shock protein 90-binding drugs: a ligand-independent approach to hormonal therapy of breast cancer. *Clinical Cancer Research* **7** 2076–2084.
- Ballinger CA, Connell P, Wu Y, Hu Z, Thompson LJ, Yin LY & Patterson C 1999 Identification of CHIP, a novel tetratricopeptide repeat-containing protein that interacts with heat shock proteins and negatively regulates chaperone functions. *Molecular and Cellular Biology* **19** 4535–4545.
- Bastien J, Adam-Stitah S, Riedl T, Egly JM, Chambon P & Rochette-Egly C 2000 TRIM28 interacts with the retinoic acid receptor γ and

- phosphorylates its AF-1-activating domain through cdk7. *Journal of Biological Chemistry* **275** 21896–21904. (doi:10.1074/jbc.M001985200)
- Beitel LK, Elhaji YA, Lumbroso R, Wing SS, Panet-Raymond V, Gottlieb B, Pinsky L & Trifiro MA 2002 Cloning and characterization of an androgen receptor N-terminal-interacting protein with ubiquitin-protein ligase activity. *Journal of Molecular Endocrinology* **29** 41–60. (doi:10.1677/jme.0.0290041)
- Bernassola F, Karin M, Ciechanover A & Melino G 2008 The HECT family of E3 ubiquitin ligases: multiple players in cancer development. *Cancer Cell* **14** 10–21. (doi:10.1016/j.ccr.2008.06.001)
- Berndsen CE & Wolberger C 2014 New insights into ubiquitin E3 ligase mechanism. *Nature Structural & Molecular Biology* **21** 301–307. (doi:10.1038/nsmb.2780)
- Bhatt S, Xiao Z, Meng Z & Katzenellenbogen BS 2012 Phosphorylation by p38 mitogen-activated protein kinase promotes estrogen receptor α turnover and functional activity via the SCFSkp2 proteasomal complex. *Molecular and Cellular Biology* **32** 1928–1943. (doi:10.1128/MCB.06561-11)
- Bloom J & Pagano M 2003 Deregulated degradation of the cdk inhibitor p27 and malignant transformation. *Seminars in Cancer Biology* **13** 41–47. (doi:10.1016/S1044-579X(02)00098-6)
- Bremm A & Komander D 2011 Emerging roles for Lys11-linked polyubiquitin in cellular regulation. *Trends in Biochemical Sciences* **36** 355–363. (doi:10.1016/j.tibs.2011.04.004)
- Bresnick EH, Dalman FC, Sanchez ER & Pratt WB 1989 Evidence that the 90-kDa heat shock protein is necessary for the steroid binding conformation of the L cell glucocorticoid receptor. *Journal of Biological Chemistry* **264** 4992–4997.
- Brown JS & Jackson SP 2015 Ubiquitylation, neddylation and the DNA damage response. *Open Biology* **5** 150018. (doi:10.1098/rsob.150018)
- Bunone G, Briand PA, Mikscek RJ & Picard D 1996 Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO Journal* **15** 2174–2183.
- Byun B & Jung Y 2008 Repression of transcriptional activity of estrogen receptor α by a Cullin3/SPOP ubiquitin E3 ligase complex. *Molecules and Cells* **25** 289–293.
- Caboni L & Lloyd DG 2013 Beyond the ligand-binding pocket: targeting alternate sites in nuclear receptors. *Medicinal Research Reviews* **33** 1081–1118. (doi:10.1002/med.21275)
- Calligé M, Kieffer I & Richard-Foy H 2005 CSN5/Jab1 is involved in ligand-dependent degradation of estrogen receptor α by the proteasome. *Molecular and Cellular Biology* **25** 4349–4358. (doi:10.1128/MCB.25.11.4349-4358.2005)
- Calvo V & Beato M 2011 BRCA1 counteracts progesterone action by ubiquitination leading to progesterone receptor degradation and epigenetic silencing of target promoters. *Cancer Research* **71** 3422–3431. (doi:10.1158/0008-5472.CAN-10-3670)
- Cardozo CP, Michaud C, Ost MC, Fliss AE, Yang E, Patterson C, Hall SJ & Caplan AJ 2003 C-terminal Hsp-interacting protein slows androgen receptor synthesis and reduces its rate of degradation. *Archives of Biochemistry and Biophysics* **410** 134–140. (doi:10.1016/S0003-9861(02)00680-X)
- Chamberlain PP, Lopez-Girona A, Miller K, Carmel G, Pagarigan B, Chie-Leon B, Rychak E, Corral LG, Ren YJ, Wang M *et al.* 2014 Structure of the human cereblon–DDB1–lenalidomide complex reveals basis for responsiveness to thalidomide analogs. *Nature Structural & Molecular Biology* **21** 803–809. (doi:10.1038/nsmb.2874)
- Chastagner P, Israël A & Brou C 2006 Itch/AIP4 mediates Deltex degradation through the formation of K29-linked polyubiquitin chains. *EMBO Reports* **7** 1147–1153. (doi:10.1038/sj.embo.7400822)
- Chau V, Tobias JW, Bachmair A, Marriott D, Ecker DJ, Gonda DK & Varshavsky A 1989 A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science* **243** 1576–1583. (doi:10.1126/science.2538923)
- Chen J & Chen ZJ 2013 Regulation of NF- κ B by ubiquitination. *Current Opinion in Immunology* **25** 4–12. (doi:10.1016/j.coi.2012.12.005)
- Chen D, Pace PE, Coombes RC & Ali S 1999 Phosphorylation of human estrogen receptor α by protein kinase A regulates dimerization. *Molecular and Cellular Biology* **19** 1002–1015.
- Chen D, Riedl T, Washbrook E, Pace PE, Coombes RC, Egly JM & Ali S 2000 Activation of estrogen receptor α by S118 phosphorylation involves a ligand-dependent interaction with TFIID and participation of CDK7. *Molecular Cell* **6** 127–137. (doi:10.1016/S1097-2765(05)00004-3)
- Chymkowitch P, May NL, Charneau P, Compe E & Egly J-M 2011 The phosphorylation of the androgen receptor by TFIID directs the ubiquitin/proteasome process. *EMBO Journal* **30** 468–479. (doi:10.1038/emboj.2010.337)
- Cirit M, Grant KG & Haugh JM 2012 Systemic perturbation of the ERK signaling pathway by the proteasome inhibitor, MG132. *PLoS ONE* **7** e50975. (doi:10.1371/journal.pone.0050975)
- Clark DE, Poteet-Smith CE, Smith JA & Lannigan DA 2001 Rsk2 allosterically activates estrogen receptor α by docking to the hormone-binding domain. *EMBO Journal* **20** 3484–3494. (doi:10.1093/emboj/20.13.3484)
- Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Höhfeld J & Patterson C 2001 The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins. *Nature Cell Biology* **3** 93–96. (doi:10.1038/35070170)
- Dace A, Zhao L, Park KS, Furuno T, Takamura N, Nakanishi M, West BL, Hanover JA & Cheng S 2000 Hormone binding induces rapid proteasome-mediated degradation of thyroid hormone receptors. *PNAS* **97** 8985–8990. (doi:10.1073/pnas.160257997)
- Davidovich S, Ben-Izhak O, Shapira M, Futerman B & Hershko DD 2008 Over-expression of Skp2 is associated with resistance to preoperative doxorubicin-based chemotherapy in primary breast cancer. *Breast Cancer Research* **10** R63. (doi:10.1186/bcr2122)
- Deng C-X 2006 BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. *Nucleic Acids Research* **34** 1416–1426. (doi:10.1093/nar/gkl010)
- Deshaies RJ & Joazeiro CAP 2009 RING domain E3 ubiquitin ligases. *Annual Review of Biochemistry* **78** 399–434. (doi:10.1146/annurev.biochem.78.101807.093809)
- Di Croce L, Okret S, Kersten S, Gustafsson JA, Parker M, Wahli W & Beato M 1999 Steroid and nuclear receptors. *EMBO Journal* **18** 6201–6210. (doi:10.1093/emboj/18.22.6201)
- Dikic I, Wakatsuki S & Walters KJ 2009 Ubiquitin-binding domains – from structures to functions. *Nature Reviews. Molecular Cell Biology* **10** 659–671. (doi:10.1038/nrm2767)
- Dizin E & Irminger-Finger I 2010 Negative feedback loop of BRCA1–BARD1 ubiquitin ligase on estrogen receptor α stability and activity antagonized by cancer-associated isoform of BARD1. *International Journal of Biochemistry & Cell Biology* **42** 693–700. (doi:10.1016/j.biocel.2009.12.025)
- Duong V, Boule N, Daujat S, Chauvet J, Bonnet S, Neel H & Cavaillès V 2007 Differential regulation of estrogen receptor α turnover and transactivation by Mdm2 and stress-inducing agents. *Cancer Research* **67** 5513–5521. (doi:10.1158/0008-5472.CAN-07-0967)
- Durcan TM, Kontogiannina M, Thorarinsdottir T, Fallon L, Williams AJ, Djarmati A, Fantaneanu T, Paulson HL & Fon EA 2011 The Machado-Joseph disease-associated mutant form of ataxin-3 regulates parkin ubiquitination and stability. *Human Molecular Genetics* **20** 141–154. (doi:10.1093/hmg/ddq452)
- Dynek JN, Goncharov T, Dueber EC, Fedorova AV, Izrael-Tomasevic A, Phu L, Helgason E, Fairbrother WJ, Deshayes K, Kirkpatrick DS *et al.* 2010 c-IAP1 and UbcH5 promote K11-linked polyubiquitination of RIP1 in TNF signalling. *EMBO Journal* **29** 4198–4209. (doi:10.1038/emboj.2010.300)
- Eakin CM, MacCoss MJ, Finney GL & Klevit RE 2007 Estrogen receptor α is a putative substrate for the BRCA1 ubiquitin ligase. *PNAS* **104** 5794–5799. (doi:10.1073/pnas.0610887104)
- Elbi C, Walker DA, Romero G, Sullivan WP, Toft DO, Hager GL & DeFranco DB 2004 Molecular chaperones function as steroid receptor

- nuclear mobility factors. *PNAS* **101** 2876–2881. (doi:10.1073/pnas.0400116101)
- El Khissi A & Leclercq G 1999 Implication of proteasome in estrogen receptor degradation. *FEBS Letters* **448** 160–166. (doi:10.1016/S0014-5793(99)00343-9)
- Emanuele S, Calvaruso G, Lauricella M, Giuliano M, Bellavia G, D'Anneo A, Vento R & Tesoriere G 2002 Apoptosis induced in hepatoblastoma HepG2 cells by the proteasome inhibitor MG132 is associated with hydrogen peroxide production, expression of Bcl-XS and activation of caspase-3. *International Journal of Oncology* **21** 857–865. (doi:10.3892/ijo.21.4.857)
- Emmerich CH, Ordureau A, Strickson S, Arthur JS, Pedrioli PG, Komander D & Cohen P 2013 Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *PNAS* **110** 15247–15252. (doi:10.1073/pnas.1314715110)
- Evans RM & Mangelsdorf DJ 2014 Nuclear receptors, RXR, and the big bang. *Cell* **157** 255–266. (doi:10.1016/j.cell.2014.03.012)
- Fan S, Wang J-A, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auburn KJ, Goldberg ID *et al.* 1999 BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science* **284** 1354–1356. (doi:10.1126/science.284.5418.1354)
- Fan M, Park A & Nephew KP 2005 CHIP (carboxyl terminus of Hsc70-interacting protein) promotes basal and geldanamycin-induced degradation of estrogen receptor- α . *Molecular Endocrinology* **19** 2901–2914. (doi:10.1210/me.2005-0111)
- Faresse N, Ruffieux-Daidie D, Salamin M, Gomez-Sanchez CE & Staub O 2010 Mineralocorticoid receptor degradation is promoted by Hsp90 inhibition and the ubiquitin-protein ligase CHIP. *American Journal of Physiology. Renal Physiology* **299** F1462–F1472. (doi:10.1152/ajprenal.00285.2010)
- Fischer ES, Böhm K, Lydeard JR, Yang H, Stadler MB, Cavadini S, Nagel J, Serluca F, Acker V, Lingaraju GM *et al.* 2014 Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide. *Nature* **512** 49–53. (doi:10.1038/nature13527)
- Freudenthal BD, Gakhar L, Ramaswamy S & Washington MT 2010 Structure of monoubiquitinated PCNA and implications for translesion synthesis and DNA polymerase exchange. *Nature Structural & Molecular Biology* **17** 479–484. (doi:10.1038/nsmb.1776)
- Gao X, Mohsin SK, Gatalica Z, Fu G, Sharma P & Nawaz Z 2005 Decreased expression of E6-associated protein in breast and prostate carcinomas. *Endocrinology* **146** 1707–1712. (doi:10.1210/en.2004-1198)
- Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ & Springer W 2010 PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nature Cell Biology* **12** 119–131. (doi:10.1038/ncb2012)
- Glauser L, Sonnay S, Stafa K & Moore DJ 2011 Parkin promotes the ubiquitination and degradation of the mitochondrial fusion factor mitofusin 1. *Journal of Neurochemistry* **118** 636–645. (doi:10.1111/j.1471-4159.2011.07318.x)
- Goto E, Yamanaka Y, Ishikawa A, Aoki-Kawasumi M, Mito-Yoshida M, Ohmura-Hoshino M, Matsuki Y, Kajikawa M, Hirano H & Ishido S 2010 Contribution of lysine 11-linked ubiquitination to MIR2-mediated major histocompatibility complex class I internalization. *Journal of Biological Chemistry* **285** 35311–35319. (doi:10.1074/jbc.M110.112763)
- Gronemeyer H, Gustafsson J-Å & Laudet V 2004 Principles for modulation of the nuclear receptor superfamily. *Nature Reviews. Drug Discovery* **3** 950–964. (doi:10.1038/nrd1551)
- Gunther JR, Parent AA & Katzenellenbogen JA 2009 Alternative inhibition of androgen receptor signaling: peptidomimetic pyrimidines as direct androgen receptor/coactivator disruptors. *ACS Chemical Biology* **4** 435–440. (doi:10.1021/cb900043e)
- Gustafsson N, Zhao C, Gustafsson J-Å & Dahlman-Wright K 2010 RBCK1 drives breast cancer cell proliferation by promoting transcription of estrogen receptor α and cyclin B1. *Cancer Research* **70** 1265–1274. (doi:10.1158/0008-5472.CAN-09-2674)
- Haglund K & Dikic I 2005 Ubiquitylation and cell signaling. *EMBO Journal* **24** 3353–3359. (doi:10.1038/sj.emboj.7600808)
- Hammes SR & Levin ER 2011 Minireview: Recent advances in extranuclear steroid receptor actions. *Endocrinology* **152** 4489–4495. (doi:10.1210/en.2011-1470)
- He Y, Xu Y, Zhang X, Gao X, Dykema KJ, Martin KR, Ke J, Hudson EA, Khoo SK, Resau JH *et al.* 2011 Identification of a lysosomal pathway that modulates glucocorticoid signaling and the inflammatory response. *Science Signaling* **4** ra44. (doi:10.1126/scisignal.4159ec44)
- Held JM, Britton DJ, Scott GK, Lee EL, Schilling B, Baldwin MA, Gibson BW & Benz CC 2012 Ligand binding promotes CDK-dependent phosphorylation of ER- α on hinge serine 294 but inhibits ligand-independent phosphorylation of serine 305. *Molecular Cancer Research* **10** 1120–1132. (doi:10.1158/1541-7786.MCR-12-0099)
- Helenius K, Yang Y, Alasaari J & Mäkelä TP 2009 Mat1 inhibits peroxisome proliferator-activated receptor γ -mediated adipocyte differentiation. *Molecular and Cellular Biology* **29** 315–323. (doi:10.1128/MCB.00347-08)
- Henrich LM, Smith JA, Kitt D, Errington TM, Nguyen B, Traish AM & Lannigan DA 2003 Extracellular signal-regulated kinase 7, a regulator of hormone-dependent estrogen receptor destruction. *Molecular and Cellular Biology* **23** 5979–5988. (doi:10.1128/MCB.23.17.5979-5988.2003)
- Hershko A & Ciechanover A 1998 The ubiquitin system. *Annual Review of Biochemistry* **67** 425–479. (doi:10.1146/annurev.biochem.67.1.425)
- Hilmi K, Hussein N, Mendoza-Sanchez R, El-Ezzy M, Ismail H, Durette C, Bail M, Rozendaal MJ, Bouvier M, Thibault P *et al.* 2012 Role of SUMOylation in full antiestrogenicity. *Molecular and Cellular Biology* **32** 3823–3837. (doi:10.1128/MCB.00290-12)
- Hoeghe C, Pfander B, Moldovan G-L, Pyrowolakis G & Jentsch S 2002 RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* **419** 135–141. (doi:10.1038/nature00991)
- Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG & Evans RM 1985 Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* **318** 635–641. (doi:10.1038/318635a0)
- Huang K-T, Pavlides SC, Lecanda J, Blank SV, Mittal KR & Gold LI 2012 Estrogen and progesterone regulate p27kip1 levels via the ubiquitin–proteasome system: pathogenic and therapeutic implications for endometrial cancer. *PLoS ONE* **7** e46072. (doi:10.1371/journal.pone.0046072)
- Husnjak K & Dikic I 2012 Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. *Annual Review of Biochemistry* **81** 291–322. (doi:10.1146/annurev-biochem-051810-094654)
- Hwang C-S, Shemorry A, Auerbach D & Varshavsky A 2010 The N-end rule pathway is mediated by a complex of the RING-type Ubr1 and HECT-type Ufd4 ubiquitin ligases. *Nature Cell Biology* **12** 1177–1185. (doi:10.1038/ncb2121)
- Ikeda F, Deribe YL, Skånland SS, Stieglitz B, Grabbe C, Franz-Wachtel M, van Wijk SJ, Goswami P, Nagy V, Terzić J *et al.* 2011 SHARPIN forms a linear ubiquitin ligase complex regulating NF- κ B activity and apoptosis. *Nature* **471** 637–641. (doi:10.1038/nature09814)
- Imhof MO & McDonnell DP 1996 Yeast RSP5 and its human homolog hRPF1 potentiate hormone-dependent activation of transcription by human progesterone and glucocorticoid receptors. *Molecular and Cellular Biology* **16** 2594–2605.
- Inoue S, Orimo A, Hosoi T, Kondo S, Toyoshima H, Kondo T, Ikegami A, Ouchi Y, Orimo H & Muramatsu M 1993 Genomic binding-site cloning reveals an estrogen-responsive gene that encodes a RING finger protein. *PNAS* **90** 11117–11121. (doi:10.1073/pnas.90.23.11117)
- Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y, Yamaguchi Y & Handa H 2010 Identification of a primary target of thalidomide teratogenicity. *Science* **327** 1345–1350. (doi:10.1126/science.1177319)
- Jackson SP & Durocher D 2013 Regulation of DNA damage responses by ubiquitin and SUMO. *Molecular Cell* **49** 795–807. (doi:10.1016/j.molcel.2013.01.017)

- Jensen EV, Desombre ER, Kawashima T, Suzuki T, Kyser K & Jungblut PW 1967 Estrogen-binding substances of target tissues. *Science* **158** 529–530. (doi:10.1126/science.158.3800.529-c)
- Jin L, Williamson A, Banerjee S, Philipp I & Rape M 2008 Mechanism of ubiquitin-chain formation by the human anaphase-promoting complex. *Cell* **133** 653–665. (doi:10.1016/j.cell.2008.04.012)
- Jing X, Infante J, Nachtman RG & Jurecic R 2008 E3 ligase FLRF (Rnf41) regulates differentiation of hematopoietic progenitors by governing steady-state levels of cytokine and retinoic acid receptors. *Experimental Hematology* **36** 1110–1120. (doi:10.1016/j.exphem.2008.04.001)
- Johnsen SA, Güngör C, Prenzel T, Riethdorf S, Riethdorf L, Taniguchi-Ishigaki N, Rau T, Tursun B, Furlow JD, Sauter G *et al.* 2009 Regulation of estrogen-dependent transcription by the LIM cofactors CLIM and RLIM in breast cancer. *Cancer Research* **69** 128–136. (doi:10.1158/0008-5472.CAN-08-1630)
- Jung SY, Malovannaya A, Wei J, O'Malley BW & Qin J 2005 Proteomic analysis of steady-state nuclear hormone receptor coactivator complexes. *Molecular Endocrinology* **19** 2451–2465. (doi:10.1210/me.2004-0476)
- Jung Y-S, Lee S-J, Yoon M-H, Ha NC & Park B-J 2012 Estrogen receptor α is a novel target of the Von Hippel-Lindau protein and is responsible for the proliferation of VHL-deficient cells under hypoxic conditions. *Cell Cycle* **11** 4462–4473. (doi:10.4161/cc.22794)
- Kamata Y, Watanabe J, Nishimura Y, Arai T, Kawaguchi M, Hattori M, Obokata A & Kuramoto H 2005 High expression of skp2 correlates with poor prognosis in endometrial endometrioid adenocarcinoma. *Journal of Cancer Research and Clinical Oncology* **131** 591–596. (doi:10.1007/s00432-005-0671-2)
- Kar G, Keskin O, Nussinov R & Gursoy A 2012 Human proteome-scale structural modeling of E2–E3 interactions exploiting interface motifs. *Journal of Proteome Research* **11** 1196–1207. (doi:10.1021/pr2009143)
- Karp SE, Tonin PN, Bégin LR, Martinez JJ, Zhang JC, Pollak MN & Foulkes WD 1997 Influence of BRCA1 mutations on nuclear grade and estrogen receptor status of breast carcinoma in Ashkenazi Jewish women. *Cancer* **80** 435–441. (doi:10.1002/(SICI)1097-0142(19970801)80:3<435::AID-CNCR11>3.0.CO;2-Y)
- Kawai H, Li H, Chun P, Avraham S & Avraham HK 2002 Direct interaction between BRCA1 and the estrogen receptor regulates vascular endothelial growth factor (VEGF) transcription and secretion in breast cancer cells. *Oncogene* **21** 7730–7739. (doi:10.1038/sj.onc.1205971)
- Kilroy G, Kirk-Ballard H, Carter LE & Floyd ZE 2012 The ubiquitin ligase Siah2 regulates PPAR γ activity in adipocytes. *Endocrinology* **153** 1206–1218. (doi:10.1210/en.2011-1725)
- Kim MY, Woo EM, Chong YTE, Homenko DR & Kraus WL 2006 Acetylation of estrogen receptor α by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activities of the receptor. *Molecular Endocrinology* **20** 1479–1493. (doi:10.1210/me.2005-0531)
- Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ *et al.* 2011 Systematic and quantitative assessment of the ubiquitin-modified proteome. *Molecular Cell* **44** 325–340. (doi:10.1016/j.molcel.2011.08.025)
- Kim J-H, Park KW, Lee E-W, Jang W-S, Seo J, Shin S, Hwang K-A & Song J 2014 Suppression of PPAR γ through MKRN1-mediated ubiquitination and degradation prevents adipocyte differentiation. *Cell Death and Differentiation* **21** 594–603. (doi:10.1038/cdd.2013.181)
- Kirisako T, Kamei K, Murata S, Kato M, Fukumoto H, Kanie M, Sano S, Tokunaga F, Tanaka K & Iwai K 2006 A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO Journal* **25** 4877–4887. (doi:10.1038/sj.emboj.7601360)
- Kirkpatrick DS, Denison C & Gygi SP 2005 Weighing in on ubiquitin: the expanding role of mass-spectrometry-based proteomics. *Nature Cell Biology* **7** 750–757. (doi:10.1038/ncb0805-750)
- Komander D 2009 The emerging complexity of protein ubiquitination. *Biochemical Society Transactions* **37** 937. (doi:10.1042/BST0370937)
- Komander D, Clague MJ & Urbé S 2009 Breaking the chains: structure and function of the deubiquitinases. *Nature Reviews. Molecular Cell Biology* **10** 550–563. (doi:10.1038/nrm2731)
- Kopf E, Plassat J-L, Vivat V, de The H, Chambon P & Rochette-Egly C 2000 Dimerization with retinoid X receptors and phosphorylation modulate the retinoic acid-induced degradation of retinoic acid receptors α and γ through the ubiquitin–proteasome pathway. *Journal of Biological Chemistry* **275** 33280–33288. (doi:10.1074/jbc.M002840200)
- Kulathu Y & Komander D 2012 Atypical ubiquitylation – the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nature Reviews. Molecular Cell Biology* **13** 508–523. (doi:10.1038/nrm3394)
- La Rosa P, Pesiri V, Marino M & Acconcia F 2011a 17 β -Estradiol-induced cell proliferation requires estrogen receptor (ER) α monoubiquitination. *Cellular Signalling* **23** 1128–1135. (doi:10.1016/j.cellsig.2011.02.006)
- La Rosa P, Marino M & Acconcia F 2011b 17 β -estradiol regulates estrogen receptor α monoubiquitination. *IUBMB Life* **63** 49–53. (doi:10.1002/iub.414)
- Lecanda J, Parekh TV, Gama P, Lin K, Liarski V, Uretsky S, Mittal K & Gold LI 2007 Transforming growth factor- β , estrogen, and progesterone converge on the regulation of p27Kip1 in the normal and malignant endometrium. *Cancer Research* **67** 1007–1018. (doi:10.1158/0008-5472.CAN-06-0235)
- Lee I & Schindelin H 2008 Structural insights into E1-catalyzed ubiquitin activation and transfer to conjugating enzymes. *Cell* **134** 268–278. (doi:10.1016/j.cell.2008.05.046)
- Lee MO, Kim EO, Kwon HJ, Kim YM, Kang HJ, Kang H & Lee JE 2002 Radicool represses the transcriptional function of the estrogen receptor by suppressing the stabilization of the receptor by heat shock protein 90. *Molecular and Cellular Endocrinology* **188** 47–54. (doi:10.1016/S0303-7207(01)00753-5)
- Le Goff P, Montano MM, Schodin DJ & Katzenellenbogen BS 1994 Phosphorylation of the human estrogen receptor. Identification of hormone-regulated sites and examination of their influence on transcriptional activity. *Journal of Biological Chemistry* **269** 4458–4466.
- Le Romancer M, Treilleux I, Leconte N, Robin-Lespinasse Y, Sentes S, Boucheikioua-Bouzaghrou K, Goddard S, Gobert-Gosse S & Corbo L 2008 Regulation of estrogen rapid signaling through arginine methylation by PRMT1. *Molecular Cell* **31** 212–221. (doi:10.1016/j.molcel.2008.05.025)
- Le Romancer M, Poulard C, Cohen P, Sentes S, Renoir J-M & Corbo L 2011 Cracking the estrogen receptor's posttranslational code in breast tumors. *Endocrine Reviews* **32** 597–622. (doi:10.1210/er.2010-0016)
- Li L, Li Z, Howley PM & Sacks DB 2006 E6AP and calmodulin reciprocally regulate estrogen receptor stability. *Journal of Biological Chemistry* **281** 1978–1985. (doi:10.1074/jbc.M508545200)
- Li H, Xu LL, Masuda K, Raymundo E, McLeod DG, Dobi A & Srivastava S 2008 A feedback loop between the androgen receptor and a NEDD4-binding protein, PMEPA1, in prostate cancer cells. *Journal of Biological Chemistry* **283** 28988–28995. (doi:10.1074/jbc.M710528200)
- Li B, Lu W, Yang Q, Yu X, Matusik RJ & Chen Z 2014 Skp2 regulates androgen receptor through ubiquitin-mediated degradation independent of Akt/mTOR pathways in prostate cancer. *Prostate* **74** 421–432. (doi:10.1002/pros.22763)
- Licchesi JD, Mieszczanek J, Mevissen TE, Rutherford TJ, Akutsu M, Virdee S, El Oualid F, Chin JW, Ovaa H, Bienz M *et al.* 2012 An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. *Nature Structural & Molecular Biology* **19** 62–71. (doi:10.1038/nsmb.2169)
- Likhite VS, Stossi F, Kim K, Katzenellenbogen BS & Katzenellenbogen JA 2006 Kinase-specific phosphorylation of the estrogen receptor changes receptor interactions with ligand, deoxyribonucleic acid, and coregulators associated with alterations in estrogen and tamoxifen activity. *Molecular Endocrinology* **20** 3120–3132. (doi:10.1210/me.2006-0068)
- Lin H-K, Wang L, Hu Y-C, Altuwaijri S & Chang C 2002 Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt

- require Mdm2 E3 ligase. *EMBO Journal* **21** 4037–4048. (doi:10.1093/emboj/cdf406)
- Loman N, Johannsson O, Bendahl PO, Borg A, Fernö M & Olsson H 1998 Steroid receptors in hereditary breast carcinomas associated with BRCA1 or BRCA2 mutations or unknown susceptibility genes. *Cancer* **83** 310–319. (doi:10.1002/(SICI)1097-0142(19980715)83:2<310::AID-CNCR15>3.0.CO;2-W)
- Lonard DM, Nawaz Z, Smith CL & O'Malley BW 2000 The 26S proteasome is required for estrogen receptor- α and coactivator turnover and for efficient estrogen receptor- α transactivation. *Molecular Cell* **5** 939–948. (doi:10.1016/S1097-2765(00)80259-2)
- Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, Wong K-K, Bradner JE & Kaelin WG 2014 The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science* **343** 305–309. (doi:10.1126/science.1244917)
- Ma Y, Fan S, Hu C, Meng Q, Fuqua SA, Pestell RG, Tomita YA & Rosen EM 2010 BRCA1 regulates acetylation and ubiquitination of estrogen receptor- α . *Molecular Endocrinology* **24** 76–90. (doi:10.1210/me.2009-0218)
- Malovannaya A, Lanz RB, Jung SY, Bulynko Y, Le NT, Chan DW, Ding C, Shi Y, Yucer N, Krenciute G *et al.* 2011 Analysis of the human endogenous coregulator complexome. *Cell* **145** 787–799. (doi:10.1016/j.cell.2011.05.006)
- Malyukova A, Brown S, Papa R, O'Brien R, Giles J, Trahair TN, Dalla Pozza L, Sutton R, Liu T, Haber M *et al.* 2013 FBXW7 regulates glucocorticoid response in T-cell acute lymphoblastic leukaemia by targeting the glucocorticoid receptor for degradation. *Leukemia* **27** 1053–1062. (doi:10.1038/leu.2012.361)
- Mao C, Patterson NM, Cherian MT, Aninye IO, Zhang C, Montoya JB, Cheng J, Putt KS, Hergenrother PJ, Wilson EM *et al.* 2008 A new small molecule inhibitor of estrogen receptor α binding to estrogen response elements blocks estrogen-dependent growth of cancer cells. *Journal of Biological Chemistry* **283** 12819–12830. (doi:10.1074/jbc.M709936200)
- Markson G, Kiel C, Hyde R, Brown S, Charalabous P, Bremm A, Semple J, Woodsmith J, Duley S, Salehi-Ashtiani K *et al.* 2009 Analysis of the human E2 ubiquitin conjugating enzyme protein interaction network. *Genome Research* **19** 1905–1911. (doi:10.1101/gr.093963.109)
- Martiniani R, Di Loreto V, Di Sano C, Lombardo A & Liberati AM 2012 Biological activity of lenalidomide and its underlying therapeutic effects in multiple myeloma. *Advances in Hematology* **2012** 842945. (doi:10.1155/2012/842945)
- Matsumoto ML, Dong KC, Yu C, Phu L, Gao X, Hannoush RN, Hymowitz SG, Kirkpatrick DS, Dixit VM & Kelley RF 2012 Engineering and structural characterization of a linear polyubiquitin-specific antibody. *Journal of Molecular Biology* **418** 134–144. (doi:10.1016/j.jmb.2011.12.053)
- McDonnell DP & Wardell SE 2010 The molecular mechanisms underlying the pharmacological actions of ER modulators: implications for new drug discovery in breast cancer. *Current Opinion in Pharmacology* **10** 620–628. (doi:10.1016/j.coph.2010.09.007)
- Métivier R, Penot G, Hübner MR, Reid G, Brand H, Kos M & Gannon F 2003 Estrogen receptor- α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. *Cell* **115** 751–763. (doi:10.1016/S0092-8674(03)00934-6)
- Michalides R, Griekspoor A, Balkenende A, Verwoerd D, Janssen L, Jalink K, Floore A, Velds A, van't Veer L & Neeffjes J 2004 Tamoxifen resistance by a conformational arrest of the estrogen receptor α after PKA activation in breast cancer. *Cancer Cell* **5** 597–605. (doi:10.1016/j.ccr.2004.05.016)
- Miesfeld R, Okret S, Wikström AC, Wrangé O, Gustafsson JA & Yamamoto KR 1984 Characterization of a steroid hormone receptor gene and mRNA in wild-type and mutant cells. *Nature* **312** 779–781. (doi:10.1038/312779a0)
- Mitsiades CS, Davies FE, Laubach JP, Joshua D, San Miguel J, Anderson KC & Richardson PG 2011 Future directions of next-generation novel therapies, combination approaches, and the development of personalized medicine in myeloma. *Journal of Clinical Oncology* **29** 1916–1923. (doi:10.1200/JCO.2010.34.0760)
- Moilanen AM, Poukka H, Karvonen U, Häkli M, Jänne OA & Palvimo JJ 1998 Identification of a novel RING finger protein as a coregulator in steroid receptor-mediated gene transcription. *Molecular and Cellular Biology* **18** 5128–5139.
- Morris JR & Solomon E 2004 BRCA1:BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Human Molecular Genetics* **13** 807–817. (doi:10.1093/hmg/ddh095)
- Mukhopadhyay D & Riezman H 2007 Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science* **315** 201–205. (doi:10.1126/science.1127085)
- Mund T, Lewis MJ, Maslen S & Pelham HR 2014 Peptide and small molecule inhibitors of HECT-type ubiquitin ligases. *PNAS* **111** 16736–16741. (doi:10.1073/pnas.1412152111)
- Nagaich AK, Walker DA, Wolford R & Hager GL 2004 Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling. *Molecular Cell* **14** 163–174. (doi:10.1016/S1097-2765(04)00178-9)
- Nakajima A, Maruyama S, Bohgaki M, Miyajima N, Tsukiyama T, Sakuragi N & Hatakeyama S 2007 Ligand-dependent transcription of estrogen receptor α is mediated by the ubiquitin ligase EFP. *Biochemical and Biophysical Research Communications* **357** 245–251. (doi:10.1016/j.bbrc.2007.03.134)
- Nawaz Z, Lonard DM, Dennis AP, Smith CL & O'Malley BW 1999a Proteasome-dependent degradation of the human estrogen receptor. *PNAS* **96** 1858–1862. (doi:10.1073/pnas.96.5.1858)
- Nawaz Z, Lonard DM, Smith CL, Lev-Lehman E, Tsai SY, Tsai M-J & O'Malley BW 1999b The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. *Molecular and Cellular Biology* **19** 1182–1189.
- Newton K, Matsumoto ML, Wertz IE, Kirkpatrick DS, Lill JR, Tan J, Dugger D, Gordon N, Sidhu SS, Fellous FA *et al.* 2008 Ubiquitin chain editing revealed by polyubiquitin linkage-specific antibodies. *Cell* **134** 668–678. (doi:10.1016/j.cell.2008.07.039)
- Nishikawa H, Ooka S, Sato K, Arima K, Okamoto J, Klevit RE, Fukuda M & Ohta T 2004 Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1–BARD1 ubiquitin ligase. *Journal of Biological Chemistry* **279** 3916–3924. (doi:10.1074/jbc.M308540200)
- Norris JD, Paige LA, Christensen DJ, Chang CY, Huacani MR, Fan D, Hamilton PT, Fowlkes DM & McDonnell DP 1999 Peptide antagonists of the human estrogen receptor. *Science* **285** 744–746. (doi:10.1126/science.285.5428.744)
- Pan X, Zhou T, Tai Y-H, Wang C, Zhao J, Cao Y, Chen Y, Zhang P-J, Yu M, Zhen C *et al.* 2011 Elevated expression of CUEDC2 protein confers endocrine resistance in breast cancer. *Nature Medicine* **17** 708–714. (doi:10.1038/nm.2369)
- Parent AA, Gunther JR & Katzenellenbogen JA 2008 Blocking estrogen signaling after the hormone: pyrimidine-core inhibitors of estrogen receptor-coactivator binding. *Journal of Medicinal Chemistry* **51** 6512–6530. (doi:10.1021/jm800698b)
- Pavlidis SC, Huang K-T, Reid DA, Wu L, Blank SV, Mittal K, Guo L, Rothenberg E, Rueda B, Cardozo T *et al.* 2013 Inhibitors of SCF–Skp2/Cks1 E3 ligase block estrogen-induced growth stimulation and degradation of nuclear p27kip1: therapeutic potential for endometrial cancer. *Endocrinology* **154** 4030–4045. (doi:10.1210/en.2013-1757)
- Peng J, Schwartz D, Elias JE, Thoreen CC, Cheng D, Marsischky G, Roelofs J, Finley D & Gygi SP 2003 A proteomics approach to understanding protein ubiquitination. *Nature Biotechnology* **21** 921–926. (doi:10.1038/nbt849)
- Peng D-J, Zeng M, Muromoto R, Matsuda T, Shimoda K, Subramaniam M, Spelsberg TC, Wei W-Z & Venuprasad K 2011 Noncanonical K27-linked polyubiquitination of TIEG1 regulates Foxp3 expression and tumor

- growth. *Journal of Immunology* **186** 5638–5647. (doi:10.4049/jimmunol.1003801)
- Pesiri V, Rosa PL, Stano P & Acconcia F 2013 Identification of an estrogen receptor α non covalent ubiquitin-binding surface: role in 17 β -estradiol-induced transcriptional activity. *Journal of Cell Science* **126** 2577–2582. (doi:10.1242/jcs.123307)
- Phu L, Izrael-Tomasevic A, Matsumoto ML, Bustos D, Dynek JN, Fedorova AV, Bakalarski CE, Arnott D, Deshayes K, Dixit VM *et al.* 2011 Improved quantitative mass spectrometry methods for characterizing complex ubiquitin signals. *Molecular & Cellular Proteomics* **10** M110.003756. (doi:10.1074/mcp.M110.003756)
- Picard N, Charbonneau C, Sanchez M, Licznar A, Busson M, Lazennec G & Tremblay A 2008 Phosphorylation of activation function-1 regulates proteasome-dependent nuclear mobility and E6-associated protein ubiquitin ligase recruitment to the estrogen receptor β . *Molecular Endocrinology* **22** 317–330. (doi:10.1210/me.2007-0281)
- Pickart CM 2001 Mechanisms underlying ubiquitination. *Annual Review of Biochemistry* **70** 503–533. (doi:10.1146/annurev.biochem.70.1.503)
- Poukka H, Aarnisalo P, Santti H, Jänne OA & Palvimo JJ 2000 Coregulator small nuclear RING finger protein (SNURF) enhances Sp1- and steroid receptor-mediated transcription by different mechanisms. *Journal of Biological Chemistry* **275** 571–579. (doi:10.1074/jbc.275.1.571)
- Powers GL, Ellison-Zelski SJ, Casa AJ, Lee AV & Alarid ET 2010 Proteasome inhibition represses ER α gene expression in ER+ cells: a new link between proteasome activity and estrogen signaling in breast cancer. *Oncogene* **29** 1509–1518. (doi:10.1038/onc.2009.434)
- Pratt WB 1997 The role of the hsp90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. *Annual Review of Pharmacology and Toxicology* **37** 297–326. (doi:10.1146/annurev.pharmtox.37.1.297)
- Pratt WB & Toft DO 1997 Steroid receptor interactions with heat shock protein and immunophilin chaperones. *Endocrine Reviews* **18** 306–360. (doi:10.1210/edrv.18.3.0303)
- Prenzel T, Begus-Nahrman Y, Kramer F, Hennion M, Hsu C, Gorsler T, Hintermair C, Eick D, Kremmer E, Simons M *et al.* 2011 Estrogen-dependent gene transcription in human breast cancer cells relies upon proteasome-dependent monoubiquitination of histone H2B. *Cancer Research* **71** 5739–5753. (doi:10.1158/0008-5472.CAN-11-1896)
- Qi J, Tripathi M, Mishra R, Sahgal N, Fazil L, Ettinger S, Placzek WJ, Claps G, Chung LW, Bowtell D *et al.* 2013 The E3 ubiquitin ligase Siah2 contributes to castration-resistant prostate cancer by regulation of androgen receptor transcriptional activity. *Cancer Cell* **23** 332–346. (doi:10.1016/j.ccr.2013.02.016)
- Rahighi S, Ikeda F, Kawasaki M, Akutsu M, Suzuki N, Kato R, Kensche T, Uejima T, Bloor S, Komander D *et al.* 2009 Specific recognition of linear ubiquitin chains by NEMO is important for NF- κ B activation. *Cell* **136** 1098–1109. (doi:10.1016/j.cell.2009.03.007)
- Rajbhandari P, Schalper KA, Solodin NM, Ellison-Zelski SJ, Ping Lu K, Rimm DL & Alarid ET 2014 Pin1 modulates ER α levels in breast cancer through inhibition of phosphorylation-dependent ubiquitination and degradation. *Oncogene* **33** 1438–1447. (doi:10.1038/onc.2013.78)
- Ramamoorthy S & Nawaz Z 2008 E6-associated protein (E6-AP) is a dual function coactivator of steroid hormone receptors. *Nuclear Receptor Signaling* **6** e006. (doi:10.1621/nrs.06006)
- Reid G, Hübner MR, Métivier R, Brand H, Denger S, Manu D, Beaudouin J, Ellenberg J & Gannon F 2003 Cyclic proteasome-mediated turnover of unliganded and liganded ER α on responsive promoters is an integral feature of estrogen signaling. *Molecular Cell* **11** 695–707. (doi:10.1016/S1097-2765(03)00090-X)
- Ren Y, Jiang H, Ma D, Nakaso K & Feng J 2011 Parkin degrades estrogen-related receptors to limit the expression of monoamine oxidases. *Human Molecular Genetics* **20** 1074–1083. (doi:10.1093/hmg/ddq550)
- Rochette-Egly C, Adam S, Rossignol M, Egly JM & Chambon P 1997 Stimulation of RAR α activation function AF-1 through binding to the general transcription factor TFIID and phosphorylation by CDK7. *Cell* **90** 97–107. (doi:10.1016/S0092-8674(00)80317-7)
- Roy R, Chun J & Powell SN 2012 BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nature Reviews. Cancer* **12** 68–78. (doi:10.1038/nrc3181)
- Sanchez M, Picard N, Sauvé K & Tremblay A 2013 Coordinate regulation of estrogen receptor β degradation by Mdm2 and CREB-binding protein in response to growth signals. *Oncogene* **32** 117–126. (doi:10.1038/onc.2012.19)
- Saville B, Poukka H, Wormke M, Janne OA, Palvimo JJ, Stoner M, Samudio I & Safe S 2002 Cooperative coactivation of estrogen receptor α in ZR-75 human breast cancer cells by SNURF and TATA-binding protein. *Journal of Biological Chemistry* **277** 2485–2497. (doi:10.1074/jbc.M109021200)
- Schulman BA & Harper JW 2009 Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nature Reviews. Molecular Cell Biology* **10** 319–331. (doi:10.1038/nrm2673)
- Searle MS, Garner TP, Strachan J, Long J, Adlington J, Cavey JR, Shaw B & Layfield R 2012 Structural insights into specificity and diversity in mechanisms of ubiquitin recognition by ubiquitin-binding domains. *Biochemical Society Transactions* **40** 404–408. (doi:10.1042/BST20110729)
- Sengupta S & Wasylyk B 2001 Ligand-dependent interaction of the glucocorticoid receptor with p53 enhances their degradation by Hdm2. *Genes and Development* **15** 2367–2380. (doi:10.1101/gad.202201)
- Sentis S, Le Romancer M, Bianchin C, Rostan M-C & Corbo L 2005 Sumoylation of the estrogen receptor α hinge region regulates its transcriptional activity. *Molecular Endocrinology* **19** 2671–2684. (doi:10.1210/me.2005-0042)
- Sheeler CQ, Singleton DW & Khan SA 2003 Mutation of serines 104, 106, and 118 inhibits dimerization of the human estrogen receptor in yeast. *Endocrine Research* **29** 237–255. (doi:10.1081/ERC-120022321)
- Shinohara K, Tomioka M, Nakano H, Toné S, Ito H & Kawashima S 1996 Apoptosis induction resulting from proteasome inhibition. *Biochemical Journal* **317** 385–388.
- Smith DF 1993 Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes. *Molecular Endocrinology* **7** 1418–1429. (doi:10.1210/mend.7.11.7906860)
- Smith DF & Toft DO 1993 Steroid receptors and their associated proteins. *Molecular Endocrinology* **7** 4–11. (doi:10.1210/mend.7.1.8446107)
- Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau LA, Xia B, Livingston DM & Greenberg RA 2007 RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* **316** 1198–1202. (doi:10.1126/science.1139516)
- Spence J, Gali RR, Dittmar G, Sherman F, Karin M & Finley D 2000 Cell cycle-regulated modification of the ribosome by a variant multi-ubiquitin chain. *Cell* **102** 67–76. (doi:10.1016/S0092-8674(00)00011-8)
- Stancato LF, Silverstein AM, Gitler C, Groner B & Pratt WB 1996 Use of the thiol-specific derivatizing agent N-iodoacetyl-3-[¹²⁵I]iodotyrosine to demonstrate conformational differences between the unbound and hsp90-bound glucocorticoid receptor hormone binding domain. *Journal of Biological Chemistry* **271** 8831–8836. (doi:10.1074/jbc.271.15.8831)
- Stavreva DA, Müller WG, Hager GL, Smith CL & McNally JG 2004 Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes. *Molecular and Cellular Biology* **24** 2682–2697. (doi:10.1128/MCB.24.7.2682-2697.2004)
- Subramanian K, Jia D, Kapoor-Vazirani P, Powell DR, Collins RE, Sharma D, Peng J, Cheng X & Vertino PM 2008 Regulation of estrogen receptor α by the SET7 lysine methyltransferase. *Molecular Cell* **30** 336–347. (doi:10.1016/j.molcel.2008.03.022)
- Sultana R, Theodoraki MA & Caplan AJ 2013 Specificity in the actions of the UBR1 ubiquitin ligase in the degradation of nuclear receptors. *FEBS Open Bio* **3** 394–397. (doi:10.1016/j.fob.2013.09.003)
- Sun J, Zhou W, Kaliappan K, Nawaz Z & Slingerland JM 2012 ER α phosphorylation at Y537 by Src triggers E6-AP-ER α binding, ER α

- ubiquitylation, promoter occupancy, and target gene expression. *Molecular Endocrinology* **26** 1567–1577. (doi:10.1210/me.2012-1140)
- Takano Y, Adachi S, Okuno M, Muto Y, Yoshioka T, Matsushima-Nishiwaki R, Tsurumi H, Ito K, Friedman SL, Moriwaki H *et al.* 2004 The RING finger protein, RNF8, interacts with retinoid X receptor α and enhances its transcription-stimulating activity. *Journal of Biological Chemistry* **279** 18926–18934. (doi:10.1074/jbc.M309148200)
- Talukder AH, Mishra SK, Mandal M, Balasenthil S, Mehta S, Sahin AA, Barnes CJ & Kumar R 2003 MTA1 interacts with MAT1, a cyclin-dependent kinase-activating kinase complex ring finger factor, and regulates estrogen receptor transactivation functions. *Journal of Biological Chemistry* **278** 11676–11685. (doi:10.1074/jbc.M209570200)
- Tateishi Y, Sonoo R, Sekiya Y, Sunahara N, Kawano M, Wayama M, Hirota R, Kawabe Y, Murayama A, Kato S *et al.* 2006 Turning off estrogen receptor β -mediated transcription requires estrogen-dependent receptor proteolysis. *Molecular and Cellular Biology* **26** 7966–7976. (doi:10.1128/MCB.00713-06)
- Teicher BA, Ara G, Herbst R, Palombella VJ & Adams J 1999 The proteasome inhibitor PS-341 in cancer therapy. *Clinical Cancer Research* **5** 2638–2645.
- Terpos E, Kanellias N, Christoulas D, Kastritis E & Dimopoulos MA 2013 Pomalidomide: a novel drug to treat relapsed and refractory multiple myeloma. *OncoTargets and Therapy* **6** 531–538. (doi:10.2147/OTT.S34498)
- Teyssier C, Ou C-Y, Khetchoumian K, Losson R & Stallcup MR 2006 Transcriptional intermediary factor 1 α mediates physical interaction and functional synergy between the coactivator-associated arginine methyltransferase 1 and glucocorticoid receptor-interacting protein 1 nuclear receptor coactivators. *Molecular Endocrinology* **20** 1276–1286. (doi:10.1210/me.2005-0393)
- Thénot S, Henriquet C, Rochefort H & Cavaillès V 1997 Differential interaction of nuclear receptors with the putative human transcriptional coactivator hTIF1. *Journal of Biological Chemistry* **272** 12062–12068.
- Thrower JS, Hoffman L, Rechsteiner M & Pickart CM 2000 Recognition of the polyubiquitin proteolytic signal. *EMBO Journal* **19** 94–102. (doi:10.1093/emboj/19.1.94)
- Toft D & Gorski J 1966 A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. *PNAS* **55** 1574–1581. (doi:10.1073/pnas.55.6.1574)
- Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T, Kamei K, Nakagawa T, Kato M, Murata S, Yamaoka S *et al.* 2009 Involvement of linear polyubiquitylation of NEMO in NF- κ B activation. *Nature Cell Biology* **11** 123–132. (doi:10.1038/ncb1821)
- Tokunaga F, Nakagawa T, Nakahara M, Saeki Y, Taniguchi M, Sakata S, Tanaka K, Nakano H & Iwai K 2011 SHARPIN is a component of the NF- κ B-activating linear ubiquitin chain assembly complex. *Nature* **471** 633–636. (doi:10.1038/nature09815)
- Totta P, Pesiri V, Marino M & Acconcia F 2014 Lysosomal function is involved in 17 β -estradiol-induced estrogen receptor α degradation and cell proliferation. *PLoS ONE* **9** e94880. (doi:10.1371/journal.pone.0094880)
- Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, Wongvipat J, Smith-Jones PM, Yoo D, Kwon A *et al.* 2009 Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* **324** 787–790. (doi:10.1126/science.1168175)
- Tzeng DZ & Klinge CM 1996 Phosphorylation of purified estradiol-liganded estrogen receptor by casein kinase II increases estrogen response element binding but does not alter ligand stability. *Biochemical and Biophysical Research Communications* **223** 554–560. (doi:10.1006/bbrc.1996.0933)
- Valley CC, Métivier R, Solodin NM, Fowler AM, Mashek MT, Hill L & Alarid ET 2005 Differential regulation of estrogen-inducible proteolysis and transcription by the estrogen receptor α N terminus. *Molecular and Cellular Biology* **25** 5417–5428. (doi:10.1128/MCB.25.13.5417-5428.2005)
- Vanaja DK, Mitchell SH, Toft DO & Young CY 2002 Effect of geldanamycin on androgen receptor function and stability. *Cell Stress & Chaperones* **7** 55–64. (doi:10.1379/1466-1268(2002)007<0055:EOGOAR>2.0.CO;2)
- Van Wijk SJ & Timmers HT 2010 The family of ubiquitin-conjugating enzymes (E2s): deciding between life and death of proteins. *FASEB Journal* **24** 981–993. (doi:10.1096/fj.09-136259)
- Vom Baur E, Zechel C, Heery D, Heine MJ, Garnier JM, Vivat V, Le Douarin B, Gronemeyer H, Chambon P & Losson R 1996 Differential ligand-dependent interactions between the AF-2 activating domain of nuclear receptors and the putative transcriptional intermediary factors mSUG1 and TIF1. *EMBO Journal* **15** 110–124.
- Wallace AD & Cidlowski JA 2001 Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. *Journal of Biological Chemistry* **276** 42714–42721. (doi:10.1074/jbc.M106033200)
- Wang X & DeFranco DB 2005 Alternative effects of the ubiquitin–proteasome pathway on glucocorticoid receptor down-regulation and transactivation are mediated by CHIP, an E3 ligase. *Molecular Endocrinology* **19** 1474–1482. (doi:10.1210/me.2004-0383)
- Wang LH, Yang XY, Zhang X, An P, Kim H-J, Huang J, Clarke R, Osborne CK, Inman JK, Appella E *et al.* 2006 Disruption of estrogen receptor DNA-binding domain and related intramolecular communication restores tamoxifen sensitivity in resistant breast cancer. *Cancer Cell* **10** 487–499. (doi:10.1016/j.ccr.2006.09.015)
- Weis KE, Ekena K, Thomas JA, Lazennec G & Katzenellenbogen BS 1996 Constitutively active human estrogen receptors containing amino acid substitutions for tyrosine 537 in the receptor protein. *Molecular Endocrinology* **10** 1388–1398. (doi:10.1210/mend.10.11.8923465)
- Whitesell L & Cook P 1996 Stable and specific binding of heat shock protein 90 by geldanamycin disrupts glucocorticoid receptor function in intact cells. *Molecular Endocrinology* **10** 705–712. (doi:10.1210/mend.10.6.8776730)
- Wickliffe KE, Williamson A, Meyer H-J, Kelly A & Rape M 2011 K11-linked ubiquitin chains as novel regulators of cell division. *Trends in Cell Biology* **21** 656–663. (doi:10.1016/j.tcb.2011.08.008)
- Wijayaratne AL & McDonnell DP 2001 The human estrogen receptor- α is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. *Journal of Biological Chemistry* **276** 35684–35692. (doi:10.1074/jbc.M101097200)
- Wu W, Nishikawa H, Hayami R, Sato K, Honda A, Aratani S, Nakajima T, Fukuda M & Ohta T 2007 BRCA1 ubiquitinates RPB8 in response to DNA damage. *Cancer Research* **67** 951–958. (doi:10.1158/0008-5472.CAN-06-3187)
- Wu-Baer F, Lagrazon K, Yuan W & Baer R 2003 The BRCA1/BARD1 heterodimer assembles polyubiquitin chains through an unconventional linkage involving lysine residue K6 of ubiquitin. *Journal of Biological Chemistry* **278** 34743–34746. (doi:10.1074/jbc.C300249200)
- Xu P & Peng J 2006 Dissecting the ubiquitin pathway by mass spectrometry. *Biochimica et Biophysica Acta* **1764** 1940–1947. (doi:10.1016/j.bbapap.2006.09.004)
- Xu P, Duong DM, Seyfried NT, Cheng D, Xie Y, Robert J, Rush J, Hochstrasser M, Finley D & Peng J 2009a Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. *Cell* **137** 133–145. (doi:10.1016/j.cell.2009.01.041)
- Xu K, Shimelis H, Linn DE, Jiang R, Yang X, Sun F, Guo Z, Chen H, Li W, Chen H *et al.* 2009b Regulation of androgen receptor transcriptional activity and specificity by RNF6-induced ubiquitination. *Cancer Cell* **15** 270–282. (doi:10.1016/j.ccr.2009.02.021)
- Xu M, Skaug B, Zeng W & Chen ZJ 2009c A ubiquitin replacement strategy in human cells reveals distinct mechanisms of IKK activation by TNF α and IL-1 β . *Molecular Cell* **36** 302–314. (doi:10.1016/j.molcel.2009.10.002)
- Yamaoka S, Courtois G, Bessia C, Whiteside ST, Weil R, Agou F, Kirk HE, Kay RJ & Israël A 1998 Complementation cloning of NEMO, a component of the I κ B kinase complex essential for NF- κ B activation. *Cell* **93** 1231–1240. (doi:10.1016/S0092-8674(00)81466-X)

- Yang CH, Gonzalez-Angulo AM, Reuben JM, Booser DJ, Puztai L, Krishnamurthy S, Esseltine D, Stec J, Broglio KR, Islam R *et al.* 2006 Bortezomib (VELCADE) in metastatic breast cancer: pharmacodynamics, biological effects, and prediction of clinical benefits. *Annals of Oncology* **17** 813–817. (doi:10.1093/annonc/mdj131)
- Ye Y & Rape M 2009 Building ubiquitin chains: E2 enzymes at work. *Nature Reviews. Molecular Cell Biology* **10** 755–764. (doi:10.1038/nrm2780)
- Yokota K, Shibata H, Kobayashi S, Suda N, Murai A, Kurihara I, Saito I & Saruta T 2004 Proteasome-mediated mineralocorticoid receptor degradation attenuates transcriptional response to aldosterone. *Endocrine Research* **30** 611–616. (doi:10.1081/ERC-200043783)
- Yudt MR, Vorojeikina D, Zhong L, Skafar DF, Sasson S, Gasiewicz TA & Notides AC 1999 Function of estrogen receptor tyrosine 537 in hormone binding, DNA binding, and transactivation. *Biochemistry* **38** 14146–14156. (doi:10.1021/bi9911132)
- Zhang P-J, Zhao J, Li H-Y, Man J-H, He K, Zhou T, Pan X, Li A-L, Gong W-L, Jin B-F *et al.* 2007 CUE domain containing 2 regulates degradation of progesterone receptor by ubiquitin–proteasome. *EMBO Journal* **26** 1831–1842. (doi:10.1038/sj.emboj.7601602)
- Zhao J, Zhang Z, Vucetic Z, Soprano KJ & Soprano DR 2009 HACE1: a novel repressor of RAR transcriptional activity. *Journal of Cellular Biochemistry* **107** 482–493. (doi:10.1002/jcb.22146)
- Zheng L, Annab LA, Afshari CA, Lee WH & Boyer TG 2001 BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. *PNAS* **98** 9587–9592. (doi:10.1073/pnas.171174298)
- Zhu J, Gianni M, Kopf E, Honoré N, Chelbi-Alix M, Koken M, Quignon F, Rochette-Egly C & de Thé H 1999 Retinoic acid induces proteasome-dependent degradation of retinoic acid receptor α (RAR α) and oncogenic RAR α fusion proteins. *PNAS* **96** 14807–14812. (doi:10.1073/pnas.96.26.14807)
- Zhu J, Zhao C, Kharman-Biz A, Zhuang T, Jonsson P, Liang N, Williams C, Lin C-Y, Qiao Y, Zendejdel K *et al.* 2014 The atypical ubiquitin ligase RNF31 stabilizes estrogen receptor α and modulates estrogen-stimulated breast cancer cell proliferation. *Oncogene* **33** 4340–4351. (doi:10.1038/onc.2013.573)

Received in final form 21 April 2015

Accepted 5 May 2015

Accepted Preprint published online 5 May 2015