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Phosphorylation: a Fundamental Regulator of Steroid Receptor Action

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Abstract

Steroid hormone receptors (SHRs) are hormone-activated transcription factors involved in numerous cellular functions and in health and disease. Their activities depend on the cellular level of the receptor, the presence of coregulator proteins and the cell signaling pathways that are active in the cell. SHRs and their coregulators are phosphorylated on multiple sites by a wide variety of kinases. Each site may contribute to multiple functions and the net effect of an individual phosphorylation depends on the activating kinase. Here we discuss functions of known SHR phosphorylation sites, kinase regulation, evidence of translational relevance, and cross-talk between SHRs and cell signaling pathways. Understanding how cell signaling pathways regulate SHRs might yield novel therapeutic targets for multiple human diseases.

Keywords

steroid receptor; phosphorylation; coactivator; cell signaling

Steroid hormone receptors: the basics

The nuclear receptor (NR) superfamily is a large family of transcription factors that includes NRs that bind steroid hormones (SHRs), other ligand-activated transcription factors such as the thyroid hormone receptor (TR) and vitamin D receptor (VDR), and orphan receptors for which endogenous ligands have yet to be identified such as RAR-related orphan receptors (RORs) and chicken ovalbumin upstream transcription factor (COUP-TF). The focus of this review is the SHR subfamily. Members of the SHR subfamily include the estrogen receptor (ER), glucocorticoid receptor (GR), progesterone receptor (PR), androgen receptor (AR) and mineralocorticoid receptor (MR) [2]. ER exists in two isoforms (ERα and ERβ) derived from distinct genes that mediate the action of estrogens in the mammary gland and the reproductive tract, as well as the central nervous, skeletal, and cardiovascular systems [1]. Cortisol and other glucocorticoids activate GR to regulate metabolism as well as the immune, central nervous, digestive, renal, and reproductive systems [2]. There are two main isoforms of PR (PR-A and PR-B) that are derived from the same gene and mediate the actions of progestins in reproduction, mammary gland development, and sexual behavior [2]. Androgens, specifically testosterone and dihydrotestosterone, activate AR to regulate

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reproduction in both males and females [3]. Aldosterone binds to MR to regulate serum levels of sodium and other electrolytes and to regulate cardiovascular function [2].

Multiple Mechanisms of SHR action

While NRs function as either homo and/or hetero-dimers with other NRs, SHRs function predominantly as homodimers. SHRs can be activated in either a ligand- dependent or ligand-independent manner to regulate transcription (Figure 1). In the former, classical genomic model of SHR action, ligands diffuse across the cell membrane and bind the receptor inducing a conformational change that promotes dissociation of the receptor from chaperone complexes, translocation to the nucleus, and binding of receptor homodimers to hormone response elements (HREs, see figure 1). This binding is followed by dynamic and sequential recruitment of coactivator complexes that modify chromatin to facilitate transcription [4]. All transcription factors depend upon coactivator complexes to regulate transcription; most, if not all, coactivators regulate many classes of transcription factors. A variety of SHR coactivators have been identified; these include the p160 steroid receptor coactivators (SRCs), chromatin remodelers such as the ATP-dependent helicase SMARCA4 (BRG1) and the androgen receptor-interacting protein (ARIP4), the histone modifiers K(lysine) acetyltransferase 2B (PCAF) among others, and the coactivator-associated arginine methyltransferase 1 (CARM1) and protein arginine N-methyltransferase 1 (PRMT1). SHR coactivators also include factors that catalyze post-translational modifications (PTMs) of the receptor itself, other coregulators, and components of the transcriptional machinery, for example protein kinases such as cyclin-dependent kinases (Cdks) and protein kinase B (Akt), regulators of desumoylation such as sentrin-specific protease 1(SENP-1), PRMT1, and regulators of ubiquitination such as E6-AP E3 ubiquitin-protein ligase [4]. SHRs also stimulate transcription through protein-protein interactions with other DNA-bound transcription factors such as activator protein 1 (AP-1), specificity protein 1 (Sp-1) and nuclear factor-kappa B (NFkB), resulting in gene transcription in the absence of direct binding of SHR to the DNA [5] (Figure 1). SHRs are also known to cause transcriptional repression; GR-mediated repression mechanisms are the best characterized and include binding to negative GREs (nGRE) resulting in displacement of other transcription factors (e.g. in the prolactin promoter) [6]. GR also represses transcription through protein-protein interactions with other transcription factors such as AP-1 and NFkB. In the case of the collagenase 3 promoter, GR binds to AP-1 and recruits TIF2/GRIP1/SRC-2 in a conformation that exposes a repressor surface rather than its better known activation function [7]. Finally, SHRs can rapidly activate kinases and resulting downstream cell signaling pathways, for example epidermal growth factor receptor (EGFR), c-Src kinase (Src), and Akt, that mediate biological responses independent of SHR nuclear localization. This form of activation, originally termed non-genomic, is often termed “rapid” and is more accurately described as extranuclear because the common characteristic of this form of signaling is extranuclear (cytoplasmic or membrane-bound) receptor mediated activation of a kinase independent of RNA or protein synthesis. The final downstream event may be altered transcription, but the SHR does not interact with the target gene [8].

Phosphorylation of SHRs

Identification of phosphorylation sites

All SHRs contain multiple phosphorylation sites. Many sites have been reported (see Figure 2) and there may be additional sites. Sites initially identified in SHRs were characterized as being phosphorylated under basal conditions or in response to hormone treatment, and investigators relied on the characterization of radiolabeled proteins and site-directed mutagenesis for site identification. Subsequent studies showed that additional sites are phosphorylated in response to activated signaling pathways. Sites also have been identified

using mass spectrometry to characterize peptides derived from purified proteins. Others have been identified in general phosphoproteomic screens, suggested by homology to sites in SHRs from other species, or identified as candidate sites from *in vitro* phosphorylation studies; it is less certain that these sites are phosphorylated *in vivo* under typical physiological conditions. Sites identified using the various methods and respective references are summarized in Figure 2. Although most sites are not positionally conserved among SHRs, there is one such conserved phosphorylation site in the hinge region of chicken PR (serine (Ser) 530), human PR-B and PR-A (Ser676), human AR (Ser650), and human ERα (Ser294) (Figure 2). Despite this apparent conservation, a common function for this site has not been identified. In chicken PR, the site sensitizes PR to lower concentrations of hormone without changing affinity for progesterone [9], and in human AR phosphorylation of the site promotes nuclear export [10].

Kinases and SHR phosphorylation

A variety of strategies have been used to identify kinases that phosphorylate SHR. These include *in vitro* phosphorylation studies and the use of kinase activators and inhibitors in cell culture studies. These studies suggest that SHR are substrates for the mitogen activated protein kinase (MAPK) family (p42/p44 MAPK, p38 MAPK, and c- Jun N-terminal kinases (JNK)), Cdks, casein kinase 2 (Ck2), glycogen synthase kinase 3 (GSK-3) and a number of other serine/threonine kinases. Although SHRs are phosphorylated on tyrosine residues in response to specific signaling pathways, more is known about the effect of site-specific phosphorylation on serine and threonine residues. It should be noted that multiple kinases can phosphorylate the same residue under various conditions (see Table 1) and this likely facilitates the integration of signals from multiple pathways. In addition, kinases can phosphorylate other proteins, so inhibition or activation of a kinase may have effects on SHR function not directly dependent on SHR phosphorylation.

Interpreting the function of SHR phosphorylation sites: approaches and caveats

Phosphorylations play roles in many functions. They may be “activating” or “inactivating”, change stability, localization, or protein/protein interactions and serve as signals for additional PTMs that alter protein function. Both direct (site-directed mutagenesis) and indirect (activation/inhibition of signaling pathways) approaches have been used to assess the role of phosphorylation. One striking feature of phosphorylation is that it can integrate signals from multiple pathways. As indicated in Table 1, SHR sites may be substrates for multiple kinases. If kinase A and B both phosphorylate a specific site, but kinase A also activates a protein that interacts with the phosphorylated SHR, the biological outcome may be different from that observed by activation of kinase B. Diverse and sometimes contradictory functions have been reported for SHR phosphorylation sites (for example, see the role of AR Ser213 below). In many cases, these differences may be due to signaling pathway or cell type specific differences. Alternatively, the amino acid substitution in mutagenesis experiments may cause a change in activity independent of the supposed change in phosphorylation. There is no perfect substitute to mimic a non-phosphorylated or phosphorylated amino acid. Substitution of an alanine (Ala) for a phospho-serine or phenylalanine (Phe) for a phospho-tyrosine is the most common approach to eliminate site-specific phosphorylation. Other choices such as glycine (Gly) for serine or Ala for tyrosine (Tyr) typically induce conformational changes that can alter activity independent of the change due to phosphorylation. Glutamic acid (Glu) or aspartic acid (Asp) substitutions can mimic a phosphorylated serine/threonine (Ser/Thr) if the major role of the phosphorylation is to introduce a negative charge. However, phosphorylations that serve as recognition motifs for binding proteins including those that serve as a signal for additional PTMs often cannot be mimicked by substitution of a negatively charged amino acid. A final caveat in interpreting these studies is that mutation of additional nearby amino acids either for

convenience in early site-directed mutagenesis strategies or “in case” there is an alternate phosphorylation site generated by the primary substitution may lead to phenotypes that are not strictly a result of elimination of SHR phosphorylation. Selected examples of roles of individual sites are presented below. For a summary of the proposed roles for additional Ser/Thr sites in SHR see Table 1 and the references therein.

Effect of SHR phosphorylation on SHR function

AR phosphorylation

The reported functions of specific AR phosphorylation are kinase-dependent. Phosphorylation of Ser81 is required for induction of endogenous AR target genes [11,12], but not for artificial promoter activation [12]. Cdk9 phosphorylates AR on Ser81 [11] leading to regulation of promoter selectivity, chromatin binding, and cellular distribution of AR [11,12]. Cdk5-dependent stabilization of AR also is dependent on Ser81 phosphorylation [13]. Phosphorylation of AR also plays a role in nuclear localization. Activation of p38 MAPK or JNK results in Ser650 phosphorylation; mutation of this site is associated with reduced nuclear export of AR [10]. The role of Ser213 phosphorylation has been controversial. Akt can phosphorylate this site, but effects of Akt on AR activity in LNCaP cells appear to be cell passage dependent [14]. More recently, two groups have shown that this site is phosphorylated by serine/threonine protein kinase pim-1 (PIM-1) [15,16]. Effects of Ser213 phosphorylation and PIM-1 expression are target gene dependent and PIM-1 isoform dependent. There are two forms of PIM-1. PIM-1S phosphorylates Ser213 leading to recruitment of the E3 ubiquitin-protein ligase Mdm2 and AR degradation. In contrast, PIM-1L phosphorylates Ser213 and Thr850, inducing recruitment of the E3 ubiquitin-protein ligase RNF6, stabilizing and activating AR [16]. Overexpression of PIM-1S inhibits R1881 (synthetic androgen)-dependent reporter activity, and the Ser213Ala mutant is resistant to this repression [15]. In contrast, over-expression of PIM-1L enhances AR-dependent reporter activity in androgen-depleted medium, and mutation of either Thr850 or Ser213 eliminates the stimulation [16]. Depletion of endogenous PIM-1 in VCaP prostate cancer cells enhances androgen-dependent PSA expression, but overexpression of PIM-1S enhances androgen-dependent interleukin 6 (IL6) expression in LNCaP prostate cancer cells [15]. Nuclear Ser213 phosphorylation is higher in castration-resistant prostate cancer compared to hormone-sensitive tumors. Interestingly, cytoplasmic phospho-Ser213 staining in prostate cancer was correlated with shorter time to progression [15].

ER phosphorylation

Phosphorylation has been implicated in many ERα functions. Substitution of Ala for serines 104,106 and 118 reduce transcriptional activity measured using an ER-responsive reporter [17], and reduces coactivation of activation function-1 (AF-1) by p160 and CREB-binding protein (CBP) coactivators [18]. The Ser118Ala mutant transfected into HeLa cells shows somewhat reduced pS2 activation and much decreased cyclin D1 and c-myc induction relative to wild type ER, as well as a different pattern of recruited coactivator complexes at a single time-point [19]. Whether these changes in coactivators are kinetic or a reflection of differential recruitment remains to be determined. There is evidence that phosphorylation of Ser118 in breast cancer tissues correlates with responsiveness to tamoxifen therapy [20]. In contrast, phosphorylation of a site in the ligand-binding domain (LBD), Ser305, mediated by p21- activated kinase 1 (PAK1) leads to tamoxifen resistance [21,22]. Mutation of Ser305 decreases activation of ERα by ~40% in the absence or presence of hormone [23]. Phosphorylation of the hinge site, Ser294, enhances ER activity measured using a reporter [24]. Cdk2-mediated phosphorylation of Ser294 provides a binding site for peptidyl-prolyl isomerase (Pin1) and a resulting increase in Ser118 and Ser167 phosphorylation [25]. In

addition, p38 MAPK-mediated phosphorylation of Ser294 stimulates ubiquitination and turnover of ER [26].

Functional studies of human ERβ phosphorylation are limited. In some contexts, a Ser105Ala mutant shows reduced reporter activity relative to wild type, and a Ser105Glu mutant exhibits enhanced reporter activity, as well as the ability to reduce migration of breast cancer cells [27].

GR phosphorylation

Although a wide variety of candidate sites in human GR have been identified directly, or suggested by phosphoproteomics or homology, the functions of relatively few sites have been examined. Ser211 in the amino-terminal domain (NTD) of GR is the best-characterized phosphorylation site; mutation to Ala decreases transcriptional activity by 25–70% measured using a GR-responsive reporter [28] or endogenous target gene expression [29], reduces its ability to induce GR-dependent apoptosis [28], and alters the conformation of the amino terminus of GR [30]. In contrast, phosphorylation of either Ser203 or Ser226 typically reduces GR activity. Ser203 phosphorylated GR is predominantly cytoplasmic and is not recruited to GR-responsive promoters as assessed by chromatin immunoprecipitation (ChIP) assays [31]. Ser226 is phosphorylated by JNK [32]; mutation to Ala increases GR transcriptional activity [29,33]. Phosphorylation of Ser404 alters target gene dependent gene regulation with both loss and gain of function. Elimination of the phosphorylation site enhances GR-dependent repression of a variety of genes [34]. In addition, phosphorylation at this site reduces GR stability, and may enhance nuclear export [34]. Cell stresses such as starvation or oxidative stress induce p38 MAPK-dependent phosphorylation of Ser134. This phosphorylation mediates GR interaction with 14-3-3 zeta, a protein associated with oxidative stress, binding to select gene promoters and alters the GR-mediated target gene profile [35]. Aberrant GR phosphorylation has been proposed to play a role in disease. For example some glucocorticoid-resistant asthma patients become responsive when p38 MAPK inhibitors are given with reduced Ser226 phosphorylation as one of the results of kinase inhibition [36]. In women, the ratio of nuclear phospho-Ser211/phospho-Ser226 measured in peripheral blood mononucleocytes (PMBCs) is inversely correlated with depression [37].

PR phosphorylation

Functions for several of the sites in PR have been reported. Substitution of Ala for Ser294 in the NTD decreases PR transcriptional activity by 50–90% and in a target gene specific manner [38], increases protein stability [39] and enhances PR sumoylation at K388 [40]. Interestingly, although this amino acid is common to both PR-B and PR-A, only the longer PR-B form is efficiently phosphorylated on this site [41]. Phosphorylation of Ser345 promotes association of PR with Sp1 in target genes that lack canonical progesterone-responsive elements; PR with Ala substitutions at both Ser 344 and Ser345 are not recruited to Sp1 binding sites in the p21 promoter [42]. A S79/81A double mutant shows no defect in activity when measured by using a PR-responsive reporter, but shows gene- specific changes in activation of target genes. For example, the levels of baculoviral IAP repeat-containing protein 3 (BIRC3) are increased when PR is expressed in PR negative cells, and expression is increased further with R5020, a synthetic progestin and a PR agonist. In contrast, both basal and hormone- induced BIRC3 levels are reduced in the S79/81A line [43]. Basal, but not R5020-dependent soft agar colony formation was reduced in the S79/81A clone relative to the wild type clone. The difference in basal activity both for target genes and colony formation is somewhat surprising since the level of Ser81 phosphorylation is quite low in the absence of hormone [43]. Ser400 is a substrate for Cdk2 and expression of constitutively active Cdk2 enhances PR activity in the absence or presence of hormone. Interestingly, a

Ser400Ala mutant fails to show the basal increase in activity, although its response to R5020 in the presence of Cdk2 is not compromised [44].

Cross-talk between cell signaling pathways and SHRs

Although it is well established that cell signaling pathways modify SHR activity as summarized above, there is evidence that under some conditions, activated cell signaling pathways can eliminate the requirement for hormone. Conversely, SHRs can activate kinases independent of classical upstream growth factor receptors. Finally, there is increasing evidence for receptor-mediated recruitment of kinases to chromatin, facilitating local phosphorylation of factors that regulate transcription.

Transcriptional activation of SHRs in steroid depleted medium

Some of the most striking evidence for cross-talk between cell signaling pathways and SHRs is the finding that some SHRs are activated in the absence of their cognate ligands by activators of cell signaling pathways such as pharmacologic activators of cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA), growth factor signaling (e.g. EGF, human epidermal growth factor 2 (HER2), insulin-like growth factor (IGF)), and cytokines (IL-6). Changes in SHR or coactivator phosphorylation have been identified in some cases, while in others the targets have not been identified. O'Malley's group first reported that 8-Bromo cAMP, a PKA activator, induced chicken PR-mediated transcription of a PR-responsive reporter, in the absence of progesterone [45]. Human PR is not activated under the same treatment conditions, but PKA signaling does cause the partial PR antagonist, RU486, to act as an agonist [46]. In some contexts, constitutively active Cdk2 stimulates basal PR activity [44] and heregulin can activate PR [47]. Human ERα responds to multiple activators of cell signaling pathways including growth factors [48]. The mechanisms have not been fully elucidated. For example, substitution of Ala for Ser118 eliminates EGF-dependent, but not hormone-dependent activation of ER in some cell types [49]. Substitution of a Glu for the Ser restores EGF-dependent activation, showing that a negative charge at Ser118 is required, but not sufficient for EGF-dependent activation. AR also is responsive to multiple signaling pathways. Activation of AR through PKA signaling [50], IL-6 [51], and growth factors have all been reported. Phosphorylation of SRC-1, a known AR coactivator, has been implicated as one of the factors in IL-6-dependent activation of AR [51].

Activation of kinases by SHR

Activation of kinases by SHR-mediated extranuclear signaling is important in many tissues. For example, rapid signaling of ER is important for bone biology, the central nervous system, and the cardiovascular system. In endothelial cells, estradiol stimulates production of nitric oxide through the rapid activation of the small G protein subunit, Gαi, by ERα, leading to activation of Erk and phosphoinositide 3-kinase (PI3K) [52,53]. This was confirmed by studies utilizing estrogen dendrimer conjugates (EDCs), bulky synthetic ligands that are excluded from the nucleus and activate only extranuclear pathways [54], and ERα mutants that cannot interact with Gαi [55]. AR also has been implicated in extranuclear signaling through Gβγ, cAMP, MAPK, and EGFR pathways to affect oocyte maturation, spermatogenesis, bone turnover, and proliferation/survival of prostate cancer cells (reviewed in [56]). Human PR mediates extranuclear signaling in breast cancer cells through direct interactions with Src or through release of EGF ligands that activate EGFR [57,58]. Extranuclear signaling of GR is important for neural progenitor cell function [59], as well as pituitary, hippocampal, cardiovascular, and immune function (reviewed in [60]). Finally, extranuclear signaling effects of aldosterone in vascular and kidney cells may be mediated, in part, by MR (reviewed in [60]). Although activation of kinases is independent of nuclear

actions of SHRs, the two forms of signaling often are integrated to mediate SHR function in target tissues.

SHR-dependent recruitment of kinases to chromatin

SHR also facilitates the phosphorylation of substrates by kinases by recruiting the kinases to chromatin. For example, PR recruits cyclin A/Cdk2 to PR-responsive promoters [61] resulting in Cdk2-dependent phosphorylation of poly (ADP-ribose) polymerase 1 (PARP-1) and subsequent displacement of histone H1 [62]. PR also recruits MAPK and mitogen- and stress-activated protein kinase 1 (MSK1) [63] as well as Ck2 [43] to promoters regulated by PR. Recruitment of kinases by SHR is not restricted to PR. ER also recruits MAPK [64] indicating that SHR-dependent recruitment of kinases to chromatin may be a common feature of SHR action.

Concluding remarks and future perspectives

SHR signaling is complex and numerous cell signaling pathways can impact SHR function. Site-specific phosphorylation modulates both ligand-dependent and ligand-independent SHR activity through the regulation of overall transcriptional activity, protein stability, cellular localization, chromatin binding and interaction with coregulators. Phosphorylation of coregulators and other DNA-binding proteins further fine-tunes SHR transcriptional activity. Thus, phosphorylation, along with other PTMs, defines a “code” that ultimately impacts biological response of SHRs under various physiological conditions. As noted above, a few studies have demonstrated the physiological relevance and/or correlation of these modifications to human disease. Studies have begun to elucidate the importance of site-specific phosphorylation but much is still unknown. Identification of phosphorylation sites in the receptors and their interacting proteins is incomplete. New, more sensitive technologies will aid in filling the gaps. Receptor phosphorylation clearly is not an on/off switch for global activity. Rather, it modulates several functions of the receptor; specific phosphorylations likely are critical for sub-sets of genes or for tissue-specific actions. Additional studies using methods that measure global gene regulation and receptor binding to chromatin followed by mechanistic studies of specific target genes are needed. Microarrays have been employed with some success [34], but global RNA-Seq and ChIP-Seq experiments using phosphorylation-specific antibodies have not been done. Phosphorylation- specific antibodies are extremely useful tools, but also have limitations particularly in immunohistochemical analyses. Many of the antibodies distinguish well between phosphorylated and unphosphorylated receptors, but may cross react with other proteins. Techniques such as proximity ligation assays (PLAs), which give a positive signal only when two antibodies (e.g. SHR and phospho-specific) are in close proximity may overcome these limitations for immunohistochemical studies. A second limitation is the inability to distinguish between an occluded epitope and failure to recruit a protein in ChIP assays. Antibodies specific to the dephosphorylated epitope would permit the investigator to distinguish between differential recruitment of the phosphorylated and dephosphorylated protein and epitope occlusion. To address the role of phosphorylation in vivo, approaches such as site-specific elimination of phosphorylation in mice are needed. In some cases, phosphorylation acts as a signal for subsequent modifications that alter function. Although there is compelling evidence that receptors are regulated by other types of PTMs, the identity, function, and regulation of these modifications is incomplete. Thus, there also is a need to identify and characterize other PTMs. This knowledge will not only yield insight into the mechanisms of cross-talk between SHR signaling and cell signaling pathways, but might also help develop novel therapeutic strategies for multiple human diseases.

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TEXT BOX 1

Structure of SHRs

Almost all NRs share a common structure comprised of a highly variable amino-terminal domain (NTD), DNA-binding domain (DBD), a flexible hinge domain, and a ligand-binding domain (LBD) responsible for binding of specific ligands (Figure 2). An additional region important for ligand-dependent receptor transcriptional activity exists within the LBD; it is termed activation function -2 (AF-2). This modular structure facilitates allosteric regulation of receptor activity [82].

In the absence of hormone, SHRs are bound to heat shock proteins and immunophilins in a heterocomplex (Figure 1). Some SHRs are predominantly cytoplasmic (GR and AR), while others are predominantly nuclear (ER and PR), but all can shuttle between the two compartments. Ligand binding induces conformational changes that alter receptor-DNA and receptor-protein interactions, ultimately regulating receptor activity. The LBD also contains sequences that serve as an interface for dimerization, particularly for ER and GR (reviewed in [82]). Ligand-induced conformational changes also expose the nuclear localization signal (NLS) that resides in the hinge region, leading to translocation of the receptor-steroid complex into the nucleus, binding to DNA, and transactivation of target genes. The DBD, which contains two zinc finger motifs, binds to hormone response elements (HREs) on the DNA that partly dictate cell or promoter-selective SHR function. This binding can also influence which coregulatory complexes are recruited by the receptor. Compared to the LBD and DBD, there is little homology among the NTDs of SHRs. The NTD contains an activation function region (AF-1) that is ligand-independent.

Intrinsically disordered SHR regions

Unlike the LBD and the DBD, the hinge and NTD are intrinsically disordered (ID) regions that are capable of undergoing disorder-to-order transitions under specific physiological conditions (reviewed in [82]). This conformational flexibility may create large interaction surfaces that allow specific, but reversible interactions with target molecules [82]. Disorder-to-order transitions may be mediated through post-translational modifications (PTMs) of the receptor, including phosphorylation [30,83]. In fact, ID regions have a higher frequency of known phosphorylation sites and there is a strong correlation between known phosphorylation residues within AR-, ERα-, GR-, and PR-NTD and predicted intrinsic disorder [82]. Pin1, an enzyme that binds to proteins and plays a role as a post phosphorylation control in regulating protein function, recognizes phosphorylated serine/threonine-proline (Ser/Thr-Pro) motifs, and induces a conformational change in target proteins. Pin1 interactions with AR phosphorylated at Ser81 [84] and ERα phosphorylated at Ser118 [85] are important for transcriptional activity of the receptors.

Highlights

- Multiple kinases phosphorylate steroid hormone receptors and their coregulators
- Site-specific phosphorylation modulates selective actions of receptors
- Activation of cell signaling pathways impacts biological response of receptors
- Regulation of receptor function by cell signaling is relevant to human disease

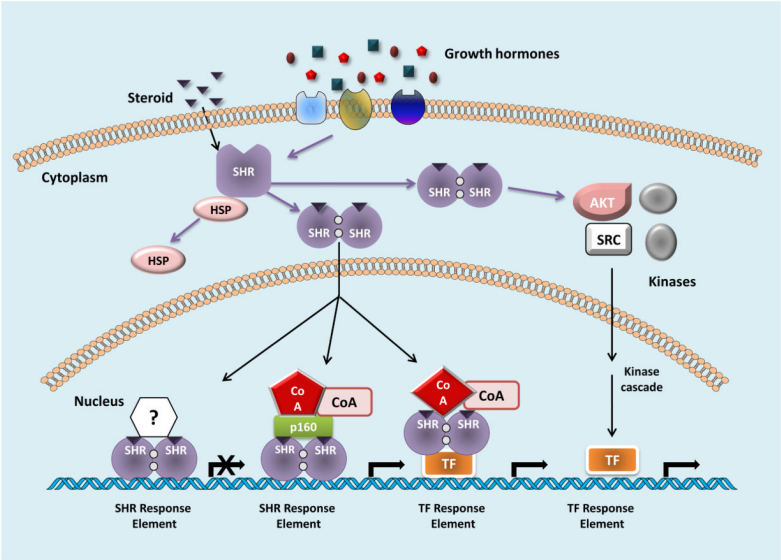

Figure 1. Mechanisms of steroid hormone receptor (SHR) action

In the absence of ligand, SHRs are bound to heat shock protein (HSP) complexes. Upon binding of ligand and/or activation of cell signaling pathways, SHRs dissociate from these complexes, form homodimers, translocate to the nucleus, and bind to SHR response elements. Following this binding, coactivator complexes (CoA) are recruited, facilitating transcription of target genes. Alternatively, SHRs can bind to other transcription factors (TF) sitting on TF response elements, resulting in the recruitment of CoAs and induction of target gene expression. SHRs can also inhibit gene transcription. Finally, SHRs also interact with and activate kinases, such as protein kinase B/Akt (AKT) and c-Src (SRC) resulting in phosphorylation and activation of other TFs bound to TF response elements and altered target gene transcription.

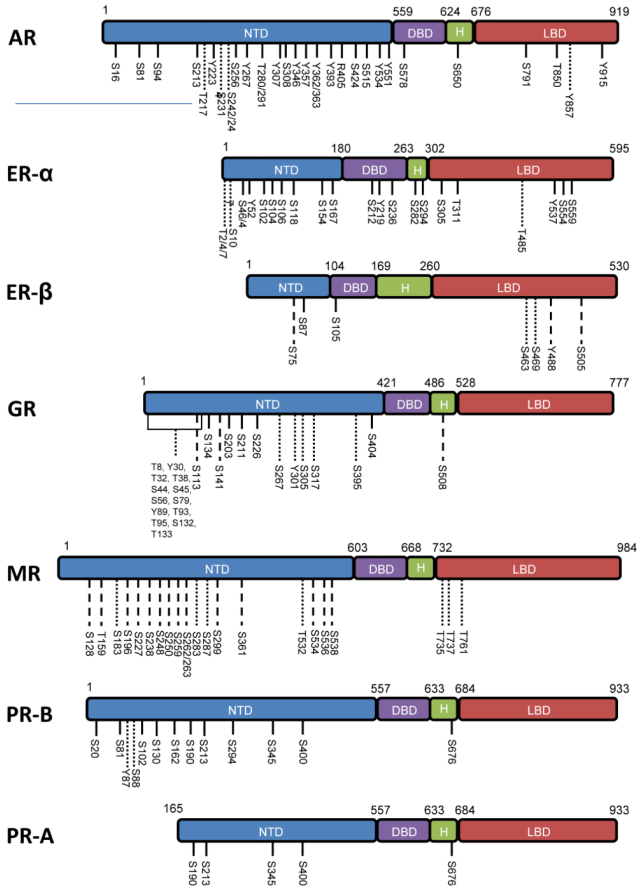

Figure 2. Structure and phosphorylation sites of SHRs

The numbers indicate the amino acids that mark the domain boundaries in the individual receptors between the NTD, DBD=, hinge region (H), and LBD. Solid lines indicate sites that have been determined using radiolabeling, protein sequencing, or mass spectrometry analysis of sites in purified proteins; dotted lines indicate sites identified by phosphoproteomic approaches, and dashed lines indicate sites identified by *in vitro* phosphorylation or by homology with sites in rodents. References for identification of the sites, with the exception of the MR sites Ser128, Thr159, and Ser250 [80] can be found at www.phosphosite.org. Note that the proposed Ser/Thr sites reported on www.phosphosite.org that have not been detected by at least one of these direct methods are not included in the figure.

AR, androgen receptor; ER, estrogen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor. S, serine; T, threonine.

Table 1

Candidate kinases and functions for some SHR Ser/Thr phosphorylation sites

SHR	Phosphorylation site	Kinase(s)	Ligand-dependent activity	Ligand-independent activity *	Activity (other)	Physiological relevance	Reference
AR	Ser81	Cdk9			Gene-specific effect on ligand- dependent transcription	Promotes prostate cancer cell growth	[11]
		Cdk5			Increased protein stability	Promotes prostate cancer cell growth	[13]
		Cdk1/Cdk9	B		DNA binding; Cytoplasmic localization	ND	[12]
	Ser213	Akt			Decreased protein stability	ND	[14]
		Pim-1	C			Possible marker of Pim-1 activity in prostate cancer	[15]
		Pim-1S	C		Decreased protein stability	Promotes prostate cancer growth (low androgen)	[16]
		Pim-1L				Promotes prostate cancer growth (low androgen); Gene-specific effect on ligand-dependent transcription	[16]
	Ser515	Cdk7	A		Decreased protein stability	ND	[65]
	Ser650	p38 MAPK			Nuclear export	ND	[10]
		JNK			Nuclear export	ND	[10]
	Thr850	Pim-1L	A		Increased protein stability	Prostate cancer cell growth (low androgen)	[16]
ERα	Ser104/106	Cyclin A- Cdk2	A			ND	[66]
		MAPK	A			Tamoxifen resistance	[67]
	Ser118	ND (hormone- mediated)	A			ND	[68]
		ND (hormone- mediated)	B			ND	[19]
		ND			Increased protein stability (Pin1)	ND	[69]
		MAPK		A		ND	[49]
		MAPK	A			ND	[67]
		Cdk7	A			ND	[70]

SHR	Phosphorylation site	Kinase(s)	Ligand-dependent activity	Ligand-independent activity *	Activity (other)	Physiological relevance	Reference
		Ikkα			DNA binding; Coregulator recruitment	ND	[71]
	Ser167	p90 RSK	A			ND	[72]
		Akt	B	B		Tamoxifen resistance	[73]
		Ikkε		A, B	DNA binding	Tamoxifen resistance	[74]
	Ser282	Ck2			C (basal transcription)	ND	[24]
	Ser294	ND (hormone- mediated)	A			ND	[24]
		Cdk (hormone-mediated)	B		Increased protein stability	ND	[75]
		p38 MAPK			Decreased protein stability (Skp2)	ND	[26]
		Cdk2	A		Mediates receptor interaction with Pin1; Regulates phosphorylation of Ser118 and Ser167	ND	[25]
	Ser305	Pak1	A		Regulation of cell proliferation (mouse mammary gland)	ND	[76]
		PKA			Blocks acetylation of K303	Enhances estrogen sensitivity of (K303R mutant)	[77]
		Akt				Aromatase inhibitor resistance (K303R mutant)	[78]
	Ser559	Ck2			C, D (basal transcription)	ND	[24]
ERβ	Ser105	ND (hormone- mediated)				Associated with good prognosis in breast cancer patients	[79]
		MAPK	A		Decreased breast cancer cell migration and invasion (phospho-mimetic)	ND	[27]
GR	Ser134	p38 MAPK			Interaction with 14-3-3-zeta; Modulates promoter selectivity; Gene-specific effect on ligand-dependent transcription	ND	[35]
	Ser203	ND (basal and hormone-mediated)			Cytoplasmic localization; DNA binding	ND	[31]
	Ser211	ND (hormone- mediated)	C, D		Receptor conformational change; Enhanced	ND	[29]

SHR	Phosphorylation site	Kinase(s)	Ligand-dependent activity	Ligand-independent activity *	Activity (other)	Physiological relevance	Reference
					interaction with MED14; Gene- specific effect on transcription		
		p38 MAPK		A	Regulation of apoptosis	ND	[28]
		p38 MAPK			Receptor conformational change; Coregulator interaction	ND	[30]
	Ser226	ND (hormone- mediated)	C, D			ND	[29]
		JNK	C		Enhanced nuclear export (ligand- independent)	ND	[32]
	Ser404	GSK3β			Enhanced nuclear export; Decreased protein stability; Decreased coregulator interaction; Gene-specific effect on transcription	ND	[34]
MR	Ser128/Thr159/Ser250	Cdk5	C			ND	[80]
PR	Ser81	Ck2	B (S79/81)		B (basal transcription; S79/81); DNA binding	ND	[43]
	Ser294	ND (hormone- mediated)			Decreased protein stability (ligand- dependent)	ND	[39]
		MAPK	A, B			ND	[38]
		MAPK			Nuclear localization (ligand- independent)	ND	[81]
	Ser345	MAPK/Src			Interaction with Sp1 (S344/345); Gene-specific effect on ligand-dependent transcription		[42]
	Ser400	Cdk2		A	Nuclear translocation (ligand- independent)	ND	[44]

Phosphorylation is associated with A = increased expression of a reporter gene; B = increased endogenous gene expression; C = decreased expression of a reporter gene; D = decreased endogenous gene expression.

* Ligand-independent activation is defined as activation by a kinase signaling cascade and does not refer to basal activity.

ND, not determined.