Review

# Estrogen receptor phosphorylation

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## Abstract

Estrogen receptor $\alpha$ (ER$\alpha$) is phosphorylated on multiple amino acid residues. For example, in response to estradiol binding, human ER$\alpha$ is predominately phosphorylated on Ser-118 and to a lesser extent on Ser-104 and Ser-106. In response to activation of the mitogen-activated protein kinase pathway, phosphorylation occurs on Ser-118 and Ser-167. These serine residues are all located within the activation function 1 region of the N-terminal domain of ER$\alpha$. In contrast, activation of protein kinase A increases the phosphorylation of Ser-236, which is located in the DNA-binding domain. The in vivo phosphorylation status of Tyr-537, located in the ligand-binding domain, remains controversial. In this review, I present evidence that these phosphorylations occur, and identify the kinases thought to be responsible. Additionally, the functional importance of ER$\alpha$ phosphorylation is discussed.
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## 1. Overview

This review will focus on the major phosphorylation sites in estrogen receptor $\alpha$ (ER$\alpha$) that occur in response to either estradiol or through the activation of second messenger signaling pathways. The following questions will be addressed: (1) What are the sites of ER phosphorylation and which kinases are responsible for these phosphorylations? (2) How does phosphorylation influence ER function? (3) What is known about ER phosphatases? (4) How does estradiol directly influence signal transduction pathways? (5) What lessons on the function of phosphorylation can be learned from other steroid receptors?

### 1.1. Introduction

The ER belongs to a superfamily of ligand-activated transcription factors [1]. However, it is now well established that ER-mediated transcription can also be stimulated by ligand-independent mechanisms involving second messenger signaling pathways [2–15]. Furthermore, there are cell-type specific differences in the ability of second messenger signaling pathways to enhance ER-mediated transcription, which may account for the differences in ER action in various cells [16].

There are two known ER isoforms, $\alpha$ and $\beta$, which differ in their ligand specificities and physiological functions [17–19]. There are also a number of splice variants for each of the isoforms, some of which influence the activity of the wild type receptor [20–23]. Like other members of the superfamily, ER$\alpha$ and ER$\beta$ are made up of several functional domains (Fig. 1) [24]. The N-terminal domains of ER$\alpha$ and ER$\beta$ are highly divergent whereas the DNA binding domain (DBD) and the ligand-binding domain (LBD) are approximately 95 and 55% homologous, respectively.

The N-terminal domain contains a ligand-independent activation function (AF1) and was originally identified by its ability to stimulate transcription in an ER$\alpha$ deletion mutant that contained only the N-terminus fused to a heterologous DBD [25]. It seems reasonable to suggest that in the context of the holoreceptor, AF1 activity is silenced because of steric hindrance by the LBD. This inhibition appears to be relieved by addition of ligand. This model is supported by the ability of the LBD to control the function, in a ligand-dependent manner, of a wide variety of proteins to which it has been fused [26–30]. AF1 activity is also enhanced by the action of second messenger signaling pathways, which presumably must relieve the inhibition by the LBD. In response to ligand, AF1 synergizes with the ligand-dependent activation function (AF2) in the LBD [31,32]. AF1 plays a role in both ligand-dependent and ligand-independent transcription. However, in some cell types and on some promoters, AF1 does not significantly contribute to the transcriptional

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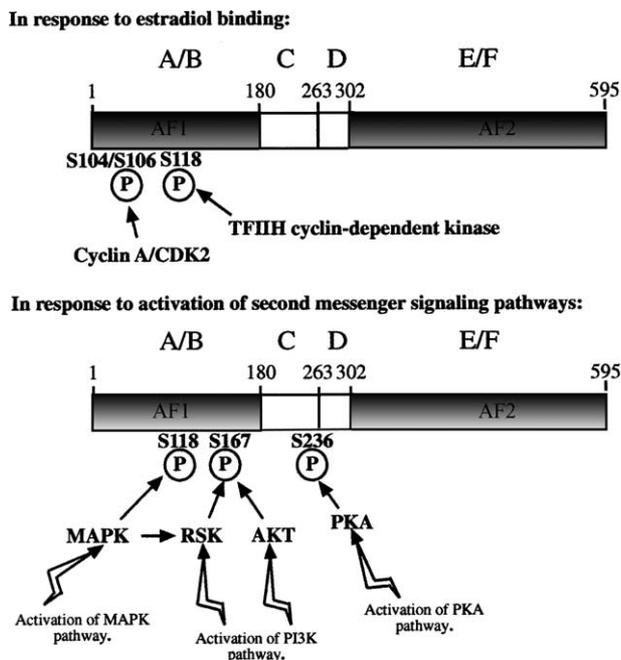*E-mail address:* dal5f@virginia.edu (D.A. Lannigan).

Fig. 1. Schematic illustrating the residues whose phosphorylations are enhanced in response to estradiol binding or activation of second messenger signaling pathways. The kinases thought to be responsible for these phosphorylations are also shown.

activation induced by ligand [33,34]. AF2 in the LBD was identified by its ability to stimulate transcription, only in response to ligand, of a mutant that contained just the LBD fused to a heterologous DBD [25,35]. In response to agonist, the receptor undergoes a conformational change in which helices 3, 5 and 12 form a surface with which co-activators interact [36–39]. The position of helix 12, relative to the body of the LBD, determines whether or not a ligand behaves as an agonist or an antagonist [38,40,41].

### 1.1.1. What are the sites of ER phosphorylation and which kinases are responsible for these phosphorylations?

Phosphorylation of ERα is enhanced in response to estradiol binding and through the action of second messenger signaling pathways [9,42–44]. Phosphorylation of ERβ in response to estradiol binding has not been examined in detail, although phosphorylation of ERβ has been shown to increase in response to activation of the MAPK pathway [45]. Several investigators have demonstrated that in a number of different cell types, in response to estradiol binding, ERα is phosphorylated only on serine residues [4,9,42,43,46–48]. Additionally, Le Goff et al. [9] demonstrated that stimulation of either the mitogen activated-protein kinase (MAPK) or cAMP-dependent kinase pathways resulted only in serine phosphorylation in ERα. However, others have reported that ERα may also be phosphorylated on tyrosine residues [49–53]. This phosphorylation does not change in response to estradiol binding. In Fig. 1, the residues whose phosphorylations are enhanced in response to ligand binding or

activation of signaling cascades are shown. Additionally, the kinases thought to be responsible for these phosphorylations are indicated.

*1.1.1.1. Serine 104, Serine 106 and Serine 118 phosphorylation.* Serine 106 (Ser-106) and Ser-118 are highly conserved residues, whereas Ser-104 appears to be conserved only in mammals. These sites were initially determined to be phosphorylated by comparison of phosphopeptide patterns generated by wild type and mutant ERαs [9]. Joel et al. then demonstrated by peptide sequencing and manual Edman degradation that Ser-118 was the major site phosphorylated in response to estradiol [44]. In response to estradiol binding, the change in Ser-118 phosphorylation is dramatically larger than the change in Ser-104 and Ser-106 phosphorylation (unpublished observations). Upon activation of the MAPK pathway by EGF, phosphorylation of Ser-118 is enhanced whereas phosphorylation of Ser-104 and Ser-106 does not appear to change significantly [54]. Thus, although Ser-104, Ser-106 and Ser-118 are each followed by Pro residues, the phosphorylation of Ser-118 appears to be regulated differently from that of Ser-104 and Ser-106. These results may be due to the action of a single kinase with Ser-118 being the preferred substrate, or the preferential action of a phosphatase for phosphoSer-104 and phosphoSer-106 compared to phosphoSer-118. On the other hand, the results may reflect that Ser-104 and Ser-106 are phosphorylated by different kinases than Ser-118. In fact there is evidence for the latter possibility because cyclin-A-cyclin dependent kinase 2 (Cdk2) complex phosphorylates Ser-104 and Ser-106 but not Ser-118 [55].

Phosphorylation of Ser-118 results in reduced ERα mobility on SDS-PAGE [14]. Using this upshift assay the stoichiometry of Ser-118 phosphorylation in MCF-7 cells, a human breast cancer cell line, in response to estradiol binding is ∼0.67 mol of phosphate/mol of ERα, whereas in response to activation of the MAPK pathway, the stoichiometry is ∼0.25. Thus ERα is significantly phosphorylated on Ser-118 in response to either estradiol binding or activation of the MAPK pathway, which suggests that this phosphorylation plays an important role in ERα function. Ser-118 is the only site in ERα in which the stoichiometry of phosphorylation is known.

There is overwhelming in vitro and in vivo evidence that activated MAPK phosphorylates Ser-118 [12,14,44,56]. However, the identity of the kinase responsible for Ser-118 phosphorylation in response to estradiol binding is controversial [14]. Migliaccio et al. have reported that in cell lines of breast, prostate or kidney origin estradiol enhances the activity of c-src resulting in activation of the MAPK pathway and the subsequent enhancement of Ser-118 phosphorylation [57–59]. However, a number of other laboratories have not observed activation of the MAPK pathway in response to estradiol in cell lines of breast or kidney origin [14,60–63]. Other groups have observed that estradiol activates MAPK in cell lines of vascular or neural origin but

there appears to be disagreement as to whether the effect is through ER [64–67]. Furthermore, inhibition of MAPK did not prevent the estradiol-induced increase in Ser-118 phosphorylation and basal MAPK activity could not account for the increase. Joel et al. have proposed that a kinase in addition to MAPK regulates Ser-118 phosphorylation [14]. One possible candidate for the estradiol-induced Ser-118 phosphorylation is TFIIH cyclin-dependent kinase [60].

*1.1.1.2. Serine 167 phosphorylation.* It has been reported that Ser-167 is a major site of phosphorylation in response to estradiol binding [68]. However, Le Goff et al. [9] did not observe Ser-167 phosphorylation in response to estradiol and these results are in agreement with observations made using a phosphospecific Ser-167 antibody [69]. However, Ser-167 does get phosphorylated in response to activation of the MAPK pathway [54]. In vivo and in vitro evidence demonstrated that the kinase downstream from MAPK, p90 ribosomal S6 kinase (Rsk) [70], can phosphorylate Ser-167 [54]. Thus ERα is phosphorylated by two different kinases in the MAPK pathway. The significance of this dual phosphorylation by kinases in the same signal transduction pathway is not understood. Ser-167 can also be phosphorylated in vitro by casein kinase II and by AKT, which is also called protein kinase B [71–74]. There is as yet no evidence that casein kinase II phosphorylates Ser-167 in vivo. In general, kinases have a broader substrate specificity in vitro than they have in vivo. Thus it is difficult to determine the physiological relevance of a kinase based solely on in vitro assays. Additionally, the extensive cross talk that exists between different signal transduction pathways in vivo makes it difficult to determine the in vivo contributions of a kinase to a particular phosphorylation event. For example, Rsk and AKT have identical consensus phosphorylation sequences that they recognize and therefore in vitro they will phosphorylate the same substrates. Furthermore, in vivo, signal transduction pathways that activate AKT also stimulate PDK1 activity. PDK1 influences the activity of both Rsk and AKT (Fig. 2). Currently, there are no known specific inhibitors of either Rsk or AKT activity that do not also inhibit the activity of upstream kinases [75]. However, a constitutively active mutant of Rsk in combination with estradiol synergistically enhances ERα-mediated transcription under conditions in which AKT activity should be extremely low [69]. These results suggest that Rsk plays an important role in ERα-mediated transcription. However, it is likely that both Rsk and AKT regulate ERα activity but their importance in this regulation may vary depending on the cell type and extracellular signal.

*1.1.1.3. Serine 236 phosphorylation.* In vitro, protein kinase A (PKA) can phosphorylate Ser-236, which is located in the DNA binding domain [76]. Additionally, 8-bromo cAMP treatment increases the phosphorylation of wild type ERα to a greater extent than a mutant in which Ser-236 is changed to Ala in vivo. These results are in agreement with
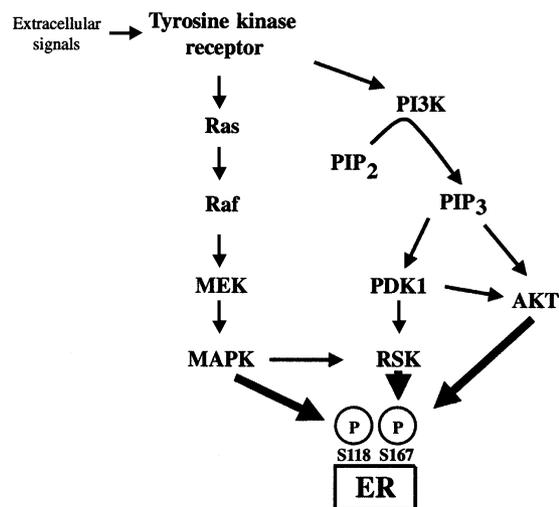


Fig. 2. Schematic demonstrating the crosstalk that occurs between the pathways that activate Rsk and AKT.

Le Goff et al. who demonstrated that in response to activation of the cAMP-dependent kinase pathway ERα is phosphorylated on serine residues not located in the N-terminal domain [9].

*1.1.1.4. Tyrosine 537 phosphorylation.* Arnold et al. [49,50] have reported that Tyr-537 of human ERα is phosphorylated in MCF7 and in insect cells and that this phosphorylation does not change in response to ligand. However, the phosphoTyrosine antibody used in these studies has also been shown to interact with a mutant ERα in which Tyr-537 has been altered to Ala [77]. Tyrosine phosphorylation has also been reported in ERα isolated from calf uterus, and from breast cancer cells that overexpress the HER-2 receptor [52,78–80] but tyrosine phosphorylation has not been observed by a number of other groups [9,42,43,46–48]. These conflicting reports may be due to the fact that tyrosine phosphorylation occurs on a small minority of the receptor molecules, is cell-specific or occurs only under conditions in which the tyrosine kinase is present in great abundance. In vitro tyrosine phosphorylation has been observed using src tyrosine kinases and a kinase purified from calf uterus [50,81].

*1.1.2. How does phosphorylation influence ER function?*
In general, phosphorylation of Ser residues in the AF1 domain appears to influence the recruitment of coactivators, resulting in enhanced ER-mediated transcription [82–84]. The importance of Ser-118 phosphorylation has been studied by a number of different investigators and there is significant discrepancy in the literature concerning the transcriptional activity of the mutant ERα, in which Ser-118 is mutated to Ala(S118-ERα), compared to the wild type ERα. A number of groups have reported, in cell lines of either fibroblast or epithelial origin, that S118A-ERα has a diminished ability to activate transcription in response to estradiol compared
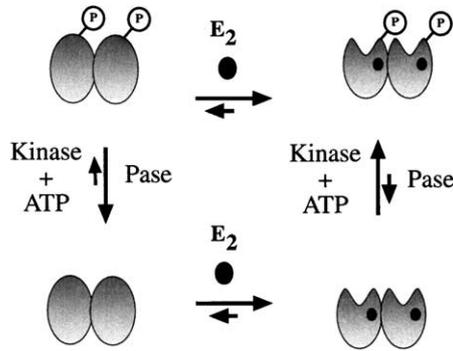
Fig. 3. Schematic showing the effect of estradiol binding on the equilibrium of the unphosphorylated and phosphorylated ERα.

to wild type ERα [12,54,56,85]. Additionally, mutation of Ser-118 to an acidic residue could enhance the transcriptional response [85]. In contrast, Le Goff et al. found that S118A-ERα had approximately the same estrogen-induced transcriptional activity compared to the wild type ERα [9]. These studies were also performed in cell lines of either fibroblast or epithelial origin. However, an ERα mutant in which Ser-104, Ser-106 and Ser-118 were all mutated to Ala did have a decreased ability to activate estrogen-induced transcription as compared to wild type ERα.

It is important to account for the possible sources of these discrepancies in order to determine the physiological significance of ERα phosphorylation. One problem is that no two groups have used reporter constructs that have identical promoters in the same cell line, which may explain the discrepancies. To account for the discrepancies that could occur we will consider a simple model (Fig. 3). In the absence of estradiol, the ER exists predominantly in a dephosphorylated form presumably because the phosphatase is more effective at dephosphorylating the ER than the kinase is at phosphorylating the ER. However, in the presence of estradiol, the receptor undergoes a conformational change, which either enhances the ability of the kinase to phosphorylate ER or decreases the ability of the phosphatase to dephosphorylate ER. Regardless, in the presence of estradiol ER phosphorylation is increased. It is possible that part of the disparity in the literature arises from the use of different transient transfection systems to study the effects of ER mutants. Overexpression of the wild type or mutant ERs from an expression plasmid may result in a mass-action-driven shift in equilibrium toward the formation of a receptor co-activator complex. Thus, differences in affinity of the phosphorylated and the unphosphorylated ER for the co-activator could be masked, resulting in similar transcriptional responses for both the wild type and mutant ERs. It is also very likely that the activity of the phosphatase and kinase and thus the extent of ER phosphorylation in response to estradiol may vary considerably between different cell types. Thus, in cells in which ER is not significantly phosphorylated because the kinase activity is low, the difference between wild type ER and phosphorylation-defective ER mutants to stim-

ulate transcription will be similar. Some of the disparity in the literature may therefore, be accounted for by variations in the stoichiometry of ER phosphorylation between cell types. Furthermore, if the function of ER phosphorylation is to enhance the ability of ER to interact with a co-activator it is possible that the ER and co-activator complex is more important for some promoters than for others.

There are also differences in the literature on the ability of the S118A-ERα mutant to transactivate in response to activation of the MAPK pathway. Some groups have reported that mutation of Ser-118 to Ala resulted in a receptor that is completely unable to transactivate in response to EGF or overexpression of ras [12,56]. However, Joel et al. [54] found that, in response to overexpression of constitutively active MAPK kinase (MEK), the S118A-ERα mutant transactivated but at a diminished level compared to wild type ERα. The discrepancy between these results might also be explained by cell type and promoter specificity. Activation of the MAPK pathway brings additional complications to the model shown in Fig. 3 because the ability of co-activators to influence transcription can be effected by their phosphorylation. For example, MAPK phosphorylation of ERβ and steroid receptor coactivator-1 enhances their ability to interact and it is thought that this increased complex formation stimulates ERβ-mediated transcription [82,86]. MAPK phosphorylation of AIB1, a member of the p160 coactivator family that stimulates ER-mediated transcription, enhances the amount of histone acetylase transferase activity associated with AIB and thus facilitates chromatin reorganization [87]. However, mutation of Ser-118 to Ala did not reduce the ability of GRIP1, another member of the p160 coactivator family, to enhance ERα-mediated transcription and so in some cases the phosphorylation of the co-activator may be more important in regulating ERα activity than phosphorylation of ERα [88,89].

It is of interest to note that the kinetics of Ser-118 phosphorylation induced by estradiol binding and activation of the MAPK pathway are quite different [44]. In MCF-7 cells, estradiol induces a steady state phosphorylation of Ser-118 within 20 min, whereas in response to epidermal growth factor (EGF) or phorbol 12-myristate 13-acetate (PMA), Ser-118 is rapidly but only transiently phosphorylated. It is possible that phosphorylation of Ser-118 performs the same function either in response to estradiol binding or activation of the MAPK pathway. However, it is also possible that the transient phosphorylation of Ser-118 in response to EGF is not as important in MCF-7 cells as the EGF-induced phosphorylation of Ser-167 in ERα and of coactivators. In support of this latter hypothesis are observations that in MCF-7 cells, the EGF-induced transient MAPK activation leads to sustained activation of Rsk and Ser-167 phosphorylation in ERα (unpublished observations). The importance of Ser-167 phosphorylation has been shown by the diminished transcriptional ability in response to activation of the MAPK pathway of an ERα mutant, which has Ser-167 replaced by Ala, compared to the activity of the wild type ERα [54,69].

Clark et al. have suggested that Rsk2 plays a more important role than MAPK in the stimulation of ERα-mediated transcription in response to EGF. They have also shown that Rsk2 does not stimulate ERβ-mediated transcription and have hypothesized that MAPK plays different roles in regulating ERα and ERβ transcriptional activity. They have suggested that direct MAPK phosphorylation of ERβ is important in ERβ-mediated transcriptional activation whereas MAPK activation of Rsk and the subsequent Rsk phosphorylation of ERα is important in ERα transcriptional activation.

The AF-2 region plays an important role in mediating transcriptional activation by cAMP [90]. However, mutagenesis of putative PKA phosphorylation sites did not prevent activation of ERα-mediated transcription by cAMP [91]. It may be that PKA regulation of co-activator function rather than the phosphorylation of ERα plays a more important role in cAMP activation of ERα-mediated transcription. This hypothesis is supported by the observations that the association of cyclin D1 with ER is regulated by cAMP-dependent pathways [92]. Cyclin D1 has been shown to stimulate ERα-mediated transcription by a mechanism that does not depend on its ability to associate with cyclin-dependent kinase 4 [93,94].

Although there have been conflicting reports on the phosphorylation of Tyr-537, a number of investigators have shown that this Tyr residue does play an important role in AF2 function. Some mutations at Tyr-537 produce constitutively active receptors that are able to recruit co-activators in a ligand-independent manner [95,96]. Tyr-537 is also thought to be involved in the association of ERα with the c-src tyrosine kinase [59]. The importance of this interaction in regulating signal transduction by the MAPK pathway will be discussed later.

### 1.1.3. What is known about ER phosphatases?

Okadaic acid, an inhibitor of protein Ser/Thr phosphatases 1 and 2, has been shown to stimulate ERα-mediated transcription; however, the identities of the ER phosphatases are unknown [2]. The phosphatase Cdc25B, which activate Cdks by removal of inhibitory phosphates, acts as an ERα co-activator but its phosphatase activity is not required for its ability to enhance ERα-mediated transcription [97]. Inhibition of protein phosphatase 5 (PP5) expression stimulates glucocorticoid receptor-mediated transcription but no studies of other steroid receptors have been described [98]. However, vanadate, a Tyr phosphatase inhibitor, has been shown to stimulate proliferation of MCF7 cells presumably through activation of ER, since antiestrogens inhibited the proliferative response [99]. Vanadate is also known to stimulate the activity of the epidermal growth factor receptor (EGFR), resulting in enhanced MAPK activity [100]. The situation is confusing because antiestrogens can also prevent the stimulation of EGFR phosphorylation in response to vanadate, but the mechanism of this inhibition is unknown [99].

### 1.1.4. How does estradiol influence signal transduction pathways?

The classical model for estrogen action is through alterations in gene expression. However, evidence is accumulating for novel paradigms of estrogen action that occur rapidly and do not involve changes in gene expression. One of the most striking examples of the new paradigms is the activation of signal transduction cascades that are the result of estradiol-induced associations of ERα with the tyrosine kinase, c-src, and with the regulatory subunit of phosphatidylinositol-3-OH (PI(3)) kinase. It is also of interest to note that addition of estradiol increases cAMP levels by enhancing adenylate cyclase activity. However, the mechanism for this activation is unknown [10]. Additionally, ERα interacts with and stimulates autophosphorylation of the insulin-like growth factor-1 receptor in an estradiol-dependent manner by a mechanism that has not yet been determined [101].

Migliaccio et al. have shown that addition of estradiol to MCF7 cells increases MAPK activity and they have proposed that phosphoTyr-537 of ERα is involved in the recruitment of c-src in a ligand-dependent manner [57,59]. However, a mutant in which Tyr-537 has been altered to Phe was used to demonstrate the importance of the Tyr-537 in c-src binding. It is not clear that the inability of the mutant to bind c-src was only due to the elimination of Tyr because the Phe mutation destabilizes ERα, most likely by altering ERα conformation [77]. There also appears to be disagreement as to whether the estradiol-induced activation of MAPK depends on src [102]. Filardo et al. have proposed that estradiol activates MAPK through the release of heparin-bound EGF, which results in activation of the EGF receptor [103,104]. This group has also proposed that estradiol-dependent activation of MAPK depends on a G protein-coupled receptor rather than on the presence of the ER.

Migliaccio et al. [58,59] have shown that hormonal stimulation of the progesterone (PR) or the androgen receptor only in the presence of co-expressed ERα stimulates c-src activity. However, Boonyaratanakornkit et al. [105] have shown that purified PR interacts and stimulates the activity of c-src whereas purified ERα interacts with but is not able to stimulate the activity of c-src. They have suggested that the binding of PR to the SH3 domain in c-src relieves c-src autoinhibition, which results in an increase in kinase activity. Interestingly, they observed that in vivo co-expression of ERα was necessary to observe the hormonal stimulation of c-src activity by the PR. It appears that ERα lowers the basal activity of c-src in vivo, but the mechanism of this inhibition is not clear. Nonetheless, enhancement of c-src activity increases the extent of Shc phosphorylation, which stimulates the formation of the complex consisting of Shc, the adapter protein, GRB2 and the GTP exchange factor, Sos. This complex increases the amount of GTP bound p21ras, which leads to activation of Raf and the subsequent activation of MAPK.

There are now a number of conflicting reports on the ability of estradiol to activate MAPK in MCF7 cells and thus there must be unexplained differences between the MCF7 sublines [14,61–63,106–108]. It is important to note that the activation of MAPK by estradiol is substantially smaller than the activation that is observed with EGF or other growth factors. This observation is important because the physiological action of MAPK has been shown to depend on the extent of MAPK activation [109–112].

Estradiol has been shown to increase endothelial nitric oxide synthase activity in vascular endothelial cells [65,113,114]. This increase is apparently mediated by an estradiol-induced increase in phosphatidylinositol-3,4,5-$P_3$ (PI(3)) levels. ER$\alpha$ is able to form a ternary complex with the regulatory subunit of PI(3) kinase (p85) and src in a ligand-dependent manner [115]. It is possible that ER$\alpha$ interacts with the SH2 domain in p85 and that this interaction relieves the inhibition of the catalytic subunit of PI(3) kinase [116]. The thyroid receptor and glucocorticoid receptors were also able to interact with the p85 regulatory subunit in a ligand-dependent manner. Estradiol also activates AKT activity in an ER-dependent manner in vascular cells but in an ER-independent manner in breast cells [65,117].

In addition to the ability of ER$\alpha$ enhancing the activity of associated kinases, Clark et al. [69] have reported that the physical interaction of Rsk with ER$\alpha$ enhances the transcriptional activity of ER$\alpha$. ER$\beta$ has also been shown to interact with the Rsk related kinase, Mnk2 but the functional consequences of this association are unknown [118].

### 1.1.5. What lessons on the function of phosphorylation can be learned from other steroid receptors?

GR phosphorylation has been shown to be regulated in a cell cycle dependent manner, which influences GR transcriptional activity through the cell cycle [119]. I am not aware of any studies demonstrating cell cycle involvement in regulating ER$\alpha$ phosphorylation. In addition to enhancing transcriptional activation there is evidence that phosphorylation of PR and glucocorticoid receptor (GR) enhances their rate of degradation by the ubiquitin/proteasome pathway [120,121]. In response to hormone binding, the rate of ER$\alpha$ destruction is also enhanced by the ubiquitin/proteasome pathway but as yet there is no evidence that phosphorylation is involved in regulating ER$\alpha$ destruction [122–124].

### 1.2. Conclusion

In summary, in response to estradiol, ER$\alpha$ becomes predominantly phosphorylated on Ser-118 and to a lesser extent on Ser-104 and Ser-106. Activation of the MAPK pathway enhances phosphorylation of Ser-118 and Ser-167. Phosphorylation of Ser-118 leads to an increased association with known ER$\alpha$ co-activators. The mechanism by which Ser-104, Ser-106 and Ser-167 phosphorylation enhances ER$\alpha$-mediated transcription is not known. Phosphorylation of Ser-236 by PKA does not appear to be involved in the activation of ER$\alpha$-mediated transcription in response to activation of the PKA pathway. However, stimulation of the PKA pathway enhances the association of ER$\alpha$ with cyclin D1, a known enhancer of ER$\alpha$-mediated transcription. Tyr-537 is thought to play a critical role in the ability of ER$\alpha$ to interact with kinases containing SH2 domains; however, the phosphorylation status of this residue in vivo remains controversial. The association of ER$\alpha$ with various kinases appears to influence both the activity of the kinase and ER$\alpha$. The functional consequences of this crosstalk have only begun to be explored.

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