Direct Acetylation of the Estrogen Receptor α Hinge Region by p300 Regulates Transactivation and Hormone Sensitivity*


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Regulation of nuclear receptor gene expression involves dynamic and coordinated interactions with histone acetyl transferase (HAT) and deacetylase complexes. The estrogen receptor (ERα) contains two transactivation domains regulating ligand-independent and -dependent gene transcription (AF-1 and AF-2 (activation functions 1 and 2)). ERα-regulated gene expression involves interactions with cointegrators (e.g. p300/CBP, P/CAF) that have the capacity to modify core histone acetyl groups. Here we show that the ERα is acetylated in vivo. p300, but not P/CAF, selectively and directly acetylated the ERα at lysine residues within the ERα hinge/ligand binding domain. Substitution of these residues with charged or polar residues dramatically enhanced ERα hormone sensitivity without affecting induction by MAPK signaling, suggesting that direct ERα acetylation normally suppresses ligand sensitivity. These ERα lysine residues also regulated transcriptional activation by histone deacetylase inhibitors and p300. The conservation of the ERα acetylation motif in a phylogenetic subset of nuclear receptors suggests that direct acetylation of nuclear receptors may contribute to additional signaling pathways involved in metabolism and development.

Nuclear receptors coordinate diverse physiological roles in metabolism and development through ligand-dependent and -independent mechanisms (1). Nuclear receptors form multicomponent complexes with coactivator and corepressor proteins to orchestrate dynamic transcriptional events in response to ligand. In the absence of ligand, nuclear receptors repress transcription through a dominant association with corepressor complexes with histone deacetylase activity (2). Conformational changes induced upon nuclear receptor ligand binding release corepressors, with subsequent transient association of coactivator proteins (2–4). Estrogen binds the estrogen receptor (ERα), thereby regulating important functions in development and reproduction and in human diseases including breast cancer, cardiovascular disease, osteoporosis, and Alzheimer’s disease. The ERα contains domains conserved with other members of the “classical” receptor subclass (termed A—F) and two activation domains, AF (activation function)-1 and AF-2.

The two activation domains of ERα contribute synergistically to transcription of target genes. The AF-1 function is both constitutive and induced by mitogen-activated protein kinases (MAPKs) induced by growth factors or oncoproteins (5). p300 (6) and a p300/CBP-binding protein, p68 RNA helicase A (7), also induce AF-1 activity. Thus, p300 binds AF-1 in the absence of ligand (6, 8) inducing ERα activity 2–3-fold in either reporter or in vitro transcription assays (6, 8). p300/CBP binding to ERα is also detectable in MCF7 cells in the absence of ligand (4). The ligand-dependent transcription activation function (AF-2) domain of ERα consists of a conserved carboxyl-terminal helix. The AF-2 domain contributes to ligand-induced activity through further recruitment of coactivator proteins including the p160 family, (SRC-1, TIF2/GRIP1, AIB1/ACTR), the cointegrators (CBP, p300), and p300/CBP-associated factor (P/CAF) (2, 8, 9). The role of p300 as an ERα cointegrator is complex; p300 contributes to ERα induction through several separable subdomains including the histone acetyl transferase (HAT) and the bromodomain (4, 8, 10), which make separate contacts to distinct domains of the ERα.

The enhancement of transcriptional activity by p300/CBP involves several different functions. The cointegrators provide a bridging function, which associates transcription factors with the basal transcription apparatus (11). Second, p300/CBP pro-

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†† The abbreviations used are: ERα, estrogen receptor α; AF, activation function; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK (extracellular signal-related kinase) kinase; CBP, CREB (cAMP-response element-binding protein)-binding protein; IP, immunoprecipitation; HAT, histone acetyl transferase; HPLC, high pressure liquid chromatography; GST, glutathione S-transferase; TSA, trichostatin A; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; E2, estradiol; P/CAF, p300/CBP-associated factor; EKLF, erythroid Kruppel-like factor; ERE, estrogen response element.
ERα (H-184) antibodies from Santa Cruz Biotechnology (Santa Cruz, CA).

In vitro [35S]methionine-labeled proteins were prepared by coupled transcription-translation with a Promega TNT-coupled reticulocyte lysate kit (Promega), using 1.0 μg of plasmid DNA in a total of 50 μl. Flag-tagged P/CAF proteins were expressed in SF9 cells by infecting with recombinant baculovirus and purified using an anti-Flag antibody (Sigma, M2) (36). Full-length recombinant baculovirus ERα was obtained from Affinity Bioreagents, Inc. (Golden, CO).

Immunoprecipitation Histone Acetyltransferase Assays—Immunoprecipitation histone acetyl transferase (IP-HAT) assays were performed using p300 as described previously (16, 37). For immunoprecipitation the protein concentration was adjusted to 1 μg/μl in 500 μl. The relevant antibodies from Santa Cruz Biotechnology (p300, N15) were added (2 μg/500 μg of extract) and incubated at 4 °C for 2 h. A standard HAT assay was performed containing 5 μg of substrate and enzyme, either 200 ng of purified histone acetyl transferase (purified baculovirus p300 or P/CAF) or immunoprecipitated p300 from cultured cells (16, 37). The mixture was incubated at 30 °C for 1 h. 90 pmol of [35S]Acetyl-CoA reaction was electrophoresed on a SDS-polyacrylamide gel and viewed following autoradiography of the gel. [35S]Acetyl incorporation into the substrates was also determined by liquid scintillation counting or filter assays.

In Vitro Protein-Protein Interactions and Mapping the ERα Acetylation Sites—The interaction between the two activation domains of the ERα is due to failed binding or substrate selectivity. We assessed whether p300 could acetylate recombinant ERα in vitro. Recombinant p300 acetylated recombinant ERα but did not acetylate GST (Fig. 1A). In contrast, recombinant baculovirus-expressed P/CAF did not acetylate ERα, although it was capable of acetylating histone H3 and itself (Fig. 1B) as shown previously (39).

RESULTS

The ERα Is Acetylated by p300 in Vitro and in Vivo—The p300/CBP coactivator proteins have been shown to regulate several promoters in a manner dependent upon their histone acetylase activity (25), and p300 can both bind and stimulate the activity of the ERα (4, 8, 10). In addition, p300/CBP and P/CAF have been shown to acetylate non-core histone-related transcription factors directly through a conserved motif. We observed that p300 could acetylate recombinant ERα in vitro. Recombinant p300 acetylated recombinant ERα but did not acetylate GST (Fig. 1A). In contrast, recombinant baculovirus-expressed P/CAF did not acetylate ERα, although it was capable of acetylating histone H3 and itself (Fig. 1B) as shown previously (39).

The ERα Is an Efficient and Selective Substrate for p300 Acetylation in Vitro—Two fundamental types of questions raised by these studies are, first, the relative efficiency of ERα acetylation and, second, whether the failure of P/CAP to acetylate the ERα is due to failed binding or substrate selectivity. To assess the relative efficiency with which p300 acetylates the ERα, a direct comparison was made between equimolar amounts of ERα and histone H3. The products acetylated by increasing amounts of p300 were electrophoresed on a SDS-polyacrylamide gel and the incorporation of [3H]Acetyl-CoA assessed (Fig. 2A). The efficiency of incorporation on an equimolar basis was ~3-fold greater for histone H3 (16 kDa) than ERα (66 kDa) (Fig. 2B), suggesting ERα is acetylated with substantial efficiency. Thus the ERα is efficiently and selectively acetylated by p300 in vitro.
P/CAF has been reported to associate with ERα in vitro (40). We examined whether the recombinant P/CAF used in the HAT assays bound to the ERα. As shown in Fig. 2C, recombinant P/CAF bound with high affinity to ERα, and binding required the HAT domain. Thus, although P/CAF acetylates histone H3 and H4, the failure of P/CAF to acetylate ERα is not due to...
equal amounts of GST-ER IP-HAT assays were performed using (C). Caacetylation sites of the ER.

Recombinant P/CAF failed to acetylate P/CAF. p300 acetylated the ER1 polypeptide but did not acet-

tylated (ER2). HAT assays were performed using recombinant p300 or and a second polypeptide including lysine 366 (ER2-(353–370)) encoding the consensus acetylation motif ER1-(293–310) (ER1) for acetylation by p300.

The products corresponding to the expected molecular weight were excised from residues 282–337 was sufficient to function as a substrate for acetylation by p300.

Acetylated Residues Regulate Basal Activation of the ERα by TSA—To examine the role of histone acetylases in the regulation of ERα activity, an estrogen-responsive luciferase reporter gene was assessed in ERα-deficient cells (MDA MB231). Inhibitors of histone deacetylase(s) trichostatin A (TSA) and sodium butyrate were added to transfected cells for 24 h. TSA induced the ER-E/LUC reporter (ERE1Tkpaluc) 4–6-fold (Fig. 5A). Similarly, sodium butyrate (1 mM) induced ER reporter activity 2-fold (Fig. 5B). To examine the functional consequence of lysines 302 and 303 in ERα function, point mutation of the ERα acetylation sites was performed. The ER-responsive reporter was assayed in ERα-deficient cells (MDA MB231 and HeLa). Activity was assessed through normalization to the internal standard β-galactosidase reporter. The 2-fold induction of wild type ERα by sodium butyrate was abolished by the ER1K302A/K303A mutant (Fig. 5C).

The abundance of the ERαK302A/K303A mutant was similar to ERα wild type in cultured cells (Fig. 5D). HeLa cells were transfected with either wild type ERα or mutants of the acetylation site and assessed for ERα activity. The wild type ERα was induced 2-fold by the addition of TSA in a dose-dependent manner (Fig. 5E). The alanine and threonine substitutions failed to respond to TSA (Fig. 5E). Together these findings suggest that direct ERα acetylation contributes to induction by histone deacetylase inhibitors.

MAPK-induced ERα Functions Independently of the ERα Acetylation Site—To investigate further the in vivo consequence of the ERα acetylation site, point mutation substitutions were introduced into the wild type ERa at the lysine residues acetylated in vitro. It was reasoned that the acetyl-

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** Mapping p300-mediated acetylation sites of the ERa. A, schematic representation of the ERα (indicating the A–F domains, DNA binding domain (DBD), the ligand binding domain (LBD), and the conserved RXKK motif) and the GST-ERα fusion proteins. B, the Coomassie-stained gel corresponding to the GST-ERα fusion proteins (upper panel) and the 14C-labeled ERα proteins (lower panel). C, p300-mediated in vitro IP-HAT assays were performed using equal amounts of GST-ERα fusion protein. The products corresponding to the expected molecular weight were excised and HAT activity quantitated by liquid scintillation counting. and assessed for ERE activity. The wild type ERα by TSA—To examine the role of histone acetylases in the regulation of ERα activity, an estrogen-responsive luciferase reporter gene was assessed in ERα-deficient cells (MDA MB231). Inhibitors of histone deacetylase(s) trichostatin A (TSA) and sodium butyrate were added to transfected cells for 24 h. TSA induced the ER-E/LUC reporter (ERE1TkpaLUC) 4–6-fold (Fig. 5A). Similarly, sodium butyrate (1 mM) induced ER reporter activity 2-fold (Fig. 5B). To examine the functional consequence of lysines 302 and 303 in ERα function, point mutation of the ERα acetylation sites was performed. The ER-responsive reporter was assayed in ERα-deficient cells (MDA MB231 and HeLa). Activity was assessed through normalization to the internal standard β-galactosidase reporter. The 2-fold induction of wild type ERα by sodium butyrate was abolished by the ER1K302A/K303A mutant (Fig. 5C). The abundance of the ERαK302A/K303A mutant was similar to ERα wild type in cultured cells (Fig. 5D). HeLa cells were transfected with either wild type ERα or mutants of the acetylation site and assessed for ERα activity. The wild type ERα was induced 2-fold by the addition of TSA in a dose-dependent manner (Fig. 5E). The alanine and threonine substitutions failed to respond to TSA (Fig. 5E). Together these findings suggest that direct ERα acetylation contributes to induction by histone deacetylase inhibitors.

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failed binding. These findings are consistent with the observation that p300 and P/CAF have distinguishable substrate specificitites (21).

**Identification of the ERα Acetylation Sites—**To identify the residues required for ERα acetylation in vitro, recombinant GST-ERα fusion fragments were expressed, their integrity was confirmed by Western blotting using a GST antibody, and equal amounts of proteins were assayed in HAT assays using recombinant p300 as a source of HAT activity and the previously described filter assay (16). As shown in Fig. 3, B and C, the ERα from residues 282–337 was sufficient to function as a substrate for acetylation by p300.

Peptides were synthesized to encompass the two lysine-containing motifs identified within the region of the ERα acetylated in vitro (Fig. 3D). We identified residues resembling an acetylation motif found in the p53 and GATA-1 transcription factors, which were conserved between species (Fig. 3D). An additional lysine, residue 362, was identified that had been implicated previously in ligand-regulated ERα function (41). Poly peptides were synthesized therefore to include residues encoding the consensus acetylation motif ER1-(293–310) (ER1) and a second polypeptide including lysine 366 (ER2-(353–370)) (ER2). HAT assays were performed using recombinant p300 or P/CAF. p300 acetylated the ER1 polypeptide but did not acetylate ER2 (Fig. 3D). Recombinant P/CAF failed to acetylate either ER polypeptides.

Mass analysis of the acetylated ER1 peptide confirmed the presence of two major ions differing by 42 mass units, with the smaller molecular weight product corresponding to the unmodified ER1 peptide and the higher molecular weight component corresponding to the acetylated ER1 product (Fig. 4A). Following in vitro acetylation of the ER1 peptide, Edman degradation assays were performed. As only monoaucetylated lysine-containing peptides were detected in the samples by MALDI-TOF mass spectrometry, the product analyzed by Edman degra-
tion of a lysine, a positively charged, hydrophobic residue, is thought to both reduce its charge and increase its polarity. If acetylation augments activity through increasing the polarity or reducing the charge, a mutation of the two ERα lysines to polar residues, ER<sub>K302Q/K303Q</sub>, may function as an activating mutant. The introduction of a large positively charged amino acid with a significant side chain (ER<sub>K302Z/K303Z</sub>) might be anticipated to mimic acetylation if increasing polarity is of greater importance. Substitution of lysine to alanine, (ER<sub>K302A/V303A</sub>) or another small hydrophobic threonine residue (ER<sub>K302T/K303T</sub>) was anticipated to result in a loss of function. If the post-translational modification of acetylation itself were important in regulating ERα activity, the substitution of the lysine residues with any of these other residues would be expected to have a similar effect. The results of these studies are shown in Fig. 6. The mutant ERα proteins were expressed equally in transfected cells (data not shown). HeLa cells were transfected with either wild type ERα or mutants of the acetylation site and assessed for their ability to regulate the activity of a synthetic ERE in the absence of ligand.

Assessment was made of the AF-1 function mediated by MAPK signaling. Growth factors induce ligand-independent activity of the ERα through activation of MAPK (5) and the p160 coactivator AIB1 (also named RAC3, ACTR, or SRC3) (42). p160 proteins bind p300 (43) and contact both the AF-1 and AF-2 of the ERα (44, 45). To determine whether the lysine substitutions within the ERα hinge region regulated MAPK-dependent ERα activity, constitutively activated MEK1 (ΔN3, ΔN3-S218E-S222D) were coexpressed with the ERα mutants (Fig. 6A). The wild type ERα was induced 3.5-fold by activated MEK1 but was not significantly induced by the catalytically defective MEK1 (K97 m). The basal activity of the ERα<sub>K302A/K303A</sub> mutant was reduced 2.5-fold; however, the magnitude of induction by activated MEK1 was not significantly changed for any of the mutants (Fig. 6A). The finding that the ERα acetylation mutants are not altered in their responsiveness to MAPK activation suggests the mechanisms governing ligand-induced ERα activity through the ERα acetylation site are distinct from those governed by ACTR.

The ERα Acetylation Site Governs Ligand Sensitivity—In previous studies of ERα activity in HeLa cells using a similar reporter assay, estradiol (10<sup>−8</sup> M) induced ERα-dependent luciferase activity 2-fold (41). In our studies the wild type ERα gave a similar 2-fold induction upon the addition of estradiol (10<sup>−8</sup> M) (Fig. 6B). This ER<sub>α</sub>_TK81LUC reporter is not induced by 10<sup>−10</sup> M E2 in HeLa cells with the wild type ERα; however, both the glutamine and arginine substitutions were induced by 2–3-fold, suggesting the positive charge of these residues may contribute to ligand sensitivity (Fig. 6B). The hinge domain mutants were compared with the wild type ERα for ligand-dependent transactivation using increasing concentrations of E2. Enhanced E2-dependent activity was observed for each of the ERα mutations of the hinge region lysines residues. Thus, uncharged, polar, or hydrophobic substitutions of the ERα enhanced ligand sensitivity. As each of the ERα mutants exhibited similar levels of expression to wild type ERα, and the wild type ERα functioned in the same manner as the ERα wild type in other studies in this cell type (41), these findings suggest that the wild type lysine residues within the ERα hinge region may play a role in normally repressing ligand-dependent ERα activity.

We next assessed the role of the hinge domain lysine residues in p300-dependent regulation of ERα function. The modest induction of wild type ERα activity by p300 in the absence of ligand (Fig. 6C) is consistent with studies by others. Binding of p300 to the ERα in the absence of ligand and a 2–3-fold induction of ERα activity in the absence of ligand were observed both in reporter assays (6) and in in vitro transcription assays (8). Conformational changes induced by the addition of estradiol recruits p160 coactivators to a hydrophobic fold in the ERαs with the p300 cointegrator (9). Because mutation of the lysine residues of the ERα enhanced ligand sensitivity, we hypothesized that substitutions of these lysines may also enhance p300-dependent transactivation of the ERα in the presence of E2. In keeping with this model each of the ERα acetylation mutants demonstrated enhanced activation by p300 in the presence of hormone (Fig. 6C). These findings raise the possibility that this region of the ERαs may serve to dock repressor proteins or that direct acetylation of the ERα may play a role in ligand-dependent transcriptional attenuation, as was recently described for the direct acetylation of ACTR by p300 (4). Crystal structural analyses showed the LXXLL motif of the coactivator GRIP1 forms the core of a short amphipathic α helix that contacts helices 3, 5/6, 11, and 12 of the ERα; however, the exact proximity of the ERα<sub>K302A/K303A</sub> residues...
required for the acetylation of ERα in cultured cells. This would seem unlikely, however, as mutations of the ERα acetylation site, which could not be acetylated in vitro, conveyed enhanced ligand sensitivity in cultured cells. To determine whether ERα is acetylated in vivo, a polyclonal antibody raised against acetylated lysines (16) was used to immunoprecipitate acetylated proteins from MCF7 cells. The IP product was subjected to SDS-polyacrylamide gel electrophoresis and probed with an ERα antibody. Fig. 6D shows that the ERα antibody specifically recognized ERα protein within the anti-acetylated lysine immunoprecipitate (upper panel). Because the coactivator ACTR is acetylated by itself (4), the coinmunoprecipitation of the ERα may potentially be due to cross-reactivity with ACTR. Therefore, a reciprocal immunoprecipitation was performed in which we used the ERα antibody to IP ERα from MCF7 cells, and Western blotting was performed with the anti-acetyl lysine antibody (Fig. 6D, lower panel). The acetyl lysine immunoreactive band corresponding to the molecular weight of the ERα was observed in the ERα IP but not with the control IgG IP. Together these studies indicated that the ERα is acetylated in cultured cells consistent with previous findings that p300 binds and regulates ERα in the absence of ligand in vivo (4, 6, 8).

DISCUSSION

The regulation of estradiol signaling by direct ERα acetylation reveals an unexpected and novel role for histone acetyltransferase in hormone signaling. Nuclear receptors have been shown to form multiprotein complexes with coregulatory proteins that possess either histone acetylase or histone deacetylase activity (4, 47). The evidence that the ERα is a direct substrate for HAT activity and may thereby regulate hormone-dependent transactivation function remained to be demonstrated. Here we have shown that ERα is acetylated in vivo and is a substrate for selective acetylation by p300 in vitro. Although cointegrators recruited to ERα share a redundant capacity to acetylate histones, herein the ERα was selectively acetylated by p300. The select enzymatic activities of p300 and P/CAF toward ERα are consistent with their structurally divergent HAT domains (36, 48). Mutagenesis demonstrated a critical role for the ERα acetylation site in regulation by histone deacetylase inhibitors. The finding that mutations with the ERα hinge domain lysine residues enhanced hormone sensitivity suggests these residues may be involved in ligand-dependent transcriptional repression or transcriptional attenuation. The finding that the lysine residues within the ERα that are substrates for the HAT activity of p300 may function in transcriptional repression suggests that cointegrator proteins acetylate several distinct substrates with distinct effects to coordinate genomic responses.

The mechanisms governing substrate specificity of HATs are not well understood at this time (49). P/CAF did not acetylate ERα but was capable of efficiently acetylating histone H3 and binding ERα. These findings suggest that p300 and P/CAF, although both capable of binding ERα, convey select enzymatic activities, consistent with the lack of sequence similarity within their HAT domains (36, 48). From previous studies of TAFII250 it is known that the bromodomain modules form selective interactions with multiple acetylated histone H4 peptides (50). To understand the mechanisms responsible for the failure of P/CAF to acetylate ERα, we performed an analysis of P/CAF domain mutants to identify the sites of interaction with the ERα lysine motif peptide. These studies revealed the surprising result that the P/CAF bromodomain was dispensable and that the HAT domain was required for binding to ERα. It is possible that the interaction surfaces may determine subsequent acetylation activity. Alternatively, the acetylation motif of the substrate may be critical. The ERα acetylation motif re-
sembles the GATA-1 and p53 acetylation sites. GATA-1, EKLF, and ACTR are selectively acetylated by p300/CBP (4, 19, 22). By contrast, P/CAF preferentially acetylates E2F-1 and MyoD in vitro (20, 51). p53 contains two acetylation sites differentially acetylated by either p300 (16) or P/CAF (21). Although the determinants of the histone acetylase substrate preference are poorly understood, this substrate specificity may form the biochemical basis for functional synergy and promoter selectivity.

In the current studies, mutation of the ERα in vitro acetylation site enhanced ligand sensitivity. The 2-fold induction of the synthetic estrogen-responsive enhancer reporter gene ER2-TK31pA-LUC at $10^{-8}$ M 17β-estradiol with the wild type ERα was identical to the induction observed by other investigators in HeLa cells using a similar luciferase reporter gene (16). Although the magnitude of induction of synthetic estrogen-responsive reporters can be enhanced by increasing the number of ERE enhancer sites, changing the type of minimal promoter, or altering the cell type (52), the high sensitivity of the assays allowed clear discrimination of basal compared with induced activity in the current studies. The expression of the acetylation site ERα mutants was identical in cultured cells, allowing a clear comparison of their functional activities. When comparing between the double point mutants, there was a tendency for the mutant with substitution of threonine (a hydrophobic polar residue) to have higher induction by E2 than other substitutions (3-fold versus 2-fold). Nonetheless, each mutation of the lysines within the acetylation motif enhanced hormone sensitivity compared with wild type ERα (p, 0.05), suggesting that the acetylation modification itself govern hormone sensitivity. These findings are consistent with recent observations in which mutation of an acetylation motif within the coactivator ACTR resulted in transcriptional attenuation of ERα signaling (4).

In the current studies, ERα acetylation site mutations that enhanced ligand sensitivity did not affect ERα activation by the MAPK signaling pathway, suggesting direct acetylation of the ERα affects a specific subset of ERα activities. MAPK regulation of ERα involves both direct phosphorylation and regulation of coactivators themselves. Our finding that the ERα acetylation mutation does not affect MAPK signaling distinguishes regulation of ERα activity from the mechanisms governing ERα regulation by the p160 coactivator ACTR/AIB1. ACTR is phosphorylated and activated by MAPK, contributing
transcription assays (8) or in reporter assays (6), together suggest that p300 conveys important ligand-dependent and -independent functions. Estradiol treatment of MCF7 for 24 h cells does not change the abundance of p300, histone deacetylase-1, or ERα (4), and the induction of histone H4 acetylation at target promoters in response to ligand is quite transient (4). Conformational changes induced by the addition of estradiol are known to recruit p160 coactivators to a hydrophobic fold in the ERα with the p300 coindicator (9). As noted above, the LXLL motif of the coactivator GRIP1 forms the core of a short amphipathic α helix that contacts helices 3, 5/6, 11, and 12 of the ERα; however, the exact proximity of the ERα(K302A/K303A) residues to the ERα hydrophobic fold remain unknown (46). Future studies will discern whether the increased ligand sensitivity of these ERα acetylation mutants is due to enhanced recruitment of coactivators within the local promoter context or to loss of binding to transcriptional repressors.

These studies raise several important new type of question regarding the direct acetylation of the ERα affects interactions with other coactivators and corepressors, DNA binding within native chromatin at estrogen-responsive promoters of target genes, the function of the ERα in vitro transcription assays, and the effect of these mutations on selectivity of estrogen signaling pathways. In the current studies, mutational analysis of the ERα acetylation site demonstrated dissociable effects of histone deacetylase inhibitors (TSA) and the addition of ligand on ERα activity. The induction of ERα activity by the histone deacetylase inhibitors TSA and sodium butyrate was abolished upon substitution of the acetylated lysine residues with small hydrophobic residues, either alanine or threonine, suggests that basal ERα activity is under constitutive repression by histone deacetylase-containing complexes and that the lysine residues may contribute to a surface recruiting such complexes. In the absence of ligand, nuclear receptors have been shown to exist in multiprotein complexes containing N-CoR (nuclear receptor corepressor) or related proteins (54) together with histone deacetylases and homologues of the yeast corepressor Sin3, which repress gene transcription (47, 55, 56). As estrogen is mitogenic in mammary epithelial cells, the enhancement of ligand-dependent transactivation induced by mutation of these ERα target lysines may be predicted to confer a growth advantage. The same mutant that we demonstrated as conveying enhanced ligand sensitivity for transactivation (ERαK302R) was recently shown to occur in 34% of premalignant human breast lesions, suggesting that these acetylated residues play an important role in ERα function and biology (57). The ERα acetylation motif is conserved between species and between phylogenetically related nuclear receptors (58) (Fig. 7). Mutations of the conserved lysine motif have been identified in the ERα in breast cancer as has the androgen receptor in prostate cancer. Because nuclear receptors that contain the candidate acetylation motif contribute to diverse roles in the regulation of growth, development, and homeostasis (1), these studies may have possible implications in understanding regulation and function of many nuclear receptors.
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