The Inhibitory Function in Human Progesterone Receptor N Termini Binds SUMO-1 Protein to Regulate Autoinhibition and Transrepression*

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Although most studies of progesterone receptors (PR) and their two isoforms, PR-A and PR-B, focus on transcriptional stimulation, the receptors exhibit important inhibitory properties. Autoinhibition refers to an inhibitory function located in the PR N terminus, whose deletion increases transcriptional activity at least 6–10-fold. Transrepression refers to the ability of PR-A to suppress the transcriptional activity of PR-B and other nuclear receptors, including estrogen receptors. Self-squelching refers to the observation in transient transfection assays that increasing receptor concentrations paradoxically decrease transcriptional activity. Using a series of N-terminal deletion mutants constructed in both PR isoforms, we have mapped their autoinhibition and transrepressor activities to a small ubiquitin-like modifier (SUMO-1) protein consensus-binding motif, 387IKEE, located in the N terminus upstream of AF1. Self-squelching does not involve this site. SUMO-1 binds PR covalently at 387IKEE, but only if the C-terminal, liganded, hormone-binding domain is also present. A single point K388R mutation within the 387IKEE motif in either PR-A or PR-B leads to a loss of autoinhibitory and transrepressor functions of the liganded, full-length receptors. We conclude that autoinhibition and transrepression involve N-terminal sumoylation combined with intramolecular N/C-terminal communication.

Human progesterone receptor A (PR-A) and B (PR-B) isoforms are members of the nuclear receptor family of ligand-activated transcription factors (1). The two PR are identical except that PR-B contains an additional 164 N-terminal amino acids in the B upstream segment (BUS) (2–4). As with other nuclear receptors, PR-A and PR-B have a multidomain structure, including a centrally located DNA-binding domain (DBD), which is flanked at its N terminus by an activation function (AF1), and at its C terminus by a nuclear localization signal (NLS) and hormone binding domain (HBD) containing a second activation function (AF2). A third activation function (AF3) in PR-B maps to critical amino acid residues within BUS (5, 6). In most exogenous contexts, as well as on many endogenous genes, PR-B are stronger transactivators than PR-A (7–10). The greater activity of PR-B has been attributed to synergism between AF3 and the downstream AFs. As a result of these isoform-specific functional differences, tissue responses to progesterone are profoundly affected by PR-A:PR-B ratios, which vary considerably among normal target tissues and breast cancers (11).

The multiplicity of regulatory effects that impact PR and other nuclear receptors is due in part to an intricate array of coactivators, corepressors, and cointegrators that are recruited to receptor-bound promoters (12). Studies of coregulators have focused mainly on transcriptional activation, but transcriptional repression is also critical to understanding gene regulation. Repression can occur over entire chromosomal loci or by global targeting of general transcription factors (13). However, transcription factor-specific repression also involves mechanisms such as direct interference with DNA binding, blockade of coactivator binding, or nucleosome remodeling by histone deacetylation (14). Of these, the interaction surfaces presented to coregulators by the receptors, possibly controlled by the DNA-binding site, are clearly important. For example, liganded glucocorticoid receptors (GR) are activators as dimers on palindromic DNA response elements but may be repressors in the monomeric state (15). Even coregulatory proteins, like RIP140, PIAS1, and SRC1, can exhibit either coactivator or corepressor activity, depending on expression levels, the nuclear receptor target, and the promoter (16, 17). Thus, transcriptional repression can result from combinatorial influences on specific transcription factors and coregulators that together reduce binding affinity to the basal transcription apparatus, induce formation of impaired preinitiation complexes, target alternative promoters, or alter protein stability (18).

Transcriptional regulation of nuclear receptors has also been variably linked to protein phosphorylation (19, 20), acetylation (21), ubiquitylation (22, 23), and sumoylation (24). These post-translational covalent modifications alter protein structure and protein–protein interactions, but in most cases, the mechanisms by which these modifications influence function are unknown. Phosphorylation of nuclear receptors regulates both their ligand-dependent and ligand-independent transcriptional activities (25). Ubiquitylation, which targets nuclear receptors for degradation, paradoxically increases their transcriptional activity (23). Sumoylation, about which little is known, involves...
covalent binding of the SUMO (small ubiquitin-like modifier) protein. The prototype, SUMO-1, is a member of a family of ubiquitin-like proteins that are post-translationally conjugated predominantly to nuclear proteins (18). Several important transcription factors, including GR, androgen receptors (AR), p53, c-Jun, and c-Myb are covalently modified by SUMO-1 binding (24, 26–29). Like ubiquitylation, sumoylation uses a battery of activating (E1), conjugating (E2), and ligating (E3) enzymes for covalent attachment (30). In contrast to ubiquitylation, polysumoylation does not occur, and sumoylation does not lead to protein degradation, at least directly. Instead, influences on a variety of cellular processes and pathways have been described, including effects on assembly of protein complexes that regulate DNA recombination and repair (31, 32), effects on subcellular localization and targeting of proteins to nuclear pores and nuclear bodies (33, 34), effects on protein stabilization by blocking ubiquitylation (35), and effects on chromatin structure and DNA binding (36).

The link between SUMO-1 binding and transcriptional repression is unclear given this multiplicity of cellular effects. However, recently, a novel synergy control (SC) motif was identified by the N-terminal region of GR (28) and other transcription factors (27) that acts as a negative regulatory region. The SC motif has no effect when the transcription factor is bound at a single DNA response element, but it blocks synergistic activity resulting from cooperative DNA binding of a transcription factor to multiple DNA response elements. Intriguingly, the SUMO-1 consensus binding motif, KXE (where X is hydrophobic and is any residue) is identical to the SC motif (28). It follows that suppression of transcriptional synergy and repressor activities might work through a common pathway.

We (37) and others (38, 39) have identified an inhibitory function (IF) in the N-terminal 165–456 amino acids between AF3 and AF1 common to both PR isoforms. Deletion of all or part of this autoinhibitory region in PR-A increases their transcriptional activity. This autoinhibition is also transferable to related nuclear receptors. When the PR IF domain is fused to the N terminus of human estrogen receptors (ER) (37) or chicken PR (39), their transcriptional activity is repressed. Similar autoinhibitory regions have been identified in other transcription factors. Examples include c-Jun, c-Fos, and other members of the AP1 protein family (27), ATF-2, a member of the ATF/CAMP regulatory element-binding protein family, and Oct-2a, a lymphoid-specific transcription factor (40). A transrepressor activity, distinct from autoinhibition, also maps to the IF domain of PR-A. This activity is defined by the ability of PR-A to inhibit the transcriptional activity of PR-B, ER, or GR (38, 39). The mechanisms that distinguish autoinhibition from transrepression, both of which originate from the IF domain, are unclear. Recent attempts to precisely map these N-terminal repressor functions have led to ambiguous conclusions, identifying multiple, nonoverlapping regions (38, 39).

We have now identified a consensus SUMO-1-binding motif, 387IKEE within the IF domain of PR that is responsible for and precisely maps both the autoinhibitory and transrepressor activities of PR-A and PR-B. Conservative K388A and K388R single point mutations of the Lys residue within this motif completely abrogate both repressor activities. Loss of repression is directly linked to the loss of SUMO-1 binding at the PR N terminus. Interestingly, SUMO-1-dependent repressor activities arising at the N terminus require the liganded C-terminal HBD, suggesting that the mechanism involves intramolecular communication between the N and C termini.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—The expression plasmids pSG5 hPR1 and pSG5 hPR2, encoding human PR-B and PR-A, respectively, and HEGO, encoding human ER, cloned into pSG5 were gifts from P. Chambon (Strasbourg, France). Wild type pE GFP-SUMO-1 and mutant pE GFP-SUMO-1G97A were gifts from J. Palvimo and O. Janne (University of Helsinki, Helsinki, Finland). To generate N-terminal deletion mutants of both hPR-A or hPR-B which contain the N terminus, DBD, and NLS of PR-A but lack the hinge and HBD), we used a 5’ sense primer containing an EcoRI site, a Kozak consensus sequence, and the ATG initiation codon. The 3’-antisense primer contained a stop codon and the BglII site. The resulting PCR fragment was cloned into pSG5 digested with EcoRI/BglII. For PR-B deletion mutants, the 5’ primer was designed in frame with RsrII site located at the PR-B/RPA-A junction and the 3’-primers were identical to those used for PR-A. The resulting PCR fragment was cloned into pSG5 hPR1 or NTB digested with RsrII/BglII. Other deletions within PR-B and PR-A (PR-BΔ375–397 and PR-AΔ375–397), were created by inserting BamHI sites in PR-A at Pro297 and Ser298 by PCR-mediated mutagenesis and ligating the two sites to produce the deletion. PR-AΔ375–397 was then digested with RsrII/BglII, and the resulting fragment was ligated into RsrII/BglII-digested hPR1 to generate PR-BΔ375–397. To mutate the PR-A ATG initiation codon in PR-B, we used a 5’ primer containing an EcoRI site and a 3’ primer in which the ATG was mutated to GCG (Ala) and linked to an RsrII site. The PCR fragment was cloned into pSG5 hPR1 cut with EcoRI/RsrII. The point mutations, K388A and K388R, were generated by PCR using a 5’ sense primer in which AAG was mutated to AGG or GGC, respectively. The mutated fragments were introduced into pSG5-hPR1 or pSG5-hPR2 using MluI/HindIII sites. All PCR-based cloning was verified by dyeoxy sequencing.

**Transient Transfection Assays**—HeLa cells were plated at a density of ~1.2 × 105 cells in 60-mm tissue culture dishes with 3 ml of minimal essential medium supplemented with 7.5% charcoal-stripped fetal calf serum. Quadruplicate plates were transfected using calcium phosphate coprecipitation with 2 μg of the reporter plasmids, PRE1-TATA-luciferase (6) or ERE1-TATA-luciferase, variable amounts of PR or ER expression vectors, 2 μg of the β-galactosidase expression plasmid pCH110 (Pharmacia Corp.) to correct for transfection efficiency and Bluescribe (Stratagene, La Jolla, CA) carrier plasmid for a total of 6 μg plate. After overnight incubation at 37 °C, the cells were washed, the medium was changed to 7.5% minimum essential medium supplemented with 7.5% charcoal-stripped fetal calf serum, and the cells were incubated with 10 nm of the synthetic progestin R5020, 10 nm 17β-estradiol, or both for an additional 24 h. The control cells received ethanol only. The cells were harvested, the lysates were normalized to β-galactosidase activity, and the luciferase activity was quantified using Promega Monolight 3010 luminometer (Analytical Luminescence Lab., Ann Arbor, MI).

**Immunoblotting**—Whole cell extracts were prepared from HeLa cells transiently transfected with PR expression vectors as described previously (37). The expressed proteins were resolved by electrophoresis on SDS-polyacrylamide gels, transferred to nitrocellulose, and probed with our anti-PR monoclonal antibodies, AB-52 and B-30 (41), and with an anti-BD polyclonal antibody, α266 (a gift of D. Toft, Rochester, MN) (42). The bands were detected by enhanced chemiluminescence (PerkinElmer Life Sciences). For detection of PR-SUMO-1 binding, HeLa cells cotransfected with PR-A, PR-B, or PR mutants and GFP-tagged SUMO-1 or SUMO-1G97A were collected in PBS containing 20 mM N-ethylmaleimide, and the cell extracts were prepared in modified RIPA buffer (50 mM Tris-HE, pH 7.5, 150 mM NaCl, 5 mM EDTA, 15 mM dithiothreitol, protease inhibitor mixture (Roche Molecular Biochemicals), and 20 mM N-ethylmaleimide). The expressed proteins were resolved on SDS-PAGE, and conjugated protein was detected by immunoblottting with AB-52 or an anti-GFP polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

**RESULTS**

**Definition of Terms**—Several studies (6, 37–39) have identified at least three different inhibitory properties of PRs. Auto-inhibition refers to an IF located in the PR N terminus whose deletion increases the activity of PR-A at least 6-fold from a tandem progesterone response element (PRE1)-containing promoter. Transrepression refers to the ability of PR-A to suppress the transcriptional activity of PR-B and other nuclear receptors at low concentrations in which self-squelching is excluded (43, 44). For example, the transcriptional activity of ER from a
promoter containing an ERE is inhibited by liganded PR-A even in the absence of a PRE. Self-squelching refers to the observation with transiently expressed PRs and other nuclear receptors (6, 37, 39) that as the concentration of exogenously transfected receptors increases, there is a paradoxical decrease in transcriptional activity from a cotransfected promoter/reporter. The studies below were designed to address the mechanisms underlying the autoinhibitory and transrepressor properties of PR.

The Autoinhibitory Region in IF Maps to Residues 375–397—We previously mapped IF to the large N-terminal region between residues 165 and 456 in the PR N terminus common to both isoforms (37). To map IF further, a series of N-terminal deletion mutants were constructed in PR-A and PR-B (Fig. 1A). A concentration (100 ng) of expression vector cDNA for each mutant that yields maximal activity without self-squelching (see Fig. 5) was cotransfected into HeLa cells together with a PRE<sub>E</sub>-TATA-luciferase reporter, followed by R5020 treatment. The transcriptional activities of the mutants are expressed as percentages of wild type PR-B activity. Stepwise deletions to residue 375 in PR-B (PR-B<sub>Δ165-315</sub>, PR-B<sub>Δ165-345</sub>, and PR-B<sub>Δ165-375</sub>) or PR-A (PR-A<sub>Δ290</sub>, PR-A<sub>Δ315</sub>, PR-A<sub>Δ345</sub>, and PR-A<sub>Δ375</sub>) had little effect on transcriptional activity compared with the wild type receptors. However, further deletion to residue 397 (PR-B<sub>Δ165-397</sub>, PR-A<sub>Δ397</sub>) resulted in 7.5-fold (PR-B) or 6-fold (PR-A) increases in transcriptional activity. These deletions map the autoinhibitory function to residues 375–397. Additional deletions to PR-B<sub>Δ456</sub> or PR-A<sub>Δ427</sub> lead to some loss of activity because of encroachment into AF1. The location of the autoinhibitory region within IF was confirmed with an internal deletion mutant (Δ375–397) in PR-B and PR-A. This 22-amino acid deletion increased the activity of PR-B 11-fold and that of PR-A 6-fold.

N-terminal Residues 375–397 Require the C-terminal HBD for Autoinhibitory Activity—Selected N-terminal deletion mutants were then constructed in the background of NTa and NTb, the constitutively active N-terminal fragments of PR-A and PR-B, respectively, which contain the entire N terminus plus the DBD and NLS but lack the HBD (Fig. 1B). Constructs (100 ng) were transfected into HeLa cells and tested for transcriptional activity on PRE<sub>E</sub>-TATA in the absence of hormone. Analogous to their full-length counterparts, the activity of NTa is ~10% that of NTb. This residual activity is due to AF1 and is entirely lost in the NTaΔAF1 construct. Interestingly, in both N-terminal backgrounds (Fig. 1B), deletion of the autoinhibitory region (NTbΔ165–397 and NTaΔ397) identified in Fig. 1A failed to increase transcription. We conclude that the C-terminally liganded HBD is required for N-terminal autoinhibitory activity in both isoforms. Thus, residues 375–397 in the N terminus are necessary but not sufficient for full expression of the autoinhibitory effect.

Fig. 2 shows immunoblots using the anti-PR monoclonal antibodies AB-52 (B-specific) and B-30 (B-specific) or the anti-DBD polyclonal antibody a266 to document protein expression levels of the wild type and mutant PR-A, PR-B, NTa, and NTb constructs used for the transcription studies of Fig. 1. They demonstrate good expression levels of all of the proteins at their expected molecular masses.

The N-terminal Autoinhibitory Region Is Covalently Modified by SUMO-1—The N-terminal autoinhibitory region defined by residues 375–397, contains an KNEE consensus SUMO-1-binding motif (Fig. 3A); the only one present in PR. Similar sites are found in GR and AR and in other transcription factors unrelated to PR. This motif binds SUMO-1 covalently. Such binding reportedly blocks synergistic transcriptional activity from promoters that contain multiple response elements for one transcription factor. To determine whether PR are sumoylated, PR-A were transiently transfected into HeLa cells together with GFP-SUMO-1 and tested for transactivation of the luciferase reporter. The studies below were designed to address the mechanisms underlying the autoinhibitory and transrepressor properties of PR.
transfected with GFP-SUMO-1 (Fig. 3). Lanes 1 and 2 show the migration of NTB (−97 kDa) and NTA (−60 kDa) at the positions expected for the unmodified receptors. No higher molecular mass bands are seen for either receptor form, and no PR-dependent band is seen with the anti-GFP antibody (lanes 3, 4). This again suggests the interesting conclusion that SUMO-1 is unable to bind the N-terminal 387IKEE motif in the absence of the C-terminal HBD. Note that free GFP-SUMO-1 (−40 kDa) was well expressed at similar levels in both studies (Fig. 3, B and C, open arrows).

To demonstrate conclusively that the N-terminal 387IKEE motif is responsible for the transcriptional phenotype of the Δ375–397 mutants (Fig. 1) and for SUMO-1 binding (Fig. 3), Lys388 was mutated to Arg in both PR-A and PR-B to generate PR-A K388R and PR-B K388R. Fig. 4 shows immunoblots of the PR-A constructs. Wild type PR-A, PR-A Δ375–397, or PR-A K388R were transfected into HeLa cells together with wild type GFP-SUMO-1 or a nonbinding GFP-SUMO-1G97A mutant (24) and treated or not with R5020. The cell extracts were probed for PR by immunoblotting with AB-52. Wild type PR-A (Fig. 4A, left panel) migrated at −97 kDa in the absence of ligand (lanes 1, 3, and 5) with characteristic molecular mass upshifts caused by hormone-dependent phosphorylation after R5020 treatment (lanes 2, 4, and 6). Additionally, a high molecular mass PR-A band (−140 kDa), seen in the presence of R5020 (lane 4) but not in its absence (lane 3), is due to covalent modification by wild type GFP-SUMO-1. This band is absent even in the presence of R5020, with the binding deficient GFP-SUMO-1G97A mutant (lane 6). Fig. 4A shows the parallel data, using mutant PR-A Δ375–397 (middle panel) or PR-A K388R (right panel). Although the mutant receptors are well expressed (lanes 1, 3, and 6) and undergo R5020-dependent phosphorylation upshifts (lanes 2, 4, and 6), they are incapable of binding wild type SUMO-1 (lanes 4 in both panels). Thus, the 387IKEE motif and particularly Lys388 are absolutely required for SUMO-1 binding to PR-A.

Fig. 4B shows a similar study, comparing wild type PR-B and the PR-B K388R mutant. Wild type PR-B (Fig. 4B, left panel) migrated at −120 kDa in the absence of R5020 (lanes 1, 3, and 5) with characteristic molecular mass upshifts caused by hormone-dependent phosphorylation (lanes 2, 4, and 6). Additionally, a high molecular mass PR-B band (−140 kDa) seen in its absence (lane 3) is due to covalent modification by wild type GFP-SUMO-1. This was confirmed by immunoblotting the GFP-SUMO-1 moiety with the anti-GFP antibody (not shown). The high molecular mass band is absent with the binding-deficient SUMO-1G97A mutant (left panel, lane 6). The PR-B K388R mutant (Fig. 4B, right panel) is well expressed but incapable of binding wild type SUMO-1 (compare lanes 4 in both panels). We conclude that the 387IKEE motif binds SUMO-1 in both PR-A and PR-B.
Autoinhibition, but Not Self-squelching, Map to \(387\)IKEE—As discussed above, PRs express three different inhibitory properties. Autoinhibition maps to residues 375–397 in the N terminus and requires the HBD (Fig. 1). Self-squelching also requires the HBD (6), but the role of the N terminus is unclear. To demonstrate whether the \(387\)IKEE motif plays a role in both properties, the cells were transiently transfected with increasing concentrations (10–1000 ng of cDNA) of PR-A, PR-B, or their respective \(\Delta 375–397\) deletion and K388R point mutants, plus the PRE2-TATA reporter, and treated without or with RU5020 (Fig. 5). Activity is expressed as fold induction over the no hormone control. The data for PR-A are shown in Fig. 5A, and those for PR-B are shown in Fig. 5B. Note the differences in the scales of the y axes caused by the much higher transcriptional activity of PR-B. At all of the cDNA concentrations tested, transcriptional activities of the PR mutants were higher than the corresponding wild type PR. Specifically, single point mutation of the Lys in \(387\)IKEE to Arg to yield PR K388R (shown) or to Ala (not shown) is sufficient to eliminate autoinhibition.

On the other hand, the bell-shaped dose-response curves seen in this study (Fig. 5 and Ref. 6) are hallmarks of self-squelching. Clearly this property is present in wild type and mutant receptors, suggesting that SUMO-1 binding at \(387\)IKEE plays no role in this property. The fact that autoinhibition occurs at cDNA concentrations far below those involved in self-squelching also suggests that these processes are mechanistically unrelated.

Transrepression Also Colocalizes to the SUMO-1-binding Motif—The third inhibitory property of PRs is transrepression. Studies related to this property have focused mainly on the ability of PR-A to inhibit transcription of PR-B, ER, and other nuclear receptors in transient transfection assays. We sought to map the transrepressor region to determine its relationship, if any, with the autoinhibitory function. Fig. 6A shows transrepression by PR-A of PR-B activity. HeLa cells were cotransfected with wild type PR-B (25 ng) and 0, 25, and 100 ng of wild type PR-A or the PR-A K388R mutant, and transcription was measured on the PRE2-TATA reporter after RU5020 treatment. PR-B activity (set at 100%) was repressed 50% by equimolar concentrations of PR-A and \(\Delta 375–397\) (closed diamonds), and PR K388A (open circles). B, relative dose-dependent activities of wild type PR-B (open squares), PR\(\Delta 375–397\) (closed diamonds), and PR K388R (open circles).
SUMO-1 Binding Represses PR Transcriptional Activity—We previously localized an inhibitory function, IF, to the 291-amino acid N-terminal region of PR bordered by BUS and AF1 (37). We now precisely map the autoinhibitory and transrepressor activities associated with IF to an 387IKEE sequence in the N terminus that fits the consensus-binding motif for the protein, SUMO-1 (35). Site-directed mutagenesis of the Lys residue in 387IKEE (K388R) eliminates autoinhibition of PR-A and PR-B, yielding receptors with 6–11-fold higher transcriptional activities than wild type PR. However, SUMO-1-mediated autoinhibition requires the liganded HBD. The increased transcriptional activity obtained with the mutants appears to be unrelated to differences in their DNA binding ability, because no leftward shift of the dose-response curve was seen (Fig. 5). The autoinhibitory effects occur at cDNA expression vector concentrations well below those associated with self-squelching (Fig. 5), further indicating that the two phenomena are mechanistically distinct.

Difficulties in Mapping the Inhibitory Function—We had previously reported that the autoinhibitory activity of IF is demonstrable with NTA lacking an HBD (37). However, based on complete dose-response data from a comprehensive series of deletion mutants constructed in NTA, NTB, and full-length receptors, it is now clear that the repressor activity requires both the N-terminal SUMO-1-binding site and the C-terminally liganded HBD. Huse et al. (38) used a systematic set of N-terminal PR deletions to map autoinhibition to residues 387–427. However, they mapped the transrepression region to residues 307–347, using GR as the target. If so, the mechanisms for ER and GR transrepression by PR differ somewhat. Interestingly, they were unable to transrepress ER with these mutants. Differences in experimental conditions including the use of microgram quantities of CMV promoter-driven cDNAs resulting in ER overexpression and self-squelching (Refs. 6 and 37 and Fig. 5) may explain these differences. Giangrande et al. (39) reported autoinhibitory and transrepressor functions in human PR based on a single 165–305 residue deletion that lies upstream of the 387IKEE SUMO-1-binding motif. The disparity with our findings is unclear. It may relate to the promoter context in which the mutant was analyzed and/or to self-squelching from high cDNA concentrations.

SUMO-1 and SC Motifs—After eliminating self-squelching artifacts, it is clear that both PR isoforms are autoinhibited and that the repression can be relieved by mutating the SUMO-1-binding site. Transcriptional activity was measured following the addition of 10⁻⁶ M estradiol and is expressed as percentages of PR-B activity alone, where 100% represents fold induction over the no hormone control. B, HeLa cells were transfected with 50 ng of the ER expression vector, HEGO, and 50 ng of expression vectors encoding PR-A, PR-B, or the corresponding K388R mutants and 2 μg of PRE₂-TATA-luciferase reporter. Transcriptional activity was measured following the addition of 10⁻⁸ M estradiol, plus 10⁻⁸ M R5020 (left panel) or RU486 (right panel) and is expressed as percentages of the activity obtained with ER alone, where 100% represents fold induction over the no hormone control.

demonstrating a role for sumoylation in transrepression. As shown here, we consistently find that wild type PR-B are also capable of transrepressing ER, albeit less effectively than PR-A. Again, the PR-B K388R mutant is ineffective. Note that PR-mediated transrepression occurs at cDNA concentrations well below those eliciting self-squelching. Transrepression of ER is also observed when PR are liganded by the antiprogestin RU486 (Fig. 6B). Interestingly NTA or NTB do not repress ER-dependent transcription from the ERE (not shown). Thus, like autoinhibition of PR, transrepression by PR also requires their liganded HBD. We conclude that the SUMO-1-binding motif in concert with the HBD is required for the transrepression properties of PR.
Alternatively, PR-A could heterodimerize with PR-B or ER in a SUMO-1-dependent manner, yielding a protein complex that is transcriptionally deficient. Although PR-A are dominant negative in the PR-A:PR-B heterodimer (45), they have never been shown to heterodimerize with ER, excluding this as a likely mechanism. Squeezing by PR of factors required for transcription by ER is another possibility. Giangrande et al. (46) recently suggested that PR-A bind to the corepressor, silencing mediator for retinoid and thyroid hormone receptor; this may also be mechanistically involved in ER transrepression. Regardless, we know very little about the ultimate mechanisms. Transrepression by nuclear receptors is not limited to family members. GR transrepresses NFκB (47) and AR transrepress NFκB/RelA (48). However, AR-mediated transrepression is SUMO-1-independent, suggesting that the effects of sumoylation are not generalizable.

Possible Implications of PR Sumoylation—the ᵇbairrox SUMO-1-binding motif, present only once in the entire PR protein, is found in a diverse array of transcription factors (18, 24, 26–35, 49). In AR, overlap of the SUMO-1-binding site and the N/C-terminal interaction region has led to the suggestion that sumoylation negatively regulates AR by sterically interfering with intramolecular associations required for activity. Interestingly, the repressor effect of SRC-1 on AR is dependent on SRC1 binding near the SUMO-1 motif (50). The apparent identity between the SUMO-1-binding site and the SC motif suggests the possibility that sumoylation is a general repressor of transcriptional synergism. However, no clear mechanism is evident. With regard to PR, only a subset of molecules appear to be sumoylated, raising the possibility that limiting enzymatic activity (18) or rapid SUMO-1 deconjugation is linked to repressor function. Like AR, N/C-terminal interactions have been reported for PR (51), and we show that both regions are required for autoinhibitory and transrepressor activities. If so, interaction sites and/or bridging proteins between the PR N and C termini need to be identified, and their relation to SUMO-1 binding needs to be defined. Alternatively, SUMO-1 may act through nontranscriptional regulatory processes involving protein stabilization, targeting, and/or trafficking (33, 49). Regardless, we anticipate that further studies of sumoylation will provide valuable insight into gene-specific repression by nuclear receptors.

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