

Review of: Human progesterone receptor displays cell cycle-dependent changes in transcriptional activity

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Abstract of the original article

The human progesterone receptor (PR) contains multiple Ser-Pro phosphorylation sites that are potential substrates for cyclin-dependent kinases, suggesting that PR activity might be regulated during the cell cycle. Using T47D breast cancer cells stably transfected with an mouse mammary tumor virus (MMTV) chloramphenicol acetyltransferase reporter (Cat0) synchronized in different phases of the cell cycle, we found that PR function and phosphorylation is remarkably cell cycle dependent, with the highest activity in S phase. Although PR expression was reduced in the G2/M phase, the activity per molecule of receptor was markedly reduced in both G1 and G2/M phases compared to the results seen with the S phase of the cell cycle. Although PR is recruited to the MMTV promoter equivalently in the G1 and S phases, recruitment of SRC-1, SRC-3, and, consequently, CBP is reduced in G1 phase despite comparable expression levels of SRC-1 and SRC-3. In G2/M phase, site-specific phosphorylation of PR at Ser162 and at Ser294, a site previously reported to be critical for transcriptional activity and receptor turnover, was abolished. Treatment with the histone deacetylase inhibitor trichostatin A elevated G1 and G2/M activity to that of the S phase, indicating that the failure to recruit sufficient levels of active histone acetyltransferase is the primary defect in PR-mediated transactivation.

Review

Progesterone receptors (PR) are ligand-activated transcription factors that are also capable of rapidly activating multiple intracellular signaling pathways initiated at or near the plasma membrane. PR exist as either 94 kDa A or 120 kDa B isoforms created by the same gene and mRNAs by the use of alternate

promoters and unique translational start sites. The A isoform is an amino-terminally truncated version of the longer B isoform, each containing a C-terminal hormone-binding domain (HBD) and DNA-binding domain (DBD), a hinge region (H), and transcriptional activation function (AF) domains located within both the HBD (AF-1) and N-termini (AF-2 in PR-A and PR-B; AF-3 in PR-B only). Like other steroid hormone receptor family members (androgen, glucocorticoid, estrogen, and mineralocorticoid receptors), PR are heavily phosphorylated by multiple protein kinases, primarily at N-terminal serine residues. Although the role of PR phosphorylation (i.e. in humans) is not fully understood, it has been shown to influence many aspects of PR transcriptional regulation, including

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promoter specificity [1], coactivator interaction [2], ligand-dependent [2,3] and independent [4,5] transcriptional activities, receptor turnover [6], and nuclear association [7].

In addition to progestin binding to PR, growth factors independently induce PR phosphorylation at specific sites and by the same or separate kinase pathways [7]. Cross-talk between PR and mitogenic growth factors occurs at multiple levels, including progestin upregulation of epidermal growth factor receptors (EGFRs) and their ligands [8,9]. In the normal breast, EGF potentiates the proliferative actions of progesterone and estrogen, and causes ductal side branching and lobuloalveolar development of the mature mammary gland [10,11]. In breast cancer cells, EGF and progestins synergistically upregulate cyclin D1 and cyclin E protein levels [12]. Cyclins, in turn, regulate progression of cells through the cell cycle by interaction with cyclin-dependent protein kinases (CDKs). For example, D-type cyclins are expressed throughout the cell cycle in response to mitogenic stimulation, while the expression of cyclins E, A, and B (the mitotic cyclin) is periodic. Cyclin D isoforms form complexes with CDK4 and CDK6, and cyclin E associates with CDK2 in S phase, as does cyclin A. Cyclin A also forms complexes with CDK1 in late S and G2, while cyclin B/CDK1 complexes are restricted to M phase [13]. Notably, 8 of 14 phosphorylated Ser residues in PR are CDK2 sites [14–17]. Thus, PR phosphorylation is predicted to be induced primarily by cyclinE/CDK2 (G1 to S transition) and/or cyclinA/CDK2 (early S phase) complexes. In addition, progestins are excellent natural synchronizers of the cell cycle, and induce increased CDK2 levels and activity, cyclin D1 expression, and precisely timed S-phase entry that is followed by cell growth inhibition at the G1/S boundary [18]. CDK2 activity is known to increase PR transcriptional activity, both in the presence and absence of progestins [2,4].

Phosphorylation of PR by activated CDK2 suggests a mechanism for the coordinate regulation of PR action during cell cycle progression. This hypothesis was recently directly tested in a paper appearing in the April 2005 issue of *Molecular and Cellular Biology* by R. Narayanan, D. P. Edwards, and N. L. Weigel entitled, 'Human progesterone receptor displays cell cycle-dependent changes in transcriptional activity.' The authors cleverly used three different treatment protocols (designed for capturing cells specifically in either G1, S, or G2/M phases) to create synchronized populations of T47D cells stably expressing an MMTV promoter-driven chloramphenicol acetyltransferase (CAT) reporter gene [19]. Cells enriched for each phase of the cell cycle were then stimulated with progestin (6 h) and CAT activity was measured in cell

lysates to determine when PR are most active. In their first set of experiments, Narayanan *et al.* [19] showed that PR transcriptional activity is highest in S phase relative to G1 and G2/M phases, in which PR are expressed but exist in a state of repressed activity.

The phospho-status of PR in each phase was also examined using available antibodies for selected sites. Notably, Ser162 and Ser294 were hormonally regulated and robustly phosphorylated in both G1 and S phase, but not in G2/M phase. The authors suggest that in G2/M phase, PR is either in an altered conformation or associated with other protein(s) that occlude these sites. An alternative interpretation of these data is that Ser294 phosphorylated PR species are active, but also rapidly degraded [3,6], perhaps preferentially in G2/M phase. This would explain low levels of phospho-Ser294 PR relative to total. It is also possible that cytosolic phosphatases (active in G2/M) mediate the 'net' dephosphorylation of 'shuttling' PRs, which the authors found to be primarily nuclear during S phase. Regardless of mechanism, a clear correlation exists between PR activity and PR phosphorylation (at Ser162 and Ser294). However, in a previous publication appearing in the same journal [2], these investigators showed that the CDK2-induced increase in PR transcriptional activity (i.e. in the presence of progestin) did not map to phosphorylation sites within PR, but instead stimulated the association of phospho-SRC-1 to PR complexes present at the MMTV promoter. They also found that cyclin A and CDK2 associated with PR in active transcription complexes. In the more recent MCB paper reviewed herein [19], the authors used CHIP assays to show that in addition to steroid receptor coactivator 1 (SRC-1) [2], both SRC-3 and CREB-binding protein (CBP) were preferentially recruited to the MMTV promoter during S phase relative to G1 or G2/M phases.

Finally, the authors showed that PR transcriptional activity in both G1 and G2/M could be restored to the S phase levels by addition of the histone deacetyltransferase (HDAC) inhibitor, trichostatin A (TSA). However, TSA-treatment did not fully restore the ability of progestin to induce PR Ser294 phosphorylation. The authors interpret these data as suggestive that phosphorylation of PR Ser294 is not involved in the mechanism of PR transcriptional activation. An alternative explanation is that histone acetylation and chromatin remodeling (i.e. unwinding) are steps that occur well after PR phosphorylation, which may act to induce persistent nuclear accumulation of PRs during S phase and early co-factor recruitment (i.e. other than SRC isoforms). That is, PR Ser294 phosphorylation may not be required after complexes with sufficient histone acetyltransferase (HAT) activity are appropriately formed.

Perhaps an under-appreciated, but exciting aspect of the Narayanan paper involves the question of hormone sensitivity and cell cycle progression. Interestingly, total levels of PR transcriptional activity in both the G1 and G2/M phases of the cell cycle were highly progesterin sensitive, but remained low relative to S phase totals. In contrast, PR transcriptional activity in S phase was heightened, but much less responsive to added hormone (i.e. the basal activity was also very high). As this activity was clearly blocked by the addition of RU486, the authors attribute this PR-dependent activity to residual ligand present after the washout of required serum during the cell cycle synchronization step for S phase enrichment. Yet, both the G1 and G2/M phase protocols required the same washout of added serum, while PR activities remained quite low in the absence of freshly added ligand. Although not addressed by Narayanan and co-workers, these studies have revealed a window (i.e. S phase) of PR 'hypersensitivity' in which phosphorylated nuclear receptors may be well-activated by sub-physiologic levels of hormone. Phosphorylation events that induce the recruitment of steroid receptor coactivators (SRC-1, SRC-3, and/or CBP) are predicted to shift the dose-response curve for receptor activation far to the left, lowering the apparent EC₅₀ for transcriptional activation [20]. This suggests that when cells are in S phase, much lower concentrations of PR ligand (i.e. agonists) are sufficient to achieve regulation of gene expression at selected promoters.

What are the implications of these studies for steroid hormone action relevant to breast cancer biology? Breast cancers notoriously display alterations in cell cycle regulation, including increased expression of cyclins D, E and A, loss of p27, and heightened CDK2 activity [21–26]. Clearly, increased proliferation as measured by several markers, including cyclin A, predicts a poor prognosis [27]. High cyclin A levels are correlated with proliferation and strongly predict a shorter time to first relapse and shortened survival from diagnosis [28]. Thus, the key question becomes, is there a role for S phase PR action in breast cancer progression? Clinical data suggest that the addition of a progestin to hormone replacement therapy increases breast cancer risk [29]. Tumors that developed in women taking estrogen plus progesterone were larger and of higher-grade relative to estrogen alone or placebo [29], suggesting that progestins actually stimulated breast cancer progression. The paper reviewed herein [19] demonstrated that in PR-positive breast cancer cells, PR activity is highest in S phase, and may be particularly ultrasensitive to low hormone levels. CDK2 can also drive ligand-independent PR activity when p27 levels are low (as in S phase) or knocked-down [4].

What are the relevant PR target genes? Little information exists on the role of PR target genes in breast cancer cell growth control or metastasis. However, nearly half of all PR regulated genes, identified in breast cancer cells, using gene-array approaches encode cell adhesion and membrane-bound proteins or proteins involved in membrane-initiated signaling [30–32]. PR is known to upregulate c-myc, STAT5A, cyclin D1, TGF-beta, and EGFR mRNAs, and progestins synergize with EGF to induce increased expression of c-myc, c-fos, p21 and cyclins D1 and E [12,30–33]. Clearly much work remains to be done. However, in light of these findings and the new studies reviewed herein [19], inclusion of antiprogestins to existing anti-estrogen and combination therapies (that block kinases) should be seriously considered, as blocking PR action in S phase may retard tumor progression and thereby prevent or delay breast cancer recurrence.

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