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# Revisiting progesterone receptor (PR) actions in breast cancer: Insights into PR repressive functions

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ABSTRACT

Progesterone receptor (PR) is a master regulator in female reproductive tissues that controls developmental processes and proliferation and differentiation during the reproductive cycle and pregnancy. PR also plays a role in progression of endocrine-dependent breast cancer. As a member of the nuclear receptor family of ligand-dependent transcription factors, the main action of PR is to regulate networks of target gene expression in response to binding its cognate steroid hormone, progesterone. Liganded-PR transcriptional activation has been thoroughly studied and associated mechanisms have been described while progesterone-mediated repression has remained less explored. The present work summarizes recent advances in the understanding of how PR-mediated repression is accomplished in breast cancer cells and highlights the significance of fully understanding the determinants of context-dependent PR action.

## 1. Introduction

Progesterone receptor (PR) is a member of the steroid receptor superfamily of ligand-activated nuclear transcription factors that is expressed primarily in female reproductive tissues and in the central nervous system. In response to binding its cognate steroid hormone, progesterone, PR regulates the expression of gene networks to control development, differentiation, and proliferation of target tissues and the pathological processes in endocrine-based cancers [1]. Progesterone is produced primarily by the corpus luteum in the ovaries during the second half of the menstrual cycle or luteal phase. Progesterone is also produced, to a lesser extent, in the adrenal glands and, during pregnancy, the placenta. Thus, cyclical hormone exposure beginning at menarche and ending in menopause occurs monthly and regulates the growth and differentiation of specialized tissues within the reproductive tract and breast tissues [2–4]. Pregnancy interrupts this process and is characterized by high progesterone levels, which are required for fetal development, breast development for lactation, maintenance of uterine/placental integrity, and myometrial quiescence [5].

Epidemiological evidence and clinical findings have demonstrated that synthetic progestins, whether given in a hormone replacement therapies (HRT) as post-menopausal treatments or as hormonal contraceptives in pre-menopausal women, confer a greater breast cancer risk (reviewed in [4]). Progestin-containing contraception is linked to an increased risk of developing breast cancer in multiple epidemiological studies [6–9]. Similarly, other epidemiological studies indicate that greater exposure to progesterone throughout an individual's lifetime leads to greater likelihood of breast cancer (reviewed in [10]). Large-scale clinical trials, including the Women's Health Initiative [11], Million Women's Study [12], E3N-EPIC cohort [13], and Finnish Cancer Registry case-controlled analysis [14], demonstrate that women taking progestins added to estrogen therapy are at greater risk of developing breast tumors. Recently, a retrospective analysis of Finnish women using the levonorgestrel-releasing intrauterine system of contraception has also demonstrated an increased risk of breast cancer [6]. However, the same regimen conferred protection from endometrial and ovarian cancers as well as lung and pancreatic cancers [6]. Together, these epidemiological and clinical findings support the notion that uncontrolled PR action in pre-neoplastic breast tissue contributes to breast cancer development. These data are corroborated by an expansive body of literature demonstrating in both *in vivo* and *in vitro* models of luminal breast cancer that exposure to progestins increases proliferation and promotes pro-survival and progression of malignant breast cells (reviewed in [15]).

## 2. Structure and function of progesterone receptor isoforms

Structurally, the PR is a modular protein composed of a C-terminal ligand-binding domain (LBD), a central DNA-binding domain (DBD), and an amino-terminal domain (NTD). There are two PR protein isoforms that arise from the same gene by utilization of two promoters: PR-

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A with a truncated NTD and full-length PR-B. PR contains two transcriptional activation domains or "functions" (AFs) that provide interaction surfaces for co-regulatory proteins; AF1 is located within the NTD and AF2 is in the LBD [16–18]. Functionally, PR isoform-specific activities (PR-A vs. PR-B) overlap but can have very disparate functions within a given target tissue and at selected gene promoters. PR-B typically acts as a more potent transcription factor relative to PR-A on hormone-induced target genes [19]. Indeed, the PR-A isoform has been linked to the repressive actions of progestins [20] but whether it exerts a particular mechanism of repression has not been detailed so far.

In its classical mechanism of action PR associates with specific progesterone response elements (PREs) on chromatin. DNA-bound PR recruits transcriptional coactivators and associated cofactors, which modify the local chromatin structure and facilitate transcriptional activation, resulting in activation or repression of PR target genes [21]. PR may also alter gene expression nonclassically, where the receptor tethers to other transcription factors bound to DNA, including activator protein 1 (AP-1), specificity protein 1 (Sp1), and signal transducer and activator of transcription 3 (Stat3) [22–24]. In addition to its direct transcriptional effects, PR activates signal transduction pathways in breast cancer cells through a rapid or nongenomic mechanism [25,26]. PR undergoes extensive post-translational modifications that include phosphorylation, acetylation, ubiquitination, SUMOylation, and methylation reviewed in [27–29] which are clearly important contributing mechanisms to the diversity and context-dependent functions of PR. Another layer of variability of the biological actions of PR involves the availability of coregulatory proteins and other cooperating factors expressed on a cell-type or tissue and on a certain developmental stage which regulates PR transcriptional effects [27,28,30,31].

## 3. PR interaction with chromatin

Interestingly, comparison of PR cistromes between different cell types revealed low overlap between genomic PR-binding sites, as might be expected as an explanation for tissue-specifications of steroid hormones. Although the genome harbors hundreds of thousands of potential PRE-binding sequences, only a small fraction of these are actually occupied by PR, indicating that the presence of a PRE alone is not sufficient for binding [32]. Additional factors required for binding to PRE*s in vivo* have been proposed, including cooperation with other transcription factors and remodeling of chromatin structures that can be a barrier for access to DNA sites [32]. In effect, coregulators are generally enzymes capable of modifying chromatin proteins, the basal transcriptional machinery, and other coregulators. The coregulators are composed of coactivators, which provide positive enhancement to gene expression, and corepressors, which are employed to suppress gene expression. These regulatory molecules provide the ability to fine-tune genes and activate them in functional combinations [33]. Bioinformatic analysis of PR cistromes has revealed enrichment of sequence motifs near PR-binding sites for several transcription factors including ETS, Stat, FoxA1, Sp1, and AP-1, with enrichment being distinct between cell types and tissues [32,34–38]. However, enrichment of motifs for other transcription factors is not significant enough to account for the differential between potential and actual receptor-binding sites. This brings into question whether other transcription factors can act as pioneer factors to modulate chromatin structure and enable binding of PR. Pioneer or licensing factors are proteins with an intrinsic ability to bind to condensed chromatin and prime specific genomic loci for subsequent receptor binding [39]. Their recruitment to the chromatin is sequence specific and can be facilitated by an epigenetic signature dependent on histone methylation [40]. Estrogen, androgen and glucocorticoid receptors (ER, AR, and GR) were shown to require pioneer factor activity [41–43]. In effect, open chromatin regions established by pre-binding of the pioneer factors FoxA1 or AP-1 have been shown, respectively, to be required for efficient binding of ER/AR and GR to genomic sites [39] but PR showed direct nucleosome binding for optimal function when activated by the synthetic progestin R5020 in a recent report [32]. Functional, genomic PR-binding sites identified by ChIP-seq are largely occupied by nucleosomes, and it appears that PR itself acts as a pioneer factor preferentially binding to nucleosomes and inducing local remodeling [32,44]. This concept for PR was initially shown with the mouse mammary tumor virus promoter, where PREs are positioned within nucleosomes in such a manner that they are accessible to PR. Upon binding PR in response to hormone, nucleosomes undergo local remodeling as a result of recruitment of chromatin-modifying enzyme complexes and displacement of histone H1 and H2A/H2B dimers without the loss of the nucleosome core particle [44]. Although the structural transitions responsible for the accessibility of PR to the PRE are complex, the local nucleosome remodeling initiated by the receptor collectively increases the accessibility of chromatin-remodeling factors [45] and basal transcriptional machinery that act synergistically with PR to maintain transcription [46]. This concept that nucleosomes encompassing PR-binding sites are remodeled, not depleted, upon hormone induction has been extended globally to genome-wide binding sites in T47D cells stably expressing the mouse mammary tumor virus (MMTV) promoter. Many PR-binding sites encompassing nucleosome-rich regions overlap with open chromatin that appears after hormone treatment, confirming that PR initiates both nucleosome binding and remodeling [32]. Interestingly however, two members of the Stat family of transcription factors were hypothesized to act as PR pioneer factors in breast cancer cells. Stat3 was endowed a novel functional role as a potential pioneer factor for PR when it was activated by Heregulinβ1, one of the ligands of the ErbB family of receptor tyrosine kinases [47]. Stat3 licensing activity on DNA was a requisite for PR transcriptional activation of bcl-X, $p21^{CIP1}$ and c-myc promoters [47]. Stat5 was postulated to function as a putative pioneer factor recruiting PR phosphorylated in its serine 81 residue to [28] selected target genes required for proliferation, stem cell maintenance, and inflammatory responses [48].

## 4. PR repressive actions

The mechanisms for PR-dependent transcriptional activation have been well studied, while PR-mediated transcriptional repression, especially direct repression in response to ligand, remains less understood. Various mechanisms have been put forth for PR-mediated transcriptional repression, and are similar to what has been well characterized for GR-mediated repression: i. squelching or sequestering of certain components of the transcriptional machinery [49], ii. mediated by the presence of negative responsive elements [50], iii. recruitment of corepressors, iv. chromatin remodeling (reviewed in [49]), v. SUMOylation of the receptor. Indeed, chromatin structure is very dynamic and undergoes extensive remodeling that leads to either activation or repression of transcription. Two highly conserved chromatin remodeling mechanisms have been found in eukaryotic cells: (i) post-translational modification of histones and (ii) ATP-dependent chromatin remodeling, which is catalyzed by enzymatic complexes containing ATPases that use the energy of ATP hydrolysis to disrupt histone–DNA interaction. There are several ATP-dependent remodeling complexes, including the SWI/SNF complex in yeast and its homologues in other higher eukaryotes, BAF and PBAF, containing Brahma-related gene 1 (BRG1) and/or BRM ATPase subunits.

In this work, we present recent advances in the field of PR function as a transcriptional repressor in breast cancer cells, both in its liganded and unliganded conditions.

## 5. Basal repression of progestin-activated genes

Recently, Vicent et al. addressed how basal repression of progestin-regulated genes is targeted to the correct sites throughout the genome [51]. In fact, they reported unliganded PR to be capable of targeting gene regulation through basal control of hormone-inducible genes by a

relief of repression mechanism. Unliganded PR can bind to selective target genes and stabilize repressive complexes that become displaced in response to hormone treatment enabling coactivator recruitment, chromatin remodeling, and gene activation [51]. The human T47D-MTVL cells which carry a single copy of the MMTV-luc transgene integrated in their genome [52] was used in the mentioned work. Regulation of the expression of the MMTV constitutes a well characterized model system for the analysis of hormone gene regulation given that promoter activity is induced by glucocorticoids, progestins, androgens, and, to a lesser extent, mineralocorticoids acting via several hormone responsive elements [53–55]. The authors described that without progesterone treatment, the MMTV promoter was associated with unliganded PR and a multiprotein complex containing heterochromatin protein 1γ (HP1γ), the silencing factor CoREST (corepressor for REST [RE1 {neuronal repressor element 1} silencing transcription factor]), the DNA binding protein BRAF35, histone deacetylases 1 and 2 (HDAC1/2), and the lysine-specific histone H3 demethylase LSD1. This complex repressed the MMTV promoter in the absence of hormone and dissociated from the promoter upon stimulation with the progestin analog R5020. Components of the HP1γ-containing repressor complex and unliganded PR were also found at the endogenous progesterone-responsive promoters *STAT5A, BIRC3, BCL-X, EGF, EGFR, DUSP1* and *CCND1*. Displacement of the HP1γ-containing repressor complex from the MMTV promoter required phosphorylation of histone H3 at Ser10 by the kinase MSK1, which is activated by hormone treatment and recruited to target promoters with ligand-bound PR. In coimmunoprecipitation assays, PR associated with components of the HP1γ-containing repressor complex in both the absence and presence of hormone. The complex was present at 20% of the endogenous PR-responsive genes, and chromatin immunoprecipitation experiments indicated that unliganded PR recruited the complex to these sites. In addition, RNase treatment of permeabilized cells reduced targeting of the repressor complex to the MMTV and endogenous promoters, and the repressor complex contained the RNA steroid receptor RNA activator (SRA), which was required for stable targeting of the complex to unliganded PR-responsive promoters. These findings suggest a model in which unliganded PR recruits a repressive complex to a subset of PR-responsive promoters to repress basal transcription of these genes. Hormone stimulation derepresses these by activating and recruiting MSK1, which phosphorylates histone H3 to displace the repressive complex. Subsequent trimethylation and acetylation of histone H3 mediate chromatin remodeling that enables ligand-bound PR-coactivator complexes to promote target gene transcription [51]. Interestingly, these endogenous upregulated genes, which have unliganded PR binding sites, were found to be functionally linked. Gene ontology (GO) analysis showed that among the top 10 more enriched terms, the functional term negative regulation of apoptosis was significantly overrepresented. Likewise, analysis of the GO terms of the genes affected by SRA knockdown and also carrying unliganded PR binding sites revealed that these genes were also associated with negative regulation of apoptosis, indicating that the HP1γ-LSD1 repressive complex is a key element involved in hormone-dependent regulation of a subset of inducible genes implicated in relevant cellular functions as regulation of apoptosis. Indeed, staurosporine-induced apoptosis of T47D cells was reduced after SRA knockdown [51]. As a whole, according to the proposed model, the unliganded receptor could be linked to the repression of a subset of genes that are activated by hormone and associated with cell proliferation and inhibition of apoptosis [51].

## 6. Ligand-activated PR transcriptional repression

The present section refers to three selected publications which describe recent advances in progestin-activated PR transcriptional repression on different target genes in breast cancer cells.

Interestingly, Nacht et al. recently disclosed the mechanism for progestin-dependent gene repression operating in 23% of the subset of progestin-downregulated genes in T47D-MTVL cells [56]. They found that ligand-activated PR recruits to the promoter of downregulated genes a repressor complex composed of HP1γ, LSD1, HDAC1/2, coREST, the RNA SRA and the ATPase BRG1. Their results are consistent with a model where upon hormone exposure, the HP1γ-LSD1 complex interacts with the ATPase BRG1 and is actively recruited to the target genes along with the kinases ERK and MSK1, responsible for PR phosphorylation in S294 and S400, two modifications associated with the "active" form of the receptor [57–59]. Once bound to the chromatin the complex promotes histone deacetylation, demethylation and chromatin remodeling via BRG1, which increase nucleosome positioning and occupancy. This arrangement of nucleosomes constitutes a suitable platform for histone variant H1.2 binding, which compacts chromatin decreasing RNA pol II loading and transcription. The pioneer factor FoxA1 was demonstrated as a strong candidate in marking the PR binding sites associated with the repressed genes, since it was found prior to hormone exposure, significantly enriched near the PR binding sites that mediate hormonal repression as compared to all PR binding sites. In addition, FoxA1 knockdown compromised BRG1 recruitment and prevented hormone-dependent gene repression [56] endowing FoxA1 a role in BRG1 targeting. The authors validated the progestin downregulated genes breast carcinoma amplified sequence 1 (*BCAS1*), keratin 23 (*KRT23*), insulin-like growth factor binding protein 5 (*IGFBP5*), Vesicle Associated Membrane Protein 1 *(VAMP1),* Coiled-Coil Domain Containing 173 (*CCDC173)* and Ras-related protein Rab-3D (*RAB3D*) as requiring the HP1γ-LSD1 complex for effective progestin regulation. Remarkably, GO analysis revealed that progestin downregulated genes are associated with relevant cell functions including intracellular signaling cascades, cell proliferation, cell adhesion and cell fate commitment [56], which are in line with the pathways associated with the upregulated genes [32], suggesting a common direction of progestin effect.

When assessing the PREs mediating the repressive effects of PR, the authors found that they were indistinguishable from the PREs involved in progestin activation of transcription [60]. However, the location of PR binding sites was different on repressed and activated genes. Indeed, the PR binding sites responsible for hormonal gene activation were preferentially located distally from the induced genes in enhancer regions [32,34,38] while the PR binding sites involved in repression were close to the Transcription Start Sites (TSS) of the target genes [51]. As a whole, these findings highlight that, at least for a subset of downregulated genes, the mechanisms for ligand-activated PR induction and repression of transcription share common factors and functions in breast cancer cells [32,56].

PR-mediated transcriptional repression was demonstrated in interferon (IFN)-stimulated genes (ISG) by an alternative mechanism from the model delineated by Nacht et al. [61]. ISG products constitute a critical response of the innate immune system and aid the cell in responding to a pathogenic threat typically following pathogen detection. Given that IFN activity is an early step required for immune-recognition and subsequent destruction of nascent tumors by immunomodulatory cells, alteration or disruption of IFN signaling pathways may contribute to the development of clinically overt tumors [61], highlighting the relevance of PR effect on this class of genes. In response to type I IFNs, such as IFNα, a heterodimeric receptor [IFNAR1 (IFNα receptor 1) and IFNAR2] complex is autophosphorylated, promoting JAK1/tyrosine kinase 2 (TYK2)-dependent phosphorylation of Stat1 and Stat2. Phosphorylated Stat1 and Stat2, together with IFN-regulatory factor 9 (IRF9), form a transcriptional complex referred to as IFN-stimulated gene factor 3 (ISGF3), which binds to DNA sequences within ISG promoter regions, referred to as IFN-stimulated response elements (ISRE). Binding of ISGF3 to ISREs leads to transcription of ISGs. Prompted by the finding of a negative correlation between progestin treatment and enrichment with IFN-related gene sets in a published microarray dataset from progestin-treated T47D cells, Walter et al. hypothesized PR repression of ISG in breast cancer [61]. They demonstrated that

progestin-activated PR is recruited to its PRE in ISG enhancers with the concomitant decreased recruitment of the ISGF3 components Stat2 and IRF9 to ISRE promoter sequences [61]. They proved that progestin-mediated ISG repression requires PR expression and could be mediated via both isoforms of PR, as T47D-Y cells stably expressing either PR-B or PR-A can both repress ISG RNA levels, although PR-B has greater transcriptional repressor activity on the selected ISGs IRF9, and IFIT (Interferon Induced proteins with Tetratricopeptide repeats) 2 and 3. Accordingly, ISG repression was not observed in PR-negative breast cancer cells. Based on the experimental results, a model involving protein displacement or steric competition between PR and Stat2/IRF9 is postulated [61] while not excluding the participation of another mechanisms of activated-PR repression. The clinical relevance of this finding resides in the fact that PR-dependent downregulation of IFN signaling may be a mechanism through which early PR-positive breast tumors evade the immune system and develop into clinically relevant tumors [61].

A novel mechanism for progestin-activated transcriptional repression was elucidated for the regulation of the master transcription factor GATA3 in breast cancer cells [62]. GATA3 is a critical regulator in both mouse and human development, since constitutive null mutations of GATA3 result in embryonic lethality [63]. In addition, GATA3 expression is necessary for the specification and maintenance of both ductal and alveolar luminal cell fate in the mammary gland [64,65]. Importantly, it was demonstrated that the loss of GATA3 expression marks the loss of tumor differentiation and the onset of tumor dissemination [66]. Accordingly, restoration of GATA3 induced differentiation of mammary ductal adenocarcinomas [66]. The clinical relevance of GATA3 is highlighted by an article which identified GATA3 as one of the only three genes carrying somatic mutations with more than 10% incidence across breast cancer subtypes defined by the mRNA expression profile [67]. Taken together, these studies underscore the role of GATA3 as a tumor suppressor and merit the study of its complex regulation. Interestingly, Izzo et al. revealed that progestin-mediated GATA3 downregulation was a required event for progestin-induced proliferation of human and murine breast cancer cells [62]. GATA3 transcriptional repression was mediated by liganded-PR recruitment of the histone methyltransferase enhancer of zeste homolog 2 (EZH2) to a PRE located in *GATA3* proximal promoter. EZH2 is the catalytic subunit of the Polycomb Repressor Complex 2 (PRC2), and catalyzes the trimethylation of lysine 27 of histone H3 (H3K27me3), a histone mark associated with chromatin compaction and transcriptional repression [68,69]. Notably, Polycomb group (PcG) target genes are mainly involved in embryonic development and cell differentiation [70], consistent with GATA3 function in the mammary gland. Overexpression of EZH2 has been detected in breast cancer, with increased EZH2 levels correlating with higher proliferation rates, neoplastic transformation and more aggressive cancer subtypes [71,72]. The mechanism described by Izzo et al. constitutes the first report showing EZH2 participation in PR repressive function. Progestin-induced loading of the PR/EZH2 complex to GATA3 promoter resulted in increased H3K27me3, chromatin compaction and transcriptional repression as evidenced by DNAse I sensitivity assays. Consonantly, progestin treatment caused a decrease in the acetylation of lysine 9 of histone H3 (H3K9ac) and in the total acetylation of histone H4 (H4ac) suggesting the involvement of additional mechanisms in the process of GATA3 repression, such as recruitment of histone deacetylases. Progestin-induced simultaneous PR and EZH2 binding to DNA was demonstrated by sequential ChIP experiments. The requirement of PR binding to DNA for EZH2 recruitment was tested using the T47D-Y-C587A cell line, which stably expresses a PR harboring a substitution of the cysteine 587 for alanine that renders the receptor unable to bind to DNA or to tether to other transcription factors bound to DNA [26]. Indeed, progestin treatment of T47D-Y-C587A cells, failed to induce EZH2 recruitment to the PRE, indicating the requirement of PR binding to this site in order to enable EZH2 recruitment upstream of the *GATA3* gene. Remarkably, EZH2 expression levels, phosphorylation of EZH2 at threonine 487 and total H3K27me3 are increased in the mammary gland during pregnancy, a condition characterized by elevated levels of progesterone [73], opening the question whether this novel mechanism could be potentially involved in the repression of other PR target genes.

## 7. Other PR repressive actions

PR is SUMOylated in a hormone-dependent manner at lysine 388 in the NTD. SUMOylation occurs via the covalent attachment of a small ubiquitin-like modifier (SUMO) peptide to lysine residues of substrate molecules, primarily at consensus SUMOylation motifs (IKxE) through an ATP-dependent enzymatic mechanism [74]. SUMOylated PR is highly stable with a longer half-life than PR Ser294 which is highly ubiquitinated and rapidly degraded by the 26S proteasome [10]. Promoter composition, namely the quantity and organization of hormone response elements, is a key factor in target gene regulation by sumoylated transcription factors, including PR [75,76]. SUMOylation has a suppressive effect on PR-mediated transcriptional activation of numerous endogenous gene loci, including heparin-binding EGF-related growth factor (*HBEGF*), insulin receptor substrate 1 *(IRS1)* and Stanniocalcin 1 (*STC1*) [77,78] all three gene products known to contribute to breast cancer cell proliferation [77,79–81], but it also enhances transcription of other targets by mechanisms that are not well defined [82]. Notably, the latter targets include many tumor suppressor genes [82]. Reversible SUMO attachment regulates aspects of PR crosstalk with other signaling pathways and also hormone sensitivity. Certainly, seminal findings by Lange and co-workers indicate that PR Ser294 phosphorylation negatively modulates PR sumoylation, thereby constituting a mechanism for “derepression” of PR [77]. In this context, activation of kinase pathways by growth factors rapidly modifies the sumoylation state of liganded PR altering both transcriptional activity on select promoters and subsequent turnover of liganded receptors. Gene expression profiling of SUMOylated and SUMOylation-deficient PR revealed a signature set of target genes regulated by hyperactive deSUMOylated PR as a result of selective recruitment of chromatin remodeling proteins such as CREB-(cAMP-response element binding protein)-binding protein (CBP) and mixed lineage leukemia 2 (MLL2) to SUMO-sensitive promoters. Given that modification of protein substrates by the addition of SUMO molecules can influence protein-protein interactions and/or alter protein stability, localization, or transcriptional activity (reviewed in [74]), the authors propose a model where the chromatin structure at the enhancer/promoter region functions in combination with PR SUMOylation to block interactions between PR and mediators of early chromatin remodeling (MLL2) as well as major coregulators, including CBP. SUMO-deficient PR gene signature was found to be associated with endocrine resistance and poor outcome in breast cancer [82], highlighting the impact of post-translational modifications in breast cancer progression.

Ligand-activated PR also exerts its repressive functions via regulation of microRNAs (miRNAs). MiRNAs are small non-coding RNAs of 20–25 nucleotides that bind target mRNAs in the 3′ untranslated region to induce mRNA degradation and inhibit translation [83]. MiRNA families regulate growth, differentiation, and metabolism and are emerging as key mediators of steroid hormone receptor signaling that may potentiate or dampen steroid hormone induction of target genes. In particular, recent studies indicate that PR may regulate several miRNAs that inhibit cell cycle and intrinsic receptor activity (reviewed in [84,85]. Acknowledged that the focus of the present work is to review recent advances in PR function as a transcriptional repressor in breast cancer cells, a detailed overview of progestin regulation of miRNAs mediating progesterone receptor action could be found in [85–87]. While it remains to be tested by a more global analysis, the dual regulation of genes at the 5′ end by hormone receptors and the 3′ end by

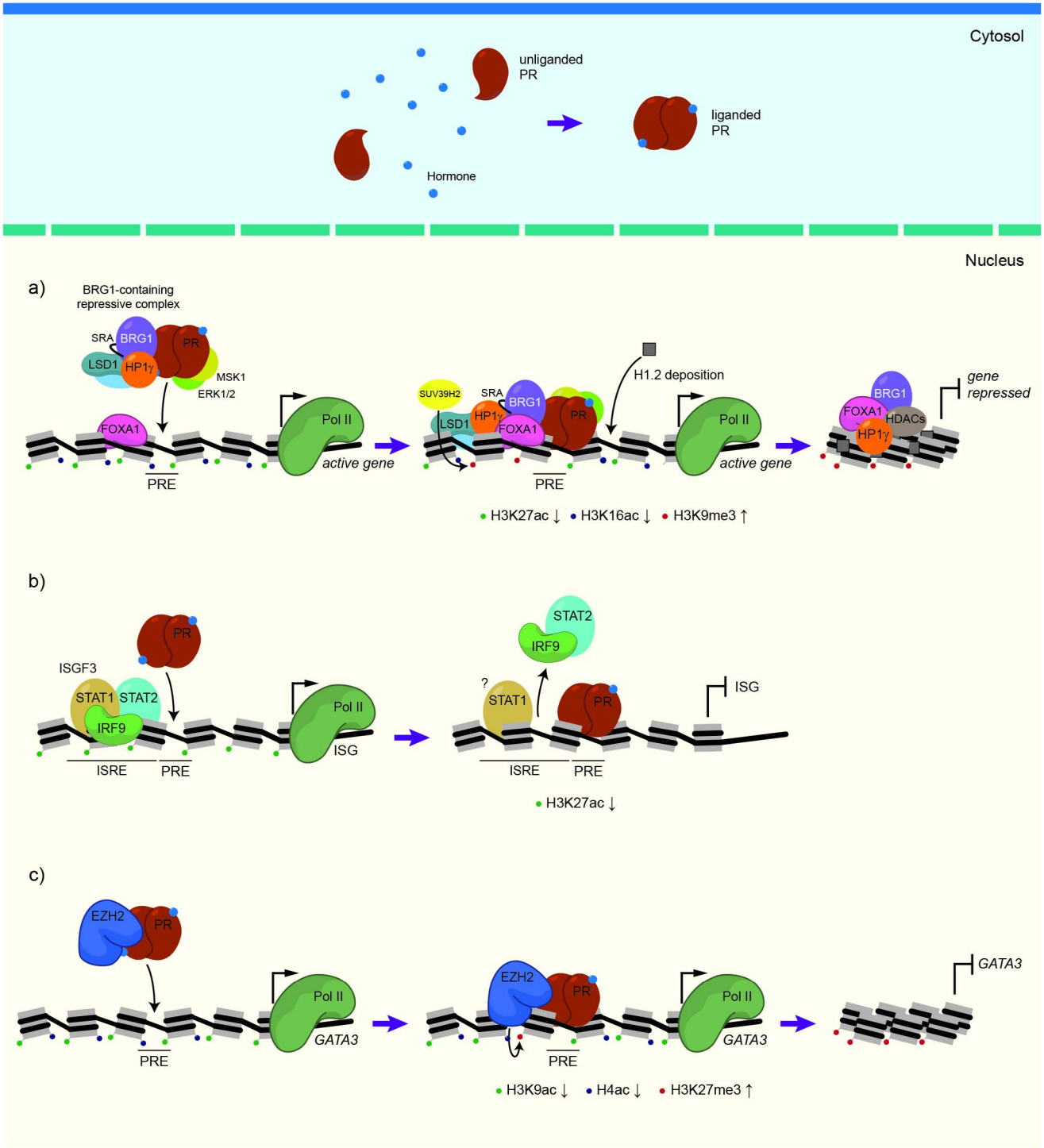


**Fig. 1.** Models of Ligand-Activated PR Transcriptional Repression. Binding of hormone results in PR nuclear localization and association with specific progesterone response elements (PREs) on chromatin. Models proposed for liganded PR-mediated transcriptional repression of target genes. **a)** In addition to ERK and MSK1 kinases, ligand-activated PR recruits a repressive complex composed of HP1γ-LSD1, the ATPase BRG1, the SRA RNA, histone deacetylases and histone demethylases. BRG1 increases linker histone H1.2 deposition and nucleosome occupancy, leading to compaction around the TSS that hinders RNA pol II loading and enables maintenance of PR binding. **b)** In response to IFNα, the transcriptional complex ISGF3 binds to IFN-stimulated response elements (ISRE) within promoter regions of IFN stimulated genes (ISG), leading to their transcription. Ligand-activated PR binds PREs in ISG enhancers, and decreases recruitment of the ISGF3 components Stat2 and IRF9, possibly by protein displacement or steric competition, thereby downregulating ISGs expression. **c)** Ligand-activated PR corecruits histone methyltransferase EZH2 to a PRE in *GATA3* promoter to induce the H3K27me3 modification and chromatin compaction around the TSS, leading to *GATA3* transcriptional repression.

hormone-regulated miRNAs may prove to be a common form of regulation designed to fine-tune the expression of hormone-responsive genes, and indeed could also be involved in tissue- and cell type specific response to hormones [87].

## 8. Conclusions and perspectives

The PR regulation of distinct target genes is mediated by complex interactions between PR itself and other regulatory factors that determine the context-dependent transcriptional action of the

progesterone receptor. In the present work we reviewed recent advances in the understanding of the mechanisms involved in PR-mediated transcriptional repression (Fig. 1). However, the described mechanisms do not account for all the repressed target genes induced by progesterone/PR. Further research is required in this arena given the relevance of the downregulated genes for breast cancer cell proliferation and apoptosis. Indeed, the factors involved in the repression mechanisms described so far could constitute potential targets for the management of hormone-dependent cancers.

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The authors have nothing to disclose.

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