Receptor mechanisms of rapid extranuclear signalling initiated by steroid hormones

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Abstract

In addition to their role as direct regulators of gene transcription mediated by classical nuclear hormone receptors, steroid hormones have also been described to exert rapid effects on intracellular signalling pathways independent of gene transcription. This chapter focuses on recent advances in our understanding of the receptors and mechanisms that mediate these rapid signalling actions of oestrogens and progesterone. Increasing evidence suggests that at least some of these rapid actions are mediated by a subpopulation of the classical nuclear oestrogen receptor (ER) and progesterone receptor (PR) that localize to the cytoplasm or associate with the plasma membrane. Human PR has been shown to mediate rapid progestin activation of the Src/Ras/Raf/mitogen-activated protein kinase signalling pathway in mammalian cells by a direct interaction with the Src homology 3 domain of Src tyrosine kinases through a Pro-Xaa-Xaa-Pro-Xaa-Arg motif located in the N-terminal domain of the receptor. Moreover, this is an extranuclear action of PR that is separable from its direct transcriptional activity. Additionally, a novel membrane protein unrelated to nuclear PR was recently identified that has

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properties of a G-protein-coupled receptor for progesterone and has been shown to be involved in mediating the extranuclear signalling actions of progesterone that promotes oocyte maturation in fish. The role of this membrane PR (mPR) in mammalian cells is less clear and the relationship of the membrane and classical nuclear PR in mediating rapid non-transcriptional signalling of progestins has not been explored. To date, a novel membrane ER unrelated to classical nuclear receptors has not been cloned and characterized, and many of the known rapid extranuclear signalling actions of oestrogen appear also to be mediated by a subpopulation of nuclear ER, or a closely related receptor. A novel protein termed modulator of non-genomic activity of ER (MNAR) has been identified that acts as an adaptor between ER and Src, and thus provides a mechanisms for coupling of oestrogen and ER with rapid oestrogen-induced activation of Src and the downstream mitogen-activated protein kinase signalling cascade. The physiological relevance of rapid extranuclear signalling by the classical ER has been provided by experiments showing that these actions contribute to the anti-apoptotic effect of oestrogen in bone \textit{in vivo} and to the rapid effects of oestrogen on vasodilation and protection of endothelial cells against injury.

\textbf{Introduction}

Steroid hormones exert many of their biological responses through nuclear receptors that directly regulate specific networks of gene expression. Nuclear receptors belong to a superfamily of ligand-dependent transcription factors that share a similar domain organization consisting of a centrally located, highly conserved DNA-binding domain, a C-terminal ligand-binding domain (LBD) and an N-terminal domain that is required for full transcription activity (Figure 1). Hormone binding activates the transcriptional potential of steroid hormone receptors by inducing a conformational change leading to dissociation from molecular chaperones, dimerization and binding to specific DNA sequences in the regulatory promoter regions of steroid-responsive genes, referred to as hormone-response elements. Upon binding to DNA, activated receptors recruit specific co-activators that are essential for assembly of a productive transcriptional complex.

However, not all biological effects of steroid hormones can be explained by the well-established roles of steroid hormone receptors as transcription factors. All classes of steroid hormone have been observed to have rapid effects that occur independently of gene transcription. This phenomenon has been termed ‘non-genomic’ action of steroids to distinguish it from the classical direct genomic actions of steroid receptors in the nucleus. These rapid actions of steroids have been described to generate intracellular second messengers, activate or inhibit cell signal-transduction cascades and modulate ion channels. However, because some of the intracellular signalling pathways activated by steroids can converge upon and activate other nuclear transcription factors, the
term ‘non-genomic’ is not very accurate. We propose the more appropriate terminology ‘extranuclear steroid-initiated signalling’.

The receptors and mechanisms that mediate extranuclear signalling by steroid hormones are generally not well defined and these are intensive on-going areas of investigation. Studies have indicated in part that a subpopulation of classical steroid receptors can mediate extranuclear signalling actions of steroids by somehow associating with the cell membrane and/or signalling complexes in the cytoplasm. Other studies suggest the existence of separate membrane receptors unrelated to the classical steroid receptors. All classes of steroid hormone have been described to exert extranuclear actions on cell signal-transduction cascades and this has recently been reviewed in detail elsewhere [1]. The focus of this review is on recent studies defining the receptors and mechanisms of rapid extranuclear signalling by oestrogen and progesterone.

Extranuclear signalling actions of progesterone: classical intracellular progesterone receptor (PR)

The PR is expressed in most target tissues as two isoforms, PR-A and PR-B, that arise from alternative uses of two promoters from the same gene. In human cells PR-A is truncated, lacking the first 164 amino acids of the N-terminal domain of full-length PR-B (Figure 1). The transcriptional activities of the two PR isoforms vary depending on the cell type and promoter context. In general, PR-B is a stronger transcriptional activator whereas PR-A can function as a ligand-dependent repressor of other steroid hormone receptors including PR-B, oestrogen receptor (ER), androgen receptor (AR), glucocorticoid receptor and mineralocorticoid receptor [2].

Rapid, extranuclear signalling actions of progesterone have been described in several biological systems. These include progesterone-induced maturation of the Xenopus oocyte, stimulation of acrosome reaction in sperm, modulation of neurotransmitter and neuron excitability, and activation of the Src/Ras/mitogen-activated protein kinase (MAPK; extracellular-signal-regulated kinase (ERK)) signal transduction pathway in breast cancer and other mammalian cells.

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**Xenopus oocyte maturation: role of classical PR**

The most thoroughly studied system of rapid extranuclear signalling by steroid hormones is progesterone induction of *Xenopus* oocyte maturation. Progesterone treatment of *Xenopus* oocytes causes a rapid decrease in cAMP and a decline in protein kinase A activity that leads to activation of a MAPK pathway and maturation-promoting factor, causing germinal vesicle breakdown and resumption of meiosis II [3]. A *Xenopus* homologue of the classical mammalian nuclear PR (XPR) was recently cloned and characterized, but shows a somewhat different cellular localization from mammalian nuclear PR [4,5]. XPR localizes predominantly to the cytoplasm [5] with small amounts in the cell membrane [7], and little or no nuclear localization [5] in *Xenopus* oocytes. However, in mammalian cells (COS cells) transiently transfected with XPR cDNA, XPR localized mainly to the nucleus and exhibited progesterone-induced transcriptional activation of a PRE reporter gene [5]. Injection of XPR mRNA increased the sensitivity of *Xenopus* oocyte to progesterone and accelerated progesterone-induced p42 MAPK activation and oocyte maturation. Injection of XPR antisense oligonucleotide inhibited progesterone-induced oocyte maturation while the transcription inhibitor, actinomycin D, did not alter the influence of XPR on oocyte maturation. In addition to XPR, injection of classical human PR has also been shown to accelerate progesterone-induced *Xenopus* oocyte maturation [6]. More recently, XPR was shown to associate with active phosphoinositide 3-kinase (PI 3-kinase) and p42 MAPK upon progesterone treatment, suggesting a direct association of XPR with cytoplasmic signalling pathways [7]. However, progesterone-induced oocyte maturation still occurs when XPR is inhibited, suggesting that XPR is involved but is not sufficient for progesterone-induced oocyte maturation. Taken together, these data suggest that progesterone-induced oocyte maturation is mediated in part by the classical PR and that a separate unrelated mPR may also be involved.

**Human PR mediates rapid progesterone induction of Src/Ras/Raf/MAPK signalling in mammalian cells**

Rapid progestin activation of the Src/Ras/MAPK pathway in the absence of transcription was first shown by Migliaccio et al. [8] to be dependent on the classical nuclear PR. Furthermore, the proliferative effect of progesterone in breast cancer cells was shown to be mediated in part by the Src/Ras/MAPK pathway. Studies by Migliaccio et al. [8] further suggested that PR activation of Src/Ras/MAPK pathway was indirect through PR association with ER, and that it was ER that directly activated Src through interaction with the Src homology 2 domain (SH2 domain). However, whether PR and ER can physically interact is controversial, and how ER interaction can activate Src has not been shown [8]. More recently, an alternative
mechanism for how PR interacts with and activates Src and the downstream MAPK cascade was reported [6].

The N-terminal region common to human PR-A and PR-B was discovered to contain a short, contiguous polyproline sequence (amino acids 421–428, Pro-Pro-Pro-Pro-Leu-Pro-Pro-Arg; Figure 1) that conforms to a consensus type II, Pro-Xaa-Xaa-Pro-Xaa-Arg (PXXPXR), motif for binding the SH3 domain of cytoplasmic signalling molecules. These polyproline sequences form a left-handed helix that interacts with the binding pocket of SH3 domains. PR, through this PXXPXR motif, interacts directly in vitro with the SH3 domain of various signalling molecules, including Src. PR interaction with the SH3 domain of Src through the PXXPXR motif also occurs in cells in a progesterone-dependent manner. The consequence of this interaction, both in vitro (cell-free) and in intact cells, is a potent activation of Src catalytic activity through an SH3-domain-displacement mechanism. The Src-family kinases are autoinhibited by an intramolecular association between the SH2 domain and a C-terminal tyrosine phosphorylation site, and between the SH3 domain and a polyproline-like motif in the linker region. These intramolecular interactions maintain the kinase in an inactive closed conformation (Figure 2A) [9]. The PXXPXR motif in PR acts as an external peptide ligand to disrupt the intramolecular SH3 domain interaction and convert Src into an active open conformation (Figure 2A). The PR concentration that gives half-maximal activation of Src kinase is in the low nanomolar range, indicating that PR is a potent activator of Src-family kinases [6].

PR interaction with SH3 domains and activation of Src appears to be of biological consequence in the cell. Progesterone-induced activation of the entire Src/Ras/MAPK signalling pathway in mammalian cells is dependent on the integrity of PXXPXR motif in PR and the ability of PR to interact with the SH3 domain of Src. Thus PR activation of Src is not spurious and is sufficient to generate activation of the entire MAPK phosphorylation cascade. In addition, the PR–SH3-domain interaction contributes to progesterone-induced inhibition of cell proliferation and cell-cycle progression of normal mammary epithelial cells in culture. Interestingly, point mutations in the PXXPXR motif that abolish progesterone-induced Src activation do not influence the transcriptional activity of PR. Conversely, point mutations in the DNA-binding domain or AF-2 (activation function 2) that compromise PR function as a transcription factor have no effect on the ability of PR to mediate progesterone-induced activation of Src [6]. These experimental data indicate that transcription and non-transcription actions of PR are separable. Taken together, these data suggest that PR is a dual-function protein capable of directly interacting with DNA in the nucleus and functioning in its well-established role as a transcription factor, and interacting with SH3 domains of Src and perhaps other signalling molecules to modulate intracellular signalling pathways (Figure 3).
Figure 2. Proposed mechanisms for PR and ER activation of Src kinases

(A) The PXXPXR motif in the N-terminal domain of PR interacts with the SH3 domain of Src and converts Src from an inactive ‘closed conformation’ into an active ‘open conformation’ by an SH3-displacement mechanism. (B) ER in the presence of oestrogen (E2) interacts with MNAR (modulator of non-genomic activity of ER) and Src to form a stable ternary complex through a specific oestrogen-induced interaction between the AF-2 domain of ER and LXXLL motifs in MNAR. Additional stabilizing interactions are provided by ER interaction (possibly through the ER LBD) with the SH2 domain of Src. The PXXPXR motif in MNAR interacts with the SH3 domain of Src and activates Src by an SH3-displacement mechanism. PG, progesterone; pY, phosphotyrosine.

Extranuclear progesterone signalling: role of a novel mPR

Some extranuclear signalling actions of progesterone have long been postulated to be mediated by a distinct membrane receptor, unrelated to classical intracellular PR. In particular, data on *Xenopus* oocyte maturation indicate the involvement of G-proteins and adenylate cyclase, and the response exhibits a steroid specificity that cannot be explained entirely by classical PR. A novel G-protein-coupled receptor (GPCR) was recently cloned and characterized from sea trout ovaries by a receptor-capture assay. A partially purified putative membrane receptor was used to generate monoclonal antibodies which in turn were used to screen a sea trout ovarian expression cDNA library [10]. There is strong evidence that the isolated clone encodes a bona fide mPR that is structurally unrelated to the classical nuclear PR. Recombinant mPR expressed in *Escherichia coli* binds specifically to progestins *in vitro* with a relatively high affinity ($K_d$, 30 nM), consistent with physiological concentrations of progesterone in sea trout ovaries. Deduced amino acid sequences from mPR cDNA predicted seven transmembrane domains, a characteristic of GPCRs,
but not similar to nuclear PR including the LBD. Moreover, mPR-mediated progesterone inhibition of adenylate cyclase can be inhibited by pertussis toxin treatment, consistent with coupling of the receptor to a G-protein, more specifically to \( G_{\alpha}\) proteins [10]. Progestin and gonadotropin increase both mRNA and protein levels of mPR in sea trout ovaries, further implicating mPR as a physiological mediator of progesterone-induced oocyte maturation. Furthermore, injection of mPR antisense oligonucleotides blocked progesterone-mediated oocyte maturation [10].

There are three isoforms of the membrane receptor, which are relatively well conserved from sea trout to human (\( \alpha, \beta \) and \( \gamma \)) [11]. Of interest are the \( \alpha \) and \( \beta \) isoforms. In humans, the \( \alpha \) isoform is expressed mainly in reproductive tissues, suggesting a role in extranuclear progesterone signalling in oocyte and sperm, whereas the \( \beta \) isoform is mainly expressed in the brain. The \( \gamma \) isoform is mainly expressed in kidney, lung and colon. However, the biological roles and the structure–function relationships of these novel mPRs have yet to be determined. The mPR so far has been studied most extensively for its role in mediating progesterone-induced maturation of ovaries in non-mammalian systems.

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**Figure 3. Model of direct transcription and extranuclear signalling pathways of PR**

(1) Nuclear transcription pathways. Progestins activate PR by inducing a conformational change(s) that leads to nuclear translocation, dimerization and binding to steroid-response elements (SRE; or hormone-response elements). Activated PR recruits co-activators that are essential for assembly of a productive transcription complex at the promoter. (2) Extranuclear signalling pathway. A subpopulation of PR associates in a progestin-dependent manner with cytoplasmic and/or cell-membrane-signalling molecules including the tyrosine kinase Src. PR–Src interaction is responsible for mediating progesterone-induced activation of the Src/Raf/MAPK phosphorylation cascade. Because MAPK can directly or indirectly activate other transcription factors (TF), progesterone activation of MAPK can potentially regulate sets of genes that are distinct from or complementary to those regulated by direct nuclear transcriptional pathways. Progestin-activated MAPK can also enhance the direct transcriptional activity of PR by an auto-feedback loop through phosphorylation of PR or PR-associated co-activators.
However, mPR may also have a role in mammalian cells. In human breast cancer cells (MDA-MB-231), which do not express classical nuclear PR, a rapid and transient progesterone-mediated MAPK activation and inhibition of adenylate cyclase was observed when cells were stably transfected with mPR cDNA [10].

It is not clear whether the classical PR, like mPR, can physically couple with and mediate its effect through G-proteins. Recent evidence suggested that an orphan GPCR, GPR30, could play a role in progesterone-induced growth inhibition of human breast cancer cells (MCF-7 cells). Overexpression of GPR30 inhibited growth of MCF-7 cells, while antisense inhibition of GPR30 expression reversed the growth-inhibitory effect of progesterone [12]. Whether or how GPR30 interacts with the classical PR is not known.

**Extranuclear signalling of oestrogen: role of the classical ER**

Classical intracellular ER exists as two isoforms synthesized from separate genes, ERα and ERβ. Selective deletion of ERα or ERβ in mice suggests that the effects of oestrogen on development and function of female reproductive tissues are mediated predominantly by ERα, while ERβ is important in the development of the ovary [13]. Both isoforms of ER are also expressed in non-reproductive tissue as well, including bone, cardiovascular tissue, central nervous system and brain. In addition to its well-established biological roles in reproductive tissue, oestrogen also has important biological roles in non-reproductive tissue including maintenance of bone density, vascular wall tone and protection against vascular injury and specific neural functions.

Oestrogen induces rapid activation of various signalling molecules and signal transduction cascades within seconds or minutes, and in a manner unaffected by RNA and protein-synthesis inhibitors. These extranuclear signalling actions of oestrogen are cell- and tissue-specific, and in many systems appear to be mediated by a subpopulation of classical ERα or ERβ. In breast cancer cells, oestrogen induces a rapid activation of the Src/Shc/Ras/Raf/MAPK [14–16] and PI 3-kinase/Akt [17] pathways in an ERα-dependent manner. Both signalling pathways have been shown to be involved in mediating the proliferative response of oestrogens. In osteoblasts and osteocytes, the anti-apoptotic effect of oestrogen is mediated by rapid ER activation of the Src/MAPK pathway [18]. This rapid extranuclear signalling by oestrogen appears to contribute significantly to the maintenance of bone density. In endothelial cells, ERα, by a mechanism that involves association with the p85 subunit of the PI 3-kinase and activation of the PI 3-kinase/Akt signalling pathway, mediates rapid oestrogen activation of the endothelial form of nitric oxide synthase (eNOS) [19]. In addition, ERα mediated rapid oestrogen activation of the p38-β isoform of MAPK in endothelial cells and has been implicated in the protective effect of oestrogen against endothelial cell injury. Activation of c-Jun N-terminal
kinase by ER has also been shown to be involved in mediating the anti-apoptotic effect of oestrogen in breast cancer cells [20].

Although an oestrogen-dependent interaction of ER with Src [6,17], Shc [16] and the p85 subunit of PI 3-kinase [19] has been reported, how ER interacts with these molecules in an oestrogen-dependent manner and how these interactions trigger a signalling response remains unclear. Recently, Wong et al. [21] identified a novel protein termed modulator of non-genomic activity of ER (MNAR) that acts as an adapter to couple ER with Src. MNAR facilitates and stabilizes oestrogen-dependent ER–Src interaction by forming a ternary ER–MNAR–Src complex. MNAR also enhances ER-dependent oestrogen activation of Src activity both in cell-free extracts and in intact cells. An interesting feature of MNAR is the presence of both LXXLL and PXXPXR motifs in the same molecule, suggesting a mechanism for how MNAR functions as an adapter between ER and Src. The AF-2 domain of ER interacts in a ligand-dependent manner with LXXLL motifs present in the p160 family of steroid hormone receptor co-activators. Interestingly, oestrogen antagonists induce an alternative conformation in AF-2 that does not permit this interaction [22], suggesting a mechanism for how ER antagonists block rapid oestrogen responses on Src-initiated signalling pathways. The PXXPXR motif present in MNAR is proposed to interact with the SH3 domain of Src to activate Src by an SH3-domain-displacement mechanism similar to the direct activation by PR. A proposed mechanism of how MNAR mediates ER activation of Src is shown in Figure 2(B). The LXXLL motifs of MNAR mediate oestrogen-dependent interaction with AF-2 in the LBD of ER [21,23], while the PXXPXR motif in MNAR interacts with the SH3 domain of Src to activate Src by an SH3-domain-displacement mechanism. Interaction of ER with the SH2 domain of Src may provide additional stabilizing interactions to contribute to Src activation.

In cell-transfection assays, MNAR potentiated the direct transcriptional activity of ER but this effect was blocked by inhibitors of Src and MAPKs [21]. These inhibitors do not affect the ER co-activation function of p160 co-activators; thus it is not likely that MNAR functions as a conventional ER co-activator. Potentiation of the transcriptional activity of ER is probably due to positive feedback between the oestrogen-stimulated Src/ERK signalling pathway and the ER transcription complex. Previous studies have shown that activation of the MAPK signalling pathway can stimulate the transcriptional activity of ER by phosphorylation and activation of AF-1. Therefore MNAR may also provide a link between transcriptional and non-transcriptional actions of ER.

**Extranuclear signalling actions of oestrogen: role of novel membrane receptors?**

The existence of a membrane ER, unrelated to classical intracellular ER, has also been long postulated. However, the cloning and characterization of such a
novel membrane ER analogous to the recently identified mPR has not yet been accomplished. An orphan GPCR, GPR30, has been implicated in rapid oestrogen signalling activation of MAPK in breast cancer cells by an undefined mechanism [23a]. Whether GPR30 can directly bind oestrogen and function as a bona fide receptor is not yet known. Recent studies inhibiting endogenous GPR30 by expressing antisense cDNA in MCF-7 breast cancer cells indicated that GPR30 had no effect on oestrogen-induced cell proliferation [12], suggesting that GPR30 may not be directly involved in oestrogen signalling. In addition to GPR30, sex-hormone-binding globulin (SHBG), the plasma carrier of sex steroid, has been implicated in rapid extranuclear oestrogen and androgen signalling. A cell-membrane high-affinity receptor (RSHBG) for SHBG has been identified that is reported to transduce a G-protein-dependent steroid activation of adenylate cyclase and the generation of cAMP [24]. However, the mechanism of how various steroid agonists or antagonists bound to SHBG elicit different cell signalling responses is not known. Interestingly, the oestradiol-induced proliferation of MCF-7 breast cancer cells was found to be inhibited by SHBG through RSHBG [25].

Oestrogen has been shown to rapidly increase cAMP production, intracellular calcium and intracellular InsP$_3$ [20,26]. These rapid effects of oestrogen are consistent with G-protein activation through a GPCR. As an alternative model to the existence of a separate or membrane GPCR for oestrogen, studies have suggested that a subpopulation of membrane-localized classical ER may physically associate with and activate G-proteins. For example, ecotopically expressed ER$\alpha$ and ER$\beta$ in Chinese hamster ovary cells were shown to physically associate with and activate various G-protein $\alpha$ subunits, including Go$\beta$$_3$ and Go$\beta$$_q$ [27]. In endothelial cells, endogenous ER$\alpha$ localized to the membrane and associated with Go$\alpha$ can mediate activation of eNOS production that is sensitive to pertussis toxin [28]. More recently, Razandi et al. [29] showed that ER localized in the cell membrane, bound to oestrogen and rapidly activated matrix metalloproteinases 2 and 9 through Go$\beta$$_q$, Go$\beta$$_i$, and G$\beta$$\gamma$-dependent mechanisms. These matrix metalloproteinases are necessary for oestrogen-induced heparin-binding epidermal growth factor (EGF) cleavage, making EGF available to transactivate EGF receptor and activate downstream signalling cascades of EGF receptors, including ERK and PI 3-kinases. These data further suggest the existence of crosstalk between ER and EGF receptor and a biological role for ER in the plasma membrane.

**Cell-membrane localization of a subpopulation of classical ERs**

There is growing evidence that a subpopulation of classical ER associates with the cell membrane. Ecotopic expression of classical nuclear ER in Chinese hamster ovary cells demonstrated that a small portion of total ER synthesized from the same transcript was localized to the cell membrane [27]. By confocal
microscopy, Song et al. [16] also showed that a portion of endogenous ERα in MCF-7 breast cancer cells translocates to areas of membrane ruffles after short-term (15 min) oestrogen treatment. Biochemical isolation of purified plasma membrane from endothelial cells showed the presence of ER in caveolae in response to oestrogen [30]. ER targeted to nuclei failed to mediate oestrogen anti-apoptotic effects in osteoblasts [18] and oestrogen activation of ERK1/2 in Chinese hamster ovary cells [31], while ER targeted to the cell membrane mediated rapid effects of oestrogen similar to that of the wild-type receptor. The ER LBD appears to be the minimum domain required for mediating rapid oestrogen signalling responses. ER LBD targeted to the cell membrane retains its ability to mediate rapid oestrogen activation of ERK [31] and to rescue bone cells from apoptosis similar to full-length ERα [18]. As further evidence that cell membrane/cytoplasmic localization of ER could play a significant biological role, Kumar et al. [32] recently reported that a naturally occurring variant of the metastatic tumour antigen 1 sequestered ER in the cytoplasm of breast cancer cells. The result of this cytoplasmic retention was reduced oestrogen-mediated transcription and enhancement of extranuclear oestrogen-initiated activation of ERK. Interestingly, the expression of metastatic tumour antigen 1 is higher in human breast tumours than in the adjacent normal tissue and a high level of metastatic tumour antigen 1 expression in tumour cells correlated with cytoplasmic localization of ER. These data further indicate that rapid oestrogen-initiated signalling is mediated through extranuclear actions of the receptor and may be of pathophysiological consequence.

How ER localizes to the cell membrane is not well understood. There is no evidence of post-translational modifications that could facilitate membrane insertion of ER, including palmitoylation, myristoylation and glycosylphosphoinositol [33]. Recent evidence suggests that ER translocation to the cell membrane is influenced by caveolin-1 [31]. ER was found to be associated with caveolin-1 in purified plasma membranes and caveolin-1 overexpression facilitated ER translocation to the cell membrane. Razandi et al. [33] recently identified Ser-522 in the LBD of mouse ERα as an important site for efficient membrane localization. A Ser-522→Ala mutation of ERα was significantly less effective in binding to caveolin-1 and localization to the plasma membrane as compared with the wild-type ERα. Furthermore, the Ser-522→Ala mutant behaved as a dominant-negative version of wild-type ER by sequestering and limiting the numbers of ER at the cell membrane, and reducing oestrogen-mediated activation of ERK and oestrogen stimulation of cell-cycle progression [31].

The role of extranuclear ER signalling in vivo

Most studies to date on extranuclear oestrogen-initiated signalling have been done in cell-culture systems. Only a few experiments have been reported, suggesting that rapid signalling actions of ER have a role in non-reproductive
target tissue in vivo. Vascular protection by oestrogen in ischaemia/reperfusion injury in vivo requires oestrogen–induced activation of eNOS, as mediated by the PI 3-kinase/Akt pathway [19]. Recently described ER ligands that dissociate the transcription from non-transcription actions of ER have been also identified. A synthetic compound termed oestren (4-oestren-3α,17β-diol) was reported to induce only non-transcriptional activities of ER, whereas another pyrazole compound induces transcriptional activities of ER with minimal effects on rapid signalling activities of ER [34]. The oestren compound administered to mice was found to be as effective as oestradiol in preventing ovariectomy-induced apoptosis of osteoblasts and in preserving bone density, but oestren treatment could not compensate for reduced uterine mass [34]. Additional evidence of an in vivo role for rapid oestrogen signalling comes from studies showing that the expression of subsets of endogenous oestrogen–regulated genes is induced through rapid ER activation of Src/ERK and PI 3-kinase/Akt signalling pathways that converge upon and activate other nuclear transcription factors, including c-Fos [35], Elk-1 [36], STAT5 and STAT3 (signal transducer and activator of transcription 5 and 3) [37]. In addition, inhibitors of PI 3-kinase influence a subset of endogenous oestrogen–regulated genes [38]. Thus extranuclear ER signalling could conceivably regulate specific gene networks that either complement or broaden gene repertoires regulated by the direct action of ER in the nucleus as a transcription factor.

Conclusions

Although significant advances in the understanding of the rapid extranuclear signalling actions of oestrogen and progesterone have been made in the past few years, several important questions remain unanswered. Classical intracellular PR can mediate both genomic and extranuclear signalling of progesterone. It will be important in future studies to determine the contribution of extranuclear signalling to biological responses to progesterone such as cell proliferation, adhesion, mobility and migration, and gene expression. The discovery of a novel mPR opens the way for study of the interrelationship between classical PR and mPR. That both mPR and classical PR can mediate rapid progesterone activation of MAPK suggests the possibility of crosstalk between the two receptors. Whether the classical PR and mPR exist in the same tissues or cells and activate the same or distinct signalling pathways remains to be determined. In addition, more work will be needed to determine the biological roles of mPR in higher vertebrates and the role of the different mPR isoforms in different tissues.

Although extranuclear signalling actions of oestrogen have been shown to be mediated in part by classical ER localized to cell membrane, how a subpopulation of ER localizes to the cell membrane remains to be determined. Unlike mPR, which has been cloned and characterized, a novel membrane ER has yet to be identified. The identification of MNAR has created exciting
opportunities for further study and provides important insights into how ER can couple with and trigger activation of signal transduction pathways. However, further experiments are needed to prove the proposed mechanism of how MNAR physically and functionally couples ER with Src (Figure 2B). How MNAR expression affects ER-mediated cell responses such as cell proliferation, survival and apoptosis also remains to be determined. It will also be important to determine how MNAR expression is regulated and if MNAR expression in breast tumours plays a role in the cellular sensitivity of ER to oestrogen and synthetic ligands. It will be interesting to determine the role of MNAR in mediating rapid signalling actions of other steroid hormones. PR is the only steroid hormone receptor that contains a PXXPXR motif, capable of direct interaction with SH3 domains [6], suggesting that it may not require an adapter such as MNAR and may have evolved a more direct mechanism for interaction with and activation of Src. Although AR has been reported to interact with SH3 of Src through a polyproline sequence in its N-terminal domain, interaction with Src does not appear to be direct. Purified recombinant AR is not able to directly bind SH3 domain of Src [6]. AR has been reported to interact and activate Src indirectly through ER [39].

It will also be important to determine whether MNAR and p160 co-activators compete for interaction of AF-2 through LXXLL motifs and, if so, how this might affect the relative balance between transcriptional and non-transcriptional activities of ER. Could MNAR also play a role in facilitating translocation of a subpopulation of ER to the cell membrane? The intracellular localization of MNAR and how it traffics in the cell with ER also needs to be determined. As we learn more about the mechanism and in vivo roles of rapid ER extranuclear-initiated signalling in different tissues, it may be possible to develop ER ligands that selectively target the transcription or non-transcription signalling pathways for different therapeutic purposes. It may turn out that MNAR participates in selectively inducing the non-transcriptional activities of ER through distinct conformational changes in the AF-2 region that favour interaction with MNAR over direct transcriptional co-activators. Finally, since PR is co-expressed with ER in target cells, it will be important to determine whether and how crosstalk between extranuclear PR and ER signalling pathways contribute to the well-known physiological interplay between oestrogen and progesterone in reproductive tissues.

Summary

- **The classical nuclear PR contains a PXXPXR motif in the N-terminal domain that interacts directly with the SH3 domain of Src and activates Src through an SH3-domain-displacement mechanism.**
• A novel mPR unrelated to nuclear PR has been cloned and characterized from sea trout ovaries. mPR binds progestin with high affinity and is required for progesterone-induced oocyte maturation. mPR appears to be a GPCR with seven transmembrane domains that mediates rapid progesterone-induced inhibition of adenylate cyclase and activation of MAPK.

• The classical nuclear ER through an adapter protein, MNAR, interacts with SH3 domain of Src and activates its kinase activity.

• A subpopulation of classical ER associated with the cell membrane can mediate certain extranuclear signalling actions of oestrogen. Sequences in the ER LBD appear to be important for membrane localization.

References


