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Regulation of Cell Signalling Cascades by Steroid Hormones

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Abstract Steroid hormones control a wide variety of cellular functions important for cell homeostasis, proliferation, differentiation, and apoptosis. Evidence collected in the last few years indicates that this regulation is mediated by a complex interface of direct control of gene expression (so-called ''transcriptional'' action, driven by receptors that are localized in cell nucleus) and by regulation of cell signaling/phosphorylation cascades (''extranuclear'' action, mediated by receptors that are localized in close proximity to cellular membrane). Regulation of gene expression takes place via ligand-dependent receptor binding to target gene promoters as part of the preinitiation complex, which leads to chromatin remodeling and ultimately regulates the rate of gene expression. Steroid-mediated regulation of cell signaling does not depend on gene transcription or protein biosynthesis. The molecular mechanism of this phenomenon is not fully understood. This review will focus on recent developments related to our understanding of the molecular mechanism of the extranuclear action of steroid hormones. J. Cell. Biochem. 93: 20–27, 2004. © 2004 Wiley-Liss, Inc.

Key words: nuclear receptors; non-genomic action of nuclear receptors; signal transduction; rapid signaling

Action of steroid hormones, as well as certain vitamins and lipid metabolites, is mediated by their intracellular receptors that are traditionally characterized as ligand-inducible transcription factors [Mangelsdorf et al., 1995; McKenna and O'Malley, 2002]. The receptors of steroid hormones include estrogen receptor (ER), androgen receptor (AR), progesterone receptor (PR), and glucocorticoid receptor (GR). AR and GR in the absence of ligand remain in the cytoplasm in association with chaperone complexes. Ligand binding by inducing conformational changes, leads to dissociation of these complexes, receptor dimerization, and translocation to the nucleus where they bind to response elements within the regulatory regions of target genes. Receptors for nonsteroidal ligands, such as the vitamin D receptor (VDR), retinoic acid receptors (RARs), retinoid X receptors (RXRs), and peroxisome proliferators activated receptors (PPARs) are primarily localized in the nucleus. In the absence of ligand, they are associated with histone deacetylase-containing complexes tethered through corepressors. This process results in silencing of the promoters. Binding of receptor agonist, reduces affinity of binding to corepressors and leads to dissociation of these complexes. Liganded receptors (either steroid or nonsteroid receptors) bring to the DNA template the coregulatory complexes, including chromatin remodeling and ATP-dependent chromatin-modification complexes. These events facilitate the recruitment of the transcription machinery in a coordinated and/or combinatorial manner leading to activation of RNA and protein biosynthesis (for review see McKenna and O'Malley [2002]).

While it has been well established that steroid receptors can act as ligand-inducible transcription factors, it has been apparent for many years that not all biological effects of steroid hormones are accomplished via direct regulation of gene expression. In addition, they induce rapid activation of diverse signal transduction

Abbreviations used: SR, steroid receptor; ER, estrogen receptor; AR, androgen receptor; PR, progesterone receptor; GR, glucocorticoid receptor; PI3K, phosphatidylinositide-3 kinase; Akt, protein kinase B; LBD, ligand-binding domain; Erk 1/2, extracellular signal-related kinases 1 and 2; E2, 17β-estradiol.

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pathways in a time frame (seconds to minutes) that is too rapid to be mediated by biosynthesis of RNA or protein and that is insensitive to inhibitors of RNA and protein synthesis. Rapid actions of steroid hormones were reported as early as 60 years ago [Sutter-Dub, 2002]. This phenomenon received major attention in 1967, when Szego and Davis reported that physiologic doses of 17β-estradiol given intravenously increased uterine adenosine 3′,5′-monophosphate (cAMP) concentration in ovariectomized mice within 15 s [Szego and Davis, 1967]. Later, Pietras and Szego described the presence of cytoplasmic membrane binding sites for estradiol (E2) in endometrial cells [Pietras and Szego, 1977]. Progress in this field has been very slow and had mostly empirical character until a few years ago, when evidence at the molecular level started accumulating to support and elucidate the mechanisms of alternative signaling action of steroid hormones. Different terms have been used to describe these mechanisms. Reflecting a relatively low level of understanding, most of them are based on a negative definition. The most popular ones have been "non-genomic," "non-transcriptional," or "non-classical." The designation "non-genomic," or "non-transcriptional" however is not satisfactory, because the activation of cell signaling cascades by steroid hormones via post-translational modification of transcription factors may affect the rate of gene expression. For simplicity, we are going to call them "extranuclear" action of steroid hormones and define them as: (1) action that is originated in cytoplasm, (2) is too rapid to be compatible with RNA or protein synthesis, and (3) is not sensitive to inhibitors of biosynthesis of RNA, or protein.

RECEPTORS THAT MEDIATE EXTRANUCLEAR ACTION OF STEROID HORMONES

An important question still remains regarding the identity of proteins that initiate these extranuclear responses. Several possibilities have been suggested so far: (i) the same classical "nuclear" receptor in a non-nuclear locations; (ii) other known membrane receptors, enzymes or channels, which are capable of binding steroids; and (iii) new membrane receptors of steroid hormones. Finally, this action may be mediated by a (iv) complex network of steroid binding proteins, which may include classical receptor as well as other steroid binding proteins.

While some studies have suggested the existence of a membrane ERs unrelated to conventional ERα, or β (for review see Wehling [1997]), confirmation of existence of the bona fide novel membrane ERs has not been accomplished. Several lines of evidence indicate that classical ER is involved not only in transcriptional action, but also in regulation of rapid extranuclear effects of estrogens. Immunohistochemical evaluations using antibodies against different epitopes of ERα demonstrated positive staining at the plasma membrane, indicating that ERα can be localized in close proximity to extracellular membrane [Pappas et al., 1995]. Confocal microscopy studies showed that treatment with E2 rapidly induces formation of membrane ruffles, pseudopodia, and ERα translocation to cell membrane in MCF-7 breast cancer cells. The E2-induced morphological changes were prevented by antiestrogens [Song et al., 2002]. Endogenous ERα was purified from plasma membrane and caveolae fraction of endothelial cells [Pyo Kim et al., 1999]. Good correlation was found between the level of expression of the classic receptor and ability of receptor' ligands to stimulate activation of cell signaling cascades. Estradiol stimulation of nitric-oxide synthase (NOS) could be further increased by overexpression of ERα. The acute response of endothelial NOS (eNOS) to 17β-estradiol could be reconstituted by ectopic expression of ERα in COS-7 cells [Shaul et al., 1997]. Finally, targeting of ERα to cell nucleus abrogated signaling action of E2 [Razandi et al., 2002].

A new membrane progesterone receptor (mPR) in spotted sea trout oocytes has been recently identified using monoclonal antibodies directed against progestin-binding membrane proteins [Zhu et al., 2003b]. Sea trout mPR has little sequence homology to GPCRs, it contains seven putative hydrophobic transmembrane domains, and thus could be considered a novel member of the GPCRs receptor family. Based on its sequence, a new family of mPR-related proteins was identified in a number of different species, including frog, human, and mouse [Zhu et al., 2003a]. Although the mPR might be critical for oocyte physiology, its existence is unlikely to explain all extranuclear effects of progestins. Compelling evidence suggests that just like ER, classical PR is also playing an important role in mediating extranuclear action in multiple tissues. In the frog oocyte,

modulation of classical PR levels has been shown to regulate progesterone-induced maturation [Bayaa et al., 2000; Boonyaratanakornkit et al., 2001], classical PRs have been shown to be associated with Src [Boonyaratanakornkit et al., 2001] and PI3K in cell membranes [Bagowski et al., 2001].

Therefore, perhaps both the classical and membrane steroid receptors are utilized, at least in case of PRs, in mediation of extranuclear action. Possibly, the expression levels of these receptors could vary in different cells, they could also have different selectivity for ligand binding. Most importantly, ligand binding by these receptors could result in activation of different signaling pathways. Therefore, receptor-specific ligands could potentially regulate distinct cellular functions.

EXTRANUCLEAR ACTION OF STEROID HORMONES

Almost all members of the steroid hormone family, from the corticosteroids (glucocorticoids and mineralocorticoids) to the gonadal hormones (estrogens, progestins, and androgens), can exhibit extranuclear effects [Falkenstein et al., 2000; Watson and Gametchu, 2003]. These effects range from activation of adenylyl cyclase (AC), mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI3K), to rise in intracellular calcium concentrations (Fig. 1). One of the earliest observed rapid actions of sex steroids is the regulation of transmembrane ion fluxes. Sex steroids act as vasodilators, and part of this effect relies on the modulation of intracellular Ca^{2+} in endothelial and smooth muscle cells. It is unclear however, whether these steroid hormones play a direct role in the control of membrane ion channels, or their action is mediated via activation of cell phosphorylation cascades (for review see Falkenstein et al., 2000).

Activation of Src/MAPK Pathways by ERα

One of the best-characterized extranuclear actions of steroids is the rapid activation of the Ras/Raf/ERK pathway. In nerve cells, E2 rapidly triggers Erk 1/2 activation, leading to cFos gene expression [Watters et al., 1997]. Rapid activation of this pathway was also found in osteoblasts [Endoh et al., 1997] and in white adipocytes [Garcia Dos Santos et al., 2002]. Estrogen activated growth of human colon carcinoma-derived Caco-2 cell is mediated through rapid and reversible stimulation of the cSrc and cYes and activation of Erk1 and Erk2 kinases [Di Domenico et al., 1996]. In the human breast cancer cell line MCF-7, estradiol triggered rapid increase in the active form of p21ras, rapid tyrosine phosphorylation of Shc

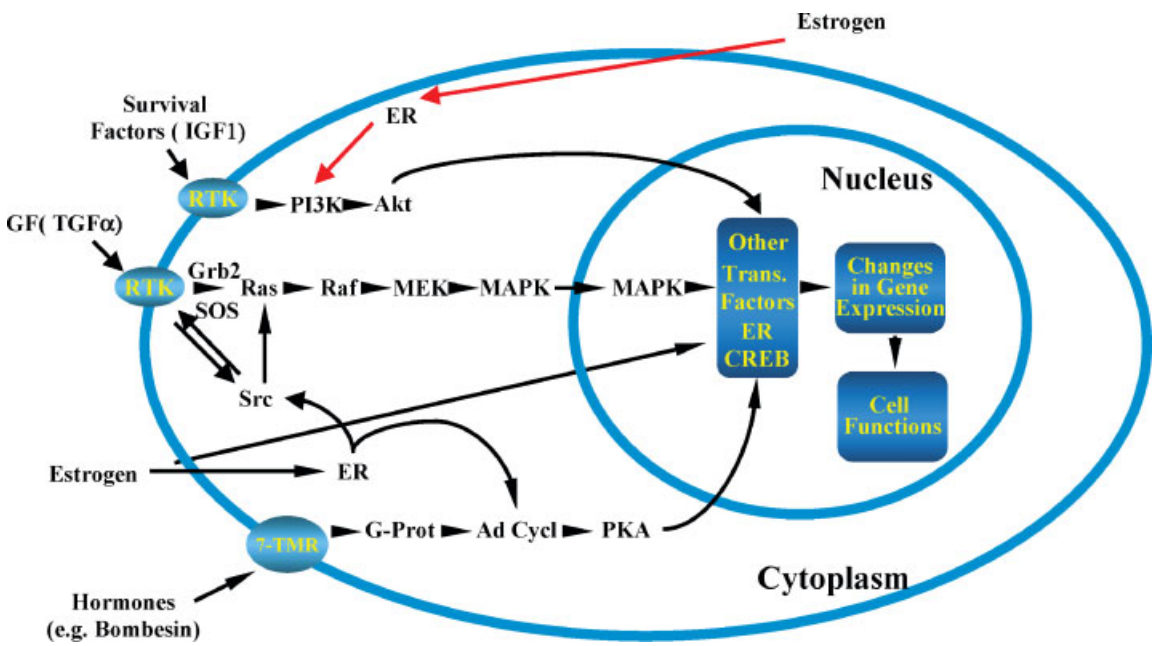

Fig. 1. Signaling pathways regulated by estrogen receptor.

and p190, and association of p190 with the guanosine triphosphatase (GTPase) activating protein (GAP). Both Shc and p190 are substrates of activated Src, and once phosphorylated, can interact with other proteins and stimulate p21ras. Estrogen-mediated stimulation of Ras/Raf/ERK pathway promotes MCF7 cell proliferation [Migliaccio et al., 1996].

Several mechanisms have been proposed to explain the initial steps that trigger this pathway. Levin and colleagues have suggested that ERα in presence of estradiol, via activation of Src, stimulates Gαq, Gαi, and Gβγ -dependent activation of matrix metalloproteinases 2 and 9 (MMP-2 and -9). Using an antisense approach they have shown that MMP-2 and MMP-9 are required for fast E_2-induced heparin-binding EGF (HB-EGF) cleavage and release, activation of EGFR, and downstream signaling to ERK and PI3K in breast cancer cells, and p38 MAPK in endothelial cells [Razandi et al., 2003]. This model however did not clarify initial steps leading to activation of Src.

It has been also demonstrated that AF1 domain of ERα can directly interact with SH2 domain of the adapter protein Shc and that this interaction is important for E2-mediated activation of the MAP kinase pathway [Song et al., 2002]. The same group has later demonstrated that Shc, ERα, and IGF-1R form a ternary complex and that this interaction leads to IGF-1R phosphorylation and activation [Song et al., 2004]. These data, however, do not explain the ability of ERα ligand binding domain (LBD), which is inactive transcriptionally, to mediate estrogen responses in regulating cellular signaling pathways [Castoria et al., 2001; Kousteni et al., 2001].

Multiple evidence suggests that activation of the tyrosine kinase, cSrc, represents one of the initial steps in ER mediated cell signaling [Migliaccio et al., 2002]. In vitro, ER is able to interact with the SH2 domain of Src [Migliaccio et al., 2000; Boonyaratanakornkit et al., 2001; Castoria et al., 2001; Wong et al., 2002], however, this interaction does not appear to be sufficient for Src activation [Boonyaratanakornkit et al., 2001; Wong et al., 2002]. Under basal conditions, the catalytic domain of Src is constrained in an inactive state through intramolecular interactions. Binding of the SH2 domain to the C-terminal phosphorylated tyrosine and the SH3 domain to the proline-rich region in the Src linker domain locks the molecule in an inhibited conformation [Matsuda et al., 1990]. Full catalytic activation requires release of these constraints. The kinase activity of Src can be enhanced by binding of phosphotyrosine-containing sequences to the SH2 domain and binding of proline-rich sequences to the SH3 domain [Hubbard et al., 1998].

Using affinity purification we [Wong et al., 2002] have recently isolated ER-interacting protein, termed MNAR (modulator of nongenomic action of ER) that promotes ligand-dependent interaction between the ERα and members of the Src family of tyrosine kinases. Sequence analysis of MNAR has revealed presence of 10 LXXLL motifs. Similar motifs in other transcription factors have been shown to interact with a hydrophobic groove on the surface of the ligand-binding domain of nuclear hormone receptors. MNAR also contains three PXXP motifs. These motifs may interact with Src homology domain 3 (SH3) present in multiple signal transducing molecules [Kay et al., 2000]. In addition to ER α and β; AR, GR, and PR also interact with MNAR in a ligand-dependent manner [Wong et al., 2002]. Interaction analysis and functional evaluation of ERα, MNAR and Src mutants, demonstrated that coordinate binding of MNAR and ER to Src's SH3 and SH2 domains, respectively, stabilized by ER–MNAR interaction through MNAR's LXXLL motifs, leads to activation of cSrc and Src-mediated signaling (Fig. 2) [Barletta et al., 2004]. MNAR therefore is a scaffold protein that mediates formation of multi-protein complexes by providing various interaction surfaces. It is possible that MNAR is just the first member of a family of adaptor proteins that integrate action of ER, and potentially some other receptors, in regulation of cell signaling cascades.

Activation of Src/MAPK Pathway by PR

Classical PR, in addition to its role as a transcription factor also regulates cellular signaling cascades. Especially well documented is its ability to activate Src/MAP kinase pathway. Two isoform of classical PR exist, A and B, where PR_A is a shorter version of receptor, which is lacking the first 168 N-terminal amino acids. It has been initially demonstrated that PR_B activation of Src and MAPK pathway requires ERα [Migliaccio et al., 1998]. However, an analysis of protein interactions provided no evidence for the existence of a ternary complex that would include PR_B, ERα, and cSrc. Authors

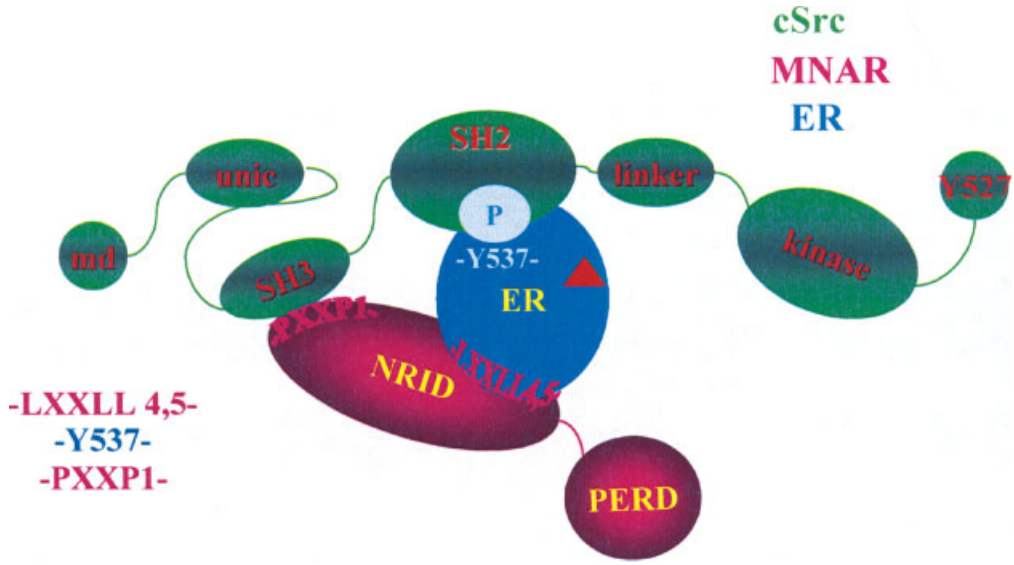

Fig. 2. Model for MNAR/ER/Src interaction. Coordinate interaction between Src SH3 and SH2 domains and MNAR and ER correspondingly, stabilized by ER-MNAR interaction leads to Src activation.

therefore postulated that ERα and PR_B interact in the absence of ligand, and that in the presence of progestin ERα is released from the complex and can now interact with cSrc. It is the ERα–cSrc interaction that triggers activation of Src and MAPK pathway. Progesterone activation of Erk1/2 was inhibited by antiprogestins, as well as by antiestrogens [Migliaccio et al., 1998].

Ballare et al. [2003] have recently extended this model by reporting that activation of cSrc by progestins depends upon the presence of unliganded ERα, which interacts with two domains that are localized in the N-terminal part of PR_B. They proposed that cSrc/MAPK activation by PR is mediated indirectly by the interaction of the Src-homology (SH2) domain of cSrc with phosphotyrosine 537 of ERα. In mammalian cells, the interaction of PR_B with ERα and the progestin-mediated activation of the Src/Erk cascade were abolished by deletion of either one of these domains of PR. Deletion of these domains did not affect PR_B transcriptional activity.

In contrast with these data, direct and hormone-dependent interaction of PR and cSrc SH3 domain was demonstrated by Boonyaratanakornkit et al. [2001]. The authors have also shown that endogenous as well as overexpressed and purified proteins interact and that this interaction is mediated by polyproline region encoded by aa 421–428 of PR_B with Src' SH3 domain. This interaction was necessary and sufficient for activation of Src and downstream targets, leading to phosphorylation/activation of Erk 1/2. In addition to cSrc, PR also interacted with SH3 domains of hematopoietic cell kinase (Hck), the regulatory subunit of PI3K, p85, Grb2, and the tyrosine kinases Fyn and Crk [Boonyaratanakornkit et al., 2001]. Reasons for this seeming incongruity between these models are presently unknown.

Activation of Src/MAPK Pathway by AR

In addition to ER and PR, androgen receptor (AR) also mediates rapid activation of MAPK pathway by direct binding and activation of cSrc [Migliaccio et al., 2000]. In agreement with these data, dihydrotestosterone (DHT)-induced activation of Erk 1 and Erk 2 kinases was blocked by Src inhibitor PP1 [Kousteni et al., 2001]. In HeLa cells, the response to a transfected ER, but not to AR, was abrogated by the cotransfection of Src mutant lacking the SH2 domain (Src δSH_2). On the other hand, the action of AR, but not of ER, was decreased by Src mutant lacking the SH3 domain (Src δSH_3) [Kousteni et al., 2001]. These findings suggest that the two receptors mediate cSrc activation by binding to different domains of Src. Indeed, the ERα interacts with SH2 domain, whereas proline-rich stretch of AR interacts with the SH3 domain of Src [Migliaccio et al., 2000]. In cells expressing AR and ERα, treatment with DHT, or E2, leads to ER–AR–Src complex formation. Antagonists of both ER and AR, prevented both assembly of ER–AR–Src

complex and activation of Src/MAPK pathway [Migliaccio et al., 2000]. In contrast with these findings, Boonyaratanakornkit et al. [2001] have recently failed to detect direct interaction between AR and Src. Potential explanation for these discrepancies could be that some scaffold proteins, such as MNAR for example [Wong et al., 2002], could potentially mediate interaction between AR and Src.

SR Mediated Activation of PI3 Kinase Pathway

In addition to Src/MAPK pathway, extranuclear action of steroid hormones can be mediated by the recruitment of lipid kinases. In presence of E2, ERα interacts with the regulatory subunit of PI3 kinase, p85, thus triggering an activation of the catalytic subunit and increasing intracellular production of phosphoinositides [Simoncini et al., 2000]. Recruitment and activation of PI3K by ER–E2 complex does not involve ER binding to SH2 domain of p85. It is therefore not clear whether ER binds to p85 alone, or as part of a multiprotein complex, components of which may interact with SH3 and SH2 domains of p85, leading to activation of PI3K. One of the principal targets of this cascade is the serine–threonine protein kinase Akt/protein kinase B. Activation of Akt mediates many of the downstream cellular effects of PI3K triggered by E2, including rapid activation of the endothelial isoform of the nitric oxide (NO) synthase (eNOS) [Simoncini et al., 2000]. Mice treated with estrogen show increased eNOS activity and decreased vascular leukocyte accumulation after ischemia and reperfusion injury. Activation of PI3K by estrogens is important also in breast cancer cells, where E2 rapidly triggers association of ERα with Src and p85 [Castoria et al., 2001]. Formation of this complex leads to activation of Src/MAP and PI3K/Akt pathways, which stimulates cell cycle progression [Castoria et al., 2001] and inhibits apoptosis [Campbell et al., 2001]. Other SRs, such as AR and GR also interact with p85 [Simoncini et al., 2000].

CONCLUSIONS

It is well established that steroid hormones, in addition to classical transcriptional action, also regulate cellular processes outside of the cell nucleus. Extranuclear signaling represents a mechanism through which they regulate activity of cellular proteins and thus rapidly fine-tune important cellular functions.

Some classical receptors trigger cell signaling via direct interaction and activation of various kinases. Interaction with PR, for example, is sufficient for activation of Src. Other receptors require adaptor proteins, like MNAR, that provide interaction surfaces for assembly of functionally distinct multi-protein complexes. ER–MNAR–Src complex is capable to phosphorylate proteins in response to changes in E2 concentration. Presence of ten LXXLL motifs in the MNAR molecule suggests that it can interact with multiple steroid receptors. Our preliminary data indeed indicate that steroid receptors may utilize distinct LXXLL motifs for their binding to MNAR (F. Barletta and B. Cheskis, unpublished). An interesting question remains, whether they can interact simultaneously, which could potentially explain why activation of Src by AR requires presence of ER. It is presently unclear also why PR interaction with Src is sufficient for Src activation, while ER requires MNAR. It is possible that PR binds to the SH3 domain with higher affinity than ER binds to the SH2 domain. Binding of MNAR could potentially stabilize the ER–Src complex. It is also possible that at different receptor expression levels steroid receptors may or may not require adaptor proteins for activation of some kinases. Extranuclear signaling is particularly important in non-traditional steroid target tissues, such as bone, cardiovascular and central nervous systems where the expression level of steroid receptor is low. It is possible that expression of adaptor proteins, like MNAR, is especially important in these tissues.

Good understanding of the molecular mechanisms through which extranuclear actions of steroids are exerted is essential as we contemplate newer and more selective pharmacological tools for endocrine therapies.

REFERENCES

Bagowski CP, Myers JW, Ferrell JE, Jr. 2001. The classical progesterone receptor associates with p42 MAPK and is involved in phosphatidylinositol 3-kinase signaling in *Xenopus* oocytes. J Biol Chem 276:37708–37714.

Ballare C, Uhrig M, Bechtold T, Sancho E, Di Domenico M, Migliaccio A, Auricchio F, Beato M. 2003. Two domains of the progesterone receptor interact with the estrogen receptor and are required for progesterone activation of the c-Src/Erk pathway in mammalian cells. Mol Cell Biol 23:1994–2008.

Barletta F, Wong CW, McNally C, Komm BS, Katzenellenbogen B, Cheskis BJ. 2004. Characterization of the interactions of estrogen receptor and MNAR in the activation of cSrc. Mol Endocrinol 18:1096–1108.

Bayaa M, Booth RA, Sheng Y, Liu XJ. 2000. The classical progesterone receptor mediates *Xenopus* oocyte maturation through a nongenomic mechanism. PNAS 97:12607–12612.

Boonyaratanakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, Miller WT, Edwards DP. 2001. Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. Mol Cell 8:269–280.

Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S, Nakshatri H. 2001. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: A new model for anti-estrogen resistance. J Bio Chem 276:9817–9824.

Castoria G, Migliaccio A, Bilancio A, Di Domenico M, de Falco A, Lombardi M, Fiorentino R, Varricchio L, Barone MV, Auricchio F. 2001. PI3-kinase in concert with Src promotes the S-phase entry of oestradiol-stimulated MCF-7 cells. EMBO J 20:6050–6059.

Di Domenico M, Castoria G, Bilancio A, Migliaccio A, Auricchio F. 1996. Estradiol activation of human colon carcinoma-derived Caco-2 cell growth. Cancer Res 56: 4516–4521.

Endoh H, Sasaki H, Maruyama K, Takeyama K, Waga I, Shimizu T, Kato S, Kawashima H. 1997. Rapid activation of MAP kinase by estrogen in the bone cell line. Biochem Biophys Res Commun 235:99–102.

Falkenstein E, Tillmann H-C, Christ M, Feuring M, Wehling M. 2000. Multiple actions of steroid hormones—a focus on rapid, nongenomic effects. Pharmacol Rev 52:513–556.

Garcia Dos Santos E, Dieudonne MN, Pecquery R, Le Moal V, Giudicelli Y, Lacasa D. 2002. Rapid nongenomic E2 effects on p42/p44 MAPK, activator protein-1, and cAMP response element binding protein in rat white adipocytes. Endocrinology 143:930–940.

Hubbard SR, Mohammadi M, Schlessinger J. 1998. Autoregulatory mechanisms in protein-tyrosine kinases. J Biol Chem 273:11987–11990.

Kay BK, Williamson MP, Sudol M. 2000. The importance of being proline: The interaction of proline-rich motifs in signaling proteins with their cognate domains. FASEB J 14:231–241.

Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC. 2001. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: Dissociation from transcriptional activity. Cell 104:719–730.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, et al. 1995. The nuclear receptor superfamily: The second decade. Cell 83:835–839.

Matsuda M, Mayer BJ, Fukui Y, Hanafusa H. 1990. Binding of transforming protein, P47gag-crk, to a broad range of phosphotyrosine-containing proteins. Science 248:1537–1539.

McKenna NJ, O'Malley BW. 2002. Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 108:465–474.

Migliaccio A, Di Domenico M, Castoria G, de Falco A, Bontempo P, Nola E, Auricchio F. 1996. Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells. EMBO J 15:1292–1300.

Migliaccio A, Piccolo D, Castoria G, Di Domenico M, Bilancio A, Lombardi M, Gong W, Beato M, Auricchio F. 1998. Activation of the Src/p21ras/Erk pathway by progesterone receptor via cross-talk with estrogen receptor. EMBO J 17:2008–2018.

Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, Barone MV, Ametrano D, Zannini MS, Abbondanza C, Auricchio F. 2000. Steroid-induced androgen receptor-oestradiol receptor {beta}-Src complex triggers prostate cancer cell proliferation. EMBO J 19:5406–5417.

Migliaccio A, Castoria G, Di DomeniIco M, De Falco A, Bilancio A, Auricchio F. 2002. Src is an initial target of sex steroid hormone action. Ann N Y Acad Sci 963:185–190.

Pappas TC, Gametchu B, Watson CS. 1995. Membrane estrogen receptors identified by multiple antibody labeling and impeded-ligand binding. FASEB J 9:404–410.

Pietras RJ, Szego CM. 1977. Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. Nature 265:69–72.

Pyo Kim H, Young Lee J, Kim Jeong J, Won Bae S, Kyu Lee H, Jo I. 1999. Nongenomic stimulation of nitric oxide release by estrogen is mediated by estrogen receptor [alpha] localized in caveolae*1. Biochem Biophys Res Commun 263:257–262.

Razandi M, Oh P, Pedram A, Schnitzer J, Levin ER. 2002. ERs associate with and regulate the production of caveolin: Implications for signaling and cellular actions. Mol Endocrinol 16:100–115.

Razandi M, Pedram A, Park ST, Levin ER. 2003. Proximal events in signaling by plasma membrane estrogen receptors. J Biol Chem 278:2701–2712.

Shaul PW, Yuhanna IS, Sherman TS, German Z, Chen Z. 1997. Role of estrogen receptor in the acute effects of estrogen on endothelial nitric oxide synthase. Circulation 96:I–48.

Simoncini T, Hafezl-Moghadam A, Brazil DP, Ley K, Chin WW, Liao JK. 2000. Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. Nature 407:538–541.

Song RX-D, McPherson RA, Adam L, Bao Y, Shupnik M, Kumar R, Santen RJ. 2002. Linkage of rapid estrogen action to MAPK activation by ER{alpha}-Shc association and Shc pathway activation. Mol Endocrinol 16:116–127.

Song RX, Barnes CJ, Zhang Z, Bao Y, Kumar R, Santen RJ. 2004. The role of Shc and insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor {alpha} to the plasma membrane. PNAS 101: 2076–2081.

Sutter-Dub M-T. 2002. Rapid non-genomic and genomic responses to progestogens, estrogens, and glucocorticoids in the endocrine pancreatic B cell, the adipocyte, and other cell types. Steroids 67:77–93.

Szego CM, Davis JS. 1967. Adenosine 3′,5′-monophosphate in rat uterus: Acute elevation by estrogen. Proc Natl Acad Sci USA 58:1711–1718.

Watson CS, Gametchu B. 2003. Proteins of multiple classes may participate in nongenomic steroid actions. Exp Biol Med 228:1272–1281.

Watters JJ, Campbell JS, Cunningham MJ, Krebs EG, Dorsa DM. 1997. Rapid membrane effects of steroids in

neuroblastoma cells: Effects of estrogen on mitogen activated protein kinase signalling cascade and c-fos immediate early gene transcription. Endocrinology 138:4030–4033.

Wehling M. 1997. Specific, nongenomic actions of steroid hormones. Annu Rev Physiol 59:365–393.

Wong CW, McNally C, Nickbarg E, Komm BS, Cheskis BJ. 2002. Estrogen receptor-interacting protein that modulates its nongenomic activity-crosstalk with Src/Erk phosphorylation cascade. PNAS 99:14783–14788.

Zhu Y, Bond J, Thomas P. 2003a. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. PNAS 100:2237–2242.

Zhu Y, Rice CD, Pang Y, Pace M, Thomas P. 2003b. From the cover: Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. PNAS 100:2231–2236.