5 Plasma Membrane Receptors for Steroid Hormones in Cell Signaling and Nuclear Function

Richard J. Pietras, PhD, MD
and Clara M. Szego, PhD

CONTENTS

INTRODUCTION
STEROID RECEPTOR SIGNALING MECHANISMS
PLASMA MEMBRANE ORGANIZATION AND STEROID HORMONE RECEPTORS
INTEGRATION OF MEMBRANE AND NUCLEAR SIGNALING IN STEROID HORMONE ACTION
MEMBRANE-ASSOCIATED STEROID RECEPTORS IN HEALTH AND DISEASE
CONCLUSION

1. INTRODUCTION

Steroid hormones play an important role in coordinating rapid, as well as sustained, responses of target cells in complex organisms to changes in the internal and external environment. The broad physiologic effects of steroid hormones in the regulation of growth, development, and homeostasis have been known for decades. Often, these hormone actions culminate in altered gene expression, which is preceded many hours earlier by enhanced nutrient uptake, increased flux of critical ions, and other preparatory changes in the synthetic machinery of the cell. Because of certain homologies of molecular structure, specific receptors for steroid hormones, vitamin D, retinoids, and thyroid hormone are often considered a receptor superfamily. The actions of ligands in this superfamily are postulated to be regulated by receptors in the cell nucleus. On binding ligand, nuclear receptors associate with target genes and permit selective transcription. This genomic mechanism is generally slow, often requiring hours or days before the consequences of hormone exposure are evident. However, steroids also elicit rapid cell responses, often within seconds (*see* Fig. 1). The time course of these acute events parallels that evoked by peptide agonists, lending support to the conclusion that they do not require precedent gene activation. Rather, many rapid effects of steroids, which have been termed nongenomic, appear to be owing to specific recognition of hormone at the cell membrane. Although the molecular identity of binding site(s) remains elusive and the signal transduction pathways require fuller delineation, there is firm evidence that primary steroid action is initiated by plasma membrane receptors.

A current challenge is to determine the precise relation of these rapid responses to intermediate and long-term effects. It appears that plasma membrane–binding sites for steroids are coupled to rapid signal transduction systems that act in concert with coactivator pro-

From: *Endocrinology: Basic and Clinical Principles, Second Edition*
(S. Melmed and P. M. Conn, eds.) © Humana Press Inc., Totowa, NJ

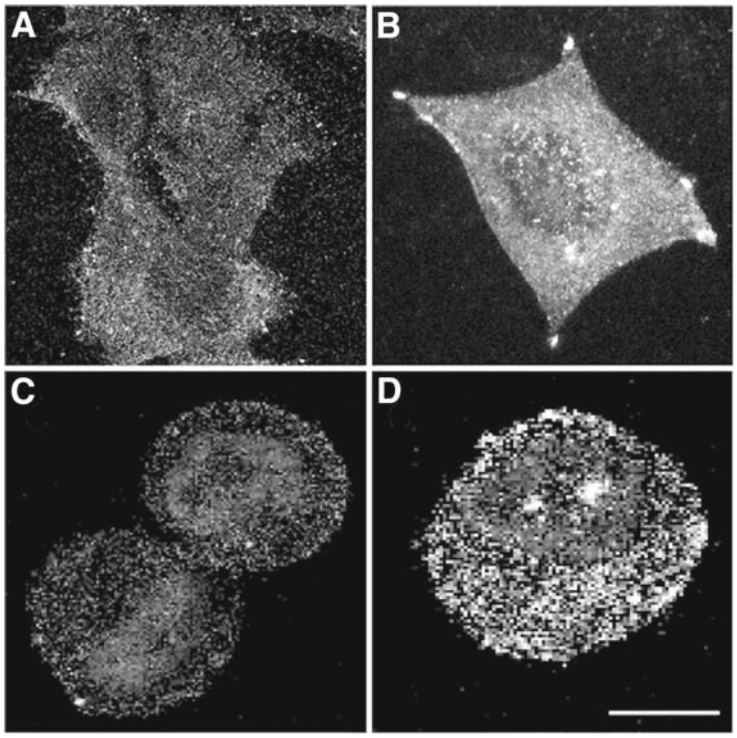

Fig. 1. Rapid response to treatment with E_2 in human breast cancer cells. Tumor cells derived from established cell lines (MCF-7), as well as those isolated from a recent tumor excision (BC), were plated on glass cover slips and grown to 60% confluency. Thereafter, cells were maintained for 24–48 h in steroid-free medium enriched with 1% dextran-coated charcoal-treated serum before the start of experiments. MCF-7 cells were treated for 2 min with control vehicle **(A)** or 2 n*M* E_2 **(B)**, and BC cells were similarly treated for 2 min with control vehicle **(C)** or 2 n*M* E_2 **(D)**. Cells were then immediately fixed and stained with a polycolonal antibody directed to phospho-p42/p44 mitogen-activated protein kinase (MAPK) (Thr203/Tyr204). Binding of primary antibody was detected by a fluorescent fluorescein isothiocyanate (FITC)-conjugated anti–rabbit IgG. Confocal microscope images of cells immunostained for phospho-MAPK (p-MAPK) allows *in situ* detection and subcellular resolution of fluorescent deposits. At baseline for both cell types (A,C), p-MAPK resides predominantly near the surface membrane and in the cortical cytoplasm. Treatment with estradiol (B,D) elicits rapid activation of p-MAPK, as evidenced by increased phosphorylation and enhanced translocation of the growth-promoting enzyme to the nucleus. Bar = 10 μ*M* (H-W Chen et al., unpublished observations.)

teins and nuclear transcription factors. Hormone-receptor interactions at the surface membrane can initiate a cascade of signaling events that regulate many cellular functions, both acute and prolonged. Steroid hormones are among the most frequently prescribed drugs in the world, yet the complete mechanism of action of this important class of compounds remains to be fully elucidated. The primary purpose of this chapter is to provide an overview of emerging evidence on the nature and function of plasma membrane–associated receptors for steroid hormones in health and disease.

2. STEROID RECEPTOR SIGNALING MECHANISMS

Members of the known steroid hormone receptor family are proteins with molecular weights ranging from about 50 to 100 kDa. These "intracellular" receptors share a series of common domains, usually referred to as A–F. Using the estrogen receptor (ER) as an example, there is evidence for the occurrence of four structurally distinct, functional domains in this receptor protein (Fig. 2). From the amino to the carboxy terminus of the ER molecule, these domains are the N-terminal A/B region that contributes to transcriptional activation and contains the activation function-1 (AF-1), the adjoining C-region that harbors the DNA-binding domain (DBD), the D-domain or hinge region, and the ligand-binding domain (LBD or E/F domain) at the carboxy terminus that contains the ligand-binding region as well as sites for cofactor binding and for transactivation functions (AF-2). On ligand binding, these receptors form dimers and modulate transcription by binding to their corresponding hormone response element (estrogen response element [ERE]) in the promoter region of target genes (*see* Fig. 3).

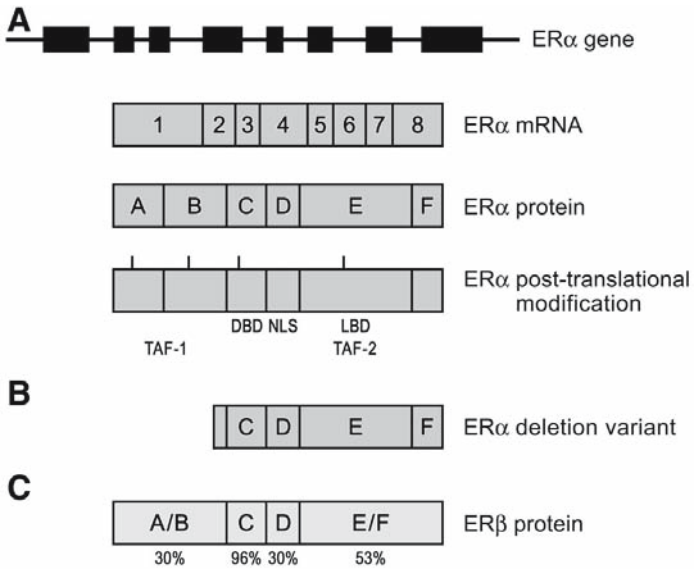

Fig. 2. Organization and functional domains of known forms of ERs compiled from the work of O'Malley, Chambon, Greene, Gustafsson, and many others. **(A)** A general diagram depicts the structure of the ERα gene; its specific transcript, ERα mRNA; and the intracellular protein product, ERα. The ERα protein has 595 amino acids, a molecular mass of 67 kDa and contains several distinct functional domains, labeled A–F. The protein product can undergo posttranslational modification, often phosphorylation of specific serine and tyrosine residues (indicated by short, vertical lines). Specific biologic activities have been localized to the different domains of the protein: transcription activation functions (TAF-1 and TAF-2), a DBD, a nuclear localization sequence (NLS), and an LBD. **(B)** Splice variant forms of ERα are known to occur. One common splice variant, a truncated 46 kDa protein with significant deletion of the A–B domains, is represented. **(C)** The ERβ protein is shown for comparison with ERα proteins. ERβ contains 530 amino acids, with the percentage of amino acid identity with that of ERα indicated in the diagram.

The first ER, termed ERα, was cloned in 1986, and the second ER, termed ERβ, the product of a gene on a different chromosome, was not discovered until 10 yr later. There is 96% amino acid identity between the two receptors in the DBD but only 53% homology in the LBD. Both ER forms bind estradiol-17β (E_2), and the bound complexes interact, in turn, with classic EREs with similar affinity. However, certain ligands show preferential binding to one or the other ER, and synthesis and testing of α- and β-selective ER ligands is currently in progress. The two ER subtypes have overlapping but distinct tissue distribution patterns and singular activation profiles at promoter elements of target genes, but it remains unclear how ERα and ERβ individually contribute to the physiologic effects of estrogens. In the case of ERs, several splice variants of ERα and ERβ have been described, and these variant receptors may have significant biologic activity in target cells (Fig. 2).

In general, the major target tissues of steroid hormones contain abundant levels of specific receptor proteins that bind and transduce downstream signals for modulating hormone action. Their relative distribution, whether plasma membrane, nuclear, or occurrence in additional cytoplasmic compartments, is a function of the metabolic history of the individual cell. Entry of receptor-bound ligand is extremely rapid and can deplete the surface membrane of native receptor, especially under conditions of excessive levels of steroid, and result in relatively high concentrations in other compartments including the nucleus.

In the example of ER, there are more than 20 coregulator proteins that bind to ERs and modulate their function, with each acting as either a positive (coactivator) or a negative (corepressor) transcriptional regulator. Coactivator proteins recruit other proteins of the transcriptional apparatus and also have histone acetyltransferase activity that may facilitate unwinding of tightly coiled DNA from its histone scaffold. The mechanisms by which corepressor proteins inhibit gene expression are less well understood. The relative and absolute levels of expression of coregulator proteins vary considerably among estrogen target cells, thus serving as a potential means of fine-tuning ultimate hormone action (*see* Fig. 3). Control of gene expression by steroids thus involves a series of specific molecular interactions among steroid hormones, their specific receptors, steroid receptor–associated

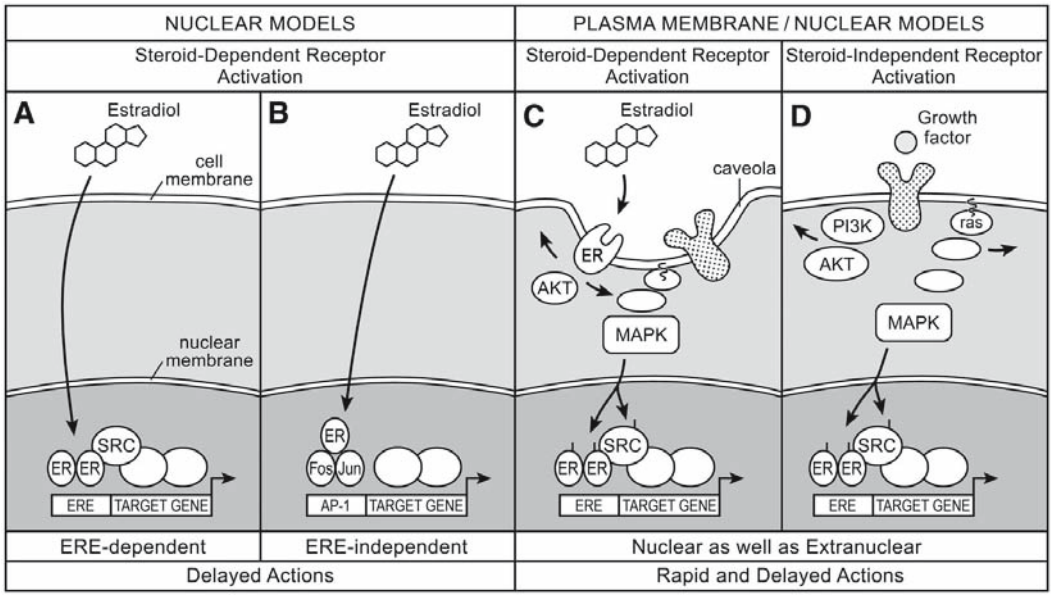

Fig. 3. Concepts of estrogen action in target cells. Nuclear, as well as plasma membrane/nuclear, models of hormone action are depicted. **(A**,**B)** In many prevailing models, the steroid freely enters all cells and, having crossed both plasma and nuclear membranes, is retained only in those cells in which it homes in on its receptor, localized within the nucleus. Therefore, steroid binding to ER is believed to promote alterations in receptor conformation favoring enhanced association with coactivator proteins and with specific EREs, leading, in turn, to selective gene transcription (A). However, this model fails to account for ERE-independent genomic activation. As shown in (B), the steroid may also interact with activating protein-1 (AP-1) to facilitate gene transcription via a pathway that does not require EREs. Neither nuclear model (A,B) can explain numerous, rapid cell responses to steroid treatment, such as the downstream signaling cascade. **(C)** In eliciting these acute responses, estrogens bind to a plasma membrane–associated ER to promote hormonal responses through a complementary pathway that cross-communicates, or interacts directly, with a genomic mechanism. **(D)** In addition, activation of ER may occur by steroid-*in*dependent signaling, involving mediation by growth factor receptors. Liganded membrane ER or growth factor receptor–activated ER may affect one or more of several pathways, including modulation of ion channels, leading to enhanced flux of ions, notably Ca^{2+}; interaction with peptide membrane receptors; and activation of G proteins, nucleotide cyclases, and/or MAPK, with resultant increases in their catalytic products. These membrane interactions may elicit phosphorylation of ER itself through steroid-promoted (C) or steroid-*in*dependent pathways (D). Accordingly, through these interrelated pathways, cytoplasmic and nuclear events respond in a coordinated manner to steroid hormone levels in the external environment. The intricate array of physiologic responses of cells to steroid hormones may occur as a consequence of a synergistic feed-forward circuit where steroids activate cell membrane signaling pathways that act, in turn, to enhance transcriptional activity of ER in the nucleus.

proteins, and the regulatory regions for the different target genes present in each cell.

Further complexity in the regulation of steroid hormone action is owing to the discovery of "nonclassic" modes of ligand-dependent effects, as well as ligand-*in*dependent modulation of steroid hormone– receptor function (*see* Fig. 3). Estrogens, e.g., can regulate the transcription of certain genes that lack functional EREs by modulating the activity of other transcription factors. Binding of the ER to subunits of AP-1 results in the formation of a novel transcription factor. Since most steroid hormone receptors are phosphoproteins, their function can also be altered by changes in their phosphorylation state in the absence of hormonal ligands (Fig. 3). Activators of protein kinases, such as peptide growth factors, can elicit steroid-independent activation of receptors, such as ER, by inducing phosphorylation of the receptor, usually at specific serine or tyrosine residues. Receptor tyrosine kinases and MAPKs generally modulate receptor phosphorylation in response to various extracellular signals initiated by membrane receptors (Fig. 3). Alternative pathways for activation of ER appear to be very important in vivo, and, in the case of ER, receptor activation can be induced independently of estrogens by epidermal growth factor (EGF), heregulin, transforming growth factor-α (TGF-α), insulin-like growth factor-1 (IGF-1), dopamine, and cyclic adenosine monophosphate (cAMP). The proximate and downstream signal transduction mechanisms available for promoting these initial interactions of steroid hormone receptors in hormone action (Fig. 3) are addressed more fully in the following sections.

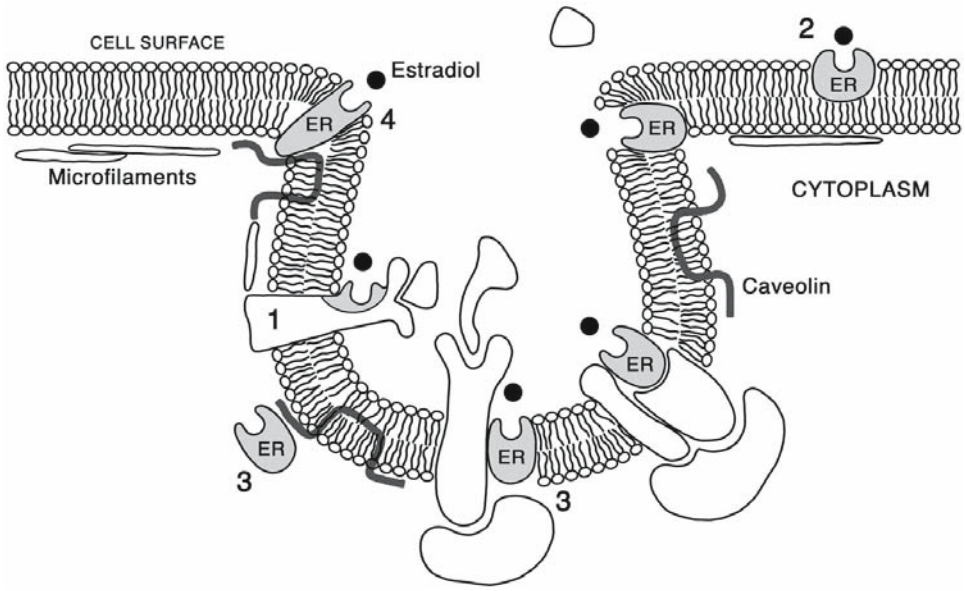

Fig. 4. Supramolecular organization of plasma membrane and occurrence of ERs. A model of the surface membrane from an estrogen-responsive cell in the region of a caveolar structure is depicted. Estradiol may interact with one of several different forms of plasma membrane–associated ERs, with examples designated as 1–4. The physical orientation and full structure of these molecules remain to be established. Of note, alternatively spliced transcripts of several steroid receptors occur, and these variant receptors give rise to proteins of different molecular mass and, possibly, modified properties (*see* Fig. 2). Membrane insertion of receptors would likely require one or more hydrophobic regions or posttranslational modification of receptor protein leading to membrane targeting. (Reprinted with permission from Szego et al., 2003.)

3. PLASMA MEMBRANE ORGANIZATION AND STEROID HORMONE RECEPTORS

As noted earlier, the "classic" mode of estrogen signaling involving nuclear interaction usually takes hours to elicit enhanced protein synthesis by activation of transcription. However, other effects of steroids cannot be fully explained by a transcriptional mechanism owing to their rapid, often instantaneous, onset. These effects can only be the result of direct steroid hormone recognition at the cell membrane.

The uptake of steroid hormones in responsive cells may occur by passive or facilitated diffusion across the plasma membrane or by one of several endocytotic mechanisms. Biophysical studies demonstrate that most steroid hormones are lipophilic molecules that partition deep within the hyrdocarbon core of lipid bilayer membranes, even those devoid of receptor proteins. However, these agonists also appear to enter target cells by a membrane-mediated process that is saturable and temperature dependent.

3.1. Membrane Models: From Fluid Mosaic to Lipid Rafts and Signaling Platforms

To understand the nature of steroid receptor association with surface membrane, it is important to consider current concepts of membrane organization (Fig. 4). The original notion of a fluid-mosaic structure of plasma membrane, wherein membrane proteins diffuse freely in a sea of lipid above a critical temperature of 15°C, is challenged by emerging evidence on the existence of macro- and microdomains that serve to concentrate key signaling molecules for more efficient coupling to specific effectors. The concept of a "signaling platform" has been advanced to characterize a supramolecular structure in which many different plasma membrane–associated receptor and effector components are assembled together in a highly coordinated hierarchical template that promotes more effective and ordered signal transduction to the cell interior.

Plasma membrane microdomains, termed *lipid rafts*, appear to arise from the phase behavior of lipid components. In the fluid bilayer of the membrane, different lipid species are asymmetrically distributed over exoplasmic and cytoplasmic leaflets of the membrane. In particular, long, saturated acyl chains of sphingolipids cluster in the presence of cholesterol to form a liquid-ordered phase relatively resistant to detergent solubilization. Saturated acyl chains of glycosylphosphatidylinositol-anchored proteins, as well as transmembrane proteins and certain tyrosine kinases, also occur within these lipid domains. Lipid raft associa-

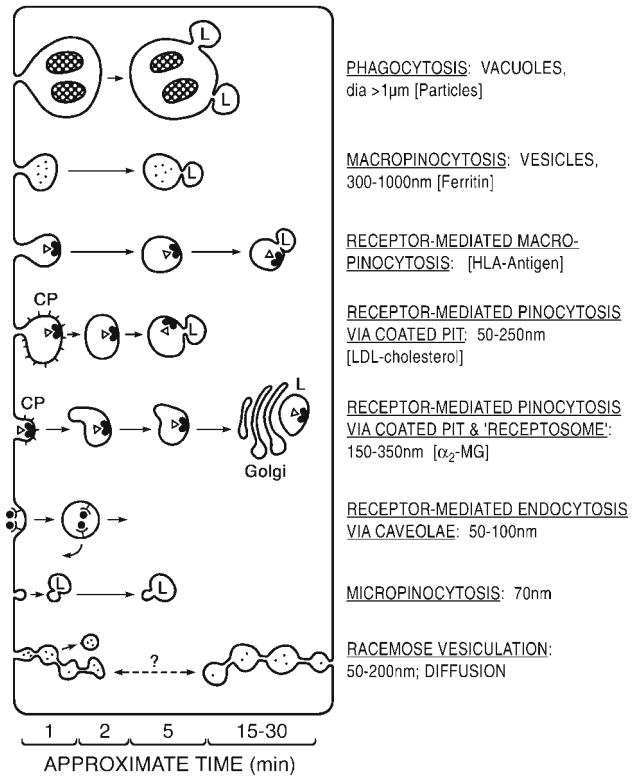

Fig. 5. Schematic representation of pathways for internalization of extracellular agonists. Such pathways may play a role in the action of steroid hormones. For example, an especially telling analysis of the [^{3}H]estradiol-17β translocation mechanism is available, using analytical cell fractionation at progressive time periods, beginning within 10 s of exposure. Estradiol-17β interacts specifically with membrane proteins in uterine cells and undergoes rapid internalization in nanometer-size endocytotic vesicles resulting in delivery of a portion of the steroid hormone and its associated receptor protein to the cell nucleus and nuclear protein matrix. Concomitant with a decline in plasmalemmal and presumptive endosomal fractions, a significant amount of labeled hormone occurs in Golgi and lysosomal compartments before the peak in nuclear accumulation. (Adapted and reprinted with permission from Szego and Pietras, 1984.)

tion may concentrate receptors for specific interaction with ligands and effectors on either side of the membrane, thus facilitating binding during signaling and suppressing inappropriate cross talk between conflicting signal transduction pathways.

3.2. Downstream Signaling Pathways

Caveolae (literally "little caves") are more specialized lipid raft microdomains that also concentrate and assemble components of many signal transduction pathways (Fig. 4). These membrane structures can be invaginated, flat within the plane of the membrane, detached vesicles, or fused together to form grapelike structures and tubules (Fig. 5). As rafts, caveolae are rich in cholesterol and sphingolipids, but, unlike lipid rafts, they are lined intracellularly with clusters of the protein caveolin, a cholesterol-binding molecule that contributes to the organization of membrane lipids. The growing list of caveolae-associated molecules constitutes a virtual catalogue of cell-signaling molecules, including receptor tyrosine kinases, G protein–coupled receptors (GPCRs), protein kinase C (PKC), components of the MAPK pathway, and endothelial nitric oxide synthase (eNOS). In one such example, subpopulations of ERs are localized to caveolae in vascular endothelial cells, and, in plasma membrane caveolae isolated from these cells, estradiol directly binds to its receptors, which are coupled to eNOS in a functional signaling module to regulate the local calcium environment and blood vessel contractility.

Clathrin-coated pits are independent membrane invaginations, decorated at the inner cell membrane surface with the protein clathrin. They mediate endocytosis of nutrients and certain receptors, such as receptor-mediated uptake of low-density lipoprotein (LDL)–cholesterol complexes, and also play an important role in cell signaling. Under certain conditions, some agonists may be internalized through either clathrin-coated pits or caveolae, with one pathway apparently providing a default entry mechanism for the other.

Lipid raft–dependent signaling is often coupled with endocytotic uptake mechanisms involving both lipid rafts and caveolae. Also important in this scheme is the cytoskeletal protein actin, considered to provide constraints for the lateral mobility of lipid microdomains and to function in endocytotic trafficking. Endocytosis itself is a diverse set of processes that promote internalization of specialized regions of plasma membrane as well as small amounts of extracellular fluid (Fig. 5). Caveolae also play an important role in potocytosis, a mechanism for uptake of small molecules across plasma membrane. Finally, some cell types can internalize larger amounts of fluid by macropinocytosis or of particulates by phagocytosis (Fig. 5). In most cells, internalized materials are first delivered to early sorting endosomes, which may mature into or be transferred to late endosomes and, ultimately, to lysosomes. The potential functions of these several membrane structures and signaling domains are considered in the following sections.

3.3. Variant Forms of Steroid Hormone Receptors in Target Cell–Surface Membranes

The exact nature of the association of steroid receptors with plasma membrane remains elusive, primarily

because full structural characterization of these molecules is largely incomplete. They may be known membrane components (e.g., enzymes, ion channel subunits, receptors for nonsteroid ligands) with previously unrecognized binding sites for steroids, new forms of steroid hormone receptors, "classic" receptors complexed with other membrane-associated proteins, or truly novel membrane proteins. As noted, the task of identifying these membrane-associated steroid receptors is made more challenging by the detection of multiple transcript variants of classic "intracellular" steroid receptors. In the case of ER, both ERα and ERβ are expressed in membranes, and both classes of receptor can elicit acute and late phases of cellular responses through activation of signal transduction cascades.

ER from target cell plasma membranes is a protein species with high-affinity, saturable binding specific for E_2. In addition, antibodies to nuclear ERα recognize surface sites, suggesting that membrane ER has antigenic homology with nuclear ER. Moreover, recent work reveals that membrane and nuclear ER may be derived from a single transcript. Likewise, properties of membrane glucocorticoid receptors closely resemble those of the intracellular receptor. On the other hand, properties of the aldosterone receptor, as well as those of the plasma membrane receptor for 1,25-dihydroxyvitamin D_3 (1, 25$[OH]_2$ D_3), suggest that membrane receptors for these steroids may be distinct from their "classic" intracellular counterparts. Finally, recent cloning, expression, and characterization of a membrane progestin receptor from fish ovaries showed evidence of a protein with seven transmembrane domains, a finding typical of GPCRs. Thus, a family of native steroid hormone receptors unrelated to nuclear steroid receptors may also occur in nature.

Collectively, current findings suggest that membrane receptors for steroid hormones may occur in different molecular forms. They may be duplicate or variant copies of nuclear receptors or products not related to nuclear receptor forms. Insertion of receptor proteins into plasma membrane would likely require one or more hydrophobic regions in the candidate protein. However, targeting of a receptor transcript to the membrane may also be promoted by posttranslational modification of receptor protein, including phosphorylation; glycosylation; and/or addition of lipid anchors or other modifications, such as palmitoylation or myristoylation. Thus, palmitoylation of a diminished variant form of ER occurs in vascular endothelial cells and represents an important structural modification for association of receptor with plasma membrane. ERα gene can encode the truncated ER form by alternative splicing in target cells, and the receptor then undergoes posttranslational palmitoylation, thus promoting targeting to the cell membrane, with a C-terminus-out and N-terminus-in topology.

It remains to be determined whether surface steroid hormone receptors may also be part of a multimeric complex including a "classic" steroid receptor bound to as-yet unidentified transmembrane protein and/or coupled to membrane-associated signaling molecules. Alternatively, plasma membrane receptors for steroids may have several common structural features with, but may be distinct from, intracellular steroid hormone receptors. In the case of retinoic acid, binding to known membrane proteins, such as mannose-6-phosphate/IGF-2 receptor (M-6-P/IGF-2R), may modulate some ligand effects. Progesterone may interact directly with oxytocin receptor, a G-linked protein, at the cell surface, with resultant inhibition of uterotonic activity of oxytocin. Progesterone congeners also bind with moderate affinity to γ-aminobutyrate type A receptors that comprise ligand-gated ion channel complexes. Similarly, acute vascular relaxation induced by pharmacologic levels of estradiol may be mediated by its binding to the regulatory subunit of Maxi-K channels in membranes, thus supporting the view that some effects of steroids, at least at high micromolar concentration, may be mediated by known membrane receptors with previously unrecognized steroid-binding sites.

In the first generation of ERα gene knockout (ERKO) mice, rapid actions of estradiol on kainate-induced currents in hippocampal neurons were noted to persist, and the effect was not inhibited by ICI 182,780, a pure antagonist of hormone binding to both ERα and ERβ. On the basis of these data, some investigators suggested that a distinct estrogen-binding site existed in neurons and appeared to be coupled to kainate receptors by a cAMP-dependent process. However, residual estrogen-binding capacity in target tissues of up to 5–10% raised doubts about whether this knockout was a true null mutation of ER. Indeed, alternatively spliced forms of ERα as well as ERβ still occur in these first-generation ERKO mice, thus complicating interpretation of such results. Nonetheless, perfection and application of this technology with the development of true, double ERα and ERβ gene knockouts should eventually prove important in deciphering the contribution of "classic" and novel receptor forms in steroid hormone action.

As with the mixed steroid hormone–binding protein systems known to occur within cells and in their extracellular fluids, it may well be that multiple forms of receptor proteins for steroids coexist in plasma membranes, thus complicating efforts to isolate and characterize *the* individual binding species in this cell

compartment. Efforts to understand ligand-receptor interactions are often limited by simplistic "lock-and-key" models that may not accurately reflect the true state of complex molecular signaling cascades. Study of the molecular organization of several neurotransmitter receptor families has shown that extraordinary biologic variability occurs, with multiple "keys" and multiple "locks" sometimes involved in ligand-receptor recognition. Researchers must consider the existence of similar high-affinity, but possibly multivalent and multifunctional, receptors in the steroid hormone superfamily. Nevertheless, available evidence suggests that a finite portion of cellular steroid receptors is associated with signaling platforms in specialized microdomains of the plasma membrane.

3.4. Specific Binding of Steroid Hormones to Surface Membranes of Responsive Cells

As postulated by Paul Ehrlich more than a century ago, the outer surface of a responsive cell is equipped with specialized components that exhibit exquisite discriminatory capacity toward potential agonists when molecular conformations are mutually complementary. Indeed, in evolutionary terms, steroid recognition at the surface membrane appears to have been the primary response pathway of the primitive cell. In plant cells, no nuclear forms of steroid receptors are currently known to exist. The only recognized response pathway to plant steroids is via a membrane-associated receptor that regulates numerous functions in the intracellular economy including growth and development. Similar membrane-associated receptors for steroids have also been described in other lower organisms, such as the unicellular *Tetrahymena*.

The concept of specific membrane-associated binding sites for steroid hormones has been supported by rigorously controlled observations from many independent laboratories. Evidence is now available for the extended steroid family, which includes the retinoids, thyroid hormone, and digitalis-like steroids (Table 1). Representative examples of several approaches used to identify membrane-associated receptors for estrogen are presented in Figs. 6 and 7. Comparable observations are also available for other members of the steroid family, especially adrenocortical steroids and vitamin D metabolites. Thus, from physical, ultrastructural, immunologic, and molecular probes, as well as direct kinetic analyses of specific binding of radiolabeled steroids to the surfaces of isolated target cells or to their purified plasma membrane fractions, a large body of evidence supports this view. Such membrane proteins constitute a fraction of total receptor molecules in the target cell and have occasionally been overlooked when methods of sufficient sensitivity were not utilized. Thus, ultrastructural studies reveal extranuclear immunoreactivity for ERα associated with membrane sites along dendritic spines and axon terminals of neurons. This modern work confirms observations by Williams and Baba in 1967, at which time they reported, using electron microscopy and an excess of labeled steroids, that [^{3}H]-aldosterone and [^{3}H]-cortisol associated with plasma membranes of their respective target cells.

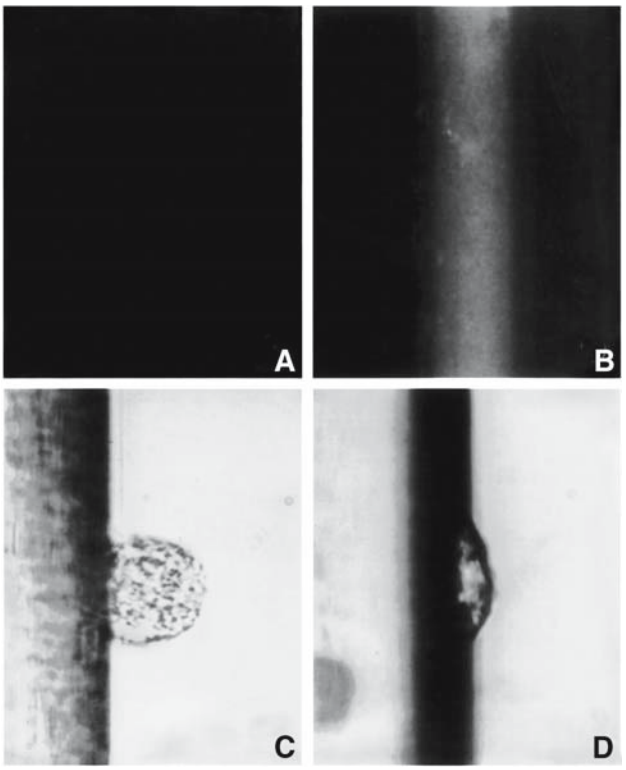

Fig. 6. Intact target cells for estrogen exhibit affinity binding to an estradiol-derivatized matrix. Estradiol was immobilized by covalent linkage to albumin-derivatized nylon fibers in order to probe for the occurrence of surface membrane–associated binding sites for estrogen. To verify the availability of steroid ligand at the fiber surface, binding of FITC-labeled antibodies to the estradiol-derivatized fiber matrix was first assessed. Experiments conducted at 22°C with **(A)** FITC-labeled nonimmune serum or **(B)** estrogen antiserum clearly demonstrated the presence of the steroid at the fiber surface, as shown in dark-field–ultraviolet fluorescence micrographs (magnification: ×100). **(C,D)** In independent experiments, estrogen target cells from liver were incubated with the estrogen-derivatized fibers in Ca^{2+}, Mg^{2+}-free Ringer solution. Washed fibers with bound cells were photographed with an immersion lens. Some cells appear fairly rounded (C), whereas others tend to flatten out at the fiber surface of the latter (D), suggesting intense interaction with immobilized estradiol (magnification: ×850). (Reprinted with permission from Pietras and Szego, 1979.)

Table 1
A Brief Chronology of Selected Reports Documenting Occurrence and Activity of Plasma Membrane Receptors for Steroid Hormones in Their Cellular Targets [a]

Steroid	*Year*	*Observation*	*Reference*
Estradiol	1967	Elevation of uterine cAMP by estrogen within seconds	Szego and Davis
	1975	Rapid endometrial cell calcium mobilization by estrogen	Pietras and Szego
Corticosterone	1975	Binding to plasma membranes of rat liver	Suyemitsu and Terayama
Estradiol	1976	Effects on electrical activity of neurons	Dufy et al.
	1977	Specific plasma membrane binding sites for estrogen	Pietras and Szego
Cortisol	1977	Electrophysiologic effects on neurons	Kelly et al.
Progesterone	1978	Induction of oocyte maturation by steroid linked to a polymer	Godeau et al.
Estradiol	1979	Increased proliferation of cells with membrane ER	Pietras and Szego
	1980	Molecular properties of ERs in liver plasma membrane	Pietras and Szego
Vitamin D	1981	Rapid intestinal cell calcium uptake	Nemere and Szego
Progestin	1982	Specific binding to oocyte surface and role in meiotic maturation	Kostellow et al.
		Steroid receptor of 110 kDa on oocyte surface by photoaffinity labeling	Sadler and Maller
Corticosterone	1983	Binding to synaptic plasma membranes	Towle and Sze
Estradiol	1983	Increased microvilli at endometrial cell surface within seconds	Rambo and Szego
	1984	Primary internalization of ER in plasma membrane vesicles	Pietras and Szego
Thyroid hormone	1985	Characterization of plasma membrane binding sites	Alderson et al.
Estradiol	1986	High-affinity binding sites in breast cancer cell plasma membranes	Berthois et al.
		Altered cell membrane potential, density of microvilli in seconds	Pourreau-Schneider et al.
Glucocorticoid	1987	Membrane receptor associated with apoptosis in lymphoma cells	Gametchu
Vitamin D	1989	Rapid activation of PLC in rat intestine	Lieberherr et al.
		Activation of calcium channels in osteoblasts	Caffrey and Farach-Carson
Thyroid hormone	1989	Rapid induction of glucose uptake	Segal
Progesterone	1990	Stimulation of calcium influx in human sperm	Blakemore et al.
	1991	Calcium uptake mediated by sperm cell-surface binding sites	
		Action at plasma membrane of human sperm	Meizel and Turner
Corticosterone	1991	Correlation of neuron membrane receptors with behavior in newts	Orchinik et al.
Aldosterone	1991	Rapid effects on Na^+/H^+ exchange	Wehling et al.

Continued on next page

Table 1 ***(Continued)***
A Brief Chronology of Selected Reports Documenting Occurrence and Activity of Plasma Membrane Receptors for Steroid Hormones in Their Cellular Targets[a]

Glucocorticoid	1993	Antigenic similarity between membrane and intracellular receptors	Gametchu et al.
Estradiol	1993	Binding and stimulation of HER-2 membrane receptor	Matsuda et al.
	1994	Activation of adenylate cyclase signaling pathways	Aronica et al.
Vitamin D	1994	Isolation of a plasma membrane receptor from chick intestine	Nemere et al.
Aldosterone	1994	Identification of membrane receptor in human lymphocytes	Eisen et al.
Estradiol	1995	Membrane receptor with antigenic identity to nuclear receptor	Pappas et al.
		Membrane receptor–enriched neural cells and nongenomic responses	
Androgen	1995	Rapid increase in cytosolic Ca^{++} in Sertoli cells	Gorczynska and Handelsman
Estradiol	1997	Membrane action and PLC regulation	LeMallay et al.
		Isolation of membrane-binding proteins from rat brain	Zheng and Ramirez
Vitamin D	1998	Hormone activation of PKC blocked by membrane receptor antibody	Nemere et al.
Estradiol	1999	Rapid Ca^{++} mobilization required for activation of MAPK	Improta-Brears et al.
		Rapid actions in neurons from ERα-knockout mice	Gu et al.
		Membrane ER expression reduced by antisense to nuclear ER	Norfleet et al.
		Membrane and nuclear ERα and ERβ each expressed from single transcript	Razandi et al.
		Activation of G proteins, IP_3, adenylate cyclase, and MAPK by membrane ER	
Androgen	1999	Rapid activation of MAPK pathway in prostate	Peterziel et al.
Progesterone	1999	Cloning, expression of binding protein from liver microsomes	Falkenstein et al.
Vitamin D	2000	Ligand-induced nuclear translocation of plasma membrane receptor	Nemere et al.
Estradiol	2000	Surface receptor in endothelial cells recognized by ERα antibody	Russell et al.
		Interaction of ER with regulatory subunit of PI3K	Simoncini et al.*
		Clustering of membrane ER in endothelial cells in caveolar domains	Chambliss et al.*
Estradiol	2001	Blockage of estrogen-induced cancer cell growth by antibody to ERα	Márquez and Pietras*
Estradiol	2003	Posttranslational palmitoylation of membrane-associated ER	Li et al.*
		Membrane ER–regulated secretion, cAMP in neurons	Navarro et al.*

[a]More than 2500 publications on membrane steroid receptors have appeared in the past 30 yr. Of these, only representative examples can be listed here. (Reprinted in expanded form with permission from Pietras et al. [2001], wherein full citations may be found, except for those references in this table followed by an asterisk, which can be found in the References at the end of this Chapter.)

Fig. 7. Electron microscopic visualization of receptor-mediated, specific binding and internalization of 17β-estradiol-17-hemisuccinate:bovine serum albumin (BSA) that had been adsorbed to colloidal gold (E17 BSA:Au) at surfaces of human hepatoblastoma (Hep G2) cells. Note the binding of ligand to plasma membrane over a prominent invagination (arrowhead) with intracellular tubulovesicular structures beneath it (arrows). In control preparations with BSA:Au (lacking derivatization with estrogen; not shown), there is minimal internalization, despite its presence in abundant extracellular concentrations. Bar = 0.250 μm. (Reprinted with permission from Moats and Ramirez, 2000.)

Loose and reversible association of steroid hormones with transport protein in the circulation is of approximately two orders of magnitude lower than the affinity of the target cell– surface receptor for the ligand, thus permitting selective binding and accumulation by the responsive tissue. By the same token, the corollary principle of competitive displacement forms the basis for the assay of trace amounts of steroids in biologic samples.

It is also important to note that different structural conformations of a given steroid hormone may, in some instances, act as specific agonists for selected cellular response pathways. For example, $1\alpha,25(OH)_2$ vitamin D_3 produces biologic responses through two distinct receptors, one predominant in the surface membrane and the other in the cell nucleus, respectively, which are able to recognize different shapes of the conformationally flexible molecule. Accordingly, it appears that utilization of responsive mechanisms depends on the immediate steroid environment, as well as selective pathway engagement for signal transduction.

4. INTEGRATION OF MEMBRANE AND NUCLEAR SIGNALING IN STEROID HORMONE ACTION

The genomic hypothesis of steroid hormone action has generally prevailed as the exclusive mechanism since 1961, the seminal year in which the novel concepts of Jacob and Monod electrified the scientific community. In the interval to the present, extraordinary accomplishments by many molecular biologists have extended and clarified the details of these concepts for the late nuclear effects of a number of steroid hormones at their cell targets. Unfortunately, the many well-documented responses owing to primary signal transduction at the cell surface, which were being characterized independently and in parallel, were often overlooked in formulating the "classic" genomic models of steroid hormone action or misinterpreted as an alternative mechanism. Communication between events at the cell surface and the relatively remote nucleus, separated as it is from all else in the cell by a double membrane, is critical for elaborating the coordinated responses of growth and differentiation. Indeed, there is rapidly growing evidence that there is close synergism between receptor-mediated, virtually instantaneous activities at the plasma membrane and delayed effects within the nucleus.

Events initiated at the cell surface may be communicated intracellularly and, thereby, modulate transcriptional events that will eventually unfold in the nucleus. In the case of estrogen, which has received the most attention among the steroid hormones in this regard, the time course of such events encompasses several orders of magnitude, leading to its general description as a continuum (Fig. 8). A similar temporal distribution pattern also prevails for responses to other steroid hormones.

Receptor-mediated signal transduction responses have been identified for all the steroid hormones. It is significant to note that the time course of some of these cellular activities, such as nucleotide cyclase reactions, can occur within seconds or less. This is particularly well illustrated in neural responses. Acute alterations in Ca^{2+} and in Na^+/K^+ flux are likewise rapid and occur within wide differences in steroid agonist and end-organ targets. Many of these changes in the cytoplasmic microenvironment, in turn, have profound effects on enzyme reactions and on cytoskeletal structure. Indeed, there is considerable evidence that microtubules and the actin cytoskeleton of the cell play an important role in endocytotic trafficking and concomitant signal transduction. Thus, amplification of the primary hormonal signal is achieved through a limited number of receptor-mediated transduction mechanisms, linked, in part,

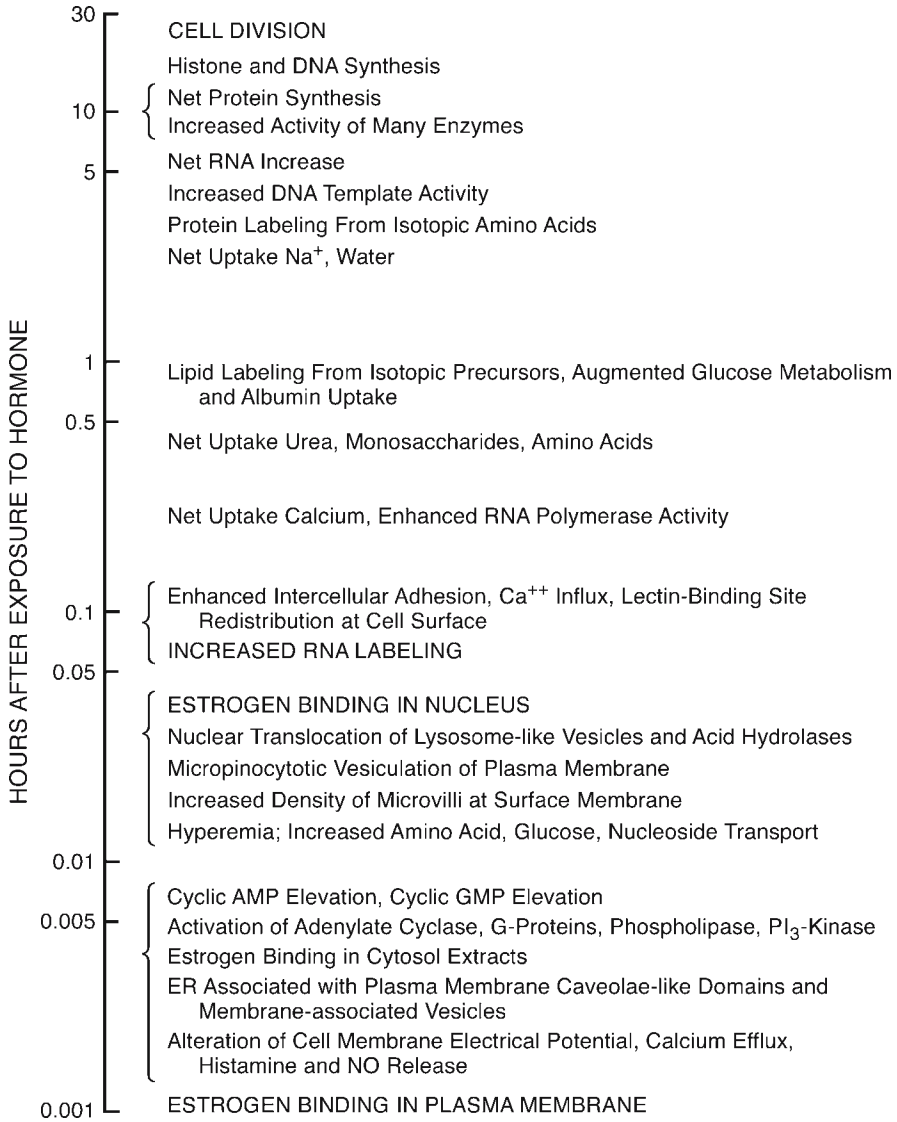

Fig. 8. Schematic representation of time course of responses of uterus to E_2. Times shown on the logarithmic scale refer to the onset of unequivocal change from baseline values. Thus, times indicated are dependent in part on sensitivities of the various analytic methods applied and on the somewhat arbitrary selection of initial time points for observation in the several experimental protocols. GMP = guanosine 5´-monophosphate; PI_3-Kinase = phosphatidylinositol 3-kinase. (Reprinted with permission from Szego and Pietras [1984], wherein additional details and sources are given.)

through heterotrimeric G proteins and other specialized signaling partners that are integral to plasma membrane.

In the case of some hormonal responses, interaction at the surface membrane may itself be sufficient to elicit an alteration in cell function. For example, estradiol can directly stimulate PKC activity in membranes isolated from chondrocytes, and the steroid also modulates calcium-dependent eNOS activity associated with its receptor in isolated plasma membranes from endothelial cells. Moreover, estrogens may enhance growth of mammary tumor cells, largely independent of ERE-dependent transcription, by stimulating membrane-associated MAPK pathways. In ER-negative cells, transfection of transcriptionally inactive, mutant forms of ERα allows full stimulation of DNA synthesis by estradiol. Ligand-independent activation of steroid hormone receptors also occurs and may represent a more primitive response pathway, whereby cross-communication with peptide signaling systems in the cell can directly modulate the activity of steroid hormone receptors. For example, ER can be activated in the absence of estradiol through phosphorylation by

EGF-stimulated MAPK. These signaling pathways may also allow for phosphorylation of important regulatory molecules, such as the steroid receptor coactivators and corepressors that play a crucial role in steroid hormone action. As is readily appreciated, the shuttling of phosphate groups in and out of proteins critical to the signal transduction cascade is a powerful means of modifying their structure, with the immediate result of altering their folding patterns and/or relative degree of their interactions with neighboring molecules. Thus, such apparently minimal changes in composition provide a means of augmenting or attenuating their catalytic functions in a virtually instantaneous manner. It is not surprising that this efficient mechanism is so widely conserved in so many biologic contexts and across so wide an evolutionary spectrum. Any comprehensive model of steroid hormone action must account for these important cellular interactions.

Accordingly, the functions of the surface membrane–associated receptors are likely twofold. Both lead to coordination of the activities of more downstream cellular organelles. One such function is *complementary* to the more distal and time-delayed events at the genome, through communication of information from the extracellular compartment. The second function *supplements* the more deferred and metabolically expensive activities at the genome, through exclusion of the latter. Instead, the cascade of signals, transduced from binding of steroids at the cell surface, are themselves converted into immediate and more readily reversible stimuli, such as those eliciting acute ion shifts and changes in vasomotor dynamics—these being of evolutionary significance for survival. In the case of some hormone responses, such primary interactions at the plasma membrane may be sufficient to trigger a cascade of intracellular signals that lead to specifically altered cell function. Thus, within seconds of estrogen binding by surface receptors of the target cell, widespread changes are communicated to the cytoarchitecture involving striking alterations in the localized assembly and disassembly of the microtubule-microfilament scaffolding of the cell. These abrupt, but transitory, changes in the subcellular cytoskeleton may allow enhanced exchanges between membrane and nuclear compartments to promote redistribution of matériel in the pretranscriptional stage of the estrogen response cascade. Indeed, these dual capacities of membrane receptor activation underlie adaptation of the target cell to processing of information from its external environment on two independent/synergistic pathways: acute and delayed. Some selected examples of the role of membrane receptors for steroids in health and disease states are provided in the following sections.

5. MEMBRANE-ASSOCIATED STEROID RECEPTORS IN HEALTH AND DISEASE

5.1. Estrogen Receptors in Bone, Neural, Cardiovascular, and Reproductive Health and in Malignancy

As with other steroid hormones, biologic activities of estrogen in breast, uterus, and other target tissues have long been considered to be fully accounted for through activation of a specific high-affinity receptor in cell nuclei. However, it is well established that estrogen can trigger in target cells rapid surges in the levels of intracellular messengers, including calcium and cAMP, as well as activation of MAPK and phospholipase (Table 1). These data have led to a growing consensus that the conventional genomic model does not explain the rapid effects of estrogen and must be expanded to include plasma membrane receptors as essential components of cellular responsiveness to this and other steroid hormones.

The first unequivocal evidence for specific membrane binding sites for E_2 was reported about 25 yr ago. Intact uterine endometrial cells equipped with ER, but not ER-deficient, control cells became bound to an inert support with covalently linked E_2. In addition, target cells so bound could be eluted selectively by free hormone, in active form, but not by the relatively inactive estradiol-17α; and cells so selected exhibited a greater proliferative response to estrogens than cells that did not bind. Further investigations have continued to provide compelling evidence for the occurrence of a plasma membrane form of ER and support for its role in mediating hormone actions (Table 1).

Selye first demonstrated that steroids at *pharmacologic* concentrations elicit acute sedative and anesthetic actions in the brain. However, electrical responses to *physiologic* levels of E_2 with rapid onset have since been reported in nerve cells from various brain regions. Estrogen has diverse effects on brain functions, including those regulating complex activities, such as hypothalamic-pituitary circuits, cognition, and memory. Some of these estrogenic actions may be attributable to regulation of cAMP signaling by G protein–coupled plasma membrane receptors for the hormone.

New caveats from randomized controlled clinical trials on the increased risk of cardiovascular disease among healthy postmenopausal women prescribed estrogen plus progestin conflict with the long-held belief that hormone therapy might reduce a woman's risk of coronary heart disease. The results of basic research and animal models had suggested the hypothesis that estrogens were beneficial for cardiovascular health. It is

likely that variations in the dose, type or timing of estrogens or the coadministration of progestogens modifies the final physiologic and clinical responses to estrogen, and these clinical variables may account for differences from the preceding laboratory and observational research studies. However, these clinical trial results also suggest that further understanding of the molecular and cellular determinants of estrogen action are required. Traditional genomic models of estrogen action in the vasculature are incomplete, but, with knowledge of the full spectrum of steroid hormone action in target cells, researchers may yet find ways to manipulate estrogenic actions that promote cardiovascular health. One starting point is to recognize that estrogen has both rapid and long-term effects on the blood vessel wall. Certain vasoprotective effects of estrogen are mediated by membrane-associated receptors. Estrogen-induced release of uterine histamine *in situ* has long been associated with rapid enhancement of the microcirculation by a process that excludes gene activation. Reinforcing these observations are data detailing the role of nitric oxide (NO) in vascular regulation by estrogen. Normal endothelium secretes NO, which relaxes vascular smooth muscle and inhibits platelet aggregation. Estrogens elicit abrupt liberation of NO by acute activation of eNOS without altering gene expression, a response that is fully inhibited by concomitant treatment with specific ER antagonists. This estrogenic effect is mediated by a receptor localized in caveolae of endothelial cell membranes. Manipulation of rapid estrogen signaling events may provide new approaches in the medical management of cardiovascular health. Direct effects of estrogen on the vasculature promote acute vasodilation and may contribute to late effects leading to inhibition of the development and progression of atherosclerosis.

Estrogen deficiency is associated with significant bone loss and is the main cause of postmenopausal osteoporosis, a disorder that affects about one-third of the postmenopausal female population. When estrogen is diminished, bone turnover increases, and bone resorption increases more than bone formation, leading to net bone loss. In randomized clinical trials, administration of estrogen plus progestin in healthy postmenopausal women increases bone mineral density and reduces the risk of fracture. However, in considering the effects of combined hormonal therapy on other important disease outcomes, such as the risk of ovarian and breast cancer, caution is recommended in the use of continuous combined hormonal therapy in the clinic. Hence, the role of membrane ER in regulating bone mass has had increasing research emphasis and could promote development of alternative treatments. Evidence for membrane-binding sites and acute effects of estrogen with an onset within 5 s has been observed in both osteoblasts and osteoclasts. The effects of estrogens on bone homeostasis also appear to involve rapid activation of MAPK, as has been demonstrated in certain other target cells. Indeed, the "classic" genotropic effects of estrogens may be dispensable for their bone-protective effects. A novel synthetic ligand, 4-estren-3α,17β-diol, stimulates transcription-independent signaling of estrogens and increases bone mass and tensile strength in ovariectomized mice. Such therapeutic agents targeted to membrane-associated receptor forms may play a role in future treatment and prevention of osteoporosis and may offer an alternative to hormone replacement therapy for this indication. Similar considerations may apply in the case of poor patient tolerance of compounds related to etidronate and calcitonin.

Estrogen stimulates the proliferation of breast epithelial cells, and endogenous and exogenous estrogens, as well as related synthetic compounds, have been implicated in the pathogenesis of breast cancer. Human breast cancer cells exhibit specific plasma membrane reactivity with antibodies directed to the nuclear form of ERα. In addition, breast cancer cells with these membrane-associated ERs show rapid responses to estradiol, including significant increments in MAPK and phosphatidylinositol 3-kinase (PI3K)/Akt kinase, enzymic molecules that are crucial in the regulation of cell proliferation and survival. There are current indications that these membrane receptors may associate with HER-2/neu growth factor receptors in lipid raft subdomains of plasma membrane and promote tumor growth. Such signaling complexes may offer a new strategy for therapeutic intervention in patients afflicted with breast cancer.

5.2. Progestogen and Androgen Receptors in Reproduction and Malignancy

As documented for estrogens, several physiologic effects of progestogens and androgens appear to be regulated, in part, by membrane-associated receptors. Progesterone controls several components of reproductive function and behavior. Some of these activities are mediated by interaction with neurons in specific brain regions, and membrane effects appear to be important in this process. Meiosis in amphibian oocytes is initiated by gonadotropins, which stimulate follicle cells to secrete progesterone. The progesterone-induced G_2/M transition in oocytes was among the first convincing examples of a steroid effect at plasma membrane, since it could be shown that exogenous, but not intracellularly injected, progesterone elicited meiosis and that many progesterone-stimulated changes occurred even in enucleated oocytes. Moreover, this process may be

related to progesterone-induced increments in intracellular Ca^{2+} and release of diacylglycerol (DAG) species that elicit a cascade of further lipid messengers.

Progesterone elicits rapid effects on the activity of second messengers and the acrosome reaction in human sperm. Assay of acute sperm responses to progesterone in subfertile patients is highly predictive of fertilization capacity. Effects of the steroid, present in the cumulus matrix surrounding the oocyte, are mediated by elevated intracellular Ca^{2+}, tyrosine phosphorylation, chloride efflux, and stimulation of phospholipases, effects attributed to activation of a membrane-initiated pathway. Indeed, two different receptors for progesterone, apparently distinct from genomic ones, have been identified at the surface of human spermatozoa; nevertheless, a monoclonal antibody against the steroid-binding domain of human *intra*cellular progesterone receptor inhibits progesterone-induced calcium influx and the acrosome reaction in sperm.

As with estrogens and progestogens, androgens promote rapid increase in cytosolic Ca^{2+} in their cellular targets. Other effects of androgens that are not attributable to genomic activation include acute stimulation of MAPK in prostate cancer cells, an action that may be important for promoting their growth. It has been demonstrated in fibroblasts that androgens can stimulate membrane-initiated signaling and the onset of DNA synthesis without activation of "classic" androgen receptor (AR)-dependent gene transcription pathways. Rather, other signaling pathways, such as those modulated by MAPK, may be operative in androgen-induced stimulation of DNA synthesis. Such observations have important implications for understanding the regulation of cell proliferation in steroid target tissues.

The androgen 5β-dihydrotestosterone induces vasodilation of aorta, which may be owing to direct action of the steroid on membranes of smooth muscle cells leading to modulation of calcium channels. In osteoblasts, membrane receptors for androgen appear to be coupled to phospholipase C (PLC) via a pertussis toxin–sensitive G protein that, after binding testosterone, mediates rapid increments in intracellular calcium and inositol triphosphate (IP_3). Testosterone also elicits Ca^{2+} mobilization in macrophages that lack a "classic" intracellular AR. These cells express an apparent G protein–coupled AR at the cell surface that undergoes agonist-induced internalization and may represent an alternative pathway of steroid hormone action.

5.3. Thyroid Hormone Receptors in Metabolic Regulation

Thyroid hormones are well known to regulate energy expenditure and development, and membrane-initiated effects may contribute to these responses. Triiodothyronine (T_3) rapidly stimulates oxygen consumption and gluconeogenesis in liver. T_3 also promotes an abrupt increase in uptake of the glucose analog 2-deoxyglucose in responsive tissues by augmenting activity of the plasma membrane transport system for glucose. In rat heart, T_3 elicits a positive inotropic effect, increasing left ventricular peak systolic pressure, as early as 15 s after hormone injection. In each tissue investigated, alterations in intracellular Ca^{2+} induced by thyroid hormone appear to modulate signal transduction to the cell interior.

Membrane-initiated effects of T_3 have been documented in bone cells by means of inositol phosphate signaling, and in brain through calcium channel activation. T_3 can also influence other cell processes, including the exocytosis of hormones and neurotransmitters, rapid effects that may be attributable to mediation by membrane receptors. Although uptake of T_3 can occur concomitantly with receptor-mediated endocytosis of LDL, and likely is accompanied by carrier proteins, direct uptake of T_3 itself is demonstrable in numerous tissues by means of a high-affinity, stereospecific, and saturable process, such as found for steroid hormones.

5.4. Glucocorticoid Receptors in Metabolic, Immune, and Neural Function

In addition to their long-established effects on mobilization of energy sources by promoting catabolism and the induction of enzymes involved in gluconeogenesis, glucocorticoids have profound effects on neuron signaling and on induction of apoptosis in lymphocytes, phenomena that appear to be membrane-initiated events. Glucocorticoids rapidly alter neuron-firing patterns. These molecular events lead to glucocorticoid modulation of specific brain functions, such as the rapid response of hypothalamic somatostatin neurons to stress. Such abrupt changes in neuron polarization are reinforced by findings of specific, saturable binding of the biologically active radioligand [^{3}H]corticosterone to neuron membranes.

Glucocorticoids play an important role in the regulation of immune function and in inflammation, especially in severe forms of hematologic, rheumatologic, and neurologic diseases. These steroids have profound anti-inflammatory and immunosuppressive actions when used at therapeutic doses. In lymphoproliferative diseases, glucocorticoids are in wide use for disease management, but the cellular mechanism leading to the therapeutic effect remains unclear. In several studies using both cell lines and freshly prepared leukemia or lymphoma cells, the presence of a membrane-associated receptor for glucocorticoids has been implicated

in modulating cell lysis and death. Moreover, in lymphocytes, the membrane-binding site is antigenically related to the intracellular glucocorticoid receptor (GR) and may be a natural splice variant of this form. Some glucocorticoids have been shown to inhibit cation transport across the plasma membrane without concomitant alterations in protein synthesis through transcription. It is postulated that the steroid may thus diminish the acute immune response by interfering with immune regulatory events such as the rise in intracellular Ca^{2+}. An important pharmacologic goal is the development of a steroid compound capable of separating detrimental side effects of glucocorticoids, such as bone loss, from their beneficial antiinflammatory activity. Future approaches aimed at discrimination of the differential activities of membrane-associated and intranuclear GRs may facilitate this prospect.

5.5. Aldosterone and Digitalis-Like Steroid Receptors in Cardiovascular Health

Beyond its classic functions of promoting renal reabsorption of sodium and excretion of excess potassium, aldosterone enhances sodium absorption from the colon and urinary bladder. In each tissue, the mineralocorticoid effect is owing to enhanced activity of amiloride-sensitive sodium channels, with aldosterone rapidly augmenting Na^+/H^+ exchange. This function is Ca^{2+} and PKC–dependent but independent of nuclear receptor activation. Similarly, nontranscriptional action of aldosterone has also been reported to underlie its acute effects on cardiac function, such as increased blood pressure and reduced cardiac output, and on sodium transport in vascular smooth muscle cells.

Digitalis-like compounds are often overlooked members of the steroid superfamily. These plant-derived agents elicit inotropic and chronotropic effects on the heart but also affect many other tissues. Endogenous steroidal ligands, termed *digitalis-like* or *ouabain-like factors*, have been found in sera of humans and other animals with blood volume expansion and hypertension and may be released from the adrenal cortex. These ligands elicit inhibition of membrane-associated Na^+,K^+-adenosine triphosphatase (ATPase), likely the principal receptor for these agonists. It is notable that the steroid-binding domain of Na^+,K^+-ATPase and that of nuclear hormone receptors share significant amino acid sequence homology. In addition to membrane actions of these compounds on Na^+,K^+-ATPase, ouabain-induced hypertrophy in myocytes is accompanied by promotion of Ca^{2+} flux and initiation of protein kinase–dependent pathways leading, in turn, to specific changes in transcription and altered expression of early and late response genes. Thus, the biologic effects of digitalis-like compounds, long considered the exception to the concept of exclusive genomic influence, may render them more closely integrated with the steroid hormone superfamily than was previously recognized.

5.6. Vitamin D Metabolite Receptors in Bone Health and Disease

Membrane-initiated effects of the secosteroid hormone $1,25(OH)_2D_3$ are well documented in bone and cartilage. In osteoblasts, interactions have been proposed between rapid effects of $1,25(OH)_2D_3$, requiring milliseconds to minutes, and longer-term effects owing to gene expression. Rapid activation of calcium channels by $1,25(OH)_2D_3$ occurs in these cells. Calcium flux, which can influence gene expression through multiple pathways, promotes key phosphorylation events in certain bone proteins. Osteoblasts exhibit rapid changes in inositol 1,4,5-triphosphate and DAG in response to vitamin D metabolites via activation of PLC. Other bone cells with rapid responses to vitamin D metabolites include osteosarcoma cells and chondrocytes. The latter system is particularly intriguing because chondrocytes elaborate matrix vesicles that appear critical in bone mineralization. Matrix vesicles, which lack nuclei, exhibit specific, saturable binding of $1,25(OH)_2D_3$, especially when derived from growth-zone chondrocytes.

Other rapid effects of vitamin D occur in a variety of cell types. Muscle cells respond within seconds to $1,25(OH)_2D_3$ via several mediators that alter cardiac output in some instances, and acute activation of calcium channels in skeletal muscle promotes contraction. Of note, in lymphoproliferative disease, $1,25(OH)_2D_3$ appears to prime monocytic leukemia cells for differentiation through acute activation or redistribution of PKC, Ca^{2+}, and MAPK. In pancreas and intestine, activation of membrane-associated signaling pathways results in vesicular exocytosis. Pancreatic β-cells respond to $1,25(OH)_2D_3$ with enhanced intracellular Ca^{2+} that is coupled to increased insulin release. In intestine, $1,25(OH)_2D_3$ stimulates exocytosis of vesicular calcium and phosphate. These cellular events may be related to vitamin D–promoted alterations in the levels of α-tubulin, thereby influencing assembly of microtubules and possibly providing a means for vectorial transport of absorbed ions. Several signal transduction pathways have been found to respond rapidly to exogenous $1,25(OH)_2D_3$, including activation of protein kinases and promotion of abrupt increments in Ca^{2+}, but integration of these signaling cascades with the physiologic response of enhanced ion absorption remains to be established.

Investigations with vitamin D congeners have recently indicated the potential hormonal nature of 24,25-dihydroxyvitamin D_3 (24,25$[OH]_2D_3$), once thought to represent merely the inactivation product of precursor 25$(OH)_2D_3$. Acute effects of 24,25$(OH)_2D_3$ have been observed in bone cells and in intestine; 24,25$(OH)_2D_3$ also inhibits rapid actions of 1,25-$(OH)_2D_3$. This may explain why abrupt effects of 1,25$(OH)_2D_3$ often fail to be observed in vivo: normal, vitamin D–replete subjects have endogenous levels of 24,25-$(OH)_2D_3$ sufficient to inhibit acute stimulation of calcium transport by 1,25$(OH)_2D_3$, thus providing a feedback regulation system.

5.7. Retinoid Receptors in Development and Malignancy

Retinoic acid exerts diverse effects in the control of cell growth during embryonic development and in oncogenesis. Effects of retinoids are widely considered to be mediated exclusively through nuclear receptors, including those for retinoic acid, as well as retinoid X receptors. However, retinoid response pathways independent of nuclear receptors appear to exist. Cellular uptake of retinol (vitamin A) may involve interaction of serum retinol-binding protein with specific surface membrane receptors followed by ligand transfer to cytoplasmic retinol-binding protein. In this regard, targeted disruption of the gene for synthesis of the major endocytotic receptor of renal proximal tubules, megalin, appears to block transepithelial transport of retinol. It is noteworthy that megalin may also be implicated in receptor-mediated endocytosis of 25-hydroxyvitamin D_3 in complex with its plasma carrier. In addition, retinoic acid binds M-6-P/IGF-2R receptor with moderate affinity and enhances its receptor function. M-6-P/IGF-2R is a membrane glycoprotein that functions in binding and trafficking of lysosomal enzymes, in activation of TGF-β, and in degradation of IGF-2, leading to suppression of cell proliferation. The concept of multiple ligands binding to and regulating the function of a single receptor is relatively novel but has important implications for modulating and integrating the activities of seemingly independent biologic pathways.

5.8. Steroidal Congeners in Regulation of Angiogenesis

Steroidal compounds are also implicated in the regulation of angiogenesis. Several steroids, including glucocorticoids, have low levels of angiostatic activity. Squalamine, a naturally occurring aminosterol, has highly potent anti-angiogenic activity. The steroidal substance does not bind to any known steroid hormone receptor, but it interacts specifically with caveolar domains at the surface membrane of vascular endothelial cells and disrupts growth factor–induced signaling for the regulation of cell proliferation. In early clinical trials, the compound showed considerable promise as a therapeutic agent to block the growth and metastatic spread of lung and ovarian cancers by interfering with tumor-associated angiogenesis. In addition, squalamine steroids may be useful for medical management of macular degeneration, a form of visual loss that is highly correlated with uncontrolled angiogenesis. At this time, there is no known cure for macular degeneration, which afflicts about 30 million people worldwide, and is the leading cause of legal blindness in adults older than 60.

6. CONCLUSION

Since the discovery of chromosomal puff induction by the insect steroid hormone ecdysone, cell regulation by steroid hormones has focused primarily on a nuclear mechanism of action. However, even ecdysone is now known to elicit rapid plasma membrane effects that may facilitate later nuclear alterations. Indeed, numerous reports of acute steroid hormone effects in diverse cell types cannot be explained by the generally prevailing theory that centers on the activity of hormone receptors located exclusively in the nucleus. Plasma membrane forms of steroid hormone receptors occur in target cells and are coupled to intracellular signaling pathways that mediate hormone action. Membrane-initiated signals appear to be the primary response of the target cell to steroid hormones and may be a prerequisite for subsequent genomic activation. Coupling of plasma membrane, cytoplasmic and nuclear responses, constitutes a progressive, ordered expansion of initial signaling events. Recent dramatic advances in this area have led to intensified efforts to delineate the nature and biologic roles of all classes of receptor molecules that function in steroid hormone–signaling pathways. Molecular details of cross-communication between steroid and peptide receptors are also beginning to emerge, and steroid receptors associated with plasma-membrane signaling platforms may be in a pivotal location to promote convergence among diverse cellular response pathways. This new synthesis has profound implications for integration of the physiology and pathophysiology of hormone action in responsive cells and may lead to development of novel approaches for the treatment of many cell proliferative, metabolic, inflammatory, reproductive, cardiovascular, and neurologic diseases.

SELECTED READING

Davis PJ, Davis FB. Nongenomic actions of thyroid hormone on the heart. *Thyroid* 2002;12:459–466.

Gruber C, Tschugguel W, Schneeberger C, Huber J. Production and actions of estrogens. *N Engl J Med* 2002;346:340–352.

Hoessli D, Llangumaran S, Soltermann A, Robinson P, Borisch B, Din N. Signaling through sphingolipid microdomians of the plasma membrane: the concept of signaling platform. *Glycoconj J* 2000;17:191–197.

Lösel R, Wehling M. Nongenomic actions of steroid hormones. *Nat Rev Mol Biol* 2003;4:46–56.

Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999;340:1801–1811.

Migliaccio A, Castoria G, Di Domenico M, de Falco A, Bilancio A, Lombardi M, Bottero D, Varicchio L, Nanayakkara M, Rotondi A, Auricchio F. Sex steroid hormones act as growth factors. *J Steroid Biochem Mol Biol* 2003;17:1–5.

Milner TA, McEwen BS, Hayashi S, Li CJ, Reagan LP, Alves SE. Ultrastructural evidence that hippocampal α-estrogen receptors are located at extranuclear sites. *J Comp Neurol* 2001;429: 355–371.

Moss RL, Gu Q, Wong M. Estrogen: nontranscriptional signaling pathway. *Recent Prog Hormone Res* 1997;52:33–70.

Nemere I, Farach-Carson M. Membrane receptors for steroid hormones: a case for specific cell surface binding sites for vitamin D metabolites and estrogen. *Biochem Biophys Res Commun* 1998;248:443–449.

Szego CM. Cytostructural correlates of hormone action: new common ground in receptor-mediated signal propagation for steroid and peptide agonists. *Endocrine* 1994;2:1079–1093.

Szego CM, Pietras R. Membrane recognition and effector sites in steroid hormone action. In: Litwack G, ed. *Biochemical Actions of Hormones*, vol. VIII. New York, NY: Academic 1981:307–463.

Watson CS, Gametchu B. Membrane-initiated steroid actions and the proteins that mediate them. *Proc Soc Exp Biol Med* 1999; 220:93–19.

Weihua Z, Andersson S, Cheng G, Simpson E, Warner M, Gustafsson J-A. Update on estrogen signaling. *FEBS Lett* 2003; 546:17–24.

REFERENCES

Chambliss KL, Yuhanna IS, Mineo C, Liu P, German Z, Sherman TS, Mendelsohn ME, Anderson RG, Shaul PW. Estrogen receptor alpha and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. *Circ Res* 2000;87: E44–E52.

Li L, Haynes MP, Bender JR. Plasma membrane localization and function of the estrogen receptor alpha variant (ER46) in human endothelial cells. *Proc Natl Acad Sci USA* 2003;100:4807–4812.

Márquez DC, Pietras RJ. Membrane-associated binding sites for estrogen contribute to growth regulation of human breast cancer cells. *Oncogene* 2001; 20: 5420–5430.

Moats RK II, Ramirez VD. Electron microscopic visualization of membrane-mediated uptake and translocation of estrogen-BSA: colloidal gold by Hep G2 cells. *J Endocrinol* 2000;166: 631–647.

Navarro CE, Saeed SA, Murdick C, Martinez-Fuentes AJ, Arora K, Krsmanovic LZ, Catt KJ. Regulation of cyclic adenosine 3′,5′-monophosphate signaling and pulsatile neurosecretion by G-coupled plasma membrane estrogen receptors in immortalized gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2003; 17:1792–1804.

Pietras RJ, Nemere I, Szego CM. Steroid hormone receptors in target cell membranes. *Endocrine* 2001;14:417–427.

Pietras RJ, Szego CM. Metabolic and proliferative responses to estrogen by hepatocytes selected for plasma membrane binding-sites specific for estradiol-17β. *J Cell Physiol* 1979;98:145–159.

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

Szego CM, Pietras RJ. Lysosomal functions in cellular activation: propagation of the actions of hormones and other effectors. *Int Rev Cytol* 1984;88:1–302.

Szego CM, Pietras RJ, Nemere I. Encyclopedia of Hormones. In: Henry H, Norman A, eds. *Plasma Membrane Receptors for Steroid Hormones: Initiation Site of the Cellular Response*. San Diego, CA: Academic 2003;657–671.