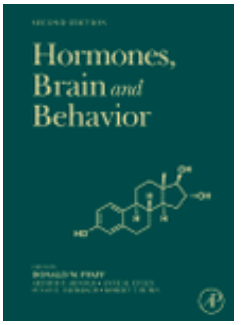

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44 Molecular Genomics of Progestin Actions

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Chapter Outline

44.1 Introduction

Progesterone is a member of a family of steroid hormones that regulate homeostasis, development, reproduction, and behavior. Many of these biological effects of progesterone are mediated through the progestin receptor (PR), which is a member of the steroid/nuclear receptor superfamily of transcriptional activators that also include receptors for estrogens, androgens, glucocorticoids, and mineralocorticoids (Evans, 1988; Mangelsdorf et al., 1995; Tsai and O'Malley, 1994). PRs function in a classical genomic mechanism by acting as ligand-dependent nuclear transcription factors. In addition, PRs can function at the membrane and/or in the cytosol as mediators of growth-factor-initiated signaling pathways. Recent observations indicate that membrane-associated PRs rapidly activate cytoplasmic signaling pathways as an alternative route for regulating PR-induced nuclear transcriptional events. Integration of these rapid cytoplasmic signaling events with PR nuclear actions has important implications for the biological functions of PR. Herein, we discuss PR-initiated classic genomic and nongenomic signaling pathways and the implications of these mechanisms for PR action in brain. While much of our discussion focuses on the molecular mechanisms of human PR action that have been elucidated from *in vitro* and cell culture studies associated with breast cancer models, studies related to PR action in brain are also presented. For a more detailed discussion of PR action in brain and behavior, including neurotransmitter-mediated ligand-independent effects of PR in brain, the reader is directed to **Chapter 45, Mechanism of Progesterone Receptor Action in the Brain**.

44.2 PR Structure and Genomic Mechanisms of Action

Similar to other nuclear receptors, PRs have a modular domain structure consisting of an

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amino-terminal region (N-domain), a central DNA-binding domain (DBD) and a carboxy-terminal ligand-binding domain (LBD) (Evans, 1988; **Figure 1**). In general, steroid receptors (SRs) have two transcriptional activation functions located in the LBD and N-terminus (Meyer et al., 1992; Tora et al., 1989; Lees et al., 1989). In a variety of species, including chicken (Gronemeyer et al., 1987; Conneely et al., 1987), rodents (Schott et al., 1991), monkeys, and humans (Kastner et al., 1990; Duffy et al., 1997; Lessey et al., 1983), PRs are expressed in two forms: the full-length PR-B and the N-terminally truncated PR-A. Thus, PR-A and PR-B have identical DBDs and LBDs and differ only in the length of the N-terminus. There is a third isoform, PR-C, that is devoid of classical transcriptional activity and can function as a dominant inhibitor of uterine PR-B in the fundal myometrium during labor (Condon et al., 2006). These PR isoforms are the product of a single gene located on chromosome 11 at q22–23 that undergoes transcription via the use of alternate promoters and internal translational start sites (Horwitz et al., 1990; Kastner et al., 1990).

PR-A and PR-B contain a ligand-dependent activation function (AF-2) in the LBD and a constitutive activation function (AF-1) in the N-domain (Meyer et al., 1990, 1992; **Figure 1**). PR-B contains an additional activation function (AF-3) in the N-terminus that is not present in PR-A (Sartorius et al., 1994; Tung et al., 2006). Under certain cell and promoter contexts, human PR-B is a stronger transcriptional activator than PR-A (Vegeto et al., 1993; McDonnell and Goldman, 1994; Tung et al., 1993; Wen et al., 1994; Giangrande et al., 1997). This difference in transcriptional activity is most likely due to conformational or other structural differences between the N-termini of PR-A and PR-B (Kastner et al., 1990; Bain et al., 2000; Giangrande et al., 1997; Hovland et al., 1998). Under certain conditions, PR-A can repress the transcriptional activity of PR-B (Vegeto et al., 1993; McDonnell and Goldman, 1994; Tung et al., 1993; Wen et al., 1994; Giangrande et al., 1997), most likely due to a transcriptional inhibitory region that has been identified in PR-A (Giangrande et al., 1997; Hovland et al., 1998). The transrepressive activity of PR-A has been shown to be dependent upon sumoylation of PR Lys388, an N-terminal modification that also represses PR transcriptional activity at selected promoters (Abdel-Hafiz et al., 2002).

While PR domains can function independently, in the context of the full receptor these domains act together to produce the complete transcriptional activity of the receptor. The PR LBD consists of 10–12 α helices that form an internal hydrophobic ligand-binding pocket. Binding of progesterone or agonists elicits a conformational change in helix 12

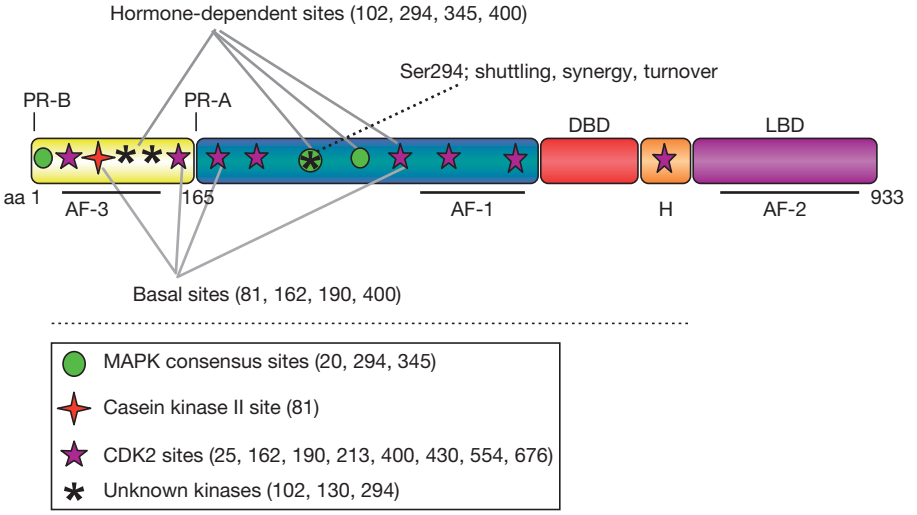

Figure 1 Human PR structure and phosphorylation sites: modular domain structure of human PR-B (aa 1–933) and PR-A (aa 165–933). Thirteen serine residues and one threonine residue in human PR are shown to represent basal (constitutive) and hormone-induced phosphorylation sites (Knotts et al., 2001) and may contribute to PR regulation by MAPK (Lange et al., 2000; Shen et al., 2001; Qiu et al., 2003), casein kinase II (Zhang et al., 1994), and CDK2 (Zhang et al., 1997; Knotts et al., 2001). Individual PR phosphorylation sites may be regulated by multiple protein kinases (Qiu et al., 2003) and/or in a sequential manner (Clemm et al., 2000), illustrating the complexity of PR regulation by phosphorylation. LBD, ligand-binding domain; H, hinge; DBD, DNA-binding domain; AF, activation functions.

to form a hydrophobic cleft that mediates interactions with nuclear receptor coactivators (Lonard et al., 2007; Rosenfeld et al., 2006). Adjacent to the LBD is the hinge region, which is critical in mediating appropriate dimerization of PR (Tetel et al., 1997). The DBD is highly conserved among the nuclear receptors and consists of two asymmetric zinc-finger-like motifs (Schwabe et al., 1990). As discussed below, the DBD is critical for target gene activation and mediates receptor binding to progestin response elements (PREs) (Beato and Sánchez-Pacheco, 1996; Bain et al., 2000). These PREs consist of partial palindromic hexanucleotide sequences that are separated by an invariant three-nucleotide spacer (Beato and Sánchez-Pacheco, 1996). The N-domain is the least conserved, and least understood, of the receptor domains. However, it is apparent that the PR N-domain mediates intramolecular interactions (Tetel et al., 1999) and protein–protein interactions (Wardell et al., 2002) and contains a number of phosphorylation sites (discussed below).

The classic, ligand-dependent, genomic mechanism of action of PR is shown in **Figure 2**. In the absence of progesterone, PRs are complexed with several chaperone molecules, including heat shock protein (hsp)90, hsp70, hsp40, Hop, and p23. These interactions are requisite for proper protein folding and assembly of stable PR–hsp90 heterocomplexes that are competent to bind ligand (Pratt et al., 2004). The hsps also function to connect PR to protein trafficking systems. Upon binding hormone, SRs undergo a conformational change that causes dissociation of these hsps and immunophilins and allow receptors to dimerize (DeMarzo et al., 1991). Activated receptors bind directly to specific PREs and PRE-like sequences in the promoter regions of target genes, including *c-myc* (Moore et al., 1997),

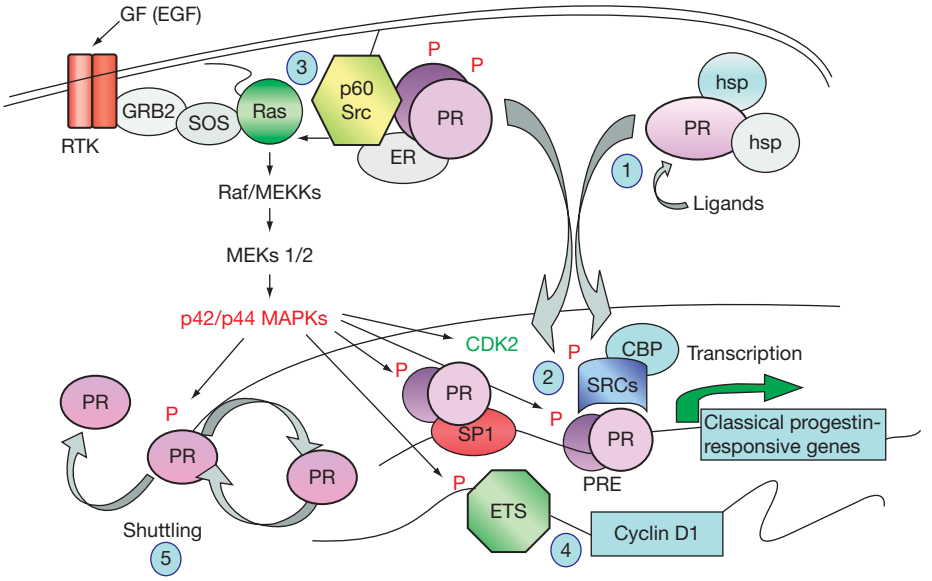

Figure 2 Mechanisms of PR action and functional significance of PR phosphorylation. Phosphorylation (P) of specific sites in PR couples multiple receptor functions, including transcriptional synergy in the presence of steroid hormones and growth factors predicted to activate MAPK and/or CDK2, and nuclear import or export (shuttling) in response to MAPK activation. Rapid ligand-dependent PR downregulation by the ubiquitin–proteasome pathway (degradation) occurs upon nuclear export. (1) Ligand binding mediates dissociation of heat-shock proteins and nuclear accumulation of PR dimers. (2) Nuclear PRs mediate gene regulation via the classical pathway; phosphorylated PRs may recruit regulatory molecules (e.g., SRC-1 and CBP) that are phosphoproteins, and function in one or more interconnected processes (transcription, localization, and turnover), perhaps linked by a common cellular machinery. (3) PRs and growth factors activate MAPKs independently via a c-Src kinase-dependent pathway, and this may result in positive regulation of PR action via feedback regulation (i.e., direct phosphorylation of liganded PR or coactivators), occurring in both the absence and presence of steroid hormone ligands and on PRE-containing or other PR-regulated gene promoters. (4) Activation of MAPKs by PR provides for regulation of gene targets whose promoters do not contain PREs and are otherwise independent of PR-transcriptional activities but utilize PR- or SR-activated MAPKs, such as regulation of the cyclin D1 promoter by Ets factors. (5) MAPK regulation of PR has been shown to mediate nuclear accumulation/shuttling and nuclear export that is coupled to regulation of PR transcriptional events.

fatty acid synthetase (Chalbos et al., 1987), and mouse mammary tumor virus (MMTV) (Krusekopf et al., 1991). Binding of receptors to DNA increases or decreases gene transcription by altering the rate of recruitment of general transcription factors and influencing the recruitment of RNA polymerase II to the initiation site (Klein-Hitpass et al., 1990; Kininis et al., 2007). Thus, in brain it is thought that progesterone acts via its receptor to alter neuronal gene transcription, resulting in changes in behavior and physiology (Pfaff, 2005; Blaustein and Mani, 2006; **Chapter 2, Feminine Reproductive Behavior and Physiology in Rodents: Integration of Hormonal, Behavioral, and Environmental Influences**; **Chapter 34, Genetic Mechanisms in Neural and Hormonal Controls over Female Reproductive Behaviors**; and **Chapter 45, Mechanism of Progesterone Receptor Action in the Brain**).

Treatment with progesterone also results in an upregulation of regulatory molecules without classical PREs in their proximal promoter regions, such as epidermal growth factor (EGF) receptor (Lange et al., 1998; Brass et al., 1995), *c-fos* (Church et al., 2005; Richer et al., 1998), and *cyclin D1* (Gregory et al., 2001; Groshong et al., 1997). Without canonical PREs, PR regulation of these genes can occur through indirect DNA-binding mechanisms, as in the example of PR binding to Specificity protein 1 to promote p21 transcription in the presence of progestin (Owen et al., 1998). PR may also regulate genes by tethering to Activating protein 1 (Tseng et al., 2003) or signal transducers and activators of transcription (STATs) (Richer et al., 1998; Proietti et al., 2005). When either directly or indirectly bound to DNA, PRs regulate the basal transcription machinery in conjunction with nuclear receptor coregulatory molecules (see below for a more detailed discussion).

44.3 Progestin-Regulated Genes

44.3.1 PR-Responsive Genes in Human Breast Cancer Cells

The biochemistry of PR action is relatively well understood, having been largely defined using PR positive human breast cancer cell line models, or PR-null cells into which wild-type (wt) or modified PR has been re-expressed. A variety of studies have focused on PR interactions with other regulatory proteins, changes in PR subcellular localization, or post-translational modifications (e.g., phosphorylation, ubiquitinylation, or sumoylation) or other conditions that affect PR transcriptional activities, usually measured on artificial gene promoters (reporter genes) that contain one or more tandem PRE sites (Lange et al., 2007). Growth factors, including EGF or heregulin, promote transcriptional synergy with progestins on PR-target genes (Qiu and Lange, 2003; Daniel et al., 2007b; Shen et al., 2001). Phosphorylation events primarily serve to augment PR action in a promoter selective manner (Daniel et al., 2007a). Despite this depth of basic understanding, gene regulation and the associated changes in cell biology in response to PR activation remain elusive. Only a handful of endogenous progesterone-responsive genes have been described in moderate detail (Moore et al., 1997; Cui et al., 2003; McGowan and Clarke, 1999). The majority of genes regulated in response to progesterone lack PREs, and the presence of one or more PREs or PRE half-sites does not predict progesterone-responsive regulation (Richer et al., 2002). A number of genes are regulated upon PR expression, but independently of progesterone (Jacobsen et al., 2002, 2005). Furthermore, many genes are downregulated in response to progesterone/PR-dependent transcriptional repression, largely by unknown mechanisms (Jacobsen et al., 2002; Richer et al., 2002). In most cases, the regulation of particular genes in response to progesterone/PR is only loosely tied (by correlation) to changes in cell biology. For example, many PR-regulated genes have been associated with aspects of tumor progression toward aggressive tumor phenotype. In addition, variation of the PR-A to PR-B ratio is a frequent occurrence in breast tumors relative to normal tissue (Graham et al., 1995), and is predicted to dramatically alter the genetic program (Jacobsen et al., 2002, 2003).

Results from expression profiling of breast cancer cells *in vitro* are consistent with the results from experimental mouse models, which suggest that the two PR isoforms serve different functions. In mice, where the PR-A to PR-B ratio is 3:1 compared to humans where it is 1:1, ablation of one or the other PR isoform leads to divergent effects on the mammary gland. PR-A knockout (leaving only PR-B) leads to normal early development (Mulac-Jericevic et al., 2000), while PR-B knockout (leaving only PR-A) leads to reduced pregnancy-associated lobuloalveolar development and reduced side-branching (Mulac-Jericevic et al., 2003). On the other hand, overexpression of PR-B causes precocious ductal arrest and inappropriate ductal development (Shyamala et al., 2000), while overexpression of PR-A causes mammary epithelial cell hyperplasia, excessive ductal branching, and a disorganized basement membrane (Shyamala et al., 1998). To explain these isoform-specific differences, gene profiling studies have been performed *in vitro* using

human breast cancer cells expressing PR-A or PR-B. The first such study used 6 h of progesterone treatment in an attempt to identify direct PR target genes (Richer et al., 2002; Jacobsen et al., 2003). Of 94 genes identified, 65 were regulated only by PR-B, 4 only by PR-A, and 25 by both PR isoforms. This regulatory pattern was confirmed in subsequent studies using breast cancer cells with inducible PR-A versus PR-B treated 6 h with progesterone (Jacobsen et al., 2005). More recent studies used progesterone-treated breast cancer cells that express both PR isoforms (Leo et al., 2005; Ghatge et al., 2005; Graham et al., 2005). Analysis of the protein pathways indicates that progesterone suppresses genes involved in proliferation and metastasis (Leo et al., 2005), supporting an anti-proliferative role for this hormone. However, a remarkable number of the genes upregulated by progestins encode proteins involved in signal transduction and cell adhesion (Jacobsen et al., 2005; Richer et al., 2002), lending some support to the concept that progestins/PR may contribute to the dysregulation of pathways important for breast cancer progression that are perhaps not well modeled *in vitro*. Additionally, the above studies address gene regulation in response to unliganded or liganded PR (i.e., single hormone exposure). We propose that PR isoforms act as sensors for signal transduction pathways (discussed above) and, thus, promoter selectivity is predicted to be highly sensitive to phosphorylation events. Further studies will be needed to address alterations in the signature of PR-regulated genes in the context of the high kinase activities characteristic of aggressive breast cancer.

44.3.2 Progestin-Regulated Genes in Brain

A classic example of a steroid-responsive gene is the induction of PR expression by estradiol (E) in a variety of hormone responsive tissues, including brain (Blaustein and Feder, 1979b; MacLusky and McEwen, 1978; Lauber et al., 1991; Simerly and Seil, 1993; Scott et al., 2002). While more is known about estrogen-regulated genes in brain (Pfaff, 1980; McEwen, 2002; Jasnow et al., 2008; Malyala et al., 2004), we are beginning to gain an understanding of genes regulated by progestins in brain. In E-primed animals, progesterone increased expression of oxytocin mRNA in the paraventricular nucleus of the hypothalamus (Kawata et al., 1991), which may play a role in the regulation of female sexual behavior. In addition, progesterone in E-primed female rats elicits an increase in hypothalamic expression of hsp70 (Krebs et al., 1999) and secretory carrier membrane protein-4 (SCAMP-4) mRNA, a gene that may contribute to female reproduction (Krebs and Pfaff, 2001). More recent studies have applied microarray technology to this question of progestin-regulated genes in brain. In males treated with progesterone, microarray analysis revealed up- and downregulation of 12 progestin-responsive genes in the hypothalamus (Auger et al., 2006). Progesterone increased expression of calmodulin and calreticulin genes, which encode for calcium-binding proteins involved in calcium signaling and steroid receptor signaling. In addition, progesterone elicited an increase in somatostatin, proliferin, and secretogranin, components important in neuroendocrine signaling. In contrast, progesterone caused a downregulation of two immediate early genes, *c-fos* and *Arc*, which may play a role in the effects of progestins on stress and anxiety (Auger et al., 2006). In the macaque dorsal raphe, estradiol and progesterone influenced the expression of a variety of genes involved in neuronal plasticity, transmitter synthesis and trafficking, and apoptosis (Reddy and Bethea, 2005). Interestingly, while estradiol had no effect, progesterone treatment dramatically increased expression of genes encoding for the gamma-aminobutyric acid-A (GABA-A) receptor (benzodiazepine site) and E2F1 (interferes with cytokine signaling) in the dorsal raphe region (Reddy and Bethea, 2005). In a rat spinal cord injury model, progesterone treatment increased expression of brain-derived neurotrophic factor (BDNF) and myelin basic protein and led to enhanced myelination (De Nicola et al., 2006). While it is known that PR-A and PR-B act in brain to differentially contribute to female sexual behavior (Mani et al., 2006; **Chapter 45, Mechanism of Progesterone Receptor Action in the Brain**), it will be important for future studies to investigate the roles of PR-A and PR-B in progestin-regulated genes in brain.

44.4 Nuclear Receptor Coregulators and PR

Coregulators are required for efficient transcriptional regulation by nuclear receptors (Lonard and O'Malley, 2006; Rosenfeld et al., 2006; O'Malley, 2006; Edwards, 2000). The importance of these coregulators in a variety of human diseases, including cancer and metabolic disorders, is becoming more apparent (Lonard et al., 2007). Coregulators consist of coactivators and corepressors that are required for efficient transcriptional regulation by nuclear receptors. Nuclear receptor coactivators dramatically enhance the transcriptional activity of nuclear receptors, including PR and ER (Lonard and O'Malley, 2006; Rosenfeld et al.,

2006; O'Malley, 2006; Edwards, 2000). Nuclear receptor coactivators influence receptor transcription through a variety of mechanisms, including acetylation, methylation, phosphorylation, and chromatin remodeling (Lonard and O'Malley, 2006; Rosenfeld et al., 2006). *In vitro* studies using antibodies against nuclear receptor coactivators indicate that recruitment of coactivators is rate limiting in steroid receptor-mediated gene transcription (McKenna et al., 1999; Torchia et al., 1997; Lonard and O'Malley, 2006; Rosenfeld et al., 2006). In further support for nuclear receptor coactivator-dependent facilitation of transcription *in vitro*, squelching, or the repression of the transcriptional activity of one steroid receptor by another, is reversed by the addition of coactivators (McKenna et al., 1999; Oñate et al., 1995). Thus, a critical component of efficient steroid receptor transcription is the recruitment of nuclear receptor coactivators, which dramatically enhance transcriptional activity (Lonard and O'Malley, 2006; Rosenfeld et al., 2006; O'Malley, 2006). Under most conditions, SRs interact with coactivators in the presence of an agonist, but not in the absence of ligand or in the presence of an antagonist or a selective receptor modulator (Oñate et al., 1995; McInerney et al., 1996; Tanenbaum et al., 1998; Shiau et al., 1998; but cf. Oñate et al., 1998; Webb et al., 1998; Dutertre and Smith, 2003). While nuclear receptor coactivators usually interact with the C-terminal AF-2 of the receptor (McKenna et al., 1999; Robyr et al., 2000; Oñate et al., 1998; Voegel et al., 1996; McInerney et al., 1996; Kobayashi et al., 2000), there are coactivators that associate with the N-terminus of PR as discussed below (Wardell et al., 2002).

Corepressors and their complexes associate with nuclear receptors when unliganded or bound to antagonists (Rosenfeld et al., 2006). These corepressors serve to repress nuclear receptor transcription by recruiting corepressor complexes to the *cis*-active elements in the promoter and enhancers of target genes. In general, corepressors and their complexes antagonize the actions of coactivators through histone deacetylase activity and phosphatase activity, and by altering chromatin modifications such as methylation of histones (Rosenfeld et al., 2006).

44.4.1 Coactivators of PR

44.4.1.1 The p160 family

Steroid receptor coactivator-1 (SRC-1/NcoA-1) was one of the first coactivators found to interact with hormone-bound PR (Oñate et al., 1995; Kamei et al., 1996). SRC-1 was found to be a member of a larger family of p160 proteins that includes SRC-2 (also known as GRIP1, TIF2 and NCoA-2; Voegel et al., 1996; Hong et al., 1997) and SRC-3 (AIB1, TRAM-1, p/CIP, ACTR, RAC3; Anzick et al., 1997; Suen et al., 1998). The SRC family of coactivators physically interacts with SRs, including PR and ER, in a ligand-dependent manner (Oñate et al., 1995; Lonard and O'Malley, 2006; Rosenfeld et al., 2006). The SRCs physically associate with agonist-bound receptors through multiple LXXLL motifs (L, leucine; X, any amino acid), or nuclear receptor (NR) boxes, that are located in the central region of the SRCs (McKenna et al., 1999; Wu et al., 2005). *In vitro* experiments reveal that depletion of SRC-1 in cultured cells by microinjection of antibodies to SRC-1 prevents receptor dependent transcription, suggesting that SRC-1 is important for transcriptional activity of SRs (Torchia et al., 1997). In cell culture, hormone-induced transactivation of PR is reduced by co-expression of ERα, presumably due to squelching or sequestering of shared coactivators (Oñate et al., 1995). This squelching effect can be reversed by overexpression of SRC-1, suggesting that coactivators are a limiting factor necessary for full transcriptional activation of receptors (Oñate et al., 1995). In further support of this concept, overexpression of SRC-1 relieves thyroid hormone receptor inhibition of ERα-mediated transcription in a neuroendocrine model (Vasudevan et al., 2001).

It has been suggested that the SRC family of coactivators acts as a platform to allow the recruitment of other coactivators, including CREB-binding protein (CBP) and p300/CBP associated factor (p/CAF), that possess histone acetyl transferase (HAT) activity and aid in chromatin remodeling (CREB – cAMP response element binding; McKenna et al., 1998; Smith et al., 1996; Kamei et al., 1996). The p160 coactivators contain two activation domains, AD1 and AD2, in the C-terminal region. AD1 mediates interactions with CBP (Chen et al., 1997), while AD2 allows binding of other proteins, including the protein arginine methyltransferase CARM1 (Chen et al., 1999, 2000).

While much is known about the molecular mechanisms of nuclear receptor coactivators from a variety of *in vitro* studies (Lonard and O'Malley, 2006; Rosenfeld et al., 2006), we are just beginning to understand their role in hormone action *in vivo*. SRC-1 knockout mice, while fertile, have partial hormone resistance in progesterone target tissues,

including uterus and mammary gland (Xu et al., 1998). In addition, SRC-1 null mice have partial resistance to thyroid hormone (Weiss et al., 1999). SRC-1 knockouts have more than a twofold increase in serum thyrotropin levels, despite a 50% increase in serum-free thyroid hormone levels compared to wt controls. Finally, recent work in SRC-1 null mice reveals that this coactivator is critical in maintaining energy balance by regulating both energy intake and expenditure (Wang et al., 2006).

As is the case with SRC-1, SRC-2 (also known as GRIP1, TIF2, and NCoA-2) enhances transcriptional activity of a variety of nuclear receptors, including PR (Voegel et al., 1996; Hong et al., 1997). The midregion of the SRC-2 protein, which mediates interactions with SRs, has relatively low homology with SRC-1, suggesting functional differences between these two proteins (Voegel et al., 1996; Hong et al., 1997). SRC-2 knockout mice reveal that this coactivator is important in fertility (Gehin et al., 2002) and is necessary for progesterone-dependent embryo implantation in uterus and ductal branching in mammary gland (Mukherjee et al., 2006, 2007; Fernandez-Valdivia et al., 2007). Generation of mice in which SRC-2 is ablated specifically in cell types that express PR ($PR^{Cre/+}SRC\text{-}2^{flox/flox}$) have allowed the investigation of the function of this coactivator in progestin action (Fernandez-Valdivia et al., 2007). While disruption of SRC-2 expression in PR-positive ovarian cells did not alter ovarian activity, $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uterine function was severely impaired. Elimination of SRC-2 expression in PR-containing uterine cells led to an early block in embryo implantation. Furthermore, removal of SRC-1 in $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ uteri caused a block in decidualization, suggesting that both SRC-1 and SRC-2 are required for complete PR-dependent decidualization. In addition, SRC-2 is important for PR action in mammary gland as demonstrated by the lack of significant branching and alveolar morphogenesis in the $PR^{Cre/+}SRC\text{-}2^{flox/flox}$ mammary gland (Mukherjee et al., 2006; Fernandez-Valdivia et al., 2007). Finally, the role of SRC-2 in the regulation of a variety of progestin-responsive genes in uterus has been investigated (Jeong et al., 2007). Microarray analysis of uteri from SRC-2 null mice revealed that this coactivator is involved in the ability of progesterone to repress specific genes involved in a variety of functions, including cell cycle and immunity (Jeong et al., 2007).

The gene for SRC-3 is amplified in 5–10% of human breast tumors and overexpressed in about 60% of tumors (Anzick et al., 1997). This amplification and overexpression of SRC-3 is thought to confer a selective growth advantage through increased steroid signaling (Anzick et al., 2003). SRC-3 (AIB1, TRAM-1, p/CIP, ACTR, RAC3) coactivates a variety of nuclear receptors, including PRs (Anzick et al., 1997; Torchia et al., 1997; Stromberg et al., 1999; Li et al., 1997). Phosphorylation of SRC-3 is thought to be important for its ability to coactivate specific SRs and has been associated with its oncogenic properties (Wu et al., 2004). The normal biological functions of SRC-3 have been studied in SRC-3 null mice. SRC-3 knockouts have a variety of deficits in the development of steroid-sensitive reproductive tissues (Xu et al., 2000a). In female SRC-3 null mice, puberty is delayed 3 days compared to wt controls. However, treatment with E can alleviate this delay, suggesting that later puberty in these animals is due to problems with E synthesis. Furthermore, although these SRC-3 knockout mice are fertile, they ovulate fewer eggs, are less likely to become pregnant, and deliver fewer pups than wt or heterozygous mice. Estrous cycles in SRC-3 knockouts were nearly twice as long as in wt mice. The authors suggest that the disrupted reproductive function in these mice may be due to defects of the ovary. Furthermore, lack of SRC-3 in oocytes may result in decreased oocyte development, leading to subfertility in knockout mice (Xu et al., 2000a). Recent studies in SRC-3 null mice reveal that this coactivator is critical for normal PR-dependent mammary gland development and function (Han et al., 2006). Another interesting study has investigated the role of gonadotropin-releasing hormones (GnRH I and GnRH II) on PR recruitment of SRC-3 (An et al., 2006). Using chromatin immunoprecipitation (ChIP) assays, progesterone stimulated recruitment of SRC-3 by PR on the PRE of a luciferase reporter gene or the gonadotropin α subunit gene promoter. However, in these same ChIP assays, GnRH stimulated more efficient recruitment of SRC-3 by PR than progesterone. The authors suggest that phosphorylation of PR and its interaction with SRC-3 and binding to DNA may play an important role in the possible ligand-independent activation of PR by GnRHs (An et al., 2006).

44.4.1.2 Other coactivators of PR

44.4.1.2(i) CREB-binding protein

While CBP was initially discovered to be a transcriptional activator CREB (Chrivia et al., 1993; Kwok et al., 1994), it is also now known to function as an integrator of nuclear receptors with other cell

signaling pathways, including CREB and activator protein 1 (AP-1) (Kwok et al., 1994; Kamei et al., 1996; Yang et al., 1996). As is the case with the SRC family, CBP is important in ligand-dependent transcriptional activity of nuclear receptors, including PR and ER (Smith et al., 1996). Interestingly, mutation of the CBP gene causes Rubinstein–Taybi syndrome, which results in severe mental retardation and a variety of physiological deformities in humans (Petrij et al., 1995). In mice, mutations of CBP lead to similar physical deformities as well as impaired memory (Oike et al., 1999). While p300 is closely related to CBP, genetic knockout mice for CBP and p300 exhibit different phenotypes, suggesting a functional distinction of these coactivators (Vo and Goodman, 2001).

A variety of *in vitro* studies indicate that SRC-1 and CBP act synergistically to enhance PR transcriptional activity (Smith et al., 1996). A synergistic effect in hormone-dependent transcriptional activity was observed when cells were transfected with both SRC-1 and CBP (Smith et al., 1996). In support of this concept, *in vitro* studies indicate that SRC-1 physically interacts with CBP and recruits CBP to the coactivator complex and forms a ternary complex at target gene promoters (Smith et al., 1996; Kamei et al., 1996). PR requires both SRC-1 and CBP for full transcriptional activity and function (Tetel et al., 1999; Liu et al., 2001; Xu et al., 2000b; Smith et al., 1996). For ligand-bound PR to induce transcription of target genes, SRC-1 must be recruited to the receptor dimer complex first, followed by CBP (Liu et al., 2001). Deletion of either the CBP/p300 binding site, or the C-terminal region containing the PR binding site, of SRC-1 dramatically reduces PR transactivation (Liu et al., 2001).

44.4.1.2(ii) Steroid receptor RNA activator

SRA is a unique coactivator in that it functions as an RNA transcript to enhance transcriptional activation of SRs (Cavarretta et al., 2002; Lanz et al., 1999). SRA was found to increase transactivation of a variety of SRs including PR, ER, glucocorticoid receptor (GR), and androgen receptor (AR) in a ligand-dependent manner, but not class II receptors such as thyroid hormone receptor (TR), retinoid X receptor (RXR), retinoic acid receptors (RAR), and peroxisome proliferator-activated receptors (PPAR) (Lanz et al., 1999). Deletion experiments revealed that this effect was mediated through SRA interactions with the N-terminal AF-1 domain of receptors. As mentioned above, coactivators can reverse squelching of one nuclear receptor by another. While liganded ER reduced PR transcriptional activation by 50%, addition of SRA reversed this squelching effect of ER (Lanz et al., 1999). The necessity of SRA for efficient PR transactivation is further demonstrated by a 70% reduction in PR target gene expression in HeLa cells by cotransfection of SRA antisense oligonucleotides (Lanz et al., 1999). In cells that were treated with SRC-1 and SRA antisense oligodeoxynucleotides (ODNs), ERα activity was decreased by 70% compared to that of control-treated cells (Cavarretta et al., 2002). Antisense to either SRA or SRC-1 alone had a less dramatic effect on ERα activity, suggesting SRA association with SRC-1 (Cavarretta et al., 2002). In further support of this association, SRA was found to copurify with SRC-1, indicating that SRA exists in a ribonucleoprotein complex containing SRC-1 (Lanz et al., 1999). Taken together, these findings further support the association of SRA and SRC-1 in a coactivator complex necessary for full steroid receptor transcriptional activity. Expression of SRA seems to be tissue specific. Using Northern blot analysis, SRA mRNA was expressed at high levels in the liver, skeletal muscle, and heart, and at lower levels in brain and placenta (Lanz et al., 1999). Finally, overexpression of SRA in a transgenic mouse model reveals a role for SRA in estrogen-induced expression of PR in mammary gland (Lanz et al., 2003).

44.4.1.2(iii) Jun dimerization protein-2

While most coactivators interact with AF-2 in the C-terminal portion of PR, some coactivators have been identified to interact with the N-terminal AF-1 of PR (Edwards et al., 2002). While Jun dimerization protein-2 (JDP-2) was first identified as a repressor of jun and other basic leucine zipper (bZIP) transcription factors, it is also a strong PR selective coactivator (Wardell et al., 2002). Interaction of PR with JDP-2 occurs through the AF-1 of PR and the bZIP of JDP-2. JDP-2 increases hormone-dependent PR transcription by stimulating AF-1 activity. Both the DBD and the AF-1 are required for JDP-2 coactivation of PR. JDP-2 interacts with other coactivators, including CBP and pCAF, but not SRC-1. These findings suggest that JDP-2 functions to stimulate AF-1 activity by providing a platform for the recruitment of other coactivators to the N-terminus of PR (Wardell et al., 2002).

Finally, it should be noted that there are a variety of other coactivators (Rowan and O'Malley, 2000), including TRAP220 (Ito et al., 1999), chromatin high-mobility group proteins 1 and 2 (Boonyaratanakornkit

et al., 1998), and TIP60 (Brady et al., 1999) known to interact with PR. With over 285 coregulators identified to date (O'Malley, 2007), there is much more to be learned about the function of coregulators in nuclear receptor action.

44.4.1.3 Chromatin remodeling and PR

One major outcome of the binding of PR, and other nuclear receptors, to their respective response elements is the modification of chromatin. Many of these modifications include post-translational changes to histones, including acetylation, methylation, phosphorylation, and ubiquitination (Iizuka and Smith, 2003). These histone modifications contribute to histone–histone and histone–DNA interactions that can result in changes in chromatin structure and, thus, lead to changes in transcription. In particular, the effects of histone acetylation on gene transcription have been well studied. Acetylation of histones occurs on the lysine residues of the N-terminal tails of histone proteins. This acetylation of histones is very dynamic and results in the opening up of the chromatin architecture (Grunstein, 1997). Histone acetylation is mediated by HATs, while deacetylation, or removal of acetyl groups, is achieved by histone deacetylases (HDACs). The majority of evidence indicates that histone acetylation results in gene activation and histone deacetylation leads to gene repression (Kurdistani and Grunstein, 2003). In support, many of the nuclear receptor coactivators discussed above, including SRC-1 and CBP, contain intrinsic HAT activity (Spencer et al., 1997; Ogryzko et al., 1996; Bannister and Kouzarides, 1996), while many corepressors have HDAC activity (Rosenfeld et al., 2006).

The profound impact of HAT activity and chromatin modification on steroid receptor function is becoming increasingly apparent. A long-standing question in steroid receptor biology has been how PR and GR can elicit distinct biological effects given that both receptors act at the same hormone response elements (Allan et al., 1991; Deroo and Archer, 2001; Vicent et al., 2006). One possible mechanism by which PR and GR could have distinct effects is through the differential recruitment of coactivators resulting in different modifications of chromatin. Li et al. (2003) used a T47D cell line with a stably integrated MMTV reporter gene to address this important question pertaining to PR and GR action. Activated PR preferentially associated with SRC-1, which recruited CBP and led to increased acetylation of the lysine residue K5 of histone4 (H4). However, ligand-bound GR preferentially interacted with SRC-2 which recruited pCAF and resulted in modification of H3. These findings suggest that different SRs can preferentially associate with coactivators that recruit distinct HATs that can ultimately result in differential gene expression (Li et al., 2003).

Another study shows the further complexity of PR activation of the MMTV promoter and the dynamic nature of histone modifications (Aoyagi and Archer, 2007). ChIP assays revealed that within 5–15 min of hormone treatment, PR activation resulted in acetylation of H4, which coincided with recruitment of RNA polymerase II, CBP, and p300 to the MMTV promoter. These events were followed by a decrease in acetylation over the next 60 min of hormone treatment. Interestingly, HDAC1 and HDAC2 were detected at the promoter prior to hormone treatment, but then were absent during the first 5–15 min of hormone treatment, perhaps allowing for the increase in acetylation of H4 (possibly by CBP and pCAF). During 15–30 min of hormone treatment, these HDACs were present again, coinciding with the overall decrease in acetylation 15 min after hormone treatment and the subsequent deacetylation of H4. This timing of acetylation in PR-mediated activation of MMTV differed from findings of GR activation of MMTV (Mulholland et al., 2003), suggesting additional differences in the kinetics of gene activation between PR and GR with regard to the use of identical hormone response elements (HREs) upstream of target genes (Aoyagi and Archer, 2007). Understanding how this ordered recruitment and exchange of cofactors is mediated, and its effects on chromatin architecture, will be essential to comprehending the specificity of steroid receptor-mediated transcription.

44.4.1.4 Function of PR coactivators in brain

While much is known about the molecular mechanisms of nuclear receptor coactivators from a variety of cell culture studies (Lonard and O'Malley, 2006; Rosenfeld et al., 2006; O'Malley, 2006), we are just beginning to understand their role in hormone action in brain (Molenda et al., 2003). SRC-1 mRNA and protein are expressed at high levels in the cortex, hypothalamus, and hippocampus of rodents (Misiti et al., 1998; Shearman et al., 1999; Martinez de Arrieta et al., 2000; Meijer et al., 2000; Auger et al., 2000; Molenda et al., 2002; Ogawa et al., 2001) and birds (Charlier et al., 2002). In order for coactivators to function with SRs, they must be expressed in the same cells. Indeed, SRC-1 is expressed in the majority of estrogen-induced PR cells in reproductively relevant brain regions, including the ventromedial

nucleus (VMN), medial preoptic area, and arcuate nucleus (Tetel et al., 2007). Given that virtually all estradiol-induced PR cells in the hypothalamus contain ERα (Blaustein and Turcotte, 1989; Warembourg et al., 1989), these findings suggest that these specialized cells represent functional sites of interaction between ovarian SRs and SRC-1 in brain (Tetel et al., 2007). The expression of the SRC family of coactivators in brain appears to be regulated by a variety of factors, including hormones (Camacho-Arroyo et al., 2005; Mitev et al., 2003; Charlier et al., 2006a; Iannacone et al., 2002; Ramos and Weiss, 2006; Maerkel et al., 2007; McGinnis et al., 2007), daylength (Tetel et al., 2004), and stress (Bousios et al., 2001; Charlier et al., 2006a; Meijer et al., 2006).

More recently, the function of nuclear receptor coactivators in hormone action in brain and behavior has been investigated. In collaboration with Tony Auger and Peg McCarthy, we investigated the role of SRC-1 in hormone-dependent sexual differentiation of the rodent sexually dimorphic nucleus (SDN) of the POA (Auger et al., 2000). On postnatal days (PN) 0–2, the hypothalami of female rat pups were bilaterally infused with antisense oligonucleotides (ODNs) to SRC-1 mRNA or scrambled control ODNs. On PN1, female pups were treated with the aromatizable androgen, testosterone propionate, to increase SDN volume. At PN13, antisense to SRC-1 was found to reduce the volume of the SDN of androgenized females by 46% compared to females receiving control ODNs. The testosterone (T) surge in males just after birth suppresses the development of female sexual behavior in adulthood (Sodersten, 1978; Booth, 1977; Whalen and Edwards, 1967; and **Chapter 62, Early-Life Experiences: Enduring Behavioral, Neurological, and Endocrinological Consequences** and **Chapter 63, Thyroid Hormones and Brain Development**). This suppression is due to E, aromatized from T, binding to ER (McCarthy et al., 1993). In addition, this T surge is critical for the development of masculine sexual behavior in the adult rat and is mediated by androgen receptors (Whalen and Edwards, 1967; van der Schoot, 1980; Ward and Renz, 1972). To test if SRC-1 was critical in development of sexual behavior, androgenized female and male rats were treated with SRC-1 antisense or control ODNs on PN 0–2 (Auger et al., 2000). Males were castrated in adulthood and, following testosterone treatment, were tested for male and female sex behavior. Males and androgenized females treated with SRC-1 antisense displayed higher levels of female sexual behavior than did rats treated with control ODNs. Interestingly, male sexual behavior in these animals did not differ. Taken together, these findings suggest that reduction of SRC-1 in brain decreases ER activity and, thus, alters brain development and inhibits the defeminizing actions of estrogen during development (Auger et al., 2000).

CBP is expressed in reproductively relevant brain areas in a dimorphic manner, and functions in the development of masculine sexual behavior (Auger et al., 2002a). On the day of birth, males express 53% more CBP-immunoreactive (CBP-IR) cells in the mPOA, while females express 83% more CBP-IR cells in the VMN than males. These findings of differential expression of CBP suggest that gonadal steroid hormones alter levels of CBP in the brain during development, which in turn influence neural steroid responsiveness. In this same study, testosterone-treated females that received CBP antisense in the hypothalamus on PN 0–2 displayed higher levels of lordosis than androgenized females treated with control ODNs (Auger et al., 2002a). However, CBP antisense treatment did not affect development of male sexual behavior in these androgenized females. Taken together with the previous study, it appears that both SRC-1 and CBP are necessary for the defeminizing actions of ER, but not the masculinizing actions of AR, during early development.

Our lab and others have investigated the role of nuclear receptor coactivators in hormone-dependent gene expression in brain and behavior in adult rodents (Molenda et al., 2002; Apostolakis et al., 2002). E-induction of PR gene expression in the VMN is necessary for hormone-dependent female sexual behavior (Pleim et al., 1989). Therefore, we tested the hypothesis that SRC-1 and CBP are critical in modulating ER-mediated transactivation of the PR gene in the VMN. Infusions of antisense ODNs to SRC-1 and CBP mRNA into one side of the VMN of adult female rats reduced the expression of ER-mediated activation of PR gene expression compared to the contralateral control ODN-treated VMN (Molenda et al., 2002). These findings are supported by previous *in vitro* studies indicating that SRC-1 and CBP function together to modulate ER activity (Smith et al., 1996). In further support of SRC-1 and CBP/p300 functioning together in brain, neurons in the rat hippocampus and dentate gyrus co-express SRC-1 and p300 (Ogawa et al., 2001). A similar study in brain supports these findings and extends them to include a role of SRC-2, but not SRC-3, in ER-mediated induction of PR in the VMN (Apostolakis et al., 2002). Finally, the p160

coactivators function in GR action in glial cells (Grenier et al., 2005) and in GR-mediated repression of the corticotropin-releasing hormone gene (van der Laan et al., 2008). Taken together, these findings indicate that nuclear receptor coactivator action in brain is essential for full steroid receptor transcriptional activity.

Given that nuclear receptor coactivators are critical for hormone-dependent gene expression in brain, we next tested the hypothesis that these coactivators act in brain to modulate the expression of hormone-dependent behaviors (Molenda et al., 2002). Female rats treated with antisense to both SRC-1 and CBP mRNA into the VMN displayed reduced levels of hormone-dependent female sexual receptivity compared to scrambled-treated controls (Molenda et al., 2002). Another study supported these findings with SRC-1 and extended them to include a role for SRC-2 in hormone-dependent behavior (Apostolakis et al., 2002). Our lab has gone on to isolate the effects of these nuclear receptor coactivators on both ER- and PR-dependent aspects of female sexual behavior. There are two modes of hormone-regulated female reproductive behavior in rats: estrogen-mediated (elicited by E alone) and progesterone-facilitated (requires E priming followed by progesterone) (see **Chapter 2, Feminine Reproductive Behavior and Physiology in Rodents: Integration of Hormonal, Behavioral, and Environmental Influences**). To test the hypothesis that nuclear receptor coactivators function in brain to modulate ER-mediated aspects of female reproductive behavior, animals were injected with E only (Molenda-Figueira et al., 2006). Antisense to SRC-1 and CBP infused into the VMN of animals treated with E alone decreased lordosis intensity, suggesting that these coactivators modulate ER-mediated aspects of female sexual behavior. Proceptive behaviors by the female, which serve to solicit interaction by the male, are PR-dependent and include ear wiggling and hopping and darting (Hardy and DeBold, 1971; Whalen, 1974; Fadem et al., 1979; Tennent et al., 1980; Edwards and Pfeifle, 1983; Erskine, 1989; Ogawa et al., 1994). Infusion of antisense ODNs to SRC-1 and CBP mRNA into the VMN around the time of P administration reduced PR-dependent ear wiggling and hopping and darting (**Figure 3**). Thus, it appears that nuclear receptor coactivators function in brain to modulate PR and ER action in brain and influence specific aspects of hormone-dependent sexual behaviors in rodents.

Recently, we have begun to explore the interactions of SRs with coactivators from rat brain. To test the hypotheses that SRC-1 from brain physically associates with PR and ER subtypes in a ligand-dependent manner, we developed pull-down assays with brain tissue from female rats (Molenda-Figueira et al., 2008). We found that SRC-1 from hypothalamic or hippocampal extracts interacted efficiently with both GST-tagged PR-A and PR-B when bound to the agonist R5020 (**Figure 4(a)**). In contrast, very little to no SRC-1 from brain associated with PR-A or PR-B in the absence of ligand or in the presence of the selective PR modulator (SPRM), RU486. **Figure 4(a)** reveals lower-molecular-weight bands labeled with the SRC-1 monoclonal antibody that appear to interact with PR-A and PR-B in a manner that is not dependent on the ligand condition, because they are present in all three ligand conditions. However, these same immunoreactive bands were observed using the polyclonal SRC-1 antibody (data not shown), suggesting that these bands are fragments of SRC-1 from brain. These findings using brain tissue are consistent with previous studies using recombinant SRC-1 and the concept that SRC-1 and PR interactions are agonist dependent (Oñate et al., 1995; Giangrande et al., 2000). Our findings using coactivators from brain support previous work indicating a role for SRC-1 action in the hypothalamus in PR-dependent female sexual behavior (Molenda-Figueira et al., 2006) and suggest that SRC-1 may contribute to progestin effects in the hippocampus on memory (Sandstrom and Williams, 2001).

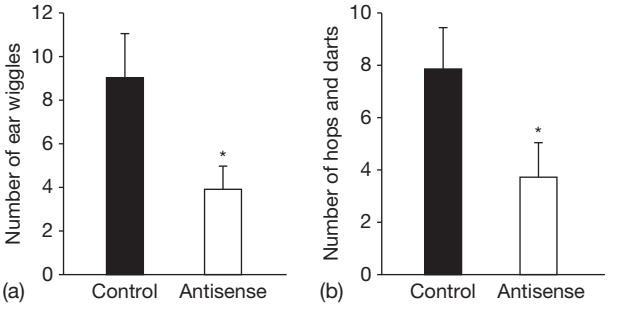

Figure 3 Nuclear receptor coactivators modulate PR action in brain. Female rats were ovariectomized, primed with estradiol and progesterone, and exposed to male rats. Infusions of antisense to both SRC-1 and CBP mRNA in the ventromedial nucleus of the hypothalamus of female rats, decreased PR-dependent (a) ear wiggling and (b) hops and darts compared to scrambled control-treated animals. *$p < 0.05$; one-tailed *t* test. Reproduced from Molenda-Figueira HA, Williams CA, Griffin AL, Rutledge EM, Blaustein JD, and Tetel MJ (2006) Nuclear receptor coactivators function in estrogen receptor- and progestin receptor-dependent aspects of sexual behavior in female rats. *Hormones and Behavior* 50: 383–392, with permission from Elsevier.

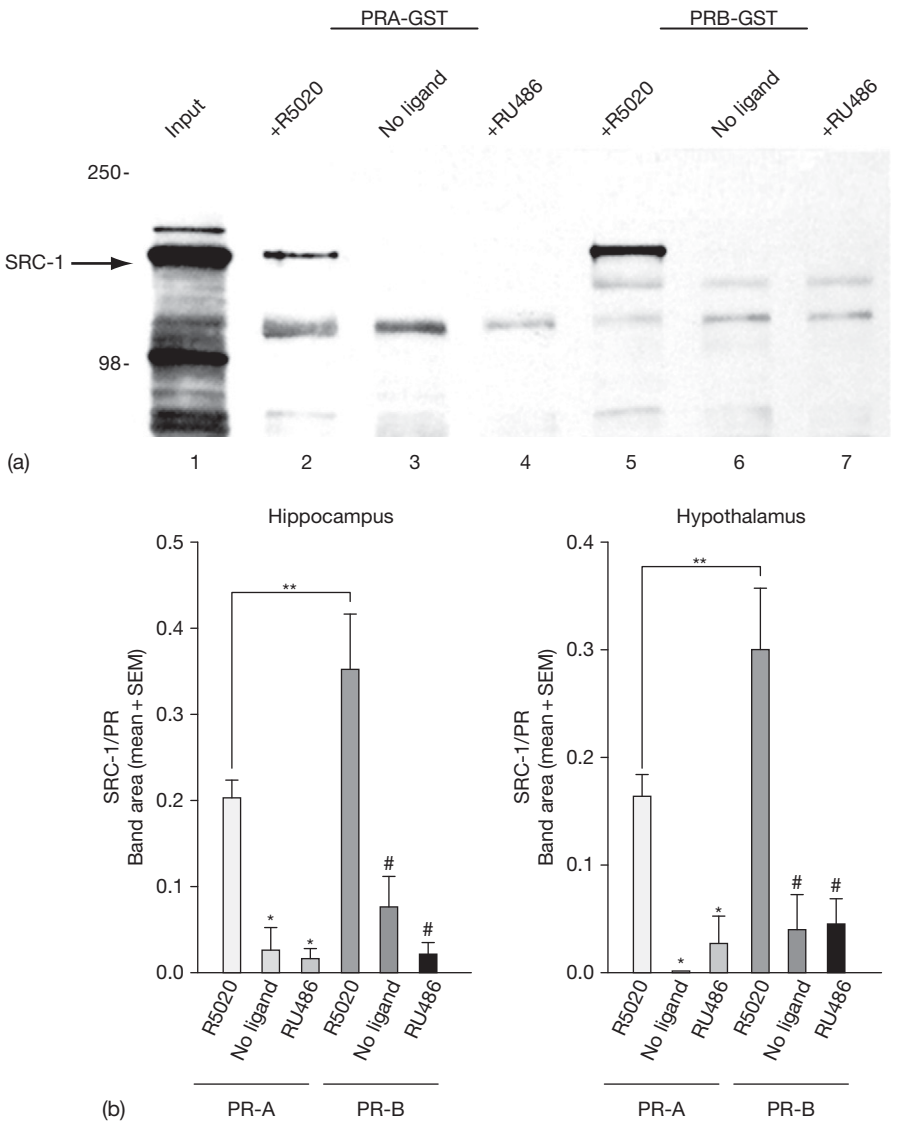

Figure 4 SRC-1 from rat brain associates with PR in a ligand-dependent and receptor isoform-specific manner. (a) SRC-1 from hippocampal whole cell extracts interacts with PR-A and PR-B in a ligand-dependent manner. SRC-1 from the hippocampus associates with PR-A and PR-B in the presence of the agonist R5020 (lanes 2 and 5), but not in the absence of ligand (lanes 3 and 6), or in the presence of the selective PR modulator, RU486 (lanes 4 and 7). Input (1% of total) of SRC-1 from hippocampal extract is shown in lane 1. (b) SRC-1 from the hippocampus and hypothalamus associates differentially with the PR isoforms. SRC-1 from hippocampal extracts interacted with both PR-A and PR-B in the presence of R5020, but not in the absence of ligand or the presence of RU486. * $p < 0.0001$, significantly different from PR-A + R5020. # $p < 0.01$, significantly different from PR-B + R5020. SRC-1 from hippocampus interacted more with PR-B, than with PR-A, when bound to R5020. ** $p < 0.05$, *t*-test. Hypothalamic SRC-1 interacts with PR-A and PR-B when bound to R5020, but little to no interactions were detected in the absence of ligand or when receptors were bound to RU486, * $p < 0.01$, significantly different from PR-A + R5020. # $p < 0.001$, significantly different from PR-B + R5020. SRC-1 from the hypothalamus interacted more with PR-B, than with PR-A, when bound to R5020. ** $p < 0.05$, *t*-test, $n = 5$–7 per treatment group. Reproduced from Molenda-Figueira HA, Muphy SD, Shea KL, et al. (2008) Steroid receptor coactivator-1 from brain physically interacts differentially with steroid receptor. *Endocrinology* 149: 5272–5279, with permission from The Endocrine Society, Copyright 2008.

Interestingly, we found that SRC-1 from hypothalamus or hippocampus interacts more with PR-B, than with PR-A, in the presence of agonist (**Figure 4(b)**). The present results are in contrast to other pull-down assays in which recombinant SRC-1 interacted equally with PR-A and PR-B (Oñate et al., 1998) or did not interact with PR-A (Giangrande et al., 2000). Taken together, our findings suggest the importance

of using biologically relevant tissue, in contrast to the use of cell lines alone, in investigating receptor–coactivator interactions (Molenda-Figueira et al., 2005). It may be that other cofactors and proteins that are present in tissue (e.g., brain) are important for appropriate SRC-1 and PR interactions. Understanding how nuclear receptor coactivators function with various SRs, and their subtypes, is critical to understanding how hormones act in different brain regions to profoundly influence physiology and behavior. Ultimately, investigation of these receptor–coactivator interactions using brain tissue may allow the identification of novel cofactors involved in the steroid receptor complex in brain.

The function of coactivators has also been studied in hormone action in bird brain. SRC-1 and CBP are expressed at high levels in steroid-sensitive brain regions of adult quail (Charlier et al., 2002) and European starlings (Auger et al., 2002b), respectively. In adult quail, infusion of antisense to SRC-1 mRNA reduced testosterone-dependent male copulatory behaviors (Charlier et al., 2005). In addition, SRC-1 was found to function in testosterone-dependent sex differences in brain volume and aromatase expression in the preoptic medial nucleus of the quail (Charlier et al., 2005, 2006b). These findings indicate that SRC-1 is important in the modulation of hormone-dependent gene expression, brain plasticity, and behavior in birds. L7-SPA has been shown to potentiate the partial agonist activity of RU486-bound PR or tamoxifen-bound ER (Jackson et al., 1997). Interestingly, careful analysis revealed a sex difference in the expression of L7/SPA in the song nuclei of zebra finch (Duncan and Carruth, 2007). In future studies, it will be important to determine the biological function of L7/SPA in brain.

44.4.2 Corepressors and PR

As stated above, corepressors function to inhibit transcriptional activity of some nuclear receptors. RARs and TRs represent a subset of NRs that bind to target DNA as heterodimers with RXR in the presence or absence of ligand. In the absence of ligand, RAR and TR recruit the corepressors, NR corepressor (N-CoR) (Horlein et al., 1995), and silencing mediator for retinoid acid and thyroid hormone receptors (SMRT) (Chen and Evans, 1995), that actively repress transcription. Binding of ligand causes an exchange of these corepressors with coactivators' complexes and the initiation of transcription. N-CoR and SMRT suppress the partial agonist activity RU486 when bound to PR (Jackson et al., 1997). Other studies have found that SMRT can repress agonist-dependent activity of PR by disrupting receptor dimer interactions, rather than recruitment of histone deacetylases (Agoulnik et al., 2003). Some have begun to investigate the role of these interesting coregulator molecules in hormone action in brain. N-CoR and SMRT are ubiquitously expressed in rodent brain, including the hippocampus, hypothalamus, cortex, and brainstem (van der Laan et al., 2005). SMRT is expressed at higher levels in hypothalamus and brainstem than N-CoR. Furthermore, coexpression of these two corepressors was detected in many of the brain regions studied (van der Laan et al., 2005). SMRT expression in the POA is altered over the estrous cycle (Camacho-Arroyo et al., 2005) and appears to be upregulated by thyroid hormone in cerebellum (Ramos and Weiss, 2006). N-CoR expression is altered by hypothyroidism in the cortex, dentate gyrus, and CA3 of the developing rat brain (Iannacone et al., 2002). Thus, N-CoR and SMRT have distinct expression patterns and are regulated in brain. It will be important for future studies to investigate the biological function of these corepressors in action of PR, and other receptors, in brain.

44.5 PR Phosphorylation

44.5.1 Direct PR Phosphorylation in Breast Cancer Models

Similar to other SR family members, phosphorylation–dephosphorylation events add multi-functionality to PR action (**Figure 1**) (Beck et al., 1992). Several protein kinases phosphorylate PR isoforms primarily on serine residues within the amino termini and, to a lesser degree, on serine residues throughout the receptor (Horwitz et al., 1990; Takimoto and Horwitz, 1993). PR contains a total of 14 known phosphorylation sites (Beck et al., 1996; Zhang et al., 1997) and is reviewed in Lange (2004) and Weigel and Moore (2007). Serines at positions 81, 162, 190, and 400 appear to be constitutively phosphorylated in the absence of hormone (Zhang et al., 1997; **Figure 1**). One to two hours after progestin treatment, serines 102, 294, and 345 are maximally phosphorylated (Zhang et al., 1995). Specific kinases have been identified that are responsible for phosphorylation of selected sites. Serines at positions 81 and 294 are phosphorylated by casein kinase II (Zhang et al., 1994) and mitogen-activated protein kinase (MAPK) (Lange et al., 2000; Shen et al., 2001), respectively. Progestins can also stimulate Ser294 phosphorylation independently of MAPKs by activation of an unknown kinase(s) (Qiu et al., 2003). Eight of the total 14 sites (i.e., Serines 25, 162, 190, 213, 400,

554, 676, and Thr430) are phosphorylated by cyclin A/cyclin-dependent protein kinase 2 (CDK2) complexes *in vitro* (Zhang et al., 1997; Knotts et al., 2001). Only five of these sites (i.e., Serines 162, 190, 213, 400, and 676) are proven *in vivo* phosphorylation sites (Zhang et al., 1997, 1994; Knotts et al., 2001). In breast cancer cells, PR Ser400 is highly sensitive to phosphorylation in response to progestins, as well as agents that activate CKD2 and/or MAPKs (Pierson-Mullany and Lange, 2004).

PRs receive signals from growth factor-initiated signal transduction pathways by way of phosphorylation–dephosphorylation events. While the function of PR phosphorylation is incompletely understood, it might influence aspects of transcriptional regulation, such as interaction with coregulators, as reported for PR (Narayanan et al., 2005a) and ERα (Font de Mora and Brown, 2000). PR phosphorylation is also involved in the regulation of ligand-dependent (Shen et al., 2001) and-independent (Labriola et al., 2003; Pierson-Mullany and Lange, 2004) PR nuclear localization, receptor turnover, hormone sensitivity, and transcriptional activities (Lange et al., 2000; Shen et al., 2001; Takimoto et al., 1992, 1996). As has been reported for ERα (Migliaccio et al., 1989; Ali et al., 1993), phosphorylated PRs are hypersensitive relative to their underphosphorylated counterparts (Qiu and Lange, 2003). For example, following a brief (5–15 min) pretreatment with EGF, phosphorylated nuclear PR-B receptors are transactivated by subphysiologic progestin levels. EGF and progestins synergistically upregulate mRNA or protein levels for a number of growth regulatory genes (Richer et al., 1998), including cyclin D1 and cyclin E (Lange et al., 1998); the regulation of cyclins by progestins is MAPK dependent. Cyclins, in turn, regulate progression of cells through the cell cycle by interaction with cyclin-dependent protein kinases. Progestins activate CDK2 (Groshong et al., 1997), and PRs are predominantly phosphorylated by CDK2 at proline-directed (S/TP) sites (Zhang et al., 1997; Knotts et al., 2001), perhaps allowing for the coordinate regulation of PR transcriptional activity during cell-cycle progression. In support of this idea, Narayanan et al. (2005a,b) found that PR activity is highest in S phase and lower in the G0/G1 phases of the cell cycle, but this activity is impaired during G2/M phases, concomitant with lowered PR phosphorylation. Overexpression of either cyclin A or CDK2 enhanced PR transcriptional activity; while cyclin A interacts with the N-terminus of PR, CDK2 seems to alter PR function indirectly by increasing the phosphorylation and recruitment of SRC-1 to liganded PR. In p27-null cells, phosphorylation of PR Ser400 mediates robust ligand-independent PR activation (Pierson–Mullany and Lange, 2004).

44.5.2 PR Ser294 Phosphorylation in Breast Cancer Models

PR Ser294 lies within a proline-directed or MAPK consensus site (PXXSP) that is rapidly phosphorylated upon exposure to ligand (Zhang et al., 1995) and considered a significant site for PR regulation by multiple protein kinases (Lange et al., 2000; Shen et al., 2001; Qiu et al., 2003; Qiu and Lange, 2003). Phosphorylation of PR Ser294 in response to progestins or MAPKs appears to mediate increased PR nucleocytoplasmic shuttling. Both rapid nuclear translocation of unliganded PR and nuclear export of liganded PR require MAPK-dependent phosphorylation of this residue (Qiu et al., 2003). PR nuclear sequestration in response to MAPK activation might serve to protect inactive or active receptors from degradation in the cytoplasm or upon nuclear export (Qiu et al., 2003). Following ligand binding, transcriptionally active PR undergoes rapid downregulation (Nardulli and Katzenellenbogen, 1988). Phosphorylation of Ser294 greatly augments this process, perhaps by making liganded PR a cytoplasmic target for ubiquitination and degradation by the 26S-proteosome pathway (Lange et al., 2000; Qiu et al., 2003). Mutant PR with alanine in place of serine at position 294 (S294A) can bind ligand and interact with regulatory elements in DNA, but fails to exit the nucleus and undergo efficient ubiquitination. As a result, the receptor remains highly stable in the presence of progestins as compared to wt PR (Lange et al., 2000; Qiu et al., 2003). Interestingly, stabilized S294A PR is a weak transcription factor and fails to respond to agents that activate MAPK (Shen et al., 2001). The mechanism of these effects was recently shown to involve reversible attachment of SUMO to PR-B Lys388 (Daniel et al., 2007a). Underphosphorylated PRs are heavily sumoylated and transcriptionally repressed. Conversely, generation of a Ser294 phospho-mimic receptor by replacement of Ser294 with aspartic acid (S294D) resulted in hyperactive progestin-induced transcription with increased PR turnover relative to wt PR (Daniel et al., 2007b); S294D PRs are predicted to be undersumoylated (Daniel et al., 2007a). Thus, reversible phosphorylation (at Ser294) determines the degree of PR modification by either ubiquitination (Lange et al., 2000) or sumoylation

(Daniel et al., 2007a) and can dramatically alter receptor location, turnover, and hormone responsiveness; ubiquitinated receptors are highly active but rapidly turnover while sumoylated receptors are transcriptionally repressed and stable in the presence of progestin relative to unmodified (at Lys388) receptors. Further investigation is required to determine details of the mechanisms of transcriptional regulation by these rapid and reversible post-translational events. Recent studies support the conclusion that EGF-induced nuclear accumulation of PR is a key step in ligand-independent transcriptional activation. Labriola et al. (2003) reported that exposure of T47D breast cancer cells to EGF family member, heregulin, can stimulate nuclear localization, DNA binding, and transcriptional activity of PR in the absence of hormone. Heregulin exposure also resulted in activation of MAPK and PR Ser294 phosphorylation. Qiu et al. (2003) reported that PR Ser294 phosphorylation results in similar nuclear activity. However, growth factors alone failed to stimulate PR transcriptional activity or alter PR downregulation in T47D cell variants (Shen et al., 2001). However, in the presence of ligand, MAPK activation greatly augmented both these events (Shen et al., 2001; Qiu et al., 2003). One explanation for these apparently conflicting results is that differential expression of EGFR family members expressed on the cell surface between T47D cell line clones might lead to differences in the activation of downstream intracellular kinases, such as CDK2 (discussed below). In any case, these exciting data (Qiu et al., 2003; Labriola et al., 2003) suggest a continuum between PR hypersensitivity to extremely low ligand concentrations and complete ligand independence, a phenomenon that is well documented for AR or ERα. Regulation of PR by alternate signaling pathways, including elevated MAPK activity often exhibited by breast tumors, may contribute to disregulated gene expression and changes in cell growth and/or survival. For example, PR-B regulation of IRS-2 expression in breast cancer cells requires phosphorylation of PR Ser294 and occurs in the absence of ligand (Qiu and Lange, 2003).

44.5.3 MAPK and PR Function in Brain

In brain, progesterone is metabolized to a variety of ring A-reduced progestins, including 5α-dihydroprogesterone (5α-DHP) and 5α,3α-prenanolone (5α,3α-Pgl) (Poletti et al., 1998). In E-primed rats, progesterone, 5α-DHP, or 5α,3α-Pgl induce lordosis and proceptivity (Gorzalka and Whalen, 1977; Etgen et al., 2006), while RU486 blocks these effects (Beyer et al., 1995). Interestingly, while P and 5α-DHP bind to intracellular PR, 5α,3α-Pgl does not, suggesting that PR may be activated by 5α,3α-Pgl via a phosphorylation-dependent pathway (Gonzalez-Flores et al., 2004b). Indeed, infusion of the MAPK inhibitor (PD98059) into the third ventricle decreased the display of lordosis and proceptivity induced by P, 5α-DHP, or 5α,3α-Pgl (Gonzalez-Flores et al., 2004b). These findings suggest that MAPK signaling in brain is required for the facilitatory actions of P, 5α-DHP, and 5α,3α-Pgl on female sexual behavior (Gonzalez-Flores et al., 2004b). cGMP can facilitate lordosis and this effect is blocked by RU486, suggesting PRs are required for this cGMP effect (Chu et al., 1999). Because cGMP-dependent kinase can activate MAPK, Etgen and colleagues asked if MAPK signaling was involved in the cGMP enhancement of lordosis (Gonzalez-Flores et al., 2004b). Inhibition of MAPK activity decreased lordosis induced by 8-bromo-cGMP, a cell-permeable cGMP analog, suggesting that cGMP enhancement of lordosis involves ligand-independent activation of PR in brain by MAPK phosphorylation (Gonzalez-Flores et al., 2004b).

While P has a facilitating effect on female sexual behavior, it also results in a refractory period to subsequent stimulation of rodent female sexual behavior by P (Dempsey et al., 1936) or E and P (Blaustein and Wade, 1977). This refractory period has been referred to as the postestrous-refractory period (Morin, 1977) or the sequential inhibitory effect of P (Blaustein and Wade, 1977). While the role of P in the termination of sexual behavior in rats during the estrous cycle and pregnancy is not completely understood (Sodersten and Hansen, 1977; Baum et al., 1979; Blaustein and Feder, 1979c), it is thought that P-dependent downregulation of hypothalamic PR is critical to this sequential inhibition by P (Moguilewsky and Raynaud, 1979; Blaustein and Feder, 1979a; Parsons et al., 1981). As discussed above, ligand-induced downregulation of PR involves MAPK-dependent phosphorylation and subsequent ubiquitination and degradation by the 26S-proteosome pathway (Lange et al., 2000; Qiu et al., 2003). Etgen and co-workers have investigated the role of MAPK and the 26S proteasome in sequential inhibition of female sexual behavior by P (Gonzalez-Flores et al., 2004a,b). As stated above, in E-primed animals, an MAPK inhibitor (PD98059) given after P reduces lordosis 4 h later (Gonzalez-Flores et al., 2004b). However, if rats were given a second injection of P 24 h later and then tested again for lordosis, an

increase in behavior was observed, suggesting that P was not as effective in eliciting sequential inhibition (Gonzalez-Flores et al., 2004b). Next, the role of 26S proteasome was explored in the regulation of sequential inhibition by progesterone and PR in brain (Gonzalez-Flores et al., 2004a). Estradiol primed rats were: (1) injected with P or P plus a proteasome inhibitor and tested for the facilitating effects of P on sex behavior 4 h later, and (2) 24 h later given a second injection of P and tested for behavior 4 h later for sequential inhibition by P. After this final behavior test, brains were removed and PRs from the hypothalamus and preoptic area were analyzed by Western blot. In the first behavior test for the facilitating effects of P, all animals showed a strong lordosis response. In the second behavior test for sequential inhibition by P, animals that received P in the first injection had decreased lordosis and proceptivity, while those animals given P and a proteasome inhibitor displayed high levels of sexual behavior. Western blot analysis revealed that the first P injection reduced estradiol-induced PR levels in the hypothalamus, while treatment with a proteasome inhibitor increased PR levels in the hypothalamus and preoptic area (Gonzalez-Flores et al., 2004a). From these findings, the authors suggest that MAPK-dependent phosphorylation of PR contributes to the facilitatory actions of P and then targets the PR for degradation by the 26S proteasome pathway leading to the sequential inhibition by P (Gonzalez-Flores et al., 2004a; Etgen et al., 2006).

44.6 Extranuclear Actions of PR

While the genomic effects of steroid hormone treatment are delayed by several minutes to hours (i.e., following transcription and translation), the extranuclear or nongenomic effects occur rapidly in only a few minutes. Progestin treatment of breast cancer cells causes a rapid and transient activation of MAPK signaling that is ER dependent, but independent of PR transcriptional activity (Migliaccio et al., 1998; Boonyaratanakornkit et al., 2001). Migliaccio et al. (1996) first reported that estradiol activates p60-Src kinase and MAPK in MCF-7 cells and that PR and ERα interact to stimulate p60-Src kinase in T47D cells (Migliaccio et al., 1998). Maximal activation of p60-Src kinase is observed within 2–5 min, and downstream activation of p42/p44 MAPKs occurs within 5–10 min of progestin treatment (Migliaccio et al., 1998; Boonyaratanakornkit et al., 2001).

Human PR contains a proline-rich (PXXP) motif that mediates direct binding to the Src-homology three (SH3) domains of signaling molecules in the p60-Src kinase family in a ligand-dependent manner (Boonyaratanakornkit et al., 2001). *In vitro* experiments demonstrate that purified liganded PR-A and PR-B activate the c-Src-related protein kinase, HcK; PR-B but not PR-A activates c-Src and MAPKs *in vivo*. PR-B with a mutated PXXP sequence prevents c-Src/PR interaction and blocks progestin-induced activation of c-Src (or HcK) and p42/p44 MAPKs. Furthermore, mutation of the PR-B DBD abolished PR transcriptional activity without affecting progestin-induced c-Src or MAPK kinase activation. Therefore, nongenomic MAPK activation by progestin/PR-B/c-Src complexes probably occurs by way of a c-Src-dependent mechanism involving Ras activation via phosphorylation of the c-Src substrate adaptor proteins p190 and/or Shc and followed by Grb-2 and Sos binding (**Figure 2**).

Others have proposed that c-Src/MAPK activation by PR is mediated indirectly by the interaction of the Src-homology two (SH2) domain of c-Src with phosphotyrosine 537 of ERα (Ballare et al., 2003). In this model, activation of c-Src and the MAPK pathway by progestins depends upon the presence of unliganded ERα, which interacts constitutively with PR-B via two domains that flank the proline-rich sequence of PR. In contrast, Boonyaratanakornkit et al. (2001) found that ectopic PR expression increased basal c-Src activity in COS-7 cells in the absence of progestins and independently of added ER; co-expression of both PR-B and ERα reduced basal levels of c-Src activity.

A variety of studies indicate that multiple interactions contribute to direct protein kinase activation by SRs and suggest that at least some nongenomic signaling functions of PR have been conserved across species (Wong et al., 2002; Haas et al., 2005; Unni et al., 2004; Zhoul et al., 2005). Interestingly, a separate gene product encoding the putative mammalian homolog of a membrane PR, a progesterone-binding G-protein-coupled receptor first identified in spotted sea trout oocytes (Zhu et al., 2003), has been described. Further studies are needed to determine if membrane PR plays a role in progestin-induced rapid signaling or if membrane PR commonly interacts with classical PR (Karteris et al., 2006). However, studies with membrane PR underscore the important concept that binding proteins other than classical SRs may regulate some nongenomic steroid-mediated signaling events.

44.7 Integration of Rapid Signaling and Nuclear SR Actions

While its role in mammalian physiology remains unclear, SR-mediated activation of cytoplasmic signaling molecules could theoretically serve to potentiate several nuclear functions of activated SRs (**Figure 2**). One mechanism by which amplification of SR nuclear functions might occur is through rapid, direct phosphorylation of SRs and/or their coregulators (discussed above) in response to activation of SR-induced cytoplasmic pathways that coincide with ligand binding. For example, phosphorylation of PR Ser345 in response to progestin requires rapid signaling events (i.e., is c-Src- and MAPK-dependent), and induces PR-Sp1 tethering and regulation of the Sp1 target genes, EGFR and p21 (Faivre et al., 2008). Clearly, such a positive feedback loop explains the dramatic influence of activated signaling pathways on PR nuclear function. Notably, several progestin-dependent functions of PR are MAPK-dependent, including upregulation of cyclins D1 and E, CDK2 activation, and S-phase entry (Lange et al., 1998; Shen et al., 2001; Pierson-Mullany and Lange, 2004; Skildum et al., 2005).

Following ligand binding, most SRs stimulate a transient (3–10 min) activation of MAPKs. However, mitogenic signaling requires sustained (hours to days) MAPK activation in fibroblast cell models (Murphy and Blenis, 2006). Recently, Faivre and Lange (2007) found that in addition to rapid and transient activation of MAPK by progestin/PR-B (5–15 min), progestin-bound PR-B-induced subsequent oscillations in MAPK activity that culminated in a sustained (hours to days) phase of MAPK activation that was EGFR and c-Src dependent. Further studies revealed the creation of an autocrine signaling loop in which PR-B triggered transcriptional upregulation of Wnt-1, leading to the activation of frizzled-dependent MMPs and shedding of EGF ligands from the cell surface. This signaling cascade implicates Wnt-1-dependent transactivation of EGFR in response to progestins; PR-induced transcriptional upregulation of Wnt-1 and EGFR mRNA was sensitive to inhibition of MAPKs. Additional experiments demonstrated that progestin-induced cyclin D1 upregulation, S-phase entry, or soft-agar growth of T47D breast cancer cells were blocked by either shRNA targeted to Wnt-1 or inhibitors of MAPK, c-Src, and EGFR. Finally, progestins failed to stimulate S-phase entry in MCF-7 cells that stably express a PXXP-mutant PR-B, which is unable to bind to the SH3-domain of c-Src and activate MAPK (Skildum et al., 2005). Taken together, these data indicate that progesterone, via robust PR-B/c-Src signaling to MAPK, can converge upon PR-dependent transcriptional events to dramatically enhance progestin action.

44.8 Integrated SR Actions in Gene Expression

An important endpoint of MAPK signaling is upregulation of cyclin D1. Cyclin D1 null mice exhibit deficiencies in mammary gland development, including specific defects in alveolar growth (Fantl et al., 1995; Sicinski et al., 1995), a phenotype similar to adult female mice lacking PR-B (Lydon et al., 1996). Cyclin D1 mRNA and protein levels increase in response to estrogen, progesterone, or androgen treatment (Groshong et al., 1997; Altucci et al., 1996; Knudsen et al., 1998) and cyclin D1 is frequently elevated in breast and prostate cancers (Gillett et al., 1994; Kaltz-Wittmer et al., 2000).

Recent evidence suggests that SRs are often recruited to distal enhancer regions far upstream or downstream of hormone-regulated gene proximal promoters; distal HRE-containing elements function in association with pioneer-factor proteins that bind nearby to recruit and tether the distant SR complex to the proximal promoter via the creation of a chromatin loop (Carroll et al., 2005; Carroll and Brown, 2006). Thus, SR recruitment to distant enhancer sites provides a mechanism of direct regulation of genes like cyclin D1 via the classical pathway (e.g., via SR-binding at putative distant HRE sites). As SR-driven tumors progress, membrane SRs may begin to function dominantly, leading to a switch in promoter regulation to MAPK-dependent induction via proximal promoter sites, or via post-transcriptional mechanisms that are also MAPK regulated (Cheng et al., 1998). This may explain how tumors escape the action of SR antagonists that primarily block transcriptional events, but may fail to inhibit the signaling functions of these receptors. In support of this idea, cyclin D1 expression is regulated by multiple SRs, perhaps via distant sites. However, transcriptional regulation of the cyclin D1 proximal promoter region by steroids (i.e., progestins or estrogens) is MAPK-dependent (Skildum et al., 2005; Marino et al., 2002), as is progestin-induced sustained upregulation of cyclin D1 protein (Faivre and Lange, 2007). Thus, the activation of cytoplasmic signaling pathways by liganded SRs not only provides enhanced SR action

at specific SR-regulated genes via HRE sequences, but couples this to the regulation of additional gene products whose gene promoters clearly use SRs, but can also utilize SR-activated MAPK pathways independently of SR transcriptional activity to achieve sustained upregulation (**Figure 2**).

44.9 Summary and Conclusions

Studies over the past decade have dramatically enhanced our knowledge of the molecular mechanisms of PR action. As reviewed here, experiments done with cell lines have revealed much about the progestin-regulated genes, the function of post-translational modifications (e.g., phosphorylation, ubiquitinylation, or sumoylation) and the distinct roles of PR-A and PR-B in these events. While recent advances have increased our knowledge of progestin-regulated genes in brain as discussed above, we need to learn much more about the role of these post-translational modifications and the PR isoforms. In particular, the recent production of PR-A and PR-B isoform specific knockouts is certain to advance our knowledge of the function of these isoforms in progestin-regulated genes in brain.

The mechanisms by which steroids act in a tissue-specific manner is a fundamental issue in steroid hormone action. Recent investigations indicate that, in addition to the bioavailability of hormone and receptor levels, nuclear receptor coregulators are critical molecules in modulating steroid receptor-mediated transcription. Studies from cell lines have revealed much about the molecular mechanisms of action of these coregulators. Furthermore, work in brain, uterus, and other progestin-sensitive tissues indicates that nuclear receptor coactivators are critical in the fine-tuning of progestin responsiveness within individual cells. Studying the order and timing of recruitment of different coactivator and corepressor complexes to the promoter, which is likely to be cell and tissue specific, will be critical to understanding hormone action at the cellular level. In addition, investigating the effects of these complexes on the chromatin architecture is essential in understanding steroid receptor-specific effects on transcription. It has been predicted that future methodologies using cell lines will allow the kinetic analysis of cofactor recruitment on a single promoter within a single cell to enable comparing transcriptional mechanisms of different genes from the same hormonal signal (Aoyagi and Archer, 2008). We hope that after achieving this monumental task, it will be possible to address this same important question in individual neurons involved in behavior.

In this review, we have also discussed the impact of phosphorylation events on PR action. Rather than acting in an obligatory or switch-like manner, phosphorylation events are considered to exert subtle effects on steroid receptor function, with kinase inputs primarily acting as a rheostat for a continuum of steroid receptor transcriptional activities. However, this conclusion is based largely on observations made with liganded receptors in the absence of controlled inhibition or activation of alternate signaling pathways. In fact, studies with human PR reviewed herein suggest that the effects of phosphorylation are quite profound in the context of multiple signaling inputs. We conclude that the phosphorylation status of a particular SR is a function of cellular kinase activities that coordinate SR responses to growth factors and steroid hormones. In the absence of alternate stimuli, independent activation of MAPKs by extranuclear-liganded SRs may result in positive regulation of receptor action via feedback regulation by direct phosphorylation of SRs or their coregulatory partners. This may theoretically occur in both the presence and absence of steroid hormone ligands and on diverse gene promoters and via distant sites in chromatin. In addition, activation of cytoplasmic kinase cascades, including MAPK modules by liganded receptors, provides for regulation of gene targets whose promoters can function entirely independently of SR transcriptional activities. This important linkage provides for well-integrated control of a large number of genes or gene subsets coordinately regulated in response to convergence of growth factor and SR signaling. Finally, the newly discovered ability of SRs to activate kinase pathways classically defined as key regulators of cell growth underscores the concept that activation of signal transduction pathways is an integral feature of SR action.

The role of phosphorylation in PR function in brain is beginning to be investigated. As addressed in detail in **Chapter 45, Mechanism of Progesterone Receptor Action in the Brain**, ligand-independent activation of PR by neurotransmitters, which may be phosphorylation dependent, has profound effects on female reproductive behavior. In addition, recent studies have revealed that MAPK is involved in facilitating effects of P on female sexual behavior and the sequential inhibition of behavior by P. It would be interesting to explore the possible differential roles of

the PR isoforms in these events influenced by MAPK. Furthermore, it will be important to investigate the function of PR phosphorylation in other PR-mediated events, such as sexual differentiation, maternal behavior, learning and memory, aggression, mood, and anxiety (Wagner, 2006, 2008; Numan et al., 1999; Sandstrom and Williams, 2001; Meisel et al., 1990; Galeeva et al., 2007; Sherwin, 1999; Halbreich et al., 1986). In addition, while the PR-A and PR-B isoform-specific knockout mice have recently revealed differential roles for these isoforms in female sexual behavior (Mani et al., 2006; see **Chapter 45, Mechanism of Progesterone Receptor Action in the Brain**), future studies will need to investigate the function of these isoforms in other progestin-mediated behaviors listed above.

It is becoming increasingly clear that, in addition to classical genomic effects, PRs elicit many of their effects by a variety of nongenomic mechanisms. As discussed earlier, many studies in cell culture indicate that these nongenomic and extranuclear mechanisms have profound effects on PR function. While some of these mechanisms have been shown to have effects on behavior (see **Chapter 45, Mechanism of Progesterone Receptor Action in the Brain**), it is essential to determine if there is integration between classical genomic mechanisms of PR action and these extranuclear signaling pathways in brain. As in the breast, such integration of these mechanisms in brain is predicted to exert profound effects on progestin-regulated genes and thus influence important aspects of behavior. Future studies will likely continue to reveal additional complexities of PR action and provide further insight into the roles of PR action in brain and behavior.

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Biographical Sketch

Dr. Marc J. Tetel received his BA in biological sciences from Northwestern University and PhD in neuroscience and behavior at the University of Massachusetts, Amherst. Working with Dr. Jeff Blaustein, he studied how estradiol and progesterone act in the brain to regulate female reproductive behavior in rats. For his postdoctoral research, Dr. Tetel studied molecular mechanisms of progestin receptor action in breast cancer with Dr. Dean Edwards at the University of Colorado Health Sciences Center. Dr. Tetel is now an assistant professor in the Neuroscience Program at Wellesley College. His lab studies molecular mechanisms of estrogen and progestin receptor action in rodent brain, with a focus on the function of nuclear receptor coactivators in hormone-dependent gene expression in brain and behavior. His lab was one of the first to show that nuclear receptor coactivators are important in steroid receptor transcriptional activity in brain and in the modulation of hormone-dependent behaviors. Recently, his lab has taken a proteomics approach and has begun to investigate protein–protein interactions between steroid receptors and nuclear receptor coactivators from brain.

Dr. Carol A. Lange completed her PhD studies in pharmaceutical science and molecular toxicology (1991; University of Colorado, Boulder). Her postdoctoral studies in two top laboratories were aimed at understanding the role of MAP kinases in cell fate (1992–95; Gary Johnson lab; National Jewish Center for Immunology) and steroid hormone receptor signaling in breast cancer (1995–99; Kathryn Horwitz lab, University of Colorado Health Sciences Center). Dr. Lange joined the University of Minnesota (Department of Medicine) faculty in 1999. Her research is focused on problems related to signal transduction and breast or ovarian cancer progression. Her lab is focused on the role of crosstalk between growth factor-mediated signaling pathways and steroid hormone receptors, using the human progesterone receptor as a model receptor. Additional research focus is aimed at understanding the role of breast tumor kinase (PTK6/Brk) in signaling pathways that mediate breast cancer progression. Dr. Lange has published 55 peer-reviewed papers and invited reviews. Dr. Lange currently serves as the basic science chair for The Endocrine Society's 2008 annual meeting. She is an editorial board member of molecular endocrinology, and has served on numerous regional and national study sections; she is a Charter Member of the Molecular Oncogenesis NIH study section.