ANNIKA VIENONEN

Nuclear Receptor and Cofactor Expression in Female Reproductive Tissues

■

ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the small auditorium of Building B,
Medical School of the University of Tampere,
Medisiinarinkatu 3, Tampere, on March 26th, 2004, at 12 o'clock.

Acta Universitatis Tamperensis 992

ACADEMIC DISSERTATION
University of Tampere, Medical School
Tampere University Hospital, Department of Clinical Chemistry
Finland

Supervised by
Professor Timo Ylikomi
University of Tampere
Professor Pentti Tuohimaa
University of Tampere

Reviewed by
Docent Veli Isomaa
University of Oulu
Docent Sari Mäkelä
University of Turku

Distribution

University of Tampere
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 3 215 6055
Fax +358 3 215 7685
taju@uta.fi
http://granum.uta.fi

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 992
ISBN 951-44-5904-0
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 324
ISBN 951-44-5905-9
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2004

To My Parents

ABSTRACT

In the female reproductive tissues the nuclear receptors (NR) have a crucial role in regulating differentiation and proliferation. NRs are transcription factors which bind to specific DNA sequences of target genes in order to regulate their transcription. Transcriptional activity of many NRs also requires ligand binding. Ligands of NRs include hormones and vitamins. Some NRs, for example progesterone receptor (PR), are expressed in two isoforms differing in their transcriptional activities and having distinct physiological roles. In addition to receptor expression, cofactors are crucial in determining NR activity. These include coactivators enhancing the transcriptional activity and corepressors mediating transcriptional silencing.

The main aim of the present study was to characterise the NR expression and regulation of it by estrogens and progestins in the female reproductive tissues. The induction of PR isoform expression by estradiol was studied in two well-defined models: chicken oviduct and breast cancer cell lines. Expression was differentially regulated by estradiol in the chicken oviduct. The preferential PR-B expression in immature oviduct was altered to PR-A dominance after estradiol-induced differentiation. In breast cancer cell lines, E2 regulated the PR isoform expression in a distinctive manner depending on the cell line. Furthermore, breast cancer cell lines were found to differ in the levels of expression of NR mRNAs and regulation of NR levels by estradiol and progestin. NR expression during the menstrual cycle was characterised in human endometrium and myometrium. Expressions of androgen receptor (AR), estrogen receptor α (ERα), ERβ, glucocorticoid receptor, PR, retinoic acid receptors (RAR) and vitamin D receptor mRNAs were detected. In the endometrium the AR, ERα, ERβ, PR and RARα mRNA levels were higher in the proliferative phase when compared to secretory phase samples. In the myometrium menstrual cycle-dependent regulation was less apparent.

Cofactor mRNA expression seemed not to be regulated by estradiol and progestin in breast cancer cell lines or during the menstrual cycle in human endometrium and myometrium. The myometrium expressed NR and cofactor mRNAs similarly to the endometrium. However, individual differences were found in the expression levels of cofactors similarly to those found in the case of NRs.

The findings suggest that the expression levels of PR isoforms, NRs and cofactors vary in breast cancer and in the uterus. In addition, variation exists in the hormonal regulation of NR expression in breast cancer cell lines. Due to these variations these tissues may respond in an individual fashion to endocrine treatment, a circumstance to be considered in developing novel therapeutic ligands.

CONTENTS

ABBREVIATIONS

aa	amino acids
AF	activating function
AIB1	amplified in breast cancer 1
AP-1	activator protein 1
AR	androgen receptor
ATG	initiation codon
bp	base pair
cAMP	cyclic 3',5'-adenosine monophosphate
CBP	cAMP-response element-binding protein
cDNA	complementary deoxyribonucleic acid
C-terminal	carboxyl terminal
CP	crossing point
cPR	chicken progesterone receptor
DBD	DNA binding domain
DNA	deoxyribonucleic acid
E2	17β-estradiol
ER	estrogen receptor
ERE	estrogen response element
GR	glucocorticoid receptor
HAT	histone acetyltransferase
HDAC	histone deacetylase
HRE	hormone response element
HRT	hormone replacement therapy
Hsp	heat shock protein
IGF-1	insulin-like growth factor-1
IgG	immunoglobulin G
kb	kilobase
kDA	kilodalton
LBD	ligand binding domain
mRNA	messenger ribonucleic acid
N-CoR	nuclear receptor corepressor
NLS	nuclear localisation signal
NR	nuclear receptor
N-terminal	amino terminal
p300	E1A binding protein p300
PBS	phosphate buffered saline
pCAF	p300/CBP-associated factor
PCR	polymerase chain reaction
PR	progesterone receptor
PR-A	A-form of progesterone receptor
PR-B	B-form of progesterone receptor
R5020	promegestone
RAR	retinoic acid receptor
RNA	ribonucleic acid
RT	room temperature
RT-PCR	reverse transcriptase polymerase chain reaction
RXR	retinoid X receptor

SDS	sodium dodecyl sulphate
SERM	selective estrogen receptor modulator
SMRT	silencing mediator of repressed transcription
SRC-1	steroid receptor coactivator 1
TIF2	transcriptional intermediary factor 2
TR	thyroid hormone receptor
VDR	vitamin D receptor

LIST OF ORIGINAL COMMUNICATIONS

The thesis is based on the following articles, referred to in the text by their Roman numerals:

I Syvala H, Vienonen A, Ylikomi T, Bläuer M, Zhuang YH and Tuohimaa P (1997): Expression of the chicken progesterone receptor forms A and B is differentially regulated by estrogen *in vivo*. Biochemical and Biophysical Research Communications 231:573-576.

II Vienonen A, Syvala H, Miettinen S, Tuohimaa P and Ylikomi T (2002): Expression of progesterone receptor isoforms A and B is differentially regulated by estrogen in different breast cancer cell lines. Journal of Steroid Biochemistry and Molecular Biology 80:307-313.

III Vienonen A, Miettinen S, Manninen T, Altucci L, Wilhelm E and Ylikomi T (2003): Regulation of nuclear receptor and cofactor expression in breast cancer cell lines. European Journal of Endocrinology 148:469-479.

IV Vienonen A, Miettinen S, Bläuer M, Martikainen PM, Tomás E, Heinonen PK, Ylikomi T. (2004): Expression of nuclear receptors and cofactors in human endometrium and myometrium. Journal of the Society for Gynecologic Investigation 11:104-112.

INTRODUCTION

In the female reproductive tissues nuclear receptors (NR) have a crucial role in regulating differentiation and proliferation during embryogenesis, puberty and reproductive years and even after the menopause. NRs are transcription factors which bind to regulatory regions of their target genes' DNA and control the transcription. The transcriptional activity of some NRs requires binding of specific ligand. Ligands of NRs include hormones and vitamins. The ligand-dependent activity of NRs provides possibilities for the pharmaceutical industry to develop therapeutic substances. For example, synthetic agonists or antagonists of estrogen receptor (ER) and progesterone receptor (PR) have been used for decades in contraception, cancer therapy and hormone replacement therapy (HRT).

In addition to ER and PR, several other NRs are involved in the regulation of cellular events such as apoptosis, differentiation and proliferation in the female reproductive tissues. Some NRs, for example PR, are expressed in two isoforms differing in their transcriptional activities and having different physiological roles. The cellular levels of cognate receptors are essential in determining target tissue response to a specific NR ligand. The regulation of NR expression occurs generally by its own ligands or by other NR ligands. For example, estrogen increases and progesterone reduces the expression of PR.

Besides receptor expression, cofactors, which consist of coactivators and corepressors, are critical in determining the ligand response. NRs form large complexes with coactivators and other transcription factors upon agonist binding, and activate transcription. Antagonist binding may lead to the recruitment of corepressors and repression of transcription. Altered expression levels of coactivators and corepressors may even change the agonist or antagonist properties of ligands. Although ER and PR expression have been widely studied in female reproductive tissues and tumors, knowledge of other NRs and cofactors is limited. The present work was undertaken to characterise the expression of several NRs and cofactors and hormonal regulation of their expression in the female reproductive tissues.

REVIEW OF THE LITERATURE

1. NUCLEAR RECEPTOR SUPERFAMILY

Members of the nuclear receptor (NR) superfamily comprise structurally-related transcription factors, which participate in the regulation of large variety of physiological, developmental and metabolic events. The NR superfamily consists of 48 members in humans (Maglich et al. 2001), divisible into three different types (reviewed in Whitfield et al. 1999, Laudet and Gronemeyer 2002, McKenna and O'Malley 2002). Type I receptors are classical steroid receptors, which upon ligand binding generally form homodimers and bind to hormone response elements (HRE) which are so-called inverted repeats or symmetrical palindromes. Type II receptors, for example thyroid and retinoic acid receptors (TR and RAR), form heterodimers with retinoic X receptor (RXR). HREs of type II receptors are so-called direct repeats. The majority of the superfamily members are type III receptors, which are "orphan" NRs, found in the late 1990s based on their sequence homology to the conserved NR sequence. The ligands of these "orphan" NRs are mostly unknown and have functions as yet unidentified. Some of these NRs may regulate transcription ligand-independently.

1.1. Structure of nuclear receptors

A typical NR consists of five to six functional domains (Figure 1). Of these the N-terminal A/B is the most variable. It participates in transcriptional activation and can contain an activation function 1 region (AF-1), which is considered constitutively active. The length of the A/B domain may be isoform-specific due to alternative splicing or differential promoter usage as described for RARα and PR (Kastner et al. 1990, Leroy et al. 1991). The A/B domain may be phosphorylated from several serine and threonine sites mediated by different signalling pathways (reviewed in Shao and Lazar 1999, Rochette-Egly 2003). For example, growth factors induce phosphorylation of AF-1 of ERα through a mitogen-activated protein kinase (MAPK) pathway (Kato et al. 1995). The A/B domain participates in interaction with coactivators, thus affecting NR activity (Onate et al. 1998, Webb et al. 1998). These interactions may also be regulated by phosphorylation, as shown for the interaction of ERβ with steroid receptor coactivator 1 (SRC-1) (Tremblay et al. 1999).

The C domain or the DNA-binding domain (DBD) is responsible for sequence specific DNA binding and is the most fully conserved structural domain throughout the NR family (reviewed in Freedman 1992). It contains N-terminal and C-terminal zinc-finger DNA-binding motifs. Both motifs contain four cysteine residues which chelate one Zn^{2+} ion. The N-terminal zinc finger contains a region called the P-box, which is involved in identifying the HREs. The C-terminal zinc finger contains a region called D-box which corresponds to the DBD dimerisation interface. DBD binds directly to HREs of target genes, which consist of two half motifs comprising six conserved nucleic acids. Mutations, extensions and duplications have created a great diversity to this conserved core sequence. Greater complexity to the response elements is usually brought by a 1-5 bp-long spacer, which separates the two core sequences. The core sequences can be oriented either as direct repeats, palindromes or everted repeats.

The D domain is called the hinge domain. It allows the DBD and the ligand-binding domain (LBD) to adopt several possible conformations, thus creating different dimerisation interfaces and allowing some receptors to bind to different type of HREs. The hinge domain is involved in binding of corepressors (Horlein et al. 1995). In addition, this domain contains a nuclear localisation signal (NLS) (Ylikomi et al. 1992).

The E domain is a multifunctional domain. It binds to receptor-specific ligands, participates in dimerisation and forms interactions with multiple proteins. In addition, LBD encloses the ligand-dependent AF-2 region, located in the C-terminal part of LBD. Crystal structures of both apo and holo LBDs of several receptors have revealed major conformational changes upon ligand binding, shifting the structure to a more compact complex (reviewed in Bourguet et al. 2000a). The LBD consists of 12 conserved α helical regions and one β turn situated in between helixes H5 and H6. Binding of the ligand induces particularly repositioning of C-terminal helixes and thus the formation of a ligand-binding pocket, which provides a hydrophobic cavity for the hydrophobic ligands. The conformational structure of LBD after binding either agonist or antagonist determines the binding of coactivators and corepressors to LBD (Brzozowski et al. 1997, Shiau et al. 1998, Xu et al. 2002). The LBD contributes as a major domain of dimerisation. For example, depending on the ligand TR and vitamin D receptor (VDR) can form either homodimers or heterodimerise with RXR *in vitro* (Cheskis and Freedman 1994, Collingwood et al. 1997, Kakizawa et al. 1997). The activity of LBD domain may be modulated by phosphorylation (reviewed in Shao and Lazar 1999, Rochette-Egly 2003).

The optional F domain located in the C-terminus of receptors is variable in both length and sequence. Of steroid receptors only ERα and ERβ acquire this domain. There are suggestions of its role as a recruiter of cofactors for the LBD domain (Montano et al. 1995, Kim et al. 2003).

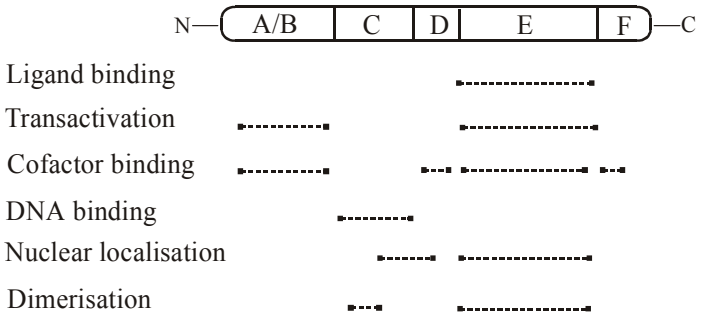

Figure 1. Structural and functional organisation of NRs. A typical NR consist of a variable N-terminal A/B domain, a conserved DNA-binding domain (C), a hinge region (D), a ligand-binding domain (E) and an optional C-terminal domain (F).

1.2. Nuclear receptors as transcription factors

Studies using green fluorescence proteins tagged to NRs have validated the conception of subcellular localisation of NRs. Most NRs partially shuttle between cytoplasm and nucleus and upon hormone binding translocate to the nucleus although, variations exist in whether the major portion of the receptor is located in the nucleus or in the cytoplasm (Georget et al. 1997, Lim et al. 1999, Racz and Barsony 1999, Poukka et al. 2000b, Tyagi et al. 2000, Maruvada et al. 2003). For example, the trafficking of PR between cytoplasm and nucleus is mediated via several NLSs located in the C, D and E domains of the receptor (Ylikomi et al. 1992). Receptor export from nucleus to cytoplasm has been suggested to involve part of the C domain and NLS (Guiochon-Mantel et al. 1994, Tyagi et al. 1998, Black et al. 2001, Saporita et al. 2003). Without ligand some of the steroid receptors form protein complexes consisting of heat shock proteins (Hsp) and associated proteins (see for review Cheung and Smith 2000, DeFranco 2002, Pratt and Toft 2003). These complexes participate in the proper folding of the receptor and participate in receptor shuttling between cytoplasm and nucleus. However, whether *in vivo* steroid receptors bind to Hsp complexes in the nucleus remains ambiguous (reviewed in Ylikomi et al. 1998).

TR and RARα are capable of gene repression by binding corepressors, while steroid receptors do not repress gene transcription in the absence of hormones (Chen and Evans 1995, Horlein et al. 1995, Farboud et al. 2003, Hauksdottir et al. 2003). Upon ligand binding corepressors dissociate from the receptor due to repositioning of helix 12 (reviewed in Hu and Lazar 2000). This is crucial for ligand response, since patients evincing thyroid hormone resistance have mutations in TR rendering the receptor incapable of dissociating from the corepressor in the presence of thyroid hormone (Yoh et al. 1997). Agonist binding changes the conformation of the ligand binding pocket in such a way that an interaction surface for coactivators is formed. Ligand-bound receptor constitutes a more compact structure than unliganded receptor, a model called mousetrap. This conformation is ligand-dependent, as has been shown for ERα and RARα (Brzozowski et al. 1997, Shiau et al. 1998, Bourguet et al. 2000b).

The first event in the initiation of transcription by NR is the formation of homo- or heterodimers and binding of liganded NR to the gene regulatory region. This is followed by cofactor recruitment, histone acetylation and chromatin remodelling (Li et al. 1999a, Dilworth et al. 2000, Shang et al. 2000, Aranda and Pascual 2001, Huang et al. 2003). Weak interaction of unliganded RAR/RXR heterodimers to cognate HREs followed by chromatin remodelling has been held to precede the ligand binding and tighter interaction with HREs (Dilworth et al. 2000). Ligand-bound NR associates directly with SRC family coactivators. This complex further recruits the coactivators cAMP-response element-binding protein (CBP), E1A binding protein p300 (p300) or p300/CBP-associated factor (pCAF), which possess histone acetyltransferase (HAT) activity. A recent model proposed by Huang and colleagues (2003) suggests that histone acetylation facilitates the recruitment of SWI/SNF and Mediator complexes. ATPase-containing SWI/SNF is involved in chromatin remodelling while Mediator may be associated with other cofactors and transcription factors. These initial steps are required for the extensive preinitiation complex to bind to the promoter area comprising general transcription factors, TFIID complexes and RNA polymerase II (reviewed in Belandia and Parker 2003).

When antagonists or partial antagonists are bound to NR, the ligand binding pocket acquires a different conformation affecting the cofactor binding (Bourguet et al. 2000a). For example,

when ERα is bound to 4-hydroxytamoxifen, helix 12 creates a conformation which inhibits coactivator binding (Shiau et al. 1998). Partially antagonist-bound to PR-B, androgen receptor (AR) and ER recruit the nuclear receptor corepressor (N-CoR) and the silencing mediator of repressed transcription (SMRT) (Jackson et al. 1997, Zhang et al. 1998, Shang et al. 2000, Shang et al. 2002). Reduced levels of N-CoR may alter the effects of tamoxifen as an antagonist towards agonist action (Lavinsky et al. 1998). Likewise the SRC-1/SMRT ratio determines the agonist/antagonist activity of RU486 for PR (Liu et al. 2002). In addition to different ligands different estrogen response elements (ERE) modulate the conformational structure of ERα or ERβ, thus affecting recruitment of coactivators and hence transcriptional activation (Loven et al. 2001, Wood et al. 2001, Hall et al. 2002, Yi et al. 2002).

The transcriptional activity of the receptors is affected by post-transcriptional modifications such as phosphorylation, acetylation, sumoylation and ubiquitination. Multiple signalling pathways participate in the phosphorylation of NRs, subsequently regulating both ligand-dependent and -independent activity, DNA binding and cofactor binding (reviewed in Shao and Lazar 1999, Rochette-Egly 2003). Acetylation, in addition to chromatin modification, may affect NR activity directly (Wang et al. 2001a, Fu et al. 2002). AR has been shown to be acetylated by p300 and pCAF in the D domain of the receptor (Fu et al. 2000). This acetylation may be required for proper release of corepressors and subsequent coactivator binding, whereas acetylation of ER diminishes ligand-dependent activation (Wang et al. 2001a, Fu et al. 2002). Sumoylation, which is a process of covalent linkage of small ubiquitin-related modifiers, modulates the transcriptional activities of at least androgen receptor (AR) and glucocorticoid receptor (GR) (Poukka et al. 2000a, Tian et al. 2002). Ligand-dependent ubiquitination of NRs targets the proteins to proteosomal degradation (Syvälä et al. 1998, Li et al. 1999b, Nawaz et al. 1999a).

1.3. Alternative signalling pathways

In addition to binding to HREs of target genes, NR can cross-talk with other signalling pathways. Several NRs have been shown to repress or initiate activating protein-1 (AP-1) mediated transcription, which is involved in the regulation of cell proliferation and anti-inflammation (reviewed in Laudet and Gronemeyer 2002). Likewise NRs modulate other signalling pathways by interacting with DNA-bound transcription factors such as SP-1 and NF-κB. A crucial role of these signalling pathways in mediating GR and ERα effects has been suggested by recent studies using mutated receptors unable to bind to their cognate HREs (Reichardt et al. 1998, Jakacka et al. 2002). Female mice heterozygous for mutated DBD of ERα are infertile, while heterozygous ERα knock-out mice are fertile (Lubahn et al. 1993, Dupont et al. 2000, Jakacka et al. 2002). This may be due to an imbalance of classical (HRE binding) and alternative signalling pathways or to an inhibitory effect of this mutated ERα on some other signalling pathways (Jakacka et al. 2002). GR knock-out mice usually die immediately after birth due to underdeveloped lungs, while mice carrying a point mutation causing inability to form GR homodimers and bind to glucocorticoid REs are viable (Cole et al. 1995, Reichardt et al. 1998). These findings emphasise that other signalling pathways are important mediators of NR effects in addition to the classical DNA binding-dependent transcriptional regulation.

The rapid non-genomic actions of NRs include regulation of cell membrane ion channels, G-protein coupled receptors and protein kinase signalling pathways. For example, the effects of

steroids on the cardiovascular system and in the central nervous system are partly mediated via rapid non-genomic mechanisms (reviewed in Simoncini and Genazzani 2003). It is still largely unknown whether these rapid effects occur through specific membrane receptors or through regular receptors located at the cell membrane.

2. COFACTORS AFFECTING NUCLEAR RECEPTOR ACTIVITY

Cofactors or coregulators comprise a large group of molecules including coactivators, cointegrators, corepressors or chromatin remodelling complexes. Over 50 different cofactors have been shown to be associated with the NR transcription complexes (reviewed in Laudet and Gronemeyer 2002). Apart from corepressors, cofactors assist in the assembly of the pre-initiation complex leading to transcriptional activation. Numerous different cofactors and transcription factors form large holocomplexes with NRs. The formation of a holocomplex depends on the receptor, ligand, cell and promoter as well as on multiple cell signalling pathways. Like NRs, cofactors are regulated via posttranslational modifications, for example via acetylation, phosphorylation and ubiquitination (Chen et al. 1999, Font de Mora and Brown 2000, Hong and Privalsky 2000, Rowan et al. 2000, Lopez et al. 2001, Zhou et al. 2001, Yan et al. 2003).

Functions of cofactors include modification and remodelling of chromatin, recruitment and stabilisation of the pre-initiation complex and recruitment of RNA polymerase II (reviewed in Aranda and Pascual 2001, Laudet and Gronemeyer 2002, Belandia and Parker 2003). In addition, cofactors may affect the cellular location of receptors, as shown for the small nuclear RING finger protein (SNURF), which interacts with the AR NLS region and assists AR nuclear localisation (Poukka et al. 2000b). Alternatively, cofactors may affect receptor proteosome degradation pathways, since a ubiquitin protein ligase E6-associated protein (E6-AP) enhances the transcriptional activity of steroid receptors (Nawaz et al. 1999b).

2.1. SRC/p160 family

One of the best-characterised groups of coactivators interacting with NRs is the SRC/p160 family. SRC-1 was the first cloned member of the SRC/p160 coactivator family (Onate et al. 1995). Although cloned as a ligand-dependent enhancer of PR-mediated transcriptional activity it also enhances the transcriptional activity mediated by other NRs such as AR, ER, GR TR, retinoid X receptor (RXR) (Onate et al. 1995, Alen et al. 1999, Bevan et al. 1999, Ma et al. 1999). It is expressed in two isoforms, SRC-1a and SRC-1e, which differ in the C-terminus (Kalkhoven et al. 1998). SRC-1e acts as a more potent enhancer of ER transcriptional activation than SRC-1a. Voegel and colleagues (1996) cloned the second member of the SRC/p160 family called transcriptional intermediary factor 2 (TIF2), and within a short period of time several groups cloned the third member, amplified in breast cancer 1 (AIB1, also known as p/CIP, ACTR, RAC3, TRAM-1) (Anzick et al. 1997, Chen et al. 1997, Li et al. 1997, Takeshita et al. 1997, Torchia et al. 1997).

SRC/p160 family coactivators function as bridging factors recruiting other cofactors such as CBP and p300 to the ligand-bound receptor. In addition, they are able to engage with another HAT pCAF and link it to the complex (Chen et al. 1997, Spencer et al. 1997). Furthermore,

they may bind directly to basal transcription machinery (Takeshita et al. 1996, Ikeda et al. 1999). Themselves SRC-1 and AIB1 have been shown to have moderate HAT activity and they may be acetylated by CBP/p300 (Chen et al. 1997, Spencer et al. 1997, Chen et al. 1999). This acetylation interrupts the interaction of the coactivator with the receptor, thus regulating hormonal response.

In addition to participating in transcriptional activation SRC/p160 family members may participate in the steroid hormone-dependent repression of transcription, as has been shown for agonist- but not antagonist-bound GR- and ER-mediated repression (An et al. 1999, Rogatsky et al. 2001). Besides regulating NR transcriptional activity SRC/p160 family members participate in a variety of other intracellular signalling pathways (reviewed in Leo and Chen 2000).

Observations on mice having null mutants for different SRC/p160 family members confirm that these coactivators are crucial in mediating steroid hormone effects and that they have distinct physiological roles despite their high level of sequence homology. SRC-1 knock-out mice are fertile but suffer from steroid hormone resistance, which may be partially compensated through increased TIF2 expression (Xu et al. 1998). AIB1 knock-out mice have decreased levels of systemic estrogens, probably due to the reduced sensitivity of granulosa cells in the ovary to follicle-stimulating hormone (Xu et al. 2000). Lack of AIB1 delays puberty, reduces fertility and diminishes mammary ductal branching and alveolar formation in response to estrogen and progesterone. In TIF2 null mutant mice the reduced fertility is due to poor development of the chorioallantoic placenta (Gehin et al. 2002).

SRC-1, TIF2 and AIB1 mRNAs are expressed in the normal human mammary gland (Anzick et al. 1997, Berns et al. 1998, Kurebayashi et al. 2000). In breast tumors AIB1 is frequently overexpressed (Anzick et al. 1997, Bouras et al. 2001). TIF2 mRNA levels seem to be upregulated and SRC-1 expression slightly downregulated in breast tumors (Berns et al. 1998, Kurebayashi et al. 2000). In the human endometrium expression of SRC-1, TIF1 and AIB1 has been reported; however, somewhat conflicting results are on record as to whether the expression levels are regulated during the menstrual cycle (Gregory et al. 2002, Wieser et al. 2002, Shiozawa et al. 2003). Women suffering from polycystic ovarian syndrome who evinced increased expression levels of ERα had elevated expression levels of both TIF2 and AIB1 (Gregory et al. 2002).

2.2. Cointegrators

CBP was first identified as a protein binding to the cAMP-response element-binding protein (CREB) (Chrivia et al. 1993). Its protein homologue p300 was found as an adenoviral E1A binding protein (Eckner et al. 1994). CBP and p300 extensively influence the proliferation, differentiation and apoptosis (reviewed in Chan and La Thangue 2001). Mutations of CBP and p300 have been found in many cancer types, suggesting that these proteins have tumor-suppressor-like activity.

They act as bridging molecules connecting other coactivators to the basal transcription machinery, or they can act as scaffold proteins enabling the other coactivators and HAT molecules to assemble in large multiprotein complexes in the transcription initiation environment. Themselves CBP and p300 have strong HAT activity and at least *in vitro* can

acetylate all four core histones (Bannister and Kouzarides 1996, Ogryzko et al. 1996). In addition they have been shown to acetylate a variety of non-chromosomal proteins (reviewed in Chan and La Thangue 2001). NRs recruit p300 and CBP more likely through interaction with SRC-1/p160 proteins, although direct interactions with NRs have also been shown (Chakravarti et al. 1996, Kamei et al. 1996, McInerney et al. 1998, Westin et al. 1998, Kraus et al. 1999, Li et al. 2000a, Sheppard et al. 2001, Shang et al. 2002).

Although CBP and p300 have strong sequence similarity and overlapping functions, the role of these proteins may be not totally overlapping (reviewed in Chan and La Thangue 2001). For example experiments with mice have revealed that CBP is required for normal hematopoietic differentiation while p300 expression is not crucial (Kung et al. 2000). Null mutant mice lacking either CBP or p300 are not viable, suggesting that these proteins cannot fully compensate each other's lost expression (Tanaka et al. 1997, Yao et al. 1998).

Cointegrator pCAF has been found to be able to bind to CBP and p300. It also possesses intrinsic HAT activity (Yang et al. 1996). It binds in ligand-dependent manner to DBD of NR and enhances transcription (Blanco et al. 1998, Korzus et al. 1998). It also interacts with SRC/p160 family members (Chen et al. 1997, Spencer et al. 1997).

CBP and p/CAF mRNAs are expressed in normal mammary glands (Kurebayashi et al. 2000). The expression of CBP is increased in intraductal carcinomas compared to normal mammary glands in correlation with increased ERα expression (Kurebayashi et al. 2000). CBP/p300 expression has been shown in human endometrial glandular and stromal cells, using antibody recognising both CBP and p300 (Shiozawa et al. 2003). The expression was slightly higher in the proliferative than in the secretory phase of the menstrual cycle.

2.3. Corepressors

Corepressors N-CoR and SMRT have been found to be associated with unliganded TR and RARα and to mediate the transcriptional silencing of TR and RAR (Chen and Evans 1995, Horlein et al. 1995, Jackson et al. 1997, Ordentlich et al. 1999, Park et al. 1999). Corepressors recruit corepressor mSin3 (mammalian Sin3) and histone deacetylases (HDAC) to histone deacetylase chromatin structures, thus causing the chromatin to adopt more a compact repressive state (Heinzel et al. 1997, Nagy et al. 1997, Li et al. 2000b). Although not binding to unliganded steroid receptors, the corepressors may bind to antagonist-bound receptors like ER and PR (Jackson et al. 1997, Smith et al. 1997, Lavinsky et al. 1998). In addition to transcriptional repression N-CoR may promote transcriptional activation, since the retinoic acid-dependent transcriptional activation of a certain type of retinoic acid response element requires N-CoR and HDAC (Jepsen et al. 2000).

Expression of N-CoR and SMRT mRNA has been shown in human mammary glands (Kurebayashi et al. 2000). Their mRNA levels were slightly upregulated in intraductal carcinomas compared to normal mammary glands concomitant with increased ERα expression. Furthermore, N-CoR levels were significantly reduced in intraductal carcinomas compared to invasive ductal carcinomas, again in correlation with increased ERα expression, which would imply that corepressor levels are altered in the course of breast tumorigenesis. Low expression of both SMRT and N-CoR has been reported in the human endometrium

(Wieser et al. 2002, Shiozawa et al. 2003). The levels of N-CoR are expressed most abundantly during menstruation (Wieser et al. 2002).

2.4. Chromatin remodeling complexes

One important function of cofactors is to participate in chromatin remodelling. At least four ATP-dependent chromatin remodeling complexes exist (reviewed in Belandia and Parker 2003). These include the human homologue of yeast SWI/SNF, which enhances ER-, RAR- and GR-mediated transcriptional activity, and the WINAC complex, which mediates recruitment of unliganded VDR to VDR target sites (Muchardt and Yaniv 1993, Chiba et al. 1994, Kitagawa et al. 2003). The recruitment of SWI/SNF by NR is probably not direct but mediated through CBP/p300 (Huang et al. 2003).

The coactivator complex Mediator (also called as VDR-interacting proteins (DRIP), TR-associated proteins (TRAP), activator recruited cofactor (ARC) or the Srb/Mediator coactivator complex (SMCC)) functions as a general transcription activation-associated protein complex (reviewed in Leo and Chen 2000). The Mediator complex consists of more than a dozen proteins. It lacks HAT activity and is not specific solely for NR signalling. It binds to the AF-2 site of NRs through its LxxLL motifs or may bind NR indirectly via association with CBP/p300 (Rachez et al. 2000, Huang et al. 2003). The precise function of the Mediator is sill rather speculative (Leo and Chen 2000). This large complex may supplementarily remodel the chromatin structure after initial chromatin acetylation by SRC-1 and CBP. This may be followed by the recruitment of the pre-initiation complex or other transcription factors, or the Mediator complex may target RNA polymerase II to the promoter region.

3. NUCLEAR RECEPTOR EXPRESSION AND FUNCTION IN FEMALE REPRODUCTIVE TISSUES

3.1. Estrogen receptor α

Estrogens have a major role in inducing proliferation and stimulating blood flow and water retention in the reproductive tissues. It was long thought that one receptor mediates the actions of estrogens, even though, disruption of this gene expression in a mouse model proved less crucial than expected (Green et al. 1986, Greene et al. 1986, Lubahn et al. 1993, Curtis et al. 1999). Soon after cloning of ERβ it became evident that the actions of estrogens are mostly results of the interplay of both ERα and ERβ (Kuiper et al. 1996).

ERα in the mammary gland

In both the normal and malignant breast epithelium estrogens are thought to promote cell proliferation. Approximately 7-15 % of luminal epithelial cells of the normal mammary gland are positive for ERα staining, but these cells are rarely proliferative, which would imply that estrogens stimulate proliferation indirectly (Clarke et al. 1997b, Russo et al. 1999, Shoker et al. 1999a). The highest expression of ERα can be found in the undifferentiated lobules and once the level of differentiation increases ERα is expressed the less (Russo et al. 1999).

During the menstrual cycle, ERα expression is higher in the follicular phase epithelial cells compared to the luteal phase cells (Soderqvist et al. 1993). In the human mammary gland only epithelial cells express ERα, while in rodents both epithelial and stromal cells express ERα (Cunha et al. 1997, Zhuang et al. 2003). ERα expression is critical for the proper development of the mammary glands, since in ERα knock-out mice only undeveloped ducts are present at the nipples (Korach 1994).

Numerous studies have elucidated the role of ERs in breast tumorigenesis, since increased exposure to circulating estrogens may augment the breast cancer risk (reviewed in Hilakivi-Clarke et al. 2002). Moreover, data from breast cancer prevention trials suggest that treatment with antiestrogens reduces the incidence of ER-positive breast cancers (reviewed in Cuzick et al. 2003). One key step towards malignancy may be the increased expression of ERα (Khan et al. 1994, Shoker et al. 1999b, Iwao et al. 2000a). The amount of proliferating cells also expressing ERα increases with age in the normal breast and during the development of malignant breast neoplasia (Clarke et al. 1997b, Shoker et al. 1999a). These findings suggest that the disruption of the hierarchical organisation in the breast, that is, ERα-positive cells regulating by paracrine factors the proliferation of ERα-negative cells is an early event in the development of malignancy.

Approximately one-third of breast cancers lack ERα (reviewed in Lapidus et al. 1998). These tumors are often of more aggressive type than ER-positive and they seldom respond to endocrine treatment. The mutations of the ERα gene are rather uncommon and may not explain all ER-negative tumors (reviewed in Dowsett et al. 1997, Lapidus et al. 1998). Another mechanism underlying the loss of ERα expression is methylation of ERα gene promoter, this resulting in a decreased expression of ERα (Yoshida et al. 2000). Histone deacetylase inhibitors re-induce the transcriptional activation of methylated ERα in human breast cancer cells, suggesting a novel therapeutic possibility (Yang et al. 2000). Abnormal ERα expression may be due to variant mRNA expression. Several ERα variants have been detected by RT-PCR in both normal and malignant tissue, but the expression at protein level is unknown (Dowsett et al. 1997, Hirata et al. 2003). Based on RT-PCR assays the clinical relevance of these variants for the progression of breast cancer has been thought to be small (Anandappa et al. 2000, Chappell et al. 2000).

ERα in the uterus

ERα knock-out mice have a hypoplastic uterus which does not respond to estrogen treatment (Lubahn et al. 1993, Couse et al. 1995). In the mouse uterus, the effects of estrogens are mediated via a paracrine mechanism, since estrogen-induced proliferation of epithelial cells and down-regulation of epithelial cell PR expression requires stromal ERα expression (Cooke et al. 1997, Kurita et al. 2000). In the human uterus, ERα protein expression is regulated concomitant with the menstrual cycle. In the proliferative phase, estrogen increases the expression of ERα in epithelial and stromal cells of the endometrium, reaching maximal levels in the mid to late proliferative phase, whereas the expression is decreased in the secretory phase (Garcia et al. 1988, Lessey et al. 1988, Snijders et al. 1992, Coppens et al. 1993, Fung et al. 1994, Noe et al. 1999). Lecce and colleagues (2001) have also reported cyclically regulated ERα expression in smooth muscle cells of the vascular wall and sporadic expression in endothelial cells. In the myometrium, ERα expression is cyclically regulated in the subendometrial part whereas the expression in the outer portion of the myometrium is not regulated (Noe et al. 1999). In myomas, ERα mRNA is expressed more abundantly than in

the surrounding myometrium (Wang et al. 2001b). During pregnancy, ERα expression in endometrium and myometrium is relatively low; however, ERα expression is increased in the labouring myometrium and this is linked to the expression of contraction-associated genes (Perrot-Applanat et al. 1994, Mesiano et al. 2002).

Endometrial cancers are often associated with hyperestrogenism. ERα expression has been reported to be associated positively with disease-free survival and tumor grade (Lindahl et al. 1992, Creasman 1993, Fukuda et al. 1998, Gehrig et al. 1999). Stage I tumors, which are ERα-negative, are more likely to relapse (Gehrig et al. 1999). In endometrial cancers as in breast cancer, methylation of the CpG island of ERα at least partly contributes to decreased ERα expression (Maeda et al. 2002, Shiozawa et al. 2002).

ERα in the other reproductive tissues

In the ovaries of ERα knock-out mice, follicle maturation arrests prior to formation of mature follicles, possibly in consequence of increased LH levels (Lubahn et al. 1993, Korach 1994, Schomberg et al. 1999, Dupont et al. 2000). Immunohistochemical results on ERα expression in the human ovaries are somewhat inconsistent. Granulosa cells of secondary follicles express ERα while primordial and primary follicles do not (Iwai et al. 1990, Suzuki et al. 1994). The expression is increased in granulosa cells of the dominant mature follicle prior to the luteinising hormone surge and decreased in samples taken at the time of the surge (Iwai et al. 1990). Furthermore, the theca interna, corpora lutea and stroma are negative for ERα expression (Iwai et al. 1990, Suzuki et al. 1994). However, Pelletier and El-Alfy have reported that granulosa cells and corpora lutea show no expression of ERα while germinal epithelial cells and theca internal cells are positive for ERα immunostaining (Pelletier and El-Alfy 2000). Some ovarian cancers express ERα, but whether the expression is of clinical relevance remains unsettled (reviewed in Perez-Gracia and Carrasco 2002). Overexpression of ERα relative to ERβ may be a marker of ovarian carcinogenesis (Pujol et al. 1998). Interestingly, only ERα and not ERβ has been seen expressed in metastatic tumors (Rutherford et al. 2000). Epithelial, stromal and muscle cells of the human oviduct express ERα and the expression is regulated during the menstrual cycle (Amso et al. 1994).

Menstrual cycle-dependent changes also occur both in the vaginal tissue and in the cervix. These tissues have been reported to evince higher ERα expression during the proliferative phase compared to secretory phase samples (Sjöberg et al. 1989, Cano et al. 1990). Both ERα and ERβ may participate in the regulation of cervical ripening during term pregnancy, since the regulation of expression occurs during term pregnacy or after parturition (Stjernholm et al. 1996, Wang et al. 2001c).

3.2. Estrogen receptor β

Cloning of rat ERβ has revealed that some estrogenic effects might be mediated through ERβ (Kuiper et al. 1996). Human ERβ has 97 % identity with the DBD of ERα and 59 % identity with the LBD sequence (Mosselman et al. 1996, Enmark et al. 1997). It binds 17β-estradiol in a comparable affinity to ERα, although ligand-binding differences also exist (Kuiper et al. 1997). In female mice most abundant ERβ expression can be found in the ovaries. ERβ knockout mice have reduced ovarian efficiency and reduced fertility (Krege et al. 1998). ERβ possibly mediates a stimulatory effect of estrogens on granulosa cell proliferation (Dupont et

al. 2000). In the uterus, ERβ modulates the effects of ERα and mediates the estrogen-induced down-regulation of PR expression in the luminal epithelium (Weihua et al. 2000). ERβ knockout mice show normal mammary gland development and lactate normally (Krege et al. 1998). However, when lactating these mice evince morphological differences compared to wild-type mice, revealing that ERβ is required for terminal differentiation of the mammary gland (Forster et al. 2002).

Several studies in addition to those made with knock-out mice have suggested that the expression of ERβ modulates the activity of ERα. ERβ forms heterodimers with ERα, diminishes the sensitivity of ERα-expressing cells to estradiol, increases the antagonist activity of tamoxifen and enhances the agonist activity of genistein (Cowley et al. 1997, Hall and McDonnell 1999, Pettersson et al. 2000, Lindberg et al. 2003).

ERβ in the mammary gland

ERβ is expressed in both normal and malignant human breast cells (Enmark et al. 1997, Fuqua et al. 1999, Järvinen et al. 2000). This expression is not regulated in relation to menstrual cycle phase (Shaw et al. 2002). Expression of ERβ in breast tumors is associated with well-differentiated and slowly proliferating tumors and with better disease-free survival rates (Järvinen et al. 2000, Omoto et al. 2001). Findings of decreased ERβ mRNA levels during breast carcinogenesis support the conception that ERβ acts as an inhibitor of cell proliferation (Leygue et al. 1999a, Iwao et al. 2000a, Iwao et al. 2000b, Roger et al. 2001). ERβ expression may be of significance in tamoxifen sensitivity, since increased expression of ERβ mRNA has been found in tamoxifen-resistant breast tumors (Speirs et al. 1999). Likewise, the expression of ERβ mRNA is higher in tamoxifen-resistant breast cancer cell lines compared to tamoxifen-sensitive lines (Speirs et al. 1999).

ERβ in the uterus

Numerous studies suggest higher expression of ERα compared to ERβ in the human endometrium, however, comparison of different RT-PCR reactions or different antibodies is problematic (Matsuzaki et al. 1999, Matsuzaki et al. 2000, Lecce et al. 2001, Fujimoto et al. 2002). In the human endometrium, expression of ERβ is regulated during the menstrual cycle in a manner similar to ERα. Immunohistochemical staining of ERβ has been stronger in glandular epithelial cells of the proliferative phase than in the secretory phase (Critchley et al. 2001, Lecce et al. 2001). Weak staining in the stromal cells has remained unchanged during the cycle or slightly increased in the secretory phase (Critchley et al. 2001, Lecce et al. 2001). Cell-specific expression of ERs has also been found. Critchley and colleagues (2001) reported ERβ but not ERα expression in vascular endothelial cells of the human endometrium and this expression was not regulated in the course of the menstrual cycle, although both ERα and ERβ are expressed in perivascular cells. Likewise only ERβ is expressed in the uterine-specific leukocytes called uterine natural killer cells (Henderson et al. 2003). In endometrial cancers, ERα has usually been found expressed more than ERβ. However, related to poor patient prognosis some lymph node metastasis-positive cases have had higher ratios of ERβ/ERα than the corresponding primary lesion (Fujimoto et al. 2002). Furthermore, increased ERβ expression is related to myometrial invasion (Takama et al. 2001).

ERβ in the other reproductive tissues

In the human ovaries, ERβ is expressed in granulosa cells of the growing follicles and in the germinal epthelium (Pelletier and El-Alfy 2000, Taylor and Al-Azzawi 2000). Contradictory

results of ERβ immunostaining in thecal cells and corpora lutea have been reported (Pelletier and El-Alfy 2000, Taylor and Al-Azzawi 2000). In ovarian cancer, a decreased expression of ERβ mRNA has been reported and loss of ERβ protein expression was linked to metastatic tumors (Brandenberger et al. 1998, Rutherford et al. 2000). In the oviduct, ERβ immunostaining is mostly located in epithelial cells (Taylor and Al-Azzawi 2000).

In the case of human vaginal tissue, conflicting results of ERβ expression have been reported (Pelletier and El-Alfy 2000, Taylor and Al-Azzawi 2000). In the human cervix, ERβ is expressed in epithelium and stroma and the levels are regulated concomitant with cervical ripening during pregnancy and parturition (Wang et al. 2001c). Furthermore, ERβ but not ERα expression was found in the stromal neutrophils, suggesting a potential role of ERβ in the cervical ripening process.

Isoforms of ERβ

Moore and colleagues (1998) have cloned 5 isoforms of human ERβ, called ERβ1-5 which differ at the C-terminal LBD domain. At the same time, Ogawa and colleagues (1998) cloned the ERβ2 and named it ERβcx. These isoforms have a tissue-specific expression pattern (Moore et al. 1998). The expression of these isoforms may have significance in gynecological disorders and breast cancer, since they can form heterodimers with ERα (Moore et al. 1998). For example, ERβcx, which does not possess ligand binding ability, functions as a dominant repressor of ERα (Ogawa et al. 1998). Expression of ERβcx linked with low PR expression in breast tumors correlates with poor response to tamoxifen therapy (Saji et al. 2002). Although expression of several ERβ isoforms at mRNA and protein level has been detected in breast cancer data concerning the clinical significance of ERβ expression are as yet rather limited (Leygue et al. 1999a, Tong et al. 2002, Chi et al. 2003). Expression of ERβcx has been detected in normal endometrial tissue and the expression was to some extent regulated with the menstrual cycle in the glands of the functional layer (Critchley et al. 2002).

3.3. Progesterone receptor

The PR-A and PR-B isoforms are generally considered to mediate the tissue-specific effects of progesterone. However, the third functional isoform, PR-C, has been suggested to be present in breast cancer cell lines (Wei and Miner 1994, Wei et al. 1996). PR-A and the 164-amino-acid-longer PR-B are most likely translated from different mRNA transcripts of the same gene due to alternative promoter usage (Kastner et al. 1990). Although *in vivo* PR-A and PR-B are expressed within the same cells, they participate partly in different physiological events (Mote et al. 1999). PR-A is crucial for normal ovarian and uterine responses while PR-B is needed for proper mammary gland morphogenesis in mice (Mulac-Jericevic et al. 2000, Mulac-Jericevic et al. 2003). Transfection assays have revealed cell and promoter type-dependent differences in the transcriptional activities of human PR-A and PR-B. PR-B is a stronger transcriptional activator than PR-A, although depending on cell and promoter context PR-A can also function as an activator. PR-A can repress the transcriptional activity of PR-B and other NRs such as AR and GR (Vegeto et al. 1993, McDonnell et al. 1994). Both receptors can repress the activity of ER (Chalbos and Galtier 1994, Kraus et al. 1995). The variations in transcriptional activity are related to the structural difference of the two isoforms. Both receptors have AF-1 and AF-2 in their N-terminus and in the LBD respectively (Meyer et al. 1990). The N-terminal sequence unique to the PR-B isoform

contains a third AF (AF3) (Sartorius et al. 1994). The first 140 amino acids of PR-A contain an inhibitory function which in PR-B is masked by the PR-B-specific N-terminal sequence (Giangrande et al. 1997, Hovland et al. 1998). Giangrande and colleagues (2000) have demonstrated that PR-A and PR-B differ in their ability to bind coactivators. Furthermore, PR-A was more efficient in binding corepressor SMRT.

PR in the mammary gland

In the mammary gland, progesterone regulates lateral ductal branching and lobular alveolar development and suppresses milk protein synthesis during pregnancy. It may be responsible for proliferation of the mammary gland during the luteal phase of the menstrual cycle, when progesterone levels are high (reviewed in Graham and Clarke 1997). PR expression has been located in the ductal and lobular epithelial cells of the human mammary gland and these cells are mainly non-proliferating (Press and Greene 1988, Clarke et al. 1997a). The expression of PR is not regulated during the menstrual cycle and both PR isoforms are expressed at similar levels within the same cells of the mammary gland (Soderqvist et al. 1993, Clarke et al. 1997a, Mote et al. 2002).

In breast cancer, PR expression has been regarded as a good predictive factor; PR-positive tumors are smaller in size and better differentiated (Stonelake et al. 1994, Osborne 1998). When the clinical outcomes in two large databases were evaluated, ER-positive/PR-positive breast tumors had better prognosis with or without endocrine therapy (Bardou et al. 2003). However, Lydon and associates have shown that murine mammary gland carcinogenesis requires PR expression (Lydon et al. 1999). Furthermore, clinical data suggest that progestins have undesirable outcomes in the breast. In postmenopausal women combined estrogen and progesterone treatment is a more potent risk for breast cancer than estrogen treatment alone (Ross et al. 2000, Schairer et al. 2000, Santen et al. 2001, Rossouw et al. 2002, Beral 2003, Li et al. 2003). Results showing that estrogen and progesterone therapy is linked to greater breast epithelial cell proliferation and greater breast epithelial cell density or to mammographic density support these findings (Greendale et al. 1999, Hofseth et al. 1999). In addition, studies using animal models support the conception that progestins can intensify estrogen-induced proliferation in the mammary gland (Said et al. 1997, Cline et al. 1998).

Transfection experiments showing PR isoform-specific transcriptional activities have elucidated many theories regarding their role in carcinogenesis. Noteworthy is that PR-A knockout mice seem to have normal mammary gland development whereas PR-B knock-out mice exhibit reduced pregnancy-associated ductal and alveolar morphogenesis (Mulac-Jericevic et al. 2000, Mulac-Jericevic et al. 2003). Mice over-expressing PR-A have abnormal features in their mammary glands and are associated more often with neoplasia (Shyamala et al. 1998). Tumors which preferentially express PR-A are less well differentiated than those expressing more PR-B than PR-A (Bamberger et al. 2000). PR-A-over-expressing breast cancer cells detach from monolayer cultures after progesterone treatment, suggesting that PR-A-over-expressing tumors might be more invasive (McGowan and Clarke 1999). Mote and group (2002) have suggested that loss of control of PR isoform expression might be an early step in breast carcinogenesis. They found a predominance of one of the isoforms, more often PR-A in ductal carcinoma *in situ* and in invasive breast lesions. In addition they reported increased inter-cell heterogeneity of PR-A and PR-B expression in association with breast lesion progression to malignancy. PR isoform expression may influence the response to tamoxifen treatment, since growth of T-47D cells solely expressing PR-A and implanted in mice as xenografts was inhibited by tamoxifen, whereas the growth of PR-B-expressing tumors was unchanged (Sartorius et al. 2003).

PR in the uterus

Progesterone with estrogen has a crucial role in the uterus during the menstrual cycle and pregnancy. Progesterone stimulates glandular epithelial secretory activity and decidual transformation of stromal fibroblasts in the luteal phase of the cycle. The expression of PR has been studied mainly using antibodies identifying both receptor isoforms. During the menstrual cycle PR expression in epithelial cells is induced by estrogen in the proliferative phase and reduced by progesterone in the secretory phase (Garcia et al. 1988, Lessey et al. 1988, Press et al. 1988, Snijders et al. 1992, Amso et al. 1994). The expression in the stromal cells is not regulated or is less markedly regulated than in the epithelial cells. Development of PR-A- and PR-B-specific antibodies has made it possible to compare the expression patterns of the two isoforms even within the same cell using the dual immunofluorescent staining method (Mote et al. 1999). In the proliferative phase, the expressions of both isoforms were increased in the glandular epithelial cells. In the stromal cells the PR-A was the predominant form, although the expression levels of both isoforms were increased. In the glandular epithelium, the expression of PR-A was decreased constantly during the secretory phase while the regulation of PR-B expression level was more complicated. PR-B expression decreased in the early secretory phase, increased again in the mid secretory phase and decreased towards the late secretory phase, causing fluctuation in the relative PR-A and PR-B expression ratio in the glandular epithelium. Throughout the secretory phase the PR-A was the predominant isoform in stromal cells. In the myometrium, like ERα expression, PR levels were regulated with the menstrual cycle in the subendometrial part but remained fairly constant in the outer part of the myometrium (Noe et al. 1999).

During pregnancy, progesterone facilitates implantation and maintains pregnancy by quieting the myometrium. The onset of labor requires progesterone withdrawal and estrogen activation. In humans this may be achieved by the modulation of PR-A and PR-B expression (Pieber et al. 2001). Although the mRNAs levels of both isoforms are increased in the laboring compared to nonlaboring myometrium, the increase in PR-A mRNA is more dramatic, suggesting that the withdrawal of progesterone action is due to the suppressive action of PR-A on PR-B (Mesiano et al. 2002). Myomas have higher PR protein levels than the adjacent myometrium. This however, was not found at mRNA level, suggesting posttranslational control (Viville et al. 1997, Englund et al. 1998).

In endometrial cancer, PR expression correlates positively with tumor grade, with the depth of myometrial tumor invasion and disease-free survival (Fukuda et al. 1998). Compared to the normal endomerium, endometrial cancer frequently evinces decreased PR expression, which is due to loss of expression of one of the isoforms (Arnett-Mansfield et al. 2001). Tumors expressing either isoform have been associated with higher grades, whereas those expressing both isoforms have been found only in lower grade tumors. One conceivable mechanism underlying the loss of PR-B expression in endometrial cancer may be the methylation of CpG islands, causing gene inactivation (Sasaki et al. 2001). Several cell culture studies suggest that the PR-B isoform mediates the growth-inhibitory effects of progestins in endometrial cancer cells, and cells expressing PR-B are more clearly differentiated and less invasive than PR-A-expressing cells (Kumar et al. 1998, Dai et al. 2002, Smid-Koopman et al. 2003).

PR in the other reproductive tissues

Epithelial and stromal cells of the human vagina and cervix express PR and the expression seems not to be regulated in the course of the menstrual cycle (Cano et al. 1990, Wolf et al.

1991). PR may have a role in cervical ripening, since PR levels are decreased during term pregnancy (Stjernholm et al. 1996).

PR-A expression is necessary and sufficient for the production of oocytes in mice (Mulac-Jericevic et al. 2000, Mulac-Jericevic et al. 2003). In the corpus luteum of the rhesus monkey, PR-B is the predominant isoform through the luteal phase (Duffy et al. 1997). Although the levels of both isoforms were decreased from the early to the very late luteal phase the levels of PR-A were decreased more dramatically, thus altering the PR-A/PR-B ratios. In the human ovary, PR immunostaining has been reported in germinal epithelium, stroma, theca internal cells and the luteinised granulosa cells (Press and Greene 1988, Iwai et al. 1990, Suzuki et al. 1994). The PR expression in the ovary is regulated during the menstrual cycle. In the human oviduct, epithelial and stromal cells express PR and the expression is higher in the follicular than in the luteal phase (Amso et al. 1994). Somewhat inconsistent results have been reported regarding the PR status as a prognostic factor in ovarian cancers (reviewed in Perez-Gracia and Carrasco 2002). A recent finding of Akahira and colleagues (2000) was that PR-B is the predominant isoform in epithelial ovarian carcinoma and PR-B expression may be a prognostic factor for overall survival.

3.4. Androgen receptor

Major physiological ligands for AR are the male hormone testosterone and its metabolite 5α-dihydrotestosterone. AR was found to be expressed in human genital skin fibroblasts in two isoforms, AR-B and its N-terminally truncated AR-A isoform (Wilson and McPhaul 1994).

AR in the mammary gland
The normal mammary gland expresses AR at low levels in both epithelial and stromal cells (Zhuang et al. 2003). In breast cancers, AR is commonly expressed and a considerable number of poorly differentiated ER-negative and PR-negative cancers are AR-positive (Kuenen-Boumeester et al. 1992, Isola 1993, Brys et al. 2002, Moinfar et al. 2003). Post-menopausal women carry an elevated risk of breast cancer if they have high estrogen levels but also if they have high testosterone levels (for a review see Liao and Dickson 2002).

AR in the uterus
AR expression has been detected in the human endometrium in epithelial and stromal cells (Horie et al. 1992, Mertens et al. 1996). More intense immunohistological staining has been observed during the proliferative than the late secretory phase (Slayden et al. 2001, Ito et al. 2002). Little staining has been observed in the myometrium (Horie et al. 1992). Estrogen treatment increases AR levels in the human endometrium (Fujimoto et al. 1994). Women suffering from polycystic ovarian syndrome and thus having high androgen levels have increased levels of AR in the endometrium (Apparao et al. 2002). Expression of AR has also been detected in adenomyosis, endometriosis and endometrial carcinoma (Horie et al. 1992, Ito et al. 2002).

AR in the other reproductive tissues
In the human ovary, Suzuki and colleagues (1994) have studied AR expression in detail. AR was expressed in stromal cells while granulosa cells of primordial and primary follicles were negatively stained. AR expression was detected in granulosa cells and some theca internal cells of maturing follicles. A majority of epithelial ovarian tumors have been reported to be

AR-positive (Kuhnel et al. 1987). AR expression is related to longer patient survival and lower tumor stage (Slotman et al. 1990). AR expression in the human vaginal mucosa correlated negatively with age and was lower in postmenopausal women than premenopausal, whereas expression levels did not differ in the vaginal submucosa (Berman et al. 2003).

3.5. Glucocorticoid receptor

The natural ligand for human GR is cortisol, which participates in the regulation of a variety of physiological actions such as metabolism and inflammation. GR is expressed in two C-terminally different isoforms, GRα and GRβ (Hollenberg et al. 1985, Weinberger et al. 1985). GRβ does not bind glucocorticoids and is transcriptionally inactive (Bamberger et al. 1995). However, it is expressed widely in human tissues and may act as a repressor of GRα and thus a potential modulator of glucocorticoid responses. Furthermore, both C-terminally different isoforms have N-terminally truncated slicing variants (reviewed in Yudt and Cidlowski 2002).

GR in the mammary gland
Glucocorticoids and prolactin act synergistically in mammary epithelial cells to activate milk protein gene expression. In virgin mice, fully active GR is required for proper ductal development of the mammary gland, whereas DNA-binding ability of GR is not crucial for the formation of alveoli during pregnancy and for normal lactation (Reichardt et al. 2001). In breast cancer, higher expression of GR mRNA has been found in grade 3 tumors than in benign breast and grades 1 and 2 tumors (Smith et al. 2003). Evidence that 17β-estradiol (E2) decreases GR expression has been obtained in MCF-7 breast cancer cells, suggesting that estrogens may affect glucocorticoid responses in some cellular context (Krishnan et al. 2001).

GR in the uterus
In the human uterus, GR is expressed in stromal and endothelial cells of the endometrium, while it is not expressed in epithelial cells (Bamberger et al. 2001). Furthermore, GR has been localised in the decidua and in ER- and PR-negative uterine natural killer cells (Henderson et al. 2003).

3.7. Vitamin D receptor

1,25-Dihydroxyvitamin D_3 is the biologically active form of vitamin D and was originally identified as a regulator of calcium and bone homeostasis. In addition it participates in the control of cell proliferation, differentiation and apoptosis. 1,25-Dihydroxyvitamin D_3 binds to VDR, which was cloned first from a chicken intestinal cDNA library (McDonnell et al. 1987) and later from human intestine and T-47D cell cDNA libraries (Baker et al. 1988). VDR forms heterodimers with RXR or forms homodimers (Carlberg et al. 1993, reviewed in Issa et al. 1998). VDR null mutant mice have impaired folliculogenesis and uterine development (Yoshizawa et al. 1997). This is due to estrogen insufficiency caused by decreased P450 aromatase activity (Kinuta et al. 2000).

VDR in the mammary gland
Vitamin D controls proliferation, differentiation and apoptosis in mammary epithelial cells (reviewed in Narvaez et al. 2001). Mammary glands of VDR null mutant mice have evinced increased growth and branching morphogenesis (Welsh et al. 2002). Furthermore, glandular

involution, a process including epithelial cell apoptosis, was delayed in VDR null mutant mice. Vitamin D and its synthetic analogues inhibit the growth of breast cancer cells and induce apoptosis (reviewed in Narvaez et al. 2001, Colston and Hansen 2002). In breast cancer VDR-positive patients have had longer disease-free survival than VDR-negative patients (Colston et al. 1989). Friedrich and colleagues (1998) found increased expression of VDR in breast tumors compared to normal breast tissue.

VDR in the other reproductive tissues
Expression of VDR has also been studied in gynecological carcinomas. Expression of VDR at protein level was observed more often in cervical carcinomas than in normal cervical tissues (Reichrath et al. 1998, Friedrich et al. 2002). VDR expression has been shown in endometrial adenocarcinomas and ovarian tumors (Saunders et al. 1992, Yabushita et al. 1996).

3.8. Retinoic acid receptors α, β and γ

The physiological ligands for RARα, β and γ are all-*trans* retinoic acid and 9-*cis* retinoic acid, which have important roles in the maintenance of epithelial differentiation, a characteristic utilised in chemotherapy (reviewed in Hansen et al. 2000). RARα and RARγ are expressed in two major isoforms, while RARβ has four major isoforms (for review see Leid et al. 1992). RAR may form either homodimers or heterodimers with RXR depending on the HREs (reviewed in (Laudet and Gronemeyer 2002). One of the target genes of RARs is RARβ itself and its expression has been suggested to be critical in the development of several types of cancer, for example breast cancer and cervical carcinoma (Geisen et al. 1997, Widschwendter et al. 1997, Xu et al. 1997).

RAR in the mammary gland
RARα, RARβ and RARγ are expressed in the normal mammary gland (Widschwendter et al. 1997). Retinoids induce apoptosis of breast cancer cells and this apoptotic effect may be mediated via RARβ (Pellegrini et al. 1995, Liu et al. 1996, Toma et al. 1997). Such a conception is supported by findings that loss of RARβ2 expression may be an important event in breast carcinogenesis (Widschwendter et al. 1997, Xu et al. 1997). This loss may be partly due to methylation of RARβ gene (Bovenzi et al. 1999, Sirchia et al. 2000, Widschwendter et al. 2000).

RAR in the uterus
RARα, RARβ and RARγ are expressed in epithelial and stromal cells of the human endometrium and the expression is decreased in the secretory compared to the proliferative phase of the menstrual cycle (Kumarendran et al. 1996, Fukunaka et al. 2001, Ito et al. 2001). RARα, RARβ and RARγ are also expressed in the human myometrium and at similar levels in leiomyoma (Tsibris et al. 1999). In cervical intraepithelial neoplasia RARα, β and γ levels are decreased, which may affect retinoid-induced growth inhibition of cervical neoplasia (Behbakht et al. 1996, Xu et al. 1999).

3.9. Retinoid X receptors α, β and γ

The physiological ligand of RXR is 9-*cis* retinoic acid. RXR participates in the regulation a large variety of NR signalling pathways, since RXRs are able to form heterodimers with several NRs, for example with RARs, VDR and thyroid hormone receptors (reviewed in Laudet and Gronemeyer 2002). In addition, it may form homodimers. Several N-terminal isoforms of RXRα, RXRβ and RXRγ exist although the function of these variant forms is still unclear (for review see Laudet and Gronemeyer 2002).

RXR in the mammary gland
RXRα, RXRβ and RXRγ are expressed in the normal mammary gland (Lawrence et al. 1998). Increased expression of RXRα, but not of RXRβ or RXRγ, is linked to an increased risk of the development of invasive breast cancer (Lawrence et al. 1998).

RXR in the uterus
Both epithelial and stromal cells of the human endometrium express RXRα, RXRβ and RXRγ and the expression appears to be higher in the proliferative phase than during the late secretory phase (Fukunaka et al. 2001). RXRα expression levels are increased in human leiomyomata compared to the normal myometrium (Tsibris et al. 1999).

4. SELECTIVE STEROID RECEPTOR MODULATORS

The ability of NRs to regulate cell growth and differentiation has drawn attention to their potential as therapeutic targets. Ligands of NRs are currently used in cancer therapy, in cancer prevention and in differentiation therapy. As noted above, multiplicity is the recurrent theme in NR signalling pathways. The activity of a given ligand at some cellular level is determined not only by the receptor, available cofactors and the target gene promoters but also by factors modifying the activity of these molecules, e.g. phosphorylation, ubiquitination etc. A compound is considered a selective NR modulator if it exerts agonist or antagonist effects selectively depending on target tissues. The basis for the concept selective estrogen receptor modulator (SERM) was generated according to the results obtained from studies with the antiestrogen tamoxifen. Clinical use of tamoxifen in the treatment of breast cancer commenced in the 1970s. Tamoxifen has antiestrogenic effects in the breast and breast cancer. In the uterus, in contrast, it acts as an agonist and increases the incidence of uterine cancer (Gottardis et al. 1988, Fornander et al. 1989). In addition to SERMs, all NR are potential targets of new tissue-selective NR modulators. The understanding of the molecular mechanism behind the tissue specificity is essential when designing new synthetic compounds having desired functions in target tissues.

Although originally thought of as reproductive hormones, estrogens cooperate in bone, cardiovascular system and central nervous system. Data derived from clinical studies suggested that long-term HRT usage might be regarded as preventive treatment for osteoporosis, heart diseases and dementia (Paganini-Hill and Henderson 1994, Ettinger et al. 1996, Grodstein et al. 1997, Torgerson and Bell-Syer 2001). Undesirable side effects of estrogen-only HRT comprise an increased risk of endometrial cancer, which may be overcome by progestin (Grady et al. 1995, 1996). Recent large HRT studies, although raising several controversial issues, suggest that combined progestin and estrogen HRT have side

effects which may offset the benefits of these therapies (Rossouw et al. 2002, Beral 2003). These studies further supported previous findings indicating that combined HRT increases the risks of breast cancer (Ross et al. 2000, Schairer et al. 2000, Chen et al. 2002, Rossouw et al. 2002, Beral 2003, Li et al. 2003). A somewhat unexpected finding in the Women's Health Initiative trial was that combined HRT slightly increased the risks of coronary heart disease and probable dementia (Rossouw et al. 2002, Shumaker et al. 2003). Beneficial outcomes of combined HRT usage were a reduced risk of colorectal cancers and hip fractures (Rossouw et al. 2002). Although this trial has been criticised for its limitations in respect of study population and regimens used, the current HRT does not answer the needs which optimal HRT would ensure, namely beneficial effects of estrogens in bone, cardiovascular system and central nervous system while antagonising the effects of estrogen in breast and uterus. Novel selective ER modulators (SERM) have been extensively developed, but only few are yet available. In spring 2003, FDA had approved three SERMS for clinical use; tamoxifen for the prevention and treatment of breast cancer, toremifene for the treatment of advanced breast cancer and raloxifene for the prevention and treatment of osteoporosis. Furthermore, several clinical trials are testing new potential SERMS.

The molecular mechanisms behind the agonist/antagonist profile of SERMs have been most intensively studied. Some of the regulation levels are briefly discussed here as examples of possible mechanisms elucidating the agonist/antagonist profile of selective NR modulators.

The tissue-specific effects of SERMs may be due to differences in ERα and ERβ expression. Some cell types express only either ERα or ERβ (Taylor and Al-Azzawi 2000). Raloxifen and tamoxifen bind to both ERα and ERβ; thus whether they exert agonist or antagonist effects depends partially on whether they bind to ERα or ERβ homodimers or to ERα/ERβ heterodimers (Cowley et al. 1997, Pace et al. 1997, Hall and McDonnell 1999). ERα works mostly as an activator whereas ERβ can repress the activity of ERα through ERE sites (Cowley et al. 1997, Hall and McDonnell 1999, Lindberg et al. 2003). Structural differences in the AF-1 and AF-2 domains of ERα and ERβ contribute partially to the different activities of these receptors. ERα has AF-1 and AF-2 (Bocquel et al. 1989, Tora et al. 1989). ERβ has a weak AF-1, which can repress the activity of the C-terminal AF-2 domain (Hall and McDonnell 1999).

Whether the ligand acts as an agonist or antagonist is also dependent on the target gene regulatory region, since receptor- and cell type-specific differences have also been shown in the activation of transcription through AP-1 response elements. Table 1 summarises the differential activities of E2 and tamoxifen depending on the receptor and target gene (Webster et al. 1988, Philips et al. 1993, Tzukerman et al. 1994, Umayahara et al. 1994, Webb et al. 1995, Paech et al. 1997). Mice which are heterozygous for mutation in the DBD of ERα exhibit hyperplasia in the uterus and hypoplasia in the mammary gland (Jakacka et al. 2002). This resembles the outcome of tamoxifen treatment, suggesting that the alternative signalling pathways such as the AP-1-mediated may have a crucial role in determining the SERM-specific responses.

Table 1. Different responses of E2 and tamoxifen or 4-hydroxytamoxifen depend on the receptor and the target gene regulatory region.

Ligand	ERα		ERβ	
	ERE	AP-1	ERE	AP-1
E2	↑	↑	↑	no change
Tamoxifen	↓↑	↓	↓	↑

↑ increased or ↓ decreased transcriptional activity

Another level regulating the SERM response is due to the conformational differences which the LBD of ERα and ERβ assume after ligand binding (Brzozowski et al. 1997, Shiau et al. 1998, Paige et al. 1999, Pike et al. 1999). When bound to E2, ERα is able to bind coactivators, while bound to 4-hydroxytamoxifen or raloxifen helix 12 of the ERα LBD adopts a position which inhibits binding of coactivators thus blocking AF-2 activity (Brzozowski et al. 1997, Shiau et al. 1998, Wong et al. 2001). In contrast, tamoxifen or raloxifen binding promotes corepressor interaction through helices H3 and H5 of the ERα LBD and this interaction is SERM-specific (Yamamoto et al. 2001, Webb et al. 2003). It is also dependent on the ligand which coactivators will be recruited by the receptor. E2 was shown to promote the interaction of ERα and ERβ with the DRIP205, CBP, SRC1 and AIB1 (Wong et al. 2001). Genistein has been shown to enhance the interaction of ERα and ERβ with SRC1 and AIB1 while having a minimal effect on interaction with DRIP205 and CBP. The cellular levels of cofactors may affect the SERM response. The estrogenic response of tamoxifen has been shown to require a high level of SRC-1 expression (Shang and Brown 2002).

The stability of ERα has also been shown to be ligand-specific (Wijayaratne and McDonnell 2001). E2 promotes degradation of ERα through the ubiquitin proteasome pathway and this is required for efficient transcriptional activity of ERα (Nawaz et al. 1999a, Lonard et al. 2000). The pure antagonist ICI 182,780 also reduces ERα stability while tamoxifen seems to stabilise ERα (Wijayaratne et al. 1999, Wijayaratne and McDonnell 2001).

One level of complexity has been suggested after findings that ERα gene polymorphism may affect response of HRT in bone and cardiovascular effects (Salmen et al. 2000, Herrington et al. 2002a, Herrington et al. 2002b). This may further lead to the identification of other associated genes having significant polymorphism, this indicating a need to chart patients' genetic background before determining the best available treatment.

AIMS OF THE PRESENT STUDY

The present work was undertaken to characterise the expression of several NRs and cofactors and the hormonal regulation of their expression in various female reproductive tissues. Broader knowledge of the receptor and the cofactor expression would help in the understanding of the responses of target tissues during hormonal treatments and when developing new ligands.

Specific aims of the study were:
1. to study the regulation of PR-isoform (PR-A and PR-B) expression by estrogen in the chicken oviduct and in breast cancer cell lines (I, II)
2. to study NR expression and hormonal regulation of expression in different breast cancer cell lines (III)
3. to characterise NR expression in proliferative and secretory phase endometrium and myometrium (IV)
4. to evaluate cofactor expression and its hormonal regulation in different breast cancer cell lines (III)
5. to characterise the expression of cofactors in proliferative and secretory phase endometrium and myometrium (IV)

MATERIALS AND METHODS

1. EXPERIMENTAL ANIMALS (I)

Two-week old White Leghorn chicks (Mäkelän Jalostukanala, Vilppula, Finland) were injected daily with 1 mg of 17β-estradiol (E2) (1,3,5(10)-estratriene-3,17β-diol, Sigma, St. Louis, MO, USA) dissolved in propylenglycol for up to 21 days. Immature control animals were not hormone-or vehicle-treated. Magnum parts of the oviducts were dissected out 24 hours after the last injection.

2. CELL CULTURE AND TRANSFECTIONS (II, III, IV)

Human breast cancer cell lines MCF-7, T-47D and ZR-75-1 were maintained in DMEM/F12 medium (Gibco BRL, N.Y., USA) supplemented with 5% fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), and bovine insulin (10 ng/ml, Sigma). MCF-7 cells were cultured with 1 nM E2 (Sigma)). The culturing medium was changed to phenol red-free DMEM/F12 medium (Gibco) containing antibiotics and 5% dextran charcoal-treated serum one week before experimentation. Thereafter the medium was changed every second day. Cells were treated with either E2, R5020 (Schering Aktiengesellschaft, Berlin, Germany), cAMP (8-bromoadenosine 3':5'-cyclic monophosphate, Sigma) or with IGF-1 (insulin-like growth factor-1, human, recombinant, Sigma). After indicated treatments cells were washed with phosphate buffered saline (PBS) and trypsinised. The cell suspension was diluted with PBS and centrifuged for 10 min at 680 x g to collect the cell pellet.

Human promyelocytic cells HL-60 were grown in RPMI medium (Gibco) supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), streptomycin (100 μg/ml), HEPES (10 mM) and glutamine (2 mM). Before trypsinisation HL-60 cells were treated for 48 h with 200 mM 8CTP-cAMP (Sigma) and 1 mM SR11237 (gift from Dr. H. Gronemeyer, IGBMC, Department of Cell Biology and Signal Transduction,, Illkirch, France).

COS cells were cultured in DMEM/F12 medium containing 5% fetal bovine serum, penicillin and streptomycin. The cells were transfected using the Lipofectamine reagent (Gibco) according to the supplier's instructions.

3. UTERINE SAMPLES (IV)

Uterine samples were obtained from 13 premenopausal women ranging in age from 38 to 50 years (mean 43 years) undergoing hysterectomy at Tampere University Hospital. The study was approved by the ethical committee of Tampere University Hospital and each patients had given fully informed consent. The donors were undergoing hysterectomy for various reasons excluding endometrial or myometrial malignancies. They had not used HRT. Instantly after removal of the uterus tissue specimens were taken from a site considered macroscopically to

be representative of the endometrium and myometrium. Endometrial and myometrial tissues were fractionated from the same tissue piece except for two endometrial samples obtained by endometrial biopsy. The samples were frozen and stored at –80 ºC until determination. Paraffin sections from each specimen were hematoxylin eosin-stained to determine the phase of the menstrual cycle. Histologically, six of the specimens represented the proliferative phase (days 5 to 19), one was from the interval phase (day 16) and six represented the secretory phase (days 17 to 31).

4. RNA ISOLATION

Total RNA was isolated from frozen chicken oviducts and human uterine samples according to Chomczynski and Sacchi (Chomczynski and Sacchi 1987). From breast cancer cells total RNA was isolated using TRIzol reagent (Gibco) according to manufacture's instructions. The amount of RNA was assayed by measuring the optical density of samples at wavelength 260 nm. Gel electrophoresis was used to confirm the integrity of the samples.

5. NORTHERN BLOTTING (I)

RNA samples were run on a 1% agarose gel containing formaldehyde and blotted to nylon filter (Magna, MSI, Westerborough, MA, USA). RNA was fixed on the nylon filter by baking it for 2 hours at 80ºC. The filter was hybridised with [^{32}P]-labelled HindIII fragment of chicken PR cDNA (Jeltsch et al. 1990). The hybridisation was continued for 18 hours at 46 ºC in a solution containing 50 % formamide, 5 X SSPE, 5 % Denhardt's solution, 0.5 % SDS and 100 µg/ml denatured herring sperm DNA. After washing the filter was exposed to autoradiography. To obtain an optimal signal Kodak MS-Biomax (Eastman Kodak Company, CT, USA) was used with an intensifying screen.

6. RIBONUCLEASE PROTECTION ASSAY (RPA) (II, III, IV)

Probes

The probe for detecting PR isoform-specific mRNAs was made by subcloning the cDNA fragment corresponding to nucleotides +618 to +971 according to Kastner and associates to transcription vector pGEM-T Easy (Promega, Madison, WI, USA) (Kastner et al. 1990). The sequence and the orientation were ensured by DNA sequencing. PR probe plasmid was digested with SalI restriction enzyme. The probe for identifying PR mRNAs was so designed that it could hybridise to both PR-A and PR-B specific mRNAs and yield three different lengths of hybrids. The longest hybrid (353 bp), called PR-B, is produced from transcripts which may encode both PR-B and PR-A. Both shorter hybrids, called PR-A/1 (220/210 bp) and PR-A/2 (129 bp), are produced from transcripts initiated after the first ATG site, thus encoding only PR-A-specific mRNAs.

The steroid receptor, VDR/RAR and cofactor probes are listed in Table 2. Probes for detecting GR, AR, PR, ERβ, cyclin A, VDR and ERα mRNAs were included in the steroid

receptor set. Probes for detecting VDR, RARα, RARβ, RARγ, RXRα, RXRβ and RXRγ mRNAs were included in the VDR/RAR set and those for N-CoR, SMRT, pCAF, CBP, TIF2, AIB1, SRC-1 and p300 in the cofactor set. Probes were made by PCR-amplifying the selected area of each gene's cDNA and further inserting the PCR product to the vectors listed in Table 1. TA-cloning was used to insert the PCR fragments to the pGEM-T Easy vector (Promega). BamHI and EcoRI digestion sites, which were included in the PCR primers, were used to subclone the PCR fragments to the pSG5 vector (Stratagene, La Jolla, CA, USA). The orientation and the sequence of the constructs were confirmed by DNA sequencing. The constructs were digested with the restriction enzymes listed in Table 2.

RPA

The RiboQuant *In vitro* transcription kit (Pharmingen, San Diego, CA, USA) was used to produce the antisense [^{32}P]-uridine triphosphate-labelled probes from linearised plasmids. The MEGAshortscript T7 kit (Ambion, Austin, TX, USA) was used to transcribe a [^{32}P]-labelled ribosomal 18S probe from plasmid pTRI RNA 18S (Ambion).

When breast cancer cell line samples were analysed, 5 μg of total RNA was hybridised with 5x10^5 cpm [^{32}P]-labelled RNA probe (PR probe) or probe set (steroid receptor, VDR/RAR or cofactor set) (specific activity 1.7x 10^9 cpm/μg) and with 340 ng of [^{32}P]-labelled 18S RNA probe (specific activity 89000 cpm/μg) using an RPA-kit (RPA III, Ambion). When uterine samples were analysed, 7.5 μg of total RNA was hybridised with 1x10^6 cpm [^{32}P]-labelled RNA probe set (steroid receptor, VDR/RAR or cofactor set) (specific activity 1.7x 10^9 cpm/μg) and with 680 ng of [^{32}P]-labelled 18S RNA probe (specific activity 56000 cpm/μg). The length of the synthesised 18S probe was 128 bp and that of the protected hybridised 18S was 80 bp. Yeast RNA was used as a negative control. In addition, either 8 μg of breast cancer cell line sample RNA or 15 μg of uterine tissue sample RNA were used to check that probes were in molar excess in hybridisation reactions. Hybridisation was carried on 43ºC for at least 16 h. Samples were resolved by running them through 5% polyacrylamide gel containing 8 M urea. Autoradiography was used for visualisation of hybrids. Gels were scanned using the PhosphoImager Storm® (Molecular Dynamics, Amersham Biosciences, Buckinghamshire, UK) or films were scanned using a Personal Densitometer SI (Molecular Dynamics). The radioactive intensities of hybrids were calculated with ImageQuant software (versions 5.0 and 5.1, Molecular Dynamics). In order to verify probe specificity each probe of the VDR/RAR and cofactor sets was labelled alone to confirm the specificity. Total RNA from COS cells transfected with the corresponding cDNA was used to verify the appropriate hybrid for ERα, ERβ, VDR, RARα, RARβ and RARγ. Furthermore, total RNA (4 μg) from HL-60 cells was hybridised with the ^{33}P-labelled cofactor set and GAPDH probe using Pharmingen's RPA kit according to the supplier's instructions to demonstrate that the cofactor set identified all the corresponding genes. The intensities of 18S hybrids were used for normalisation of loading between samples. Comparisons were made only with samples run along the same RPA assay and gel. To assess whether insulin, estradiol and progestin regulated the expression levels, the relative intensity of the hormone-treated sample was divided by the relative intensity of the control.

Table 2. Probes for RPA (AC# symbolises for GeneBank accession number, [1]basepairs)

Gene	Size[1]	mRNA region	AC#	Vector	Restriction enzyme
GR	500	1741-2240	M10901	pGEM-T Easy	SalI
AR	460	2641-3100	M23263	pGEM-T Easy	SalI
PR	400	3945-4344	X51730	pGEM-T Easy	SalI
ERβ	316	503-818	X99101	pGEM-T Easy	SalI
Cyclin A	300	598-897	A32139	pGEM-T Easy	SalI
ERα	220	1846-2065	M12674	pGEM-T Easy	SalI
VDR	326	1-326	AF026260	pSG5	XbaI
RXRα	290	83-372	X52773	pSG5	XbaI
RXRβ	259	141-399	M84820	pSG5	XhoI
RXRγ	232	9-240	U38480	pSG5	XhoI
RARγ	202	404-605	M24857	pSG5	XhoI
RARβ	183	333-515	X07282	pSG5	XhoI
RARα	166	309-474	X06538	pSG5	XbaI
N-CoR	361	72-432	AA564930	pSG5	HindIII
SMRT	311	600-910	U37146	pSG5	HindIII
pCAF	266	901-1166	U57317	pSG5	XhoI
CBP	234	598-831	U47741	pSG5	XhoI
TIF2	200	3111-3310	X97674	pSG5	XhoI
AIB1	179	2594-2772	AF010227	pSG5	BamHI
SRC-1e	160	1421-1580	U19179	pSG5	BamHI
SRC-1a	145	4053-4197	U90661	pSG5	BamHI
p300	130	7404-7533	U01877	pSG5	BamHI

7. RT-PCR (III, IV)

The expression of ERα and VDR mRNAs from breast cancer cell line samples were also analysed using real-time RT-PCR (LightCycler, Roche Diagnostics, Mannheim, Germany). Acidic ribosomal phosphoprotein P0 (RPLP0) was used as an endogenous RNA control and primers for it were selected as described by Bièche and colleagues (2001). All primer pairs were selected in different exons in order to avoid amplification from possible contaminating genomic DNA. Table 3 summarises the sequences of the primers.

Table 3. Primers, product size and reaction conditions applied in real time PCR using LightCycler instrument.

Primer	Primer sequence	Annealing temp.	$Mn(OAc)_2$ concentration	Product size
ERα-F	5'-CCACTCAACAGCGTGTCTC-3'	55ºC	2.5 mM	242 bp
ERα-R	5'-GGCAGATTCCATAGCCATAC-3			
VDR-F	5'-CTGACCCTGGAGACTTTGAC-3'	55ºC	3.0 mM	276 bp
VDR-R	5'-TTCCTCTGCACTTCCTCATC-3'			
RPLP0-F	5'-GGCGACCTGGAAGTCCAACT-3'	58ºC	3.25 mM	149 bp
RPLP0-R	5'-CCATCAGCACCACAGCCTTC-3'			

One step RT-PCR was performed using a LightCycler RNA Master SYBR Green I kit (Roche) according to manufacturer's instructions. The total reaction volume was 20 μl containing 200 ng of total RNA and 0.5 μM of each primer. The $Mn(OAc)_2$ concentration varied depending on the assay (listed in Table 3). Reaction conditions in the LightCycler instrument were chosen according to the manufacturer's recommendations. Reverse transcription was performed at 61°C for 20 min followed by denaturation at 95°C for 30 s. PCR cycles consisted of 3 steps; denaturation at 95°C for 1s, annealing at 55°C - 58°C for 5 s and elongation at 72°C for 11 s (ERα), 12 s (VDR) or 7 s (RPLP0). A total of 38 cycles were carried out. Fluorescence was measured at the end of each elongation step at 72°C. However, when amplifying RPLP0 the fluorescence was measured at 81°C to avoid measurement of fluorescence due to an undesired faintly amplified product, which had a Tm lower than 81°C. The specificities of PCR products were verified by a melting curve analysis using the LightCycler instrument. In addition, the lengths of the PCR products were verified by agarose gel electrophoresis. The Fit Points Method determined the crossing point (CP) at which the fluorescence of each sample rose appreciably above the background fluorescence. Relative quantification of the target gene in comparison to the reference gene RPLP0 (ratio) was calculated using the CP and the efficiencies (E) of the PCR reactions.

ratio= $(E_{target})^{\Delta CP_{target}(control-sample)}/(E_{RPLP0})^{\Delta CP_{RPLP0}(control-sample)}$ (Pfaffl 2001).

Relative quantification of VDR mRNA levels from uterine tissue samples was also done using real time PCR (AbiPrism 7000, Applied Biosystems, Foster City, CA, USA). A High-Capacity cDNA Archive Kit (Applied Biosystems) was used to convert 8 μg of total RNA to cDNA as described by the manufacturer. RPLP0 was used as an endogenous RNA control. Primers, which are listed in Table 4, were designed using Primer Express 2.0 (Applied Biosystems) and selected in different exons. Real time PCR was done using 20 ng of sample cDNA and SYBR® Green PCR Master Mix (Applied Biosystems) according to producer's protocol. As a calibrator we utilised total RNA from epithelial cells isolated from the endometrium basically as described by Laird and associates (Laird et al. 1997) (proliferative

phase, day 7). The results were expressed as N-fold differences in VDR gene expression (N_{VDR}) relative to RPLP0 expression and the calibrator. N_{VDR} (ratio) was calculated using the above equation except that control values were replaced with the calibrator values.

Table 4. Primers used in analysing mRNA expression with the AbiPrism instrument.

Primer	Primer sequence
VDR-F	5'-CCTTCACCATGGACGACATG-3'
VDR-R	5'-CGGCTTTGGTCACGTCACT-3'
RPLP0-F	5'-AATCTCCAGGGGCACCATT-3'
RPLP0-R	5'-CGCTGGCTCCCACTTTGT-3'

8. WESTERN BLOT ANALYSIS (I, II)

Chicken oviducts were homogenised in 3 volumes of homogenisation buffer containing 10 mM Tris-HCl, 1.5 mM EDTA, 12 mM monothioglycerol, 10 mM Na_2MoO_4, pH 7.4. In addition, the homogenisation buffer contained 0.3- 0.9 mM phenylmethyl-sulphonyl fluoride, 0.1 mM leupeptin, 77 µg/ml aprotinin, 1.5 mM pepstatin and 100 µg/ml bacitracin. Nuclear material was fractionated by centrifuging the homogenate at 800 g for 5 min. The supernatant was further centrifuged at 100 000 g (+4°C) for 1 hour and the consequent supernatant was used as cytosolic extract. The pellet obtained after low-speed centrifugation was washed with homogenisation buffer containing 10 mM Na_2MoO_4. It was further incubated with buffer containing 10 mM Tris, 1.5 mM EDTA, 12 mM monothioglycerol, 0.4 M KCl, pH 8.0 for 1 hour at +4°C and then centrifuged at 4000 g for 30 min. The resulting supernatant was precipitated at a final volume of 50% saturated ammonium sulphate. Both cytosolic and nuclear fractions were dissolved in 4 volumes of sample buffer (63 mM Tris-HCl, 2% SDS, 10% glycerol, 1.5 mM EDTA, 5% β-mercaptoethanol, 0.05% bromophenol blue, pH 7.4) and boiled for 5 min.

Cell pellets from breast cancer cells were lysed using 4 volumes of sample buffer, which contained 63 mM Tris-HCl, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.025% bromophenol blue. After homogenisation of the cell pellet each sample was drawn several times through a 23 G needle in order to reduce the viscosity of the samples. Before storing the samples at –70°C they were boiled for 5 min.

Western blots were done according to standard procedures (Laemmli 1970). Samples were run through 6.5 % polyacrylamide gels containing 0.1 % SDS followed by electrophoretic transfer to nitrocellulose membranes (Protran, Schleicher & Schuell, Dassel, Germany). The transfer buffer contained 25 mM Tris, 192 mM glycin, 20 % (v/v) methanol. Transfer was done either for 2 hours (I) or overnight (II). Membranes were blocked with 5 % bovine serum albumin or 1% non-fat milk milk TBS (50 mM Tris, 0.9% NaCl, pH 8.0) for 1 hour at +37°C. Membranes were incubated with primary monoclonal antibodies PR22 (I)(Sullivan et al. 1986) and NCL-PGR (II) (Novocastra Laboratories, Newcastle, UK) or with polyclonal IgG-

RB (I) (Tuohimaa et al. 1984) at +4°C overnight. The final concentrations were 1 μg/ml for PR22, 150 ng/ml for NCL-PGR and 5 μg/ml for IgG-RB. After washings with TBS the membranes were incubated with secondary antibodies, peroxidase-conjugated anti-mouse or anti rabbit antibodies (Cappel, West Chester, PA, USA) at RT 1 hour. These antibodies were used in a dilution of 1:10000 (I) or 1:50000 (II). After washings with TBS the peroxidase was visualised using an enhanced chemiluminescence (ECL) detection system according to the instructions of the manufacturer (Amersham International). The intensities of subsequent bands were quantified using either digitalised images of X-ray films or densitometric scanning of X-ray films (Personal Densitometer, Molecular Dynamics).

9. STATISTICAL METHODS (III, IV)

The statistical significance of differences after hormonal treatments of breast cancer cell lines was assessed using the non-parametric Mann-Whitney U test (SPSS 10.1 for Windows). The same test was also used to determine the statistical significance of differences in the expression levels of proliferative and secretory phase endometrium and myometrium samples. Results were considered statistically significant at $p<0.05$. The difference in expression levels between endometrium and myometrium was assayed using Wilcoxon signed ranks test (SPSS 10.1 for Windows). Results were considered statistically significant at $p<0.05$. Spearman's bivariate correlation analysis (SPSS 10.1 for Windows) was used to analyse correlations between the ratio of cyclin A endometrium to cyclin A myometrium and the same ratios of steroid receptors, VDR, RARs and cofactors N-CoR, pCAF, AIB1 and p300. Correlation was considered statistically significant at $r>0.6$ and $p<0.05$.

RESULTS

1. PR ISOFORM EXPRESSION (I, II)

1.1. Expression and regulation by estrogen in chicken oviduct

The chicken oviduct is a classical model system for study of the hormonal regulation of PR expression, since its differentiation and maturation can be initiated in immature animals by hormonal administration. Chicken PR (cPR) is expressed in two PR isoforms (Gronemeyer et al. 1987, Jeltsch et al. 1990). The molecular weight of cPR-A is 79 kD and of cPR-B is 110 kD. Western blot analysis was used to quantitate the isoform expression at protein level in the immature chicken oviduct as well as after estrogen stimulation. Both cytosolic and nuclear extracts were analysed. PR-B was the preferentially expressed isoform in immature oviducts but PR-A expression was also detected. When estrogen was administered for 3 days to 2-week-old chickens, the preferential expression of PR-B isoform had changed to almost equal expression of both isoforms. Longer estrogen exposure, from 7 up to 21 days, preferentially increased the PR-A isoform expression.

The expression of PR was also studied at mRNA level using Northern blotting. The [^{32}P]-labelled probe recognised the sequence encoding the C-terminal end of the receptor. Jeltsch and colleagues (1990) have used the same probe and identified three polyadenylation variant mRNA species (sizes 8.2 kb, 4.5 kb and 3.3 kb) and a 5' truncated mRNA (size 4.1 kb). This 5' truncated mRNA does not contain the first ATG codon and thus can only be translated to PR-A protein. In the present study, four PR-specific bands were detected approximately corresponding to the mRNA sizes described by Jeltsch and colleagues (1990). In the immature oviduct, 8.0 kb, 4.4 kb, 3.1 kb mRNAs were strongly expressed while the PR-A-specific 3.9 kb mRNA was barely detected. Estrogen treatment markedly induced the PR-A-specific mRNA. The other 3 mRNA variants were also detected. Notable was that the signal for 8.0 kb mRNA was reduced compared to other mRNA signals.

1.2. Expression and regulation by E2 in breast cancer cell lines

The finding that E2 preferentially increases the expression of PR-A in the chicken oviduct differed from the results reported for breast cancer cell line T-47D. In this cell line estrogen has been shown to preferentially induce PR-B expression (Graham et al. 1995a). We further studied whether estrogen regulates PR isoform expression uniformly in different breast cancer cell lines. Western blot analysis was used to quantitate the relative expression of PR-A (85-94 kDa) and PR-B (116-120 kDa) in three breast cancer cell lines (Clarke and Satyaswaroop 1985, Horwitz et al. 1985). When cultured in phenol red-free medium with 5 % DCC serum, MCF-7, T-47D and ZR-75-1 cells expressed PR isoforms at fairly equal levels. When cells were treated with 10 nM E2 for up to 48 hours differential responses were detected. In T-47D cells both PR isoforms were induced by E2 in a similar way. The PR-A/PR-B ratio of control cells was 1.07 ± 0.35 and when the cells were treated with E2 for 48h the ratio was 1.10 ± 0.45. In MCF-7 cells E2 induced a preferential increase in the PR-A form and expression of both isoforms reached maximal levels in 24 hours. The PR-A/PR-B ratio of control cells was

1.03 ± 0.06 and changed to 1.71± 0.09 after 48 hours' E2 treatment. In ZR-75-1 cells the expression of PR-B was preferentially increased and the maximal expression of PR-B was observed 24 hours after E2 addition. However, PR-A expression increased up to 48 hours. The PR-A/PR-B ratio of ZR-75-1 control cells was 0.90 ± 0.30 and the ratio changed to 0.40 ± 0.03 after 24 hours' E2 treatment.

To test whether this differential regulation of PR isoforms by E2 also occurs at mRNA level, the expression of PR-A and PR-B specific mRNAs was analysed using RPA. Two different hybrids were detected. The longer of them (353 bp) was obtained from transcripts which may encode both isoforms, but are most probably translated to PR-B (Kastner et al. 1990). The shorter hybrid (220/210) was obtained from mRNA which can only be translated to PR-A. The third possible hybrid, also encoding PR-A, was not detected, suggesting either that those transcripts were not expressed or that the expression was too weak to be detected. E2 induced PR mRNA expression in all cell lines in a way similar to that at protein level. In T-47D cells no clear dominance of either hybrid was detected. In MCF-7 cells preferential up-regulation of the 220/210 bp hybrid was detected in three out of four experiments. In ZR-75-1 cells the 353 bp hybrid was induced more in three out of four experiments.

1.3. Regulation by insulin, IGF-1 and cAMP

We further studied whether insulin, IGF-1 or cAMP alter the PR isoform ratio, since these agents have been reported to induce PR expression (Katzenellenbogen and Norman 1990, Cho et al. 1994). All three cell lines were cultured with 5 % DCC serum and treated with 10 nM E2 and insulin (10 ng/ml or 1 μg/ml) or IGF-1 (1 or 100 ng) for 24 hours. Insulin or IGF-1 did not affect the PR protein expression when compared to control cells treated with E2. When cell lines were cultured with 5 % DCC serum and treated with 10 μM 8-bromo-cAMP for 48 hours no changes in expression level could be detected.

2. NUCLEAR RECEPTOR EXPRESSION IN BREAST CANCER CELL LINES (III)

A lot of studies have surveyed the hormonal regulation of ER and PR in breast cancer cell lines. However, much less is known about the expression and regulation of expression of other NRs. In order to provide comparable information the expression levels of NR mRNAs in three commonly used breast cancer cell lines were characterised. The mRNA levels of steroid receptors, VDR, RARs and RXRs were quantitated using RPA. The regulation of expression by insulin, E2 and progesterone was also studied in these cell lines.

2.1. Expression of nuclear receptors

The expression of GR, AR, PR, ERβ, ERα, VDR, RARα, RARβ, RARγ, RXRα, RXRβ and RXRγ mRNAs was analysed in MCF-7, T-47D and ZR-75-1 cells. There were differences in the expression levels, but all cell lines expressed mRNAs for steroid receptors, VDR, RARα, RARγ and RXRα. For example, T-47D cells expressed most abundantly PR and VDR. MCF-

7 cells expressed most abundantly RARγ. The expression of AR and GR was most abundant in ZR-75-1 cells. No expression of RARβ, RXRβ and RXRγ mRNAs was detected.

2.2. Regulation of expression by insulin, E2 or progestin

Regulation of NR expression was analysed using control cells grown without E2 and insulin. Cells were treated with either 1 μg/ml insulin, 1 nM E2 or a combination of both for 24 hours. In addition, samples treated for one week with 1 nM E2 and 1 μg/ml insulin were analysed. Regulation of NR expression by progestin was analysed using cells grown for one week with 1 nM E2 and 1 μg/ml insulin. Cells were treated with either ethanol (control) or 10 nM R5020 for 24 hours. Experiments were repeated 3 to 5 times. The regulation was considered significant if the following criteria were met: the expression was changed 2-fold compared to control and the p values in Mann-Whitney U test were less than 0.05. Table 5 summarises all the statistically significant results. Insulin alone did not significantly regulate the expression of any receptor analysed. Expression of cyclin A was checked for to evaluate that the hormonal treatments had the expected effects on cellular proliferation.

Table 5. Hormonal regulation of NR expression in breast cancer cell lines (mean ± standard deviation).

Cell line	mRNA	Estradiol (24h)	Estradiol+insulin (24h)	Estradiol+insulin (1 week)	R5020 (24h)
MCF-7	PR	↑ 5.83±1.77	↑ 4.37 ± 2.01	↑ 3.19 ± 0.91	↓ 0.52 ± 0.10
	ERβ	↑ 7.55±2.89	↑ 5.39 ± 3.52[1]	↑ 3.01 ± 1.78	↓ 0.55 ± 0.11
	ERα^{RT-PCR}			↓ 0.21 ± 0.14	
	AR			↓ 0.37 ± 0.20	
	GR			↓ 0.48 ± 0.22	
	VDR^{RT-PCR}				↑ 8.02 ± 0.99
T-47D	PR	↑ 3.01±0.80	↑ 2.31 ± 0.93	↑ 3.69 ± 2.31	↓ 0.53 ± 0.16
	ERβ	↑ 3.87±1.50	↑ 2.43 ± 0.94	↑ 4.04 ± 1.95	↓ 0.47 ± 0.28
	ERα^{RT-PCR}	↑ 2.29±0.29	↑ 2.20 ± 0.45	↑ 2.03 ± 0.90	↓ 0.40 ± 0.18
	AR		↓ 0.32 ± 0.29[2]		
	VDR^{RT-PCR}			↓ 0.48 ± 0.08	↑ 4.70 ± 1.11
	RARα		↑ 2.07 ± 0.68		↓ 0.46 ± 0.12
ZR-75-1	PR	↑ 4.06±2.16	↑ 3.14 ± 1.19	↑ 3.37 ± 1.18	↓ 0.36 ± 0.34
	ERβ	↑ 3.95±1.93	↑ 3.07 ± 1.47	↑ 3.05 ± 1.31	↓ 0.33 ± 0.39
	ERα^{RT-PCR}		↓ 0.45 ± 0.17	↓ 0.43 ± 0.19	↓ 0.31 ± 0.19
	RARα	↑ 2.22±0.50	↑ 2.70 ± 0.55	↑ 2.31 ± 0.69	
	RARγ			↑ 2.87 ± 2.40	

↑ denotes for up-regulation. ↓ denotes for down-regulation. $^{RT-PCR}$Result from quantitative RT-PCR. [1]Mean based on two values, third repeat up-regulated but the intensity of the control sample below detection limit. [2]Mean based on two values, not statistically significant.

3. COFACTOR EXPRESSION IN BREAST CANCER CELL LINES (III)

3.1. Expression of cofactors

Large group of cofactors have been shown to affect the transcriptional activity of NRs. The expression levels of some cofactors have been shown to be altered in breast cancer and proposed thus to modulate the response to estrogen (Anzick et al. 1997, Leygue et al. 1999b, Kurebayashi et al. 2000, Murphy et al. 2000, Hudelist et al. 2003). We wanted to compare the mRNA expression levels s of cofactors N-CoR, SMRT, pCAF, CBP, TIF2, AIB1, SRC-1a, SRC-1e and p300 mRNAs in MCF-7, T-47D and ZR-75-1 cell lines. The hybrids encoding N-CoR, SMRT, pCAF, CBP, TIF2, AIB1 and p300 were detected. No expressions of SRC-1a and SRC-1e were detected although the probe yielded proper hybrids when hybridised to total RNA from HL-60 cells. The expression of AIB1 varied most; it had highest expression in MCF-7 cells while T-47D and ZR-75-1 cells had much weaker expression.

3.2. Hormonal regulation of cofactor expression

The same samples were analysed as described in section 2.2. Insulin, E2 or a combination of both did not regulate the expression of N-CoR, AIB1, CBP or pCAF. Neither were they regulated after one week E2 and insulin treatment. Likewise, the mRNAs of N-CoR, AIB1, CBP or pCAF were not regulated after 24 hours' R5020 treatment. The expressions of SMRT, TIF2 and p300 were too low to be quantified accurately.

4. NUCLEAR RECEPTOR EXPRESSION IN HUMAN UTERUS (IV)

NR expression was studied in human uterine tissue in addition to breast cancer cell lines since mammary gland and uterus are the most remarkable target tissues of estrogen and progesterone. In addition, the human uterine tissue provides a model to study the physiological hormonal regulation of NR expression. RPA was used to analyse the mRNA expression levels of different steroid receptors, VDR, RARs and RXRs in human endometrium and myometrium from hysterectomy samples. Six of the samples represented the proliferative phase (6 endometrium and 4 myometrium samples), one represented the interval phase and six the secretory phase. The expression of mRNAs encoding for AR, ERα, ERβ, GR, PR, RARα, RARβ, RARγ and VDR was detected using the RPA. The assay was not sufficiently sensitive to detect RXRα, RXRβ and RXRγ mRNAs, although at protein level these receptors have been detected in the human endometrium (Fukunaka et al. 2001).

To characterise the proliferation status of the samples, the cyclin A mRNA levels were quantitated. As expected, cyclin A mRNA levels were higher in the proliferative phase endometrium samples compared to the secretory phase (Mann-Whitney U test: $p=0.010$). Most myometrium samples from the proliferative phase had higher cyclin A expression levels than the secretory phase samples, but this phenomenon was not statistically significant ($p=0.088$).

The expression levels of ERβ (Mann-Whitney U test, p=0.004), PR (p=0.004), AR (p=0.016) and RARα (p=0.004) were higher in the proliferative-phase endometriums than in the secretory-phase endometrium. Also the expression of ERα (p=0.055) was higher in most of the proliferative-phase endometrial samples than in the secretory phase. Like cyclin A expression, the levels of ERα (p=0.055), ERβ (p=0.088) and PR (p=0.055) mRNAs appeared to be higher in most of the proliferative-phase myometrium samples but this trend was not statistically significant. The expression of GR, RARβ and RARγ mRNAs were not regulated during the menstrual cycle. Since the expression of VDR was hardly detectable the quantitation was confirmed using RT-PCR. All samples expressed VDR, but the expression was not regulated during the menstrual cycle.

The mRNA expression levels of endometrium and myometrium from the same patient were also compared. AR (Wilcoxon, p=0.013, AR_{End}/AR_{Myom} mean ± S.D. 0.66 ± 0.46) and RARβ (p=0.013, $RAR\beta_{End}/RAR\beta_{Myom}$ 0.77 ± 0.25) mRNAs were expressed more abundantly in the myometrium than in the endometrium. Likewise, GR (p=0.021) expression was more abundant in the myometrium than in the endometrium except for two patients from the late secretory phase who had less GR mRNA in the myometrium than in the endometrium (GR_{End}/GR_{Myom} ratios 15.87 and 2.32).

Besides the menstrual cycle-dependent changes, there were substantial individual differences in the expression levels of cyclin A and NRs. Since the expression ratio of cyclin A in the endometrium did not correlate with the expression ratio in the myometrium (Figure 2A) (correlation coefficient r=0.445, p=0.17), Spearman's bivariate correlation analyses were made in order to establish whether the receptor expression would explain this phenomenon. Patient-to patient variation in the $cycA_{End}/cycA_{Myom}$ ratio was detected which could not be explained by menstrual cycle phase (Figure 2B). $CycA_{End}/cycA_{Myom}$ correlated with the endometrium vs. myometrium ratios of AR (r=0.809, p=0.003), ERα (r=0.838, p=0.001), ERβ (r=0.941, p<0.001), PR (r=0.992, p<0.001) and RARβ (r=0.627, p=0.039). The correlations with ERα and PR are shown in Figures 2C and D.

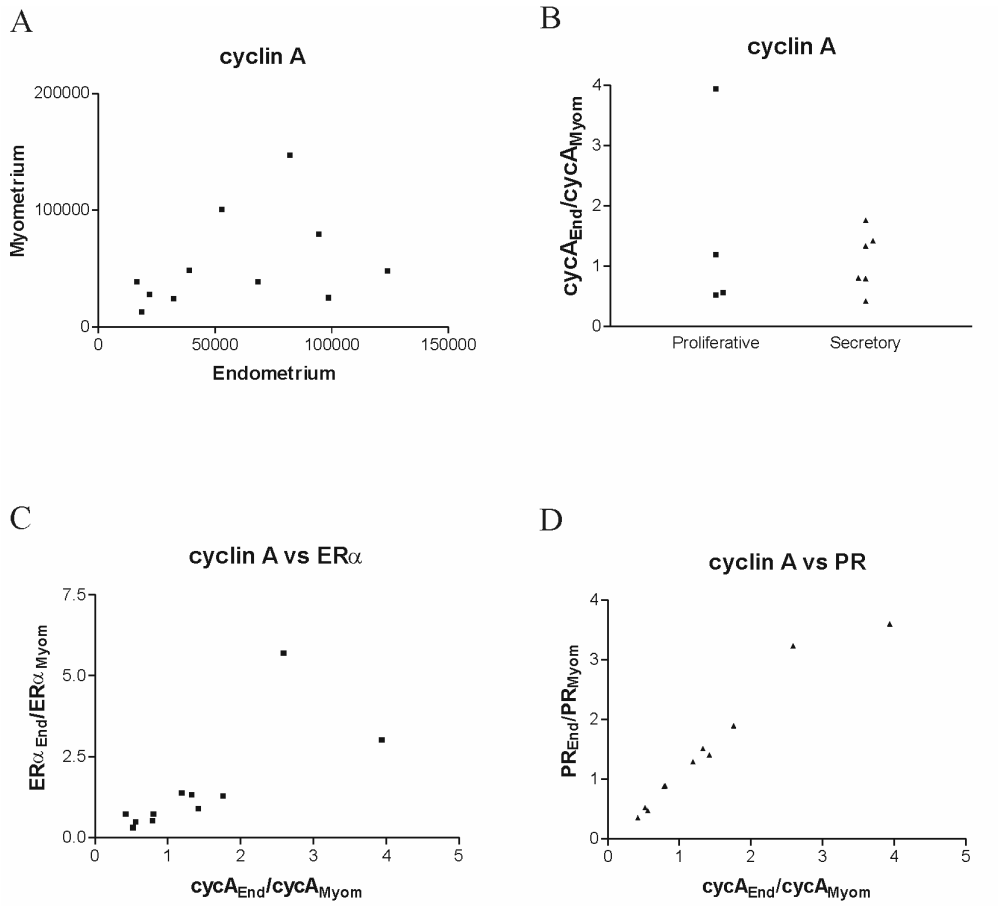

Figure 2. (**A**) Expression levels of cyclin A in the endometrium did not correlate with the expression levels in the myometrium. (**B**) $CycA_{End}/cycA_{Myom}$ in the proliferative and secretory phase samples. $CycA_{End}/cycA_{Myom}$ correlated with the same ratios of ERα (**C**) and PR (**D**).

5. COFACTOR EXPRESSION IN HUMAN UTERUS (IV)

Due to the essential role of cofactors on the NR transcriptional activity RPA was also used to analyse the mRNA expression levels of different cofactors from the uterine samples. N-CoR, SMRT, pCAF, CBP, TIF2, AIB1and p300 mRNAs were expressed in all samples in both the endometrium and myometrium. Expression levels of N-CoR, pCAF, AIB1 and p300 did not differ between the proliferative and secretory phases. No statistically significant trends were observed in the endometrium vs. myometrium ratios of N-CoR, pCAF, AIB1 and p300. Due to the detection limit for RPA, the expression levels of SMRT, CBP and TIF2 were not reliably quantified. No expression whatsoever of SRC-1e and SRC-1a mRNAs was detected although SRC-1 expression in the endometrium has been reported (Gregory et al. 2002, Wieser et al. 2002, Shiozawa et al. 2003).

The expression levels of N-CoR, pCAF, AIB1 and p300 appeared to vary between different patients regardless of their menstrual phase. Spearman's bivariate correlation analyses were made in order to test whether the $cycA_{End}/cycA_{Myom}$ ratio was associated with the expression levels of cofactors. As with steroid receptors, the $cycA_{End}/cycA_{Myom}$ ratio correlated with the endometrium vs. myometrium ratios of corepressor N-CoR ($r=0.664$, $p=0.026$) and coactivators AIB1 ($r=0.682$, $p=0.021$) and p300 ($r=0.729$, $p=0.011$).

DISCUSSION

1. METHODOLOGICAL CONSIDERATIONS

Three mRNA detection methods were used for the relative quantification of mRNA expression. The expression of cPR-specific mRNAs was analysed using traditional Northern blot analysis, since cPR is expressed as several different transcripts (Gronemeyer et al. 1987, Jeltsch et al. 1990). The benefit of this method is that all transcripts containing the sequence the probe is able to hybridise will be detected according to their size, if expressed abundantly enough. This is beneficial when analysing the expression of genes whose transcription and transcripts have not yet been thoroughly characterised. In addition, Northern blot does not require expensive equipment. The sensitivity of this method is poor in comparison with RT-PCR. However, if carefully designed it is nonetheless suitable for quantitation of mRNA.

The RPA method was chosen to quantify the mRNA expression of human PR isoforms, NRs and cofactors. As a method it is more sensitive than Northern blot, but cannot compete with the sensitivity of RT-PCR. Benefits of RPA include high capacity, since in a single RPA reaction mRNA transcripts of several different genes can be detected simultaneously. In addition, with proper probe designing this method is suitable for characterising transcripts of the same gene which vary in length.

Due to low signal levels ERα and VDR mRNA levels in breast cancer cell lines and VDR mRNA level in uterine samples were also analysed using real-time RT-PCR. This method offers the most sensitive means of analysing mRNA expression. In real-time RT-PCR quantitation is based on measurement of fluorescent dyes after every cycle. The detection method used in all analyses was SYBR Green I. The fluorescence of this dye is enhanced upon binding to the minor groove of double-stranded DNA. Thus the amount of fluorescence increases upon an increase in PCR product. The quantitation is based on a value called the crossing point (CP), which is the point at which the fluorescence of each sample, rises appreciably above the background fluorescence and the reaction enters exponential amplification. The fact that the measurement is made during the early cycles of the assay provides several benefits. For example, none of the reaction components is limiting at this point, the ongoing loss of polymerase activity and accumulation of substances which inhibit the PCR reaction are less marked than at the end of the exponential phase.

2. HORMONAL REGULATION OF PR ISOFORM EXPRESSION

Estrogens commonly increase PR expression in reproductive tissues. Although in the normal mammary gland PR expression is not regulated during the menstrual cycle, it is induced by E2 *in vitro* (Soderqvist et al. 1993, Clarke et al. 1997a, Mote et al. 2002, Zhuang et al. 2003). Whether the expressions of both PR isoforms are similarly regulated is less clear. We elucidated the E2-dependent up-regulation in two well-defined model systems, the chicken oviduct and breast cancer cell lines. Differential regulation of PR isoforms by E2 was found in both these models.

The PR expression in the immature chicken is thought to be constitutive and not induced by estrogen (Tanabe et al. 1979). We demonstrated here that this constitutive expression comprises preferential PR-B expression. Estrogen was found to induce the expression of both isoforms, but the expression of PR-A was preferentially induced. The expression ratio altered after 7 days of estrogen treatment to PR-A predominance. The preferential change was also seen at mRNA level. This may be due to stabilisation of PR-A-specific mRNA, since estrogen has been held to regulate PR expression in the chicken oviduct at post-transcriptional level (Turcotte et al. 1991). Preferential up-regulation of PR-A protein in the chicken oviduct has also been shown in newly-hatched chicks treated with follicle-stimulating hormone or luteinising hormone during embryonic development (Gonzalez-Moran and Camacho-Arroyo 2003). This up-regulation is probably mediated indirectly by induction of estrogen synthesis in the ovary. Estrogen-induced cytodifferentiation is required for the induction of egg white proteins by progesterone (Palmiter and Wrenn 1971, Moen and Palmiter 1980). *In vitro* studies have suggested that the synthesis of egg white protein ovalbumin may be PR-A-mediated or PR-B-repressed (Tora et al. 1988). Differential regulation of PR isoforms by E2 has also been found in the rat forebrain; estrogen induces PR-B mRNA expression in preferentially the preoptic area, PR-A mRNA again, in the hippocampus (Camacho-Arroyo et al. 1998). In the hypothalamus both PR forms were induced similarly. Using dual antibody staining, Mote and colleagues (1999) have studied the isoform-specific regulation of PR expression in the human endometrium during the menstrual cycle. They report that fluctuations in estrogen and progesterone concentrations differentially regulate PR isoform expression in glandular epithelium and stroma. They confirm that cell type-specific regulation of human PR isoform expression occurs in normal physiological conditions.

Preferential PR-B induction by E2 had previously been shown in T-47D cells (Graham et al. 1995a). We further studied whether this phenomenon is characteristic of other PR-positive breast cancer cell lines. We found that E2 induces PR isoform expression in a cell line-specific manner. In MCF-7 cells E2 was found to preferentially induce the expression of PR-A, while in ZR-75-1 cells PR-B was preferentially up-regulated. We could not confirm the result that E2 preferentially stimulates PR-B in T-47D cells. In our cell culture system, E2 induced the expression of both isoforms similarly in T-47D cells. However, these cell lines were not grown under the same selective pressures. Our cells were grown in steroid depleted medium for only one week while Graham and colleagues (1995a) used steroid-depleted medium for several weeks to increase the sensitivity of cells to E2. Like cancer cells in general, the T-47D cell line is genetically unstable and easily forms colonies which differ in their hormonal response (Reddel et al. 1988, Graham et al. 1989). Differential receptor regulation by E2 in breast cancer cell lines has been previously shown for ERα (Saceda et al. 1988, Read et al. 1989, Clayton et al. 1997). In addition to E2, cAMP, insulin and IGF-1 have been shown to induce PR expression in MCF-7 cells under low-serum-containing medium or in serum-free medium (Katzenellenbogen and Norman 1990, Cho et al. 1994). In the present study, no induction of PR was seen in any cell line studied. This may be due to the higher serum concentration (5%) used. Serum may contain factors which repress basal PR expression and suppress the stimulatory effects of cAMP and IGF-1, while E2 induces PR expression more effectively in the presence of a high serum concentration (5%) (Katzenellenbogen and Norman 1990, Cho et al. 1994).

Breast tumors exhibit wide variation in their PR-A/PR-B ratios, while in normal mammary gland the PR isoforms are expressed at similar levels (Graham et al. 1995b, Mote et al. 2002). Adjacent cells of the normal mammary gland express homogeneous PR isoform ratios, while premalignant and malignant lesions evince heterogeneity in this ratio (Mote et al. 2002). The

authors suggest that the loss of co-ordinated expression of PR isoforms is an early event in the development of breast tumors. The dominance of PR-A expression may affect the breast tumor response to endocrine therapy, since PR isoforms differ in their transcriptional activities; for example PR-A may repress the transcriptional activity of PR-B and AR (Vegeto et al. 1993, Chalbos and Galtier 1994, McDonnell et al. 1994, Kraus et al. 1995). High PR-A expression compared to PR-B expression may lead to altered gene expression. Indeed T-47D cells engineered to express solely either PR-B or PR-A exhibit differential gene expression profiles (Richer et al. 2002). Based on gene expression profiles the authors propose that three different types of progesterone-responsive genes exist. One group is regulated by PR-A, one by PR-B and one group consists of genes which PR-B induces while PR-A diminishes this induction. Consequently, the PR-A/PR-B ratio of breast tumors may be critical for endocrine therapy. Several studies have reported more frequent preferential expression of PR-A than PR-B in breast tumors (Graham et al. 1995b, Bamberger et al. 2000, Mote et al. 2002). Progesterone induces cancer-associated cytoskeletal changes only in PR-A over-expressing breast cancer cells (McGowan et al. 2003). Moreover, in response to progestin PR-A over-expressing breast cancer cells detach from the monolayer cultures (McGowan and Clarke 1999). Thus preferentially PR-A-expressing tumors may be associated with more invasive phenotypes. In addition to breast cancer, preferential PR-A expression has been found in endometrial cancer (Arnett-Mansfield et al. 2001, Sasaki et al. 2001). Endometrial cell culture models suggest that PR-B has greater influence on progesterone-induced growth inhibition and PR-A expression may be associated with less fully differentiated and more invasive phenotypes (Kumar et al. 1998, Dai et al. 2002, Smid-Koopman et al. 2003). An aberrant PR isoform ratio in general may be linked to higher-grade endometrial tumors (Arnett-Mansfield et al. 2001).

3. DIFFERENTIAL EXPRESSION OF NUCLEAR RECEPTORS

One aspect of cell or tissue-specific responses of estrogen and progesterone is the cross-talk with other NRs. In addition to regulating the expression levels of their own receptors, E2 and progesterone regulate the levels of other NRs. Some ligands may not bind specifically to one receptor but have cross-reactivity with other receptors as well. For example, PR antagonist RU486 may bind to AR, GR, and ERβ (Cadepond et al. 1997, Zou et al. 1999). The receptor levels thus may have influence when considering endocrine treatments of breast cancer. Broader knowledge of NR expression would help in the development of new selective NR modulators such as SERMs. RPA analysis was used to compare the mRNA levels of steroid receptors, VDR, RARs and RXRs in different breast cancer cell lines and in uterine samples of different individuals.

The MCF-7, T-47D and ZR-75-1 breast cancer cell lines expressed mRNAs for GR, AR, PR, ERα, ERβ, VDR, RARα, RARγ and RXRα. Variations in their expression levels were observed when comparing the control samples of each cell line, which would imply that these cell lines may respond to endocrine treatment differently.

The expression of NR mRNAs was also studied in the human endometrium and myometrium. AR, ERα, ERβ, GR, PR, RARα, RARβ, RARγ and VDR mRNAs were expressed in both endometrium and myometrium. Although these two tissues are functionally and structurally

different, NRs transcripts were expressed in similar manner in both except for AR, GR and RARβ mRNA, which in most cases were expressed more abundantly in the myometrium than in the endometrium. A novel finding of this study was also that VDR is expressed in normal human uterine tissue.

Patient-to-patient variations in the expression levels of NRs could be detected when comparing endometrium and myometrium samples within the same cycle phase. Cyclin A expression is related to the proliferation status of the uterine tissue. As expected, cyclin A was clearly regulated in the endometrium samples during the menstrual cycle; higher expression levels were detected in the proliferative than in the secretory phase samples. Patient-to-patient variation was also observed in cyclin A mRNA levels. Noteworthy was that although presumably both endometrium and myometrium are predisposed to the same hormone concentrations, the cyclin A mRNA level in the endometrium did not correlate with the expression level in the corresponding myometrium. When calculating patients' cyclin A endometrium vs. myometrium ratio marked variation prevailed which could not be explained by menstrual cycle phases. This finding was further studied by comparing each patient's $cycA_{End}/cycA_{Myom}$ to the same ratios of NRs. Strong correlations were found between $cycA_{End}/cycA_{Myom}$ and the ratios of AR, ERα, ERβ and PR. Interestingly, $cycA_{End}/cycA_{Myom}$ ratio correlated with the same ratio of RARβ, although RARβ was not found to be regulated during the menstrual cycle in the endometrium. Altogether this finding would suggest that the proliferation status of the endometrium and myometrium is associated with the expression level of AR, ERα, ERβ, PR and RARβ.

4. HORMONAL REGULATION OF NUCLEAR RECEPTOR EXPRESSION

Mammary gland and uterus are the two most notable target tissues of estrogen and progesterone. Due to difficulties of obtaining samples of normal mammary gland breast cancer cell lines were used as an *in vitro* model system to characterise how estrogen and progesterone regulate the mRNA expression levels of steroid receptors, VDR, RARs and RXRs. The endometrial and myometrial samples from hysterectomies were used as a model of physiological regulation.

As with the PR isoforms, a cell line-specific regulation pattern was also observed for other NRs after E2 treatment. Likewise progestin differently regulated some of the NRs. Such differential regulation has already been reported for ERα expression. The ERα mRNA levels are decreased in MCF-7 cells and increased in T-47D and ZR-75-1 cells after E2 treatment (Saceda et al. 1988, Read et al. 1989, Clayton et al. 1997). In our experimental conditions E2 increased ERα mRNA levels in T-47D cells and a trend towards decreased expression was observed in MCF-7 cells. However, in ZR-75-1 cells E2 reduced the ERα mRNA level, which is contradictory to previous results (Clayton et al. 1997). This may be due to different cell clones, since as noted above, breast cancer cells are genetically somewhat unstable and easily form colonies differing in their hormonal response. Cell line-specific regulation of AR, RARα, RARγ and VDR by E2 was also observed. RARα mRNA levels were decreased only in T-47D cells after progestin treatment, as previously shown (Roman et al. 1992). VDR mRNA levels were increased several-fold by progestin treatment in MCF-7 and T47D cells and a minor increase was observed in ZR-75-1 cells. Thus, in addition to regulating their own receptor levels, estrogens and progestins regulate other NR levels which are involved in

controlling cell proliferation and differentiation. The mechanisms behind the cell line-specific hormonal regulation call for further analysis. However, our results imply that tumors from different individuals or even different clones of the same tumor might respond differentially to endocrine therapy.

In the endometrium AR, ERα, ERβ and PR mRNAs were more abundantly expressed in the proliferative than in the secretory phase, as earlier reported (Lau et al. 1996, Matsuzaki et al. 1999, Matsuzaki et al. 2000, Slayden et al. 2001). RARα was also expressed more abundantly during the proliferative phase. Previously Kumarendran and colleagues (1996) have reported that mRNAs for RARs are not markedly regulated during the menstrual cycle, although Fukunaka and colleagues (2001) have found higher protein expression levels of all RARs during the proliferative compared to the secretory phase. In the myometrium none of the NR mRNAs studied was regulated statistically significantly, although ERα, ERβ and PR mRNA levels were higher in most of the proliferative phase samples compared to the secretory phase samples. These results, indicating the myometrium as a more insensitive tissue in response to hormonal fluctuations, are in accord with those of a study by Noe and colleagues (1999). They reported that in the myometrium ERα and PR levels are regulated in the layer adjacent to the endometrium in an endometrial-like pattern during the menstrual cycle, while expression patterns in the outer part of the myometrium are more stable. In the present study the myometrial sample was not separated into different layers and the myometrial samples may contain both the layer reactive for cyclical hormonal fluctuations as well as the hormonally less active part.

When comparing the hormonal regulation of NR mRNA expression in breast cancer cells and in the human endometrium the expected similarities were seen, but also tissue-specific hormonal responses. This comparison cannot be made without keeping in mind that the hormonal regulation of the endometrium is based on physiological changes, thought to be due to increased levels of estrogen in the proliferative phase and increased levels of progesterone and decreasing levels of estrogen in the secretory phase. Thus in general, estrogens seemed to up-regulate and progestins to reduce PR and ERβ mRNA expression in both breast cancer cells and the human endometrium. ERα mRNA expression in the endometrium exhibited a trend towards decreased expression in the secretory phase. In breast cancer cells the ERα mRNA levels were either increased or decreased by E2 depending on the cell line. Progestin treatment reduced ERα expression in T-47D cells and in ZR-75-1 cells, while in MCF-7 cells regulation was not observed at all. Increased expression of AR was detected in the proliferative phase endometrium compared to the secretory phase. Progestin treatment or short-term E2 treatment did not regulate AR mRNA levels in any breast cancer cell lines studied, but longer-term E2 treatment reduced the expression in MCF-7 cells. A higher expression level of RARα mRNA was observed in the proliferative phase endometrium. E2 also seemed to increase RARα expression in T-47D and ZR-75-1 cells. Long-term E2 treatment induced RARγ mRNA expression in ZR-75-1 cells, while no regulation was observed in other cell lines or in endometrium samples.

5. COFACTOR EXPRESSION

Cell and tissue type differences exist in the expression levels of cofactors (Misiti et al. 1998, Shang and Brown 2002). In the present work two different types of tissues were used to determine cofactor expression and regulation of expression by estrogen and progesterone. In the breast cancer cell lines, the AIB1 mRNA level varied most when comparing different cell lines. It was expressed most abundantly in MCF-7 cells, previously described as containing AIB1 gene amplification (Anzick et al. 1997). Though no regulation of cofactor expression was noted during the menstrual cycle, individual cofactor mRNA expression levels varied markedly. Since the relative expression levels of cofactors determine whether the ligand acts as agonist or antagonist, the expression levels may affect the individual response to endocrine therapy (Lavinsky et al. 1998, Wagner et al. 1998, Liu et al. 2002, Shang and Brown 2002).

Levels of cofactors seemed not to be regulated either in breast cancer cell lines by estrogens and progestins or in the human uterus during the menstrual cycle. This is in line with other studies reporting fairly stable expression of different cofactors in breast cancer cell lines after estrogen treatment as well as in the human endometrium during the menstrual cycle (Thenot et al. 1999, Gregory et al. 2002, Shiozawa et al. 2003). In contrast, the protein expression levels of cofactors AIB1, SRC-1 and N-CoR may be altered during the cycle in the endometrium, although reports on N-CoR and SRC-1 expression are partly ambiguous (Gregory et al. 2002, Wieser et al. 2002, Shiozawa et al. 2003). A further novel finding in this present study was that also the myometrium expresses cofactors N-CoR, SMRT, pCAF, CBP, TIF2, AIB1 and p300 similarly to the endometrium.

The finding that cofactor levels varied individually was further studied by comparing each patient's $cycA_{End}/cycA_{Myom}$ to the same ratios of cofactors. Interestingly, the $cycA_{End}/cycA_{Myom}$ correlated with the same ratios of N-CoR, AIB1 and p300, although the expression levels of these cofactors were not regulated during the menstrual cycle. This correlation may indicate that the proliferation status of the endometrium and myometrium is associated with the expression levels of N-CoR, AIB1 and p300.

6. CELL-OR TISSUE-SPECIFIC EFFECTS OF NUCLEAR RECEPTOR LIGANDS

The present study consists of three different types of female reproductive tissues. We found that they all had complexities in their hormonal response to E2 and progesterone. These complexities consisted of PR isoform expression, NR and cofactor expression and hormonal regulation of NR expression. These results augment the knowledge about ligand-specific cellular or tissue responses. These responses are dependent on several factors in addition to pharmacokinetic factors such as metabolism and bioavailability (Figure 3).

The activity depends on the target gene regulatory regions. NRs may bind directly to DNA through cognate HREs or bind DNA indirectly through binding of other transcription factors. The activity of SERMs has been shown to vary depending on whether it binds directly to ERE or indirectly to DNA through AP-1 and transcription factor Sp1 (Webb et al. 1995, Paech et al. 1997, Zou et al. 1999).

Furthermore the ligand-specific cellular or tissue response is dependent on the expression of cognate receptors. ERα and ERβ bind partly the same ligands and ERβ may modulate the activity of ERα (Hall and McDonnell 1999, Weihua et al. 2000). Some ligands exhibit cross-reactivity with several steroid receptors; in particular antagonists of PR may bind to AR, GR and ERβ, as shown for RU486 (Cadepond et al. 1997, Zou et al. 1999). The results of the present study imply that levels of NRs in different breast cancer cell lines and in the human uterus of different individuals differ. Due to this variation, breast cancers in different individuals or uterine tissue may respond in a selective fashion to endocrine treatment.

Some of the NRs are expressed in different isoforms. These may differ in their transcriptional activities and may have diverse physiological roles, as shown for PR-A and PR-B (Mulac-Jericevic et al. 2000, Mulac-Jericevic et al. 2003). As noted above, PR isoform expression has been reported to be abnormal in breast and endometrial cancers. The isoform expression may be associated with antiestrogen resistance, since estrogen-dependent human breast xenografts expressing solely PR-A were growth-inhibited by tamoxifen while PR-B-expressing xenografts were unresponsive (Sartorius et al. 2003). The results of the present study suggest that PR isoforms are regulated by E2 differently in different breast cancer cell lines. This selective regulation may be of physiological relevance in the treatment of breast cancer.

A ligand-bound receptor adopts a ligand-specific conformation and recruits cofactors. Which cofactors are recruited depends on the ligand but also the HRE which the receptor binds to affects the conformation and cofactor recruitment (Brzozowski et al. 1997, Shiau et al. 1998, Takeyama et al. 1999, Loven et al. 2001, Wong et al. 2001, Wood et al. 2001, Hall et al. 2002, Yi et al. 2002). The level of cofactors has been thought to regulate the agonist/antagonist activity of selective NR modulators (Lavinsky et al. 1998, Wagner et al. 1998, Liu et al. 2002, Shang and Brown 2002). The results presented here and elsewhere suggest that the expression levels of cofactors in general are not hormonally regulated (Thenot et al. 1999, Nephew et al. 2000, Gregory et al. 2002, Shiozawa et al. 2003). Cofactor expression is cell-type dependent and is probably related to cell differentiation. Altered expression of several cofactors has been proposed in breast cancer (Anzick et al. 1997, Leygue et al. 1999b, Kurebayashi et al. 2000, Murphy et al. 2000, Hudelist et al. 2003). For example, AIB1 expression is increased in human breast carcinomas and the high expression level has been found to be associated with high tumor grade (Bouras et al. 2001, Hudelist et al. 2003). SRC-1 expression may be lower in breast tumors than in the normal breast and the low expression may be associated with tamoxifen resistance (Berns et al. 1998). Our results imply that in breast cancer cell lines and even in normal uterine tissues individual differences prevail in the expression levels of cofactors. This individual variation may bring an additional level of complexity to the biological response to certain selective NR modulators.

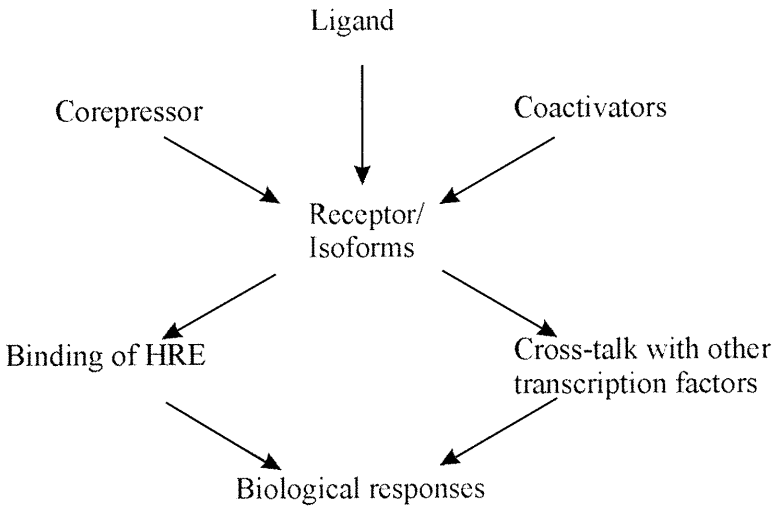

Figure 3. Cell or tissue specific biological responses of NR ligands are dependent on several factors.

SUMMARY AND CONCLUSIONS

The PR isoform expression and regulation by E2 were studied in the chicken oviduct and in breast cancer cell lines at both mRNA and protein level. The expression in the immature chicken oviduct was found to consist in preferential PR-B protein expression, although PR-A was also detected. Estrogen treatment, which is known to induce growth and differentiation in the oviduct, altered the PR isoform ratios by preferentially increasing the expression of PR-A protein as against expression of PR-B. This phenomenon was also noted at mRNA level. Considering the different biological activities of these receptor isoforms the results of this study would suggest that progesterone response in the chicken oviduct is dependent on the hormonal and developmental status of the organ.

In breast cancer cell lines E2 increases the expression of PR. In the present work, the regulation of PR isoform expression by E2 was characterised in three different breast cancer cell lines both at mRNA and at protein level. Differential regulation of PR-A and PR-B was observed when comparing the cell lines. In T-47D cells both isoforms were increased similarly upon E2 treatment, in MCF-7 cells PR-A was preferentially increased, while in ZR-75-1 cells the preferentially increased isoform was PR-B. This regulation was observed at both protein and mRNA level. The results of this study suggest that since E2 regulates PR isoform expression in distinct manner in different breast cancer cell lines, breast tumors in general may evince differential regulation of PR isoform expression and may thus have altered responses to hormonal therapy.

In addition to the differential regulation of PR isoforms, MCF-7, T-47D and ZR-75-1 breast cancer cell lines were found to differ in their expression levels of NR mRNAs and in the regulation of NR mRNA levels by E2 and progestin. These findings further indicate that breast tumors in general might differ in their responses to endocrine therapy.

NR expression during the menstrual cycle was characterised in the human endometrium and myometrium. Expression of AR, ERα, ERβ, GR, PR, RARα, RARβ, RARγ and VDR mRNAs were detected in both endometrium and myometrium. NR transcripts were expressed in similar manner in both tissue types except for AR, GR and RARβ mRNA, which in most cases were expressed more abundantly in the myometrium than in the endometrium. In the endometrium the AR, ERα, ERβ, PR and RARα mRNA levels were higher in the proliferative phase when compared to secretory phase samples. In the myometrium no statistically significant changes were observed; however, most of the proliferative samples evinced higher expression of ERα, ERβ and PR than the secretory phase samples.

An interesting finding was the patient-to-patient variation in the expression levels of cyclin A, NRs and cofactors, which occurred both in the endometrium and in the myometrium. Furthermore, the cyclin A expression level in the endometrium did not correlate with the expression level in the corresponding myometrium. To test which factors could explain differences in proliferation, the endometrium vs. myometrium ratio of cyclin A was compared to the same ratios of NRs and cofactors and the ratios of AR, ERα, ERβ, PR, RARβ, N-CoR, AIB1 and p300 was found to correlate with the cyclin A ratio. The expression levels of these receptors and cofactors may thus be associated with the hormone sensitivity of endometrium and myometrium.

Both E2 and progestin treatments appeared not to regulate the expression of AIB1, pCAF, CBP, and N-CoR in breast cancer cell lines. Furthermore, cofactor mRNA expression was evidently not regulated during the menstrual cycle in the human endometrium and myometrium. The myometrium expressed the mRNAs encoding N-CoR, SMRT, pCAF, CBP, TIF2, AIB1 and p300 similarly to the endometrium.

Altogether the present study consists of three different type of female reproductive tissues which all have complexities in their hormonal response to E2 and progesterone. These complexities consist of several aspects, PR isoform expression, NR and cofactor expression and hormonal regulation of NR expression. The results broaden out the knowledge of NR and cofactor expression in these well-studied models. Furthermore, they suggest that individual variation exist in the expression levels of NRs and cofactors. Due to these variations these tissues may respond in an individual fashion to endocrine treatment, a circumstance to be reckoned with in when developing novel therapeutic ligands.

ACKNOWLEDGEMENTS

This study was carried out at the Departments of Cell Biology and Anatomy, Medical School, University of Tampere as part of projects in the Graduate School of Steroid Research and the Tampere Graduate School of Biomedicine.

I wish to express my deepest gratitude to my supervisor Professor Timo Ylikomi, , M.D., Ph.D., for patient guidance and encouragement during these years. His broad knowledge on all aspects of research and enthusiastic attitude towards science is deeply acknowledged.

I am very grateful to my second supervisor Professor Pentti Tuohimaa, M.D., Ph.D., for offering me the possibility to start working in his group. His kind support and interest on my research has been invaluable help during these years.

The official reviewers of my thesis, Docent Veli Isomaa, Ph.D., and Docent Sari Mäkelä, M.D., Ph.D., are thanked for their careful review and valuable criticism.

I wish to thank Heimo Syvälä, Ph.D., for his patient help throughout the years. His deep interest in science and encouraging attitude were especially important during the first years of my study. I want to thank Susanna Miettinen, M.Sc., for the fruitful collaboration and friendship. Her enthusiasm towards research is greatly acknowledged. I wish to thank Tommi Manninen, B.Sc., and Sami Purmonen, M.Sc., for their endless support and help. I had so much fun working with them due to their humorous way of thinking and continuous flow of jokes about everyday life and lab work. I thank Merja Bläuer, Ph.D. and Ya-Hua Zhuang, Ph.D., for sharing their scientific expertise whenever I needed help.

I owe my sincere thanks to my other co-authors, Lucia Altucci, M.D., Ph.D., Emmanuelle Wilhelm, Ph.D., Docent Paula Martikainen, M.D., Ph.D., Eija Tomás, M.D., Ph.D., and Professor Pentti Heinonen, M.D., Ph.D., for their contribution to this work. Also I wish to thank Heini Huhtala, M.Sc., for helping me with the statistical analyses.

I am very grateful to Ms. Taina Eskola and Ms. Hilkka Mäkinen for helping me with so many ways. Their skilful technical help, their warm friendship and endless encouragement were invaluable to this work.

I want to express my warm thanks to my all co-workers during these years at the Medical School for their friendship, help and collaboration. Especially, I wish to thank Professor Markku Pelto-Huikko, M.D., Ph.D., Tytti Ahola, M.D., Merja Ahonen, Ph.D., Marjo Aitola, M.D., Ph.D., Marjaana Haverinen, M.Sc., Pirjo Koikkalainen, M.Sc., Ilkka Laaksi, M.D., Yan-Ru Lou, M.Sc., Susanna Pasanen, M.D, Ph.D., Satu Passinen, M.D., Pasi Pennanen, M.Sc., Riina Sarkanen, M.Sc., Nanna Sarvilinna, M.D., Pekka Vilja, Ph.D. and Johanna Väliaho, M.Sc. The help and warm support of Ms. Arja Ahola, Ms. Tarja Arvola, Ms. Marja Eloranta, Ms. Janette Hinkka, Ms. Mirja Hyppönen, Ms. Ullamargit Jukarainen and Ms. Maarit Pehkonen are greatly appreciated.

Finally, my deepest thanks go to my dear family and all my friends. Especially, I am gratitude to Teemu for his love and patient encouragement. I thank my brother Panu and his wife Auli for their endless support and friendship. I owe my deepest gratitude to my mother Kirsti and father Matti for their loving support. Without them this project would have not been achievable.

I wish to thank Robert MacGilleon, M. A., for revising the language of this thesis. This study was financially supported by grants from the Medical Research Fund of Tampere University Hospital, the Pirkanmaa Cancer Society, the University of Tampere, the Scientific Foundation of the City of Tampere, European Community (PL 963433) and TEKES Drug 2000. Permissions to reproduce the original articles were kindly granted by Elsevier Science (I, II, IV) and the Society of the European Journal of Endocrinology (III).

Tampere, January 2004

Annika Vienonen

REFERENCES

Akahira J, Inoue T, Suzuki T, Ito K, Konno R, Sato S, Moriya T, Okamura K, Yajima A and Sasano H (2000): Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies. Br J Cancer 83:1488-1494.

Alen P, Claessens F, Verhoeven G, Rombauts W and Peeters B (1999): The androgen receptor amino-terminal domain plays a key role in p160 coactivator-stimulated gene transcription. Mol Cell Biol 19:6085-6097.

Amso NN, Crow J and Shaw RW (1994): Comparative immunohistochemical study of oestrogen and progesterone receptors in the fallopian tube and uterus at different stages of the menstrual cycle and the menopause. Hum Reprod 9:1027-1037.

An J, Ribeiro RC, Webb P, Gustafsson JA, Kushner PJ, Baxter JD and Leitman DC (1999): Estradiol repression of tumor necrosis factor-alpha transcription requires estrogen receptor activation function-2 and is enhanced by coactivators. Proc Natl Acad Sci U S A 96:15161-15166.

Anandappa SY, Sibson R, Platt-Higgins A, Winstanley JH, Rudland PS and Barraclough R (2000): Variant estrogen receptor alpha mRNAs in human breast cancer specimens. Int J Cancer 88:209-216.

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM and Meltzer PS (1997): AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965-968.

Apparao KB, Lovely LP, Gui Y, Lininger RA and Lessey BA (2002): Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod 66:297-304.

Aranda A and Pascual A (2001): Nuclear hormone receptors and gene expression. Physiol Rev 81:1269-1304.

Arnett-Mansfield RL, deFazio A, Wain GV, Jaworski RC, Byth K, Mote PA and Clarke CL (2001): Relative expression of progesterone receptors A and B in endometrioid cancers of the endometrium. Cancer Res 61:4576-4582.

Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J and O'Malley BW (1988): Cloning and expression of full-length cDNA encoding human vitamin D receptor. Proc Natl Acad Sci U S A 85:3294-3298.

Bamberger AM, Milde-Langosch K, Schulte HM and Loning T (2000): Progesterone receptor isoforms, PR-B and PR-A, in breast cancer: correlations with clinicopathologic tumor parameters and expression of AP-1 factors. Horm Res 54:32-37.

Bamberger AM, Milde-Langosch K, Loning T and Bamberger CM (2001): The glucocorticoid receptor is specifically expressed in the stromal compartment of the human endometrium. J Clin Endocrinol Metab 86:5071-5074.

Bamberger CM, Bamberger AM, de Castro M and Chrousos GP (1995): Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. J Clin Invest 95:2435-2441.

Bannister AJ and Kouzarides T (1996): The CBP co-activator is a histone acetyltransferase. Nature 384:641-643.

Bardou VJ, Arpino G, Elledge RM, Osborne CK and Clark GM (2003): Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol 21:1973-1979.

Behbakht K, DeGeest K, Turyk ME and Wilbanks GD (1996): All-trans-retinoic acid inhibits the proliferation of cell lines derived from human cervical neoplasia. Gynecol Oncol 61:31-39.

Belandia B and Parker MG (2003): Nuclear receptors: a rendezvous for chromatin remodeling factors. Cell 114:277-280.

Beral V (2003): Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 362:419-427.

Berman JR, Almeida FG, Jolin J, Raz S, Chaudhuri G and Gonzalez-Cadavid NF (2003): Correlation of androgen receptors, aromatase, and 5-alpha reductase in the human vagina with menopausal status. Fertil Steril 79:925-931.

Berns EM, van Staveren IL, Klijn JG and Foekens JA (1998): Predictive value of SRC-1 for tamoxifen response of recurrent breast cancer. Breast Cancer Res Treat 48:87-92.

Bevan CL, Hoare S, Claessens F, Heery DM and Parker MG (1999): The AF1 and AF2 domains of the androgen receptor interact with distinct regions of SRC1. Mol Cell Biol 19:8383-8392.

Bieche I, Parfait B, Laurendeau I, Girault I, Vidaud M and Lidereau R (2001): Quantification of estrogen receptor alpha and beta expression in sporadic breast cancer. Oncogene 20:8109-8115.

Black BE, Holaska JM, Rastinejad F and Paschal BM (2001): DNA binding domains in diverse nuclear receptors function as nuclear export signals. Curr Biol 11:1749-1758.

Blanco JC, Minucci S, Lu J, Yang XJ, Walker KK, Chen H, Evans RM, Nakatani Y and Ozato K (1998): The histone acetylase PCAF is a nuclear receptor coactivator. Genes Dev 12:1638-1651.

Bocquel MT, Kumar V, Stricker C, Chambon P and Gronemeyer H (1989): The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. Nucleic Acids Res 17:2581-2595.

Bouras T, Southey MC and Venter DJ (2001): Overexpression of the steroid receptor coactivator AIB1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/neu. Cancer Res 61:903-907.

Bourguet W, Germain P and Gronemeyer H (2000a): Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. Trends Pharmacol Sci 21:381-388.

Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H and Moras D (2000b): Crystal structure of a heterodimeric complex of RAR and RXR ligand- binding domains. Mol Cell 5:289-298.

Bovenzi V, Le NL, Cote S, Sinnett D, Momparler LF and Momparler RL (1999): DNA methylation of retinoic acid receptor beta in breast cancer and possible therapeutic role of 5-aza-2'-deoxycytidine. Anticancer Drugs 10:471-476.

Brandenberger AW, Tee MK and Jaffe RB (1998): Estrogen receptor alpha (ER-alpha) and beta (ER-beta) mRNAs in normal ovary, ovarian serous cystadenocarcinoma and ovarian cancer cell lines: down-regulation of ER-beta in neoplastic tissues. J Clin Endocrinol Metab 83:1025-1028.

Brys M, Wojcik M, Romanowicz-Makowska H and Krajewska WM (2002): Androgen receptor status in female breast cancer: RT-PCR and Western blot studies. J Cancer Res Clin Oncol 128:85-90.

Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA and Carlquist M (1997): Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 389:753-758.

Cadepond F, Ulmann A and Baulieu EE (1997): RU486 (mifepristone): mechanisms of action and clinical uses. Annu Rev Med 48:129-156.

Camacho-Arroyo I, Guerra-Araiza C and Cerbon MA (1998): Progesterone receptor isoforms are differentially regulated by sex steroids in the rat forebrain. Neuroreport 9:3993-3996.

Cano A, Serra V, Rivera J, Monmeneu R and Marzo C (1990): Expression of estrogen receptors, progesterone receptors, and an estrogen receptor-associated protein in the human cervix during the menstrual cycle and menopause. Fertil Steril 54:1058-1064.

Carlberg C, Bendik I, Wyss A, Meier E, Sturzenbecker LJ, Grippo JF and Hunziker W (1993): Two nuclear signalling pathways for vitamin D. Nature 361:657-660.

Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, Juguilon H, Montminy M and Evans RM (1996): Role of CBP/P300 in nuclear receptor signalling. Nature 383:99-103.

Chalbos D and Galtier F (1994): Differential effect of forms A and B of human progesterone receptor on estradiol-dependent transcription. J Biol Chem 269:23007-23012.

Chan HM and La Thangue NB (2001): p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J Cell Sci 114:2363-2373.

Chappell SA, Johnson SM, Shaw JA and Walker RA (2000): Expression of oestrogen receptor alpha variants in non-malignant breast and early invasive breast carcinomas. J Pathol 192:159-165.

Chen CL, Weiss NS, Newcomb P, Barlow W and White E (2002): Hormone replacement therapy in relation to breast cancer. JAMA 287:734-741.

Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y and Evans RM (1997): Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90:569-580.

Chen H, Lin RJ, Xie W, Wilpitz D and Evans RM (1999): Regulation of hormone-induced histone hyperacetylation and gene activation via acetylation of an acetylase. Cell 98:675-686.

Chen JD and Evans RM (1995): A transcriptional co-repressor that interacts with nuclear hormone receptors. Nature 377:454-457.

Cheskis B and Freedman LP (1994): Ligand modulates the conversion of DNA-bound vitamin D3 receptor (VDR) homodimers into VDR-retinoid X receptor heterodimers. Mol Cell Biol 14:3329-3338.

Cheung J and Smith DF (2000): Molecular chaperone interactions with steroid receptors: an update. Mol Endocrinol 14:939-946.

Chi A, Chen X, Chirala M and Younes M (2003): Differential expression of estrogen receptor beta isoforms in human breast cancer tissue. Anticancer Res 23:211-216.

Chiba H, Muramatsu M, Nomoto A and Kato H (1994): Two human homologues of Saccharomyces cerevisiae SWI2/SNF2 and Drosophila brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. Nucleic Acids Res 22:1815-1820.

Cho H, Aronica SM and Katzenellenbogen BS (1994): Regulation of progesterone receptor gene expression in MCF-7 breast cancer cells: a comparison of the effects of cyclic adenosine 3',5'-monophosphate, estradiol, insulin-like growth factor-I, and serum factors. Endocrinology 134:658-664.

Chomczynski P and Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate- phenol-chloroform extraction. Anal Biochem 162:156-159.

Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR and Goodman RH (1993): Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 365:855-859.

Clarke CL and Satyaswaroop PG (1985): Photoaffinity labeling of the progesterone receptor from human endometrial carcinoma. Cancer Res 45:5417-5420.

Clarke RB, Howell A and Anderson E (1997a): Estrogen sensitivity of normal human breast tissue in vivo and implanted into athymic nude mice: analysis of the relationship between estrogen-induced proliferation and progesterone receptor expression. Breast Cancer Res Treat 45:121-133.

Clarke RB, Howell A, Potten CS and Anderson E (1997b): Dissociation between steroid receptor expression and cell proliferation in the human breast. Cancer Res 57:4987-4991.

Clayton SJ, May FE and Westley BR (1997): Insulin-like growth factors control the regulation of oestrogen and progesterone receptor expression by oestrogens. Mol Cell Endocrinol 128:57-68.

Cline JM, Soderqvist G, von Schoultz E, Skoog L and von Schoultz B (1998): Effects of conjugated estrogens, medroxyprogesterone acetate, and tamoxifen on the mammary glands of macaques. Breast Cancer Res Treat 48:221-229.

Cole TJ, Blendy JA, Monaghan AP, Krieglstein K, Schmid W, Aguzzi A, Fantuzzi G, Hummler E, Unsicker K and Schutz G (1995): Targeted disruption of the glucocorticoid receptor gene blocks adrenergic chromaffin cell development and severely retards lung maturation. Genes Dev 9:1608-1621.

Collingwood TN, Butler A, Tone Y, Clifton-Bligh RJ, Parker MG and Chatterjee VK (1997): Thyroid hormone-mediated enhancement of heterodimer formation between thyroid hormone receptor beta and retinoid X receptor. J Biol Chem 272:13060-13065.

Colston KW and Hansen CM (2002): Mechanisms implicated in the growth regulatory effects of vitamin D in breast cancer. Endocr Relat Cancer 9:45-59.

Colston KW, Berger U and Coombes RC (1989): Possible role for vitamin D in controlling breast cancer cell proliferation. Lancet 1:188-191.

Cooke PS, Buchanan DL, Young P, Setiawan T, Brody J, Korach KS, Taylor J, Lubahn DB and Cunha GR (1997): Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. Proc Natl Acad Sci U S A 94:6535-6540.

Coppens MT, Dhont MA, De Boever JG, Serreyn RF, Vandekerckhove DA and Roels HJ (1993): The distribution of oestrogen and progesterone receptors in the human endometrial basal and functional layer during the normal menstrual cycle. An immunocytochemical study. Histochemistry 99:121-126.

Couse JF, Curtis SW, Washburn TF, Lindzey J, Golding TS, Lubahn DB, Smithies O and Korach KS (1995): Analysis of transcription and estrogen insensitivity in the female mouse after targeted disruption of the estrogen receptor gene. Mol Endocrinol 9:1441-1454.

Cowley SM, Hoare S, Mosselman S and Parker MG (1997): Estrogen receptors alpha and beta form heterodimers on DNA. J Biol Chem 272:19858-19862.

Creasman WT (1993): Prognostic significance of hormone receptors in endometrial cancer. Cancer 71:1467-1470.

Critchley HO, Brenner RM, Henderson TA, Williams K, Nayak NR, Slayden OD, Millar MR and Saunders PT (2001): Estrogen receptor beta, but not estrogen receptor alpha, is present in the vascular endothelium of the human and nonhuman primate endometrium. J Clin Endocrinol Metab 86:1370-1378.

Critchley HO, Henderson TA, Kelly RW, Scobie GS, Evans LR, Groome NP and Saunders PT (2002): Wild-type estrogen receptor (ERbeta1) and the splice variant (ERbetacx/beta2) are both expressed within the human endometrium throughout the normal menstrual cycle. J Clin Endocrinol Metab 87:5265-5273.

Cunha GR, Young P, Hom YK, Cooke PS, Taylor JA and Lubahn DB (1997): Elucidation of a role for stromal steroid hormone receptors in mammary gland growth and development using tissue recombinants. J Mammary Gland Biol Neoplasia 2:393-402.

Curtis SW, Clark J, Myers P and Korach KS (1999): Disruption of estrogen signaling does not prevent progesterone action in the estrogen receptor alpha knockout mouse uterus. Proc Natl Acad Sci U S A 96:3646-3651.

Cuzick J, Powles T, Veronesi U, Forbes J, Edwards R, Ashley S and Boyle P (2003): Overview of the main outcomes in breast-cancer prevention trials. Lancet 361:296-300.

Dai D, Wolf DM, Litman ES, White MJ and Leslie KK (2002): Progesterone inhibits human endometrial cancer cell growth and invasiveness: down-regulation of cellular adhesion molecules through progesterone B receptors. Cancer Res 62:881-886.

DeFranco DB (2002): Navigating steroid hormone receptors through the nuclear compartment. Mol Endocrinol 16:1449-1455.

Dilworth FJ, Fromental-Ramain C, Yamamoto K and Chambon P (2000): ATP-driven chromatin remodeling activity and histone acetyltransferases act sequentially during transactivation by RAR/RXR In vitro. Mol Cell 6:1049-1058.

Dowsett M, Daffada A, Chan CM and Johnston SR (1997): Oestrogen receptor mutants and variants in breast cancer. Eur J Cancer 33:1177-1183.

Duffy DM, Wells TR, Haluska GJ and Stouffer RL (1997): The ratio of progesterone receptor isoforms changes in the monkey corpus luteum during the luteal phase of the menstrual cycle. Biol Reprod 57:693-699.

Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P and Mark M (2000): Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. Development 127:4277-4291.

Eckner R, Ewen ME, Newsome D, Gerdes M, DeCaprio JA, Lawrence JB and Livingston DM (1994): Molecular cloning and functional analysis of the adenovirus E1A- associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev 8:869-884.

Englund K, Blanck A, Gustavsson I, Lundkvist U, Sjoblom P, Norgren A and Lindblom B (1998): Sex steroid receptors in human myometrium and fibroids: changes during the menstrual cycle and gonadotropin-releasing hormone treatment. J Clin Endocrinol Metab 83:4092-4096.

Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjold M and Gustafsson JA (1997): Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. J Clin Endocrinol Metab 82:4258-4265.

Ettinger B, Friedman GD, Bush T and Quesenberry CP, Jr. (1996): Reduced mortality associated with long-term postmenopausal estrogen therapy. Obstet Gynecol 87:6-12.

Farboud B, Hauksdottir H, Wu Y and Privalsky ML (2003): Isotype-restricted corepressor recruitment: a constitutively closed helix 12 conformation in retinoic acid receptors beta and gamma interferes with corepressor recruitment and prevents transcriptional repression. Mol Cell Biol 23:2844-2858.

Font de Mora J and Brown M (2000): AIB1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor. Mol Cell Biol 20:5041-5047.

Fornander T, Rutqvist LE, Cedermark B, Glas U, Mattsson A, Silfversward C, Skoog L, Somell A, Theve T, Wilking N and et al. (1989): Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. Lancet 1:117-120.

Forster C, Makela S, Warri A, Kietz S, Becker D, Hultenby K, Warner M and Gustafsson JA (2002): Involvement of estrogen receptor beta in terminal differentiation of mammary gland epithelium. Proc Natl Acad Sci U S A 99:15578-15583.

Freedman LP (1992): Anatomy of the steroid receptor zinc finger region. Endocr Rev 13:129-145.

Friedrich M, Rafi L, Tilgen W, Schmidt W and Reichrath J (1998): Expression of 1,25-dihydroxy vitamin D3 receptor in breast carcinoma. J Histochem Cytochem 46:1335-1337.

Friedrich M, Meyberg R, Axt-Fliedner R, Villena-Heinsen C, Tilgen W, Schmidt W and Reichrath J (2002): Vitamin D receptor (VDR) expression is not a prognostic factor in cervical cancer. Anticancer Res 22:299-304.

Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati ML and Pestell RG (2000): p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 275:20853-20860.

Fu M, Wang C, Wang J, Zhang X, Sakamaki T, Yeung YG, Chang C, Hopp T, Fuqua SA, Jaffray E, Hay RT, Palvimo JJ, Janne OA and Pestell RG (2002): Androgen receptor acetylation governs trans activation and MEKK1-induced apoptosis without affecting in vitro sumoylation and trans-repression function. Mol Cell Biol 22:3373-3388.

Fujimoto J, Nishigaki M, Hori M, Ichigo S, Itoh T and Tamaya T (1994): The effect of estrogen and androgen on androgen receptors and mRNA levels in uterine leiomyoma, myometrium and endometrium of human subjects. J Steroid Biochem Mol Biol 50:137-143.

Fujimoto J, Sakaguchi H, Aoki I, Toyoki H and Tamaya T (2002): Clinical implications of the expression of estrogen receptor-alpha and - beta in primary and metastatic lesions of uterine endometrial cancers. Oncology 62:269-277.

Fukuda K, Mori M, Uchiyama M, Iwai K, Iwasaka T and Sugimori H (1998): Prognostic significance of progesterone receptor immunohistochemistry in endometrial carcinoma. Gynecol Oncol 69:220-225.

Fukunaka K, Saito T, Wataba K, Ashihara K, Ito E and Kudo R (2001): Changes in expression and subcellular localization of nuclear retinoic acid receptors in human endometrial epithelium during the menstrual cycle. Mol Hum Reprod 7:437-446.

Fung HY, Wong YL, Wong FW and Rogers MS (1994): Study of oestrogen and progesterone receptors in normal human endometrium during the menstrual cycle by immunocytochemical analysis. Gynecol Obstet Invest 38:186-190.

Fuqua SA, Schiff R, Parra I, Friedrichs WE, Su JL, McKee DD, Slentz-Kesler K, Moore LB, Willson TM and Moore JT (1999): Expression of wild-type estrogen receptor beta and variant isoforms in human breast cancer. Cancer Res 59:5425-5428.

Garcia E, Bouchard P, De Brux J, Berdah J, Frydman R, Schaison G, Milgrom E and Perrot-Applanat M (1988): Use of immunocytochemistry of progesterone and estrogen receptors for endometrial dating. J Clin Endocrinol Metab 67:80-87.

Gehin M, Mark M, Dennefeld C, Dierich A, Gronemeyer H and Chambon P (2002): The function of TIF2/GRIP1 in mouse reproduction is distinct from those of SRC-1 and p/CIP. Mol Cell Biol 22:5923-5937.

Gehrig PA, Van Le L, Olatidoye B and Geradts J (1999): Estrogen receptor status, determined by immunohistochemistry, as a predictor of the recurrence of stage I endometrial carcinoma. Cancer 86:2083-2089.

Geisen C, Denk C, Gremm B, Baust C, Karger A, Bollag W and Schwarz E (1997): High-level expression of the retinoic acid receptor beta gene in normal cells of the uterine cervix is regulated by the retinoic acid receptor alpha and is abnormally down-regulated in cervical carcinoma cells. Cancer Res 57:1460-1467.

Georget V, Lobaccaro JM, Terouanne B, Mangeat P, Nicolas JC and Sultan C (1997): Trafficking of the androgen receptor in living cells with fused green fluorescent protein-androgen receptor. Mol Cell Endocrinol 129:17-26.

Giangrande PH, Pollio G and McDonnell DP (1997): Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. J Biol Chem 272:32889-32900.

Giangrande PH, E AK, Edwards DP and McDonnell DP (2000): The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. Mol Cell Biol 20:3102-3115.

Gonzalez-Moran G and Camacho-Arroyo I (2003): Changes in the presence of progesterone receptor isoforms in the oviduct magnum of newly-hatched chicks after gonadotropins treatment. Life Sci 73:871-882.

Gottardis MM, Robinson SP, Satyaswaroop PG and Jordan VC (1988): Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athymic mouse. Cancer Res 48:812-815.

Grady D, Gebretsadik T, Kerlikowske K, Ernster V and Petitti D (1995): Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol 85:304-313.

Graham JD and Clarke CL (1997): Physiological action of progesterone in target tissues. Endocr Rev 18:502-519.

Graham JD, Roman SD, McGowan E, Sutherland RL and Clarke CL (1995a): Preferential stimulation of human progesterone receptor B expression by estrogen in T-47D human breast cancer cells. J Biol Chem 270:30693-30700.

Graham JD, Yeates C, Balleine RL, Harvey SS, Milliken JS, Bilous AM and Clarke CL (1995b): Characterization of progesterone receptor A and B expression in human breast cancer. Cancer Res 55:5063-5068.

Graham ML, 2nd, Dalquist KE and Horwitz KB (1989): Simultaneous measurement of progesterone receptors and DNA indices by flow cytometry: analysis of breast cancer cell mixtures and genetic instability of the T47D line. Cancer Res 49:3943-3949.

Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P and Chambon P (1986): Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature 320:134-139.

Greendale GA, Reboussin BA, Sie A, Singh HR, Olson LK, Gatewood O, Bassett LW, Wasilauskas C, Bush T and Barrett-Connor E (1999): Effects of estrogen and estrogen-progestin on mammographic parenchymal density. Postmenopausal Estrogen/Progestin Interventions (PEPI) Investigators. Ann Intern Med 130:262-269.

Greene GL, Gilna P, Waterfield M, Baker A, Hort Y and Shine J (1986): Sequence and expression of human estrogen receptor complementary DNA. Science 231:1150-1154.

Gregory CW, Wilson EM, Apparao KB, Lininger RA, Meyer WR, Kowalik A, Fritz MA and Lessey BA (2002): Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. J Clin Endocrinol Metab 87:2960-2966.

Grodstein F, Stampfer MJ, Colditz GA, Willett WC, Manson JE, Joffe M, Rosner B, Fuchs C, Hankinson SE, Hunter DJ, Hennekens CH and Speizer FE (1997): Postmenopausal hormone therapy and mortality. N Engl J Med 336:1769-1775.

Gronemeyer H, Turcotte B, Quirin-Stricker C, Bocquel MT, Meyer ME, Krozowski Z, Jeltsch JM, Lerouge T, Garnier JM and Chambon P (1987): The chicken progesterone receptor: sequence, expression and functional analysis. EMBO J 6:3985-3994.

Guiochon-Mantel A, Delabre K, Lescop P and Milgrom E (1994): Nuclear localization signals also mediate the outward movement of proteins from the nucleus. Proc Natl Acad Sci U S A 91:7179-7183.

Hall JM and McDonnell DP (1999): The estrogen receptor beta-isoform (ERbeta) of the human estrogen receptor modulates ERalpha transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. Endocrinology 140:5566-5578.

Hall JM, McDonnell DP and Korach KS (2002): Allosteric regulation of estrogen receptor structure, function, and coactivator recruitment by different estrogen response elements. Mol Endocrinol 16:469-486.

Hansen LA, Sigman CC, Andreola F, Ross SA, Kelloff GJ and De Luca LM (2000): Retinoids in chemoprevention and differentiation therapy. Carcinogenesis 21:1271-1279.

Hauksdottir H, Farboud B and Privalsky ML (2003): Retinoic acid receptors beta and gamma do not repress, but instead activate target gene transcription in both the absence and presence of hormone ligand. Mol Endocrinol 17:373-385.

Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo SD, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK and Rosenfeld MG (1997): A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature 387:43-48.

Henderson TA, Saunders PT, Moffett-King A, Groome NP and Critchley HO (2003): Steroid receptor expression in uterine natural killer cells. J Clin Endocrinol Metab 88:440-449.

Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Meyers DA and Bleecker ER (2002a): Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. Circulation 105:1879-1882.

Herrington DM, Howard TD, Hawkins GA, Reboussin DM, Xu J, Zheng SL, Brosnihan KB, Meyers DA and Bleecker ER (2002b): Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. N Engl J Med 346:967-974.

Hilakivi-Clarke L, Cabanes A, Olivo S, Kerr L, Bouker KB and Clarke R (2002): Do estrogens always increase breast cancer risk? J Steroid Biochem Mol Biol 80:163-174.

Hirata S, Shoda T, Kato J and Hoshi K (2003): Isoform/variant mRNAs for sex steroid hormone receptors in humans. Trends Endocrinol Metab 14:124-129.

Hofseth LJ, Raafat AM, Osuch JR, Pathak DR, Slomski CA and Haslam SZ (1999): Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast. J Clin Endocrinol Metab 84:4559-4565.

Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, Thompson EB, Rosenfeld MG and Evans RM (1985): Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 318:635-641.

Hong SH and Privalsky ML (2000): The SMRT corepressor is regulated by a MEK-1 kinase pathway: inhibition of corepressor function is associated with SMRT phosphorylation and nuclear export. Mol Cell Biol 20:6612-6625.

Horie K, Takakura K, Imai K, Liao S and Mori T (1992): Immunohistochemical localization of androgen receptor in the human endometrium, decidua, placenta and pathological conditions of the endometrium. Hum Reprod 7:1461-1466.

Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK and et al. (1995): Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377:397-404.

Horwitz KB, Francis MD and Wei LL (1985): Hormone-dependent covalent modification and processing of human progesterone receptors in the nucleus. DNA 4:451-460.

Hovland AR, Powell RL, Takimoto GS, Tung L and Horwitz KB (1998): An N-terminal inhibitory function, IF, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors. J Biol Chem 273:5455-5460.

Hu X and Lazar MA (2000): Transcriptional repression by nuclear hormone receptors. Trends Endocrinol Metab 11:6-10.

Huang ZQ, Li J, Sachs LM, Cole PA and Wong J (2003): A role for cofactor-cofactor and cofactor-histone interactions in targeting p300, SWI/SNF and Mediator for transcription. EMBO J 22:2146-2155.

Hudelist G, Czerwenka K, Kubista E, Marton E, Pischinger K and Singer CF (2003): Expression of sex steroid receptors and their co-factors in normal and malignant breast tissue: AIB1 is a carcinoma-specific co-activator. Breast Cancer Res Treat 78:193-204.

Ikeda M, Kawaguchi A, Takeshita A, Chin WW, Endo T and Onaya T (1999): CBP-dependent and independent enhancing activity of steroid receptor coactivator-1 in thyroid hormone receptor-mediated transactivation. Mol Cell Endocrinol 147:103-112.

Isola JJ (1993): Immunohistochemical demonstration of androgen receptor in breast cancer and its relationship to other prognostic factors. J Pathol 170:31-35.

Issa LL, Leong GM and Eisman JA (1998): Molecular mechanism of vitamin D receptor action. Inflamm Res 47:451-475.

Ito K, Suzuki T, Moriya T, Utsunomiya H, Sugawara A, Konno R, Sato S and Sasano H (2001): Retinoid receptors in the human endometrium and its disorders: a possible modulator of 17 beta-hydroxysteroid dehydrogenase. J Clin Endocrinol Metab 86:2721-2727.

Ito K, Suzuki T, Akahira J, Moriya T, Kaneko C, Utsunomiya H, Yaegashi N, Okamura K and Sasano H (2002): Expression of androgen receptor and 5alpha-reductases in the human normal endometrium and its disorders. Int J Cancer 99:652-657.

Iwai T, Nanbu Y, Iwai M, Taii S, Fujii S and Mori T (1990): Immunohistochemical localization of oestrogen receptors and progesterone receptors in the human ovary throughout the menstrual cycle. Virchows Arch A Pathol Anat Histopathol 417:369-375.

Iwao K, Miyoshi Y, Egawa C, Ikeda N and Noguchi S (2000a): Quantitative analysis of estrogen receptor-beta mRNA and its variants in human breast cancers. Int J Cancer 88:733-736.

Iwao K, Miyoshi Y, Egawa C, Ikeda N, Tsukamoto F and Noguchi S (2000b): Quantitative analysis of estrogen receptor-alpha and -beta messenger RNA expression in breast carcinoma by real-time polymerase chain reaction. Cancer 89:1732-1738.

Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L and Horwitz KB (1997): The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. Mol Endocrinol 11:693-705.

Jakacka M, Ito M, Martinson F, Ishikawa T, Lee EJ and Jameson JL (2002): An estrogen receptor (ER)alpha deoxyribonucleic acid-binding domain knock-in mutation provides evidence for nonclassical ER pathway signaling in vivo. Mol Endocrinol 16:2188-2201.

Järvinen TA, Pelto-Huikko M, Holli K and Isola J (2000): Estrogen receptor beta is coexpressed with ERalpha and PR and associated with nodal status, grade, and proliferation rate in breast cancer. Am J Pathol 156:29-35.

Jeltsch JM, Turcotte B, Garnier JM, Lerouge T, Krozowski Z, Gronemeyer H and Chambon P (1990): Characterization of multiple mRNAs originating from the chicken progesterone receptor gene. Evidence for a specific transcript encoding form A. J Biol Chem 265:3967-3974.

Jepsen K, Hermanson O, Onami TM, Gleiberman AS, Lunyak V, McEvilly RJ, Kurokawa R, Kumar V, Liu F, Seto E, Hedrick SM, Mandel G, Glass CK, Rose DW and Rosenfeld MG (2000): Combinatorial roles of the nuclear receptor corepressor in transcription and development. Cell 102:753-763.

Kakizawa T, Miyamoto T, Kaneko A, Yajima H, Ichikawa K and Hashizume K (1997): Ligand-dependent heterodimerization of thyroid hormone receptor and retinoid X receptor. J Biol Chem 272:23799-23804.

Kalkhoven E, Valentine JE, Heery DM and Parker MG (1998): Isoforms of steroid receptor co-activator 1 differ in their ability to potentiate transcription by the oestrogen receptor. EMBO J 17:232-243.

Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK and Rosenfeld MG (1996): A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85:403-414.

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H and Chambon P (1990): Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J 9:1603-1614.

Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H, Masushige S, Gotoh Y, Nishida E, Kawashima H and et al. (1995): Activation of the estrogen receptor through phosphorylation by mitogen- activated protein kinase. Science 270:1491-1494.

Katzenellenbogen BS and Norman MJ (1990): Multihormonal regulation of the progesterone receptor in MCF-7 human breast cancer cells: interrelationships among insulin/insulin-like growth factor-I, serum, and estrogen. Endocrinology 126:891-898.

Khan SA, Rogers MA, Obando JA and Tamsen A (1994): Estrogen receptor expression of benign breast epithelium and its association with breast cancer. Cancer Res 54:993-997.

Kim K, Thu N, Saville B and Safe S (2003): Domains of estrogen receptor alpha (ERalpha) required for ERalpha/Sp1-mediated activation of GC-rich promoters by estrogens and antiestrogens in breast cancer cells. Mol Endocrinol 17:804-817.

Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S and Seino Y (2000): Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology 141:1317-1324.

Kitagawa H, Fujiki R, Yoshimura K, Mezaki Y, Uematsu Y, Matsui D, Ogawa S, Unno K, Okubo M, Tokita A, Nakagawa T, Ito T, Ishimi Y, Nagasawa H, Matsumoto T, Yanagisawa J and Kato S (2003): The chromatin-remodeling complex WINAC targets a nuclear receptor to promoters and is impaired in Williams syndrome. Cell 113:905-917.

Korach KS (1994): Insights from the study of animals lacking functional estrogen receptor. Science 266:1524-1527.

Korzus E, Torchia J, Rose DW, Xu L, Kurokawa R, McInerney EM, Mullen TM, Glass CK and Rosenfeld MG (1998): Transcription factor-specific requirements for coactivators and their acetyltransferase functions. Science 279:703-707.

Kraus WL, Manning ET and Kadonaga JT (1999): Biochemical analysis of distinct activation functions in p300 that enhance transcription initiation with chromatin templates. Mol Cell Biol 19:8123-8135.

Kraus WL, Weis KE and Katzenellenbogen BS (1995): Inhibitory cross-talk between steroid hormone receptors: differential targeting of estrogen receptor in the repression of its transcriptional activity by agonist- and antagonist-occupied progestin receptors. Mol Cell Biol 15:1847-1857.

Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA and Smithies O (1998): Generation and reproductive phenotypes of mice lacking estrogen receptor beta. Proc Natl Acad Sci U S A 95:15677-15682.

Krishnan AV, Swami S and Feldman D (2001): Estradiol inhibits glucocorticoid receptor expression and induces glucocorticoid resistance in MCF-7 human breast cancer cells. J Steroid Biochem Mol Biol 77:29-37.

Kuenen-Boumeester V, Van der Kwast TH, van Putten WL, Claassen C, van Ooijen B and Henzen-Logmans SC (1992): Immunohistochemical determination of androgen receptors in relation to oestrogen and progesterone receptors in female breast cancer. Int J Cancer 52:581-584.

Kuhnel R, de Graaff J, Rao BR and Stolk JG (1987): Androgen receptor predominance in human ovarian carcinoma. J Steroid Biochem 26:393-397.

Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S and Gustafsson JA (1997): Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 138:863-870.

Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S and Gustafsson JA (1996): Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 93:5925-5930.

Kumar NS, Richer J, Owen G, Litman E, Horwitz KB and Leslie KK (1998): Selective down-regulation of progesterone receptor isoform B in poorly differentiated human endometrial cancer cells: implications for unopposed estrogen action. Cancer Res 58:1860-1865.

Kumarendran MK, Loughney AD, Prentice A, Thomas EJ and Redfern CP (1996): Nuclear retinoid receptor expression in normal human endometrium throughout the menstrual cycle. Mol Hum Reprod 2:123-129.

Kung AL, Rebel VI, Bronson RT, Ch'ng LE, Sieff CA, Livingston DM and Yao TP (2000): Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. Genes Dev 14:272-277.

Kurebayashi J, Otsuki T, Kunisue H, Tanaka K, Yamamoto S and Sonoo H (2000): Expression levels of estrogen receptor-alpha, estrogen receptor-beta, coactivators, and corepressors in breast cancer. Clin Cancer Res 6:512-518.

Kurita T, Lee KJ, Cooke PS, Taylor JA, Lubahn DB and Cunha GR (2000): Paracrine regulation of epithelial progesterone receptor by estradiol in the mouse female reproductive tract. Biol Reprod 62:821-830.

Laemmli UK (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Laird SM, Tuckerman EM, Dalton CF, Dunphy BC, Li TC and Zhang X (1997): The production of leukaemia inhibitory factor by human endometrium: presence in uterine flushings and production by cells in culture. Hum Reprod 12:569-574.

Lapidus RG, Nass SJ and Davidson NE (1998): The loss of estrogen and progesterone receptor gene expression in human breast cancer. J Mammary Gland Biol Neoplasia 3:85-94.

Lau TM, Witjaksono J, Affandi B and Rogers PA (1996): Expression of progesterone receptor mRNA in the endometrium during the normal menstrual cycle and in Norplant users. Hum Reprod 11:2629-2634.

Laudet V and Gronemeyer H. (2002). *The Nuclear Receptor Facts Book*, Academic Press, London.

Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen TM, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG and Rose DW (1998): Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. Proc Natl Acad Sci U S A 95:2920-2925.

Lawrence JA, Merino MJ, Simpson JF, Manrow RE, Page DL and Steeg PS (1998): A high-risk lesion for invasive breast cancer, ductal carcinoma in situ, exhibits frequent overexpression of retinoid X receptor. Cancer Epidemiol Biomarkers Prev 7:29-35.

Lecce G, Meduri G, Ancelin M, Bergeron C and Perrot-Applanat M (2001): Presence of estrogen receptor beta in the human endometrium through the cycle: expression in glandular, stromal, and vascular cells. J Clin Endocrinol Metab 86:1379-1386.

Leid M, Kastner P, Lyons R, Nakshatri H, Saunders M, Zacharewski T, Chen JY, Staub A, Garnier JM, Mader S and et al. (1992): Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. Cell 68:377-395.

Leo C and Chen JD (2000): The SRC family of nuclear receptor coactivators. Gene 245:1-11.

Leroy P, Krust A, Zelent A, Mendelsohn C, Garnier JM, Kastner P, Dierich A and Chambon P (1991): Multiple isoforms of the mouse retinoic acid receptor alpha are generated by alternative splicing and differential induction by retinoic acid. EMBO J 10:59-69.

Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL and McCarty KS, Jr. (1988): Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab 67:334-340.

Leygue E, Dotzlaw H, Watson PH and Murphy LC (1999a): Expression of estrogen receptor beta1, beta2, and beta5 messenger RNAs in human breast tissue. Cancer Res 59:1175-1179.

Leygue E, Dotzlaw H, Watson PH and Murphy LC (1999b): Expression of the steroid receptor RNA activator in human breast tumors. Cancer Res 59:4190-4193.

Li CI, Malone KE, Porter PL, Weiss NS, Tang MT, Cushing-Haugen KL and Daling JR (2003): Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. JAMA 289:3254-3263.

Li H, Gomes PJ and Chen JD (1997): RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. Proc Natl Acad Sci U S A 94:8479-8484.

Li J, O'Malley BW and Wong J (2000a): p300 requires its histone acetyltransferase activity and SRC-1 interaction domain to facilitate thyroid hormone receptor activation in chromatin. Mol Cell Biol 20:2031-2042.

Li J, Wang J, Nawaz Z, Liu JM, Qin J and Wong J (2000b): Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. EMBO J 19:4342-4350.

Li Q, Imhof A, Collingwood TN, Urnov FD and Wolffe AP (1999a): p300 stimulates transcription instigated by ligand-bound thyroid hormone receptor at a step subsequent to chromatin disruption. EMBO J 18:5634-5652.

Li XY, Boudjelal M, Xiao JH, Peng ZH, Asuru A, Kang S, Fisher GJ and Voorhees JJ (1999b): 1,25-Dihydroxyvitamin D3 increases nuclear vitamin D3 receptors by blocking ubiquitin/proteasome-mediated degradation in human skin. Mol Endocrinol 13:1686-1694.

Liao DJ and Dickson RB (2002): Roles of androgens in the development, growth, and carcinogenesis of the mammary gland. J Steroid Biochem Mol Biol 80:175-189.

Lim CS, Baumann CT, Htun H, Xian W, Irie M, Smith CL and Hager GL (1999): Differential localization and activity of the A- and B-forms of the human progesterone receptor using green fluorescent protein chimeras. Mol Endocrinol 13:366-375.

Lindahl B, Ferno M, Gullberg B, Norgren A and Willen R (1992): 5-year survival rate in endometrial carcinoma stage I-II related to steroid receptor concentration, degree of differentiation, age and myometrial invasion. Anticancer Res 12:409-412.

Lindberg MK, Moverare S, Skrtic S, Gao H, Dahlman-Wright K, Gustafsson JA and Ohlsson C (2003): Estrogen Receptor (ER)-beta Reduces ERalpha-Regulated Gene Transcription,

Supporting a "Ying Yang" Relationship between ERalpha and ERbeta in Mice. Mol Endocrinol 17:203-208.

Liu Y, Lee MO, Wang HG, Li Y, Hashimoto Y, Klaus M, Reed JC and Zhang X (1996): Retinoic acid receptor beta mediates the growth-inhibitory effect of retinoic acid by promoting apoptosis in human breast cancer cells. Mol Cell Biol 16:1138-1149.

Liu Z, Auboeuf D, Wong J, Chen JD, Tsai SY, Tsai MJ and O'Malley BW (2002): Coactivator/corepressor ratios modulate PR-mediated transcription by the selective receptor modulator RU486. Proc Natl Acad Sci U S A 99:7940-7944.

Lonard DM, Nawaz Z, Smith CL and O'Malley BW (2000): The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation. Mol Cell 5:939-948.

Lopez GN, Turck CW, Schaufele F, Stallcup MR and Kushner PJ (2001): Growth factors signal to steroid receptors through mitogen-activated protein kinase regulation of p160 coactivator activity. J Biol Chem 276:22177-22182.

Loven MA, Likhite VS, Choi I and Nardulli AM (2001): Estrogen response elements alter coactivator recruitment through allosteric modulation of estrogen receptor beta conformation. J Biol Chem 276:45282-45288.

Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS and Smithies O (1993): Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci U S A 90:11162-11166.

Lydon JP, Ge G, Kittrell FS, Medina D and O'Malley BW (1999): Murine mammary gland carcinogenesis is critically dependent on progesterone receptor function. Cancer Res 59:4276-4284.

Ma H, Hong H, Huang SM, Irvine RA, Webb P, Kushner PJ, Coetzee GA and Stallcup MR (1999): Multiple signal input and output domains of the 160-kilodalton nuclear receptor coactivator proteins. Mol Cell Biol 19:6164-6173.

Maeda K, Tsuda H, Hashiguchi Y, Yamamoto K, Inoue T, Ishiko O and Ogita S (2002): Relationship between p53 pathway and estrogen receptor status in endometrioid-type endometrial cancer. Hum Pathol 33:386-391.

Maglich JM, Sluder A, Guan X, Shi Y, McKee DD, Carrick K, Kamdar K, Willson TM and Moore JT (2001): Comparison of complete nuclear receptor sets from the human, Caenorhabditis elegans and Drosophila genomes. Genome Biol 2.

Maruvada P, Baumann CT, Hager GL and Yen PM (2003): Dynamic shuttling and intranuclear mobility of nuclear hormone receptors. J Biol Chem 278:12425-12432.

Matsuzaki S, Fukaya T, Suzuki T, Murakami T, Sasano H and Yajima A (1999): Oestrogen receptor alpha and beta mRNA expression in human endometrium throughout the menstrual cycle. Mol Hum Reprod 5:559-564.

Matsuzaki S, Uehara S, Murakami T, Fujiwara J, Funato T and Okamura K (2000): Quantitative analysis of estrogen receptor alpha and beta messenger ribonucleic acid levels in normal endometrium and ovarian endometriotic cysts using a real-time reverse transcription-polymerase chain reaction assay. Fertil Steril 74:753-759.

McDonnell DP, Mangelsdorf DJ, Pike JW, Haussler MR and O'Malley BW (1987): Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. Science 235:1214-1217.

McDonnell DP, Shahbaz MM, Vegeto E and Goldman ME (1994): The human progesterone receptor A-form functions as a transcriptional modulator of mineralocorticoid receptor transcriptional activity. J Steroid Biochem Mol Biol 48:425-432.

McGowan EM and Clarke CL (1999): Effect of overexpression of progesterone receptor A on endogenous progestin-sensitive endpoints in breast cancer cells. Mol Endocrinol 13:1657-1671.

McGowan EM, Weinberger RP, Graham JD, Hill HD, Hughes JA, O'Neill GM and Clarke CL (2003): Cytoskeletal responsiveness to progestins is dependent on progesterone receptor A levels. J Mol Endocrinol 31:241-253.

McInerney EM, Rose DW, Flynn SE, Westin S, Mullen TM, Krones A, Inostroza J, Torchia J, Nolte RT, Assa-Munt N, Milburn MV, Glass CK and Rosenfeld MG (1998): Determinants of coactivator LXXLL motif specificity in nuclear receptor transcriptional activation. Genes Dev 12:3357-3368.

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

Mertens HJ, Heineman MJ, Koudstaal J, Theunissen P and Evers JL (1996): Androgen receptor content in human endometrium. Eur J Obstet Gynecol Reprod Biol 70:11-13.

Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G and Smith R (2002): Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. J Clin Endocrinol Metab 87:2924-2930.

Meyer ME, Pornon A, Ji JW, Bocquel MT, Chambon P and Gronemeyer H (1990): Agonistic and antagonistic activities of RU486 on the functions of the human progesterone receptor. EMBO J 9:3923-3932.

Misiti S, Schomburg L, Yen PM and Chin WW (1998): Expression and hormonal regulation of coactivator and corepressor genes. Endocrinology 139:2493-2500.

Moen RC and Palmiter RD (1980): Changes in hormone responsiveness of chick oviduct during primary stimulation with estrogen. Dev Biol 78:450-463.

Moinfar F, Okcu M, Tsybrovskyy O, Regitnig P, Lax SF, Weybora W, Ratschek M, Tavassoli FA and Denk H (2003): Androgen receptors frequently are expressed in breast carcinomas: potential relevance to new therapeutic strategies. Cancer 98:703-711.

Montano MM, Muller V, Trobaugh A and Katzenellenbogen BS (1995): The carboxy-terminal F domain of the human estrogen receptor: role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists. Mol Endocrinol 9:814-825.

Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL, Su JL, Kliewer SA, Lehmann JM and Willson TM (1998): Cloning and characterization of human estrogen receptor beta isoforms. Biochem Biophys Res Commun 247:75-78.

Mosselman S, Polman J and Dijkema R (1996): ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 392:49-53.

Mote PA, Balleine RL, McGowan EM and Clarke CL (1999): Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. J Clin Endocrinol Metab 84:2963-2971.

Mote PA, Bartow S, Tran N and Clarke CL (2002): Loss of co-ordinate expression of progesterone receptors A and B is an early event in breast carcinogenesis. Breast Cancer Res Treat 72:163-172.

Muchardt C and Yaniv M (1993): A human homologue of Saccharomyces cerevisiae SNF2/SWI2 and Drosophila brm genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J 12:4279-4290.

Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP and Conneely OM (2000): Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. Science 289:1751-1754.

Mulac-Jericevic B, Lydon JP, DeMayo FJ and Conneely OM (2003): Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. Proc Natl Acad Sci U S A 100:9744-9749.

Murphy LC, Simon SL, Parkes A, Leygue E, Dotzlaw H, Snell L, Troup S, Adeyinka A and Watson PH (2000): Altered expression of estrogen receptor coregulators during human breast tumorigenesis. Cancer Res 60:6266-6271.

Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL and Evans RM (1997): Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. Cell 89:373-380.

Narvaez CJ, Zinser G and Welsh J (2001): Functions of 1alpha,25-dihydroxyvitamin D(3) in mammary gland: from normal development to breast cancer. Steroids 66:301-308.

Nawaz Z, Lonard DM, Dennis AP, Smith CL and O'Malley BW (1999a): Proteasome-dependent degradation of the human estrogen receptor. Proc Natl Acad Sci U S A 96:1858-1862.

Nawaz Z, Lonard DM, Smith CL, Lev-Lehman E, Tsai SY, Tsai MJ and O'Malley BW (1999b): The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. Mol Cell Biol 19:1182-1189.

Nephew KP, Ray S, Hlaing M, Ahluwalia A, Wu SD, Long X, Hyder SM and Bigsby RM (2000): Expression of estrogen receptor coactivators in the rat uterus. Biol Reprod 63:361-367.

Noe M, Kunz G, Herbertz M, Mall G and Leyendecker G (1999): The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial-subendometrial unit. Hum Reprod 14:190-197.

Ogawa S, Inoue S, Watanabe T, Orimo A, Hosoi T, Ouchi Y and Muramatsu M (1998): Molecular cloning and characterization of human estrogen receptor betacx: a potential inhibitor ofestrogen action in human. Nucleic Acids Res 26:3505-3512.

Ogryzko VV, Schiltz RL, Russanova V, Howard BH and Nakatani Y (1996): The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell 87:953-959.

Omoto Y, Inoue S, Ogawa S, Toyama T, Yamashita H, Muramatsu M, Kobayashi S and Iwase H (2001): Clinical value of the wild-type estrogen receptor beta expression in breast cancer. Cancer Lett 163:207-212.

Onate SA, Tsai SY, Tsai MJ and O'Malley BW (1995): Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354-1357.

Onate SA, Boonyaratanakornkit V, Spencer TE, Tsai SY, Tsai MJ, Edwards DP and O'Malley BW (1998): The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. J Biol Chem 273:12101-12108.

Ordentlich P, Downes M, Xie W, Genin A, Spinner NB and Evans RM (1999): Unique forms of human and mouse nuclear receptor corepressor SMRT. Proc Natl Acad Sci U S A 96:2639-2644.

Osborne CK (1998): Steroid hormone receptors in breast cancer management. Breast Cancer Res Treat 51:227-238.

Pace P, Taylor J, Suntharalingam S, Coombes RC and Ali S (1997): Human estrogen receptor beta binds DNA in a manner similar to and dimerizes with estrogen receptor alpha. J Biol Chem 272:25832-25838.

Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ and Scanlan TS (1997): Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. Science 277:1508-1510.

Paganini-Hill A and Henderson VW (1994): Estrogen deficiency and risk of Alzheimer's disease in women. Am J Epidemiol 140:256-261.

Paige LA, Christensen DJ, Gron H, Norris JD, Gottlin EB, Padilla KM, Chang CY, Ballas LM, Hamilton PT, McDonnell DP and Fowlkes DM (1999): Estrogen receptor (ER) modulators each induce distinct conformational changes in ER alpha and ER beta. Proc Natl Acad Sci U S A 96:3999-4004.

Palmiter RD and Wrenn JT (1971): Interaction of estrogen and progesterone in chick oviduct development. 3. Tubular gland cell cytodifferentiation. J Cell Biol 50:598-615.

Park EJ, Schroen DJ, Yang M, Li H, Li L and Chen JD (1999): SMRTe, a silencing mediator for retinoid and thyroid hormone receptors- extended isoform that is more related to the nuclear receptor corepressor. Proc Natl Acad Sci U S A 96:3519-3524.

Pellegrini R, Mariotti A, Tagliabue E, Bressan R, Bunone G, Coradini D, Della Valle G, Formelli F, Cleris L, Radice P and et al. (1995): Modulation of markers associated with tumor aggressiveness in human breast cancer cell lines by N-(4-hydroxyphenyl) retinamide. Cell Growth Differ 6:863-869.

Pelletier G and El-Alfy M (2000): Immunocytochemical localization of estrogen receptors alpha and beta in the human reproductive organs. J Clin Endocrinol Metab 85:4835-4840.

Perez-Gracia JL and Carrasco EM (2002): Tamoxifen therapy for ovarian cancer in the adjuvant and advanced settings: systematic review of the literature and implications for future research. Gynecol Oncol 84:201-209.

Perrot-Applanat M, Deng M, Fernandez H, Lelaidier C, Meduri G and Bouchard P (1994): Immunohistochemical localization of estradiol and progesterone receptors in human uterus throughout pregnancy: expression in endometrial blood vessels. J Clin Endocrinol Metab 78:216-224.

Pettersson K, Delaunay F and Gustafsson JA (2000): Estrogen receptor beta acts as a dominant regulator of estrogen signaling. Oncogene 19:4970-4978.

Pfaffl MW (2001): A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:E45-E45.

Philips A, Chalbos D and Rochefort H (1993): Estradiol increases and anti-estrogens antagonize the growth factor- induced activator protein-1 activity in MCF7 breast cancer cells without affecting c-fos and c-jun synthesis. J Biol Chem 268:14103-14108.

Pieber D, Allport VC, Hills F, Johnson M and Bennett PR (2001): Interactions between progesterone receptor isoforms in myometrial cells in human labour. Mol Hum Reprod 7:875-879.

Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, Ljunggren J, Gustafsson JA and Carlquist M (1999): Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. EMBO J 18:4608-4618.

Poukka H, Karvonen U, Jänne OA and Palvimo JJ (2000a): Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad Sci U S A 97:14145-14150.

Poukka H, Karvonen U, Yoshikawa N, Tanaka H, Palvimo JJ and Jänne OA (2000b): The RING finger protein SNURF modulates nuclear trafficking of the androgen receptor. J Cell Sci 113 (Pt 17):2991-3001.

Pratt WB and Toft DO (2003): Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med (Maywood) 228:111-133.

Press MF and Greene GL (1988): Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor. Endocrinology 122:1165-1175.

Press MF, Udove JA and Greene GL (1988): Progesterone receptor distribution in the human endometrium. Analysis using monoclonal antibodies to the human progesterone receptor. Am J Pathol 131:112-124.

Pujol P, Rey JM, Nirde P, Roger P, Gastaldi M, Laffargue F, Rochefort H and Maudelonde T (1998): Differential expression of estrogen receptor-alpha and -beta messenger RNAs as a potential marker of ovarian carcinogenesis. Cancer Res 58:5367-5373.

Rachez C, Gamble M, Chang CP, Atkins GB, Lazar MA and Freedman LP (2000): The DRIP complex and SRC-1/p160 coactivators share similar nuclear receptor binding determinants but constitute functionally distinct complexes. Mol Cell Biol 20:2718-2726.

Racz A and Barsony J (1999): Hormone-dependent translocation of vitamin D receptors is linked to transactivation. J Biol Chem 274:19352-19360.

Read LD, Greene GL and Katzenellenbogen BS (1989): Regulation of estrogen receptor messenger ribonucleic acid and protein levels in human breast cancer cell lines by sex steroid hormones, their antagonists, and growth factors. Mol Endocrinol 3:295-304.

Reddel RR, Alexander IE, Koga M, Shine J and Sutherland RL (1988): Genetic instability and the development of steroid hormone insensitivity in cultured T 47D human breast cancer cells. Cancer Res 48:4340-4347.

Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, Gass P, Schmid W, Herrlich P, Angel P and Schutz G (1998): DNA binding of the glucocorticoid receptor is not essential for survival. Cell 93:531-541.

Reichardt HM, Horsch K, Grone HJ, Kolbus A, Beug H, Hynes N and Schutz G (2001): Mammary gland development and lactation are controlled by different glucocorticoid receptor activities. Eur J Endocrinol 145:519-527.

Reichrath J, Rafi L, Muller SM, Mink D, Reitnauer K, Tilgen W, Schmidt W and Friedrich M (1998): Immunohistochemical analysis of 1,25-dihydroxyvitamin D3 receptor in cervical carcinoma. Histochem J 30:561-567.

Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM and Horwitz KB (2002): Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem 277:5209-5218.

Rochette-Egly C (2003): Nuclear receptors: integration of multiple signalling pathways through phosphorylation. Cell Signal 15:355-366.

Rogatsky I, Zarember KA and Yamamoto KR (2001): Factor recruitment and TIF2/GRIP1 corepressor activity at a collagenase- 3 response element that mediates regulation by phorbol esters and hormones. EMBO J 20:6071-6083.

Roger P, Sahla ME, Makela S, Gustafsson JA, Baldet P and Rochefort H (2001): Decreased expression of estrogen receptor beta protein in proliferative preinvasive mammary tumors. Cancer Res 61:2537-2541.

Roman SD, Clarke CL, Hall RE, Alexander IE and Sutherland RL (1992): Expression and regulation of retinoic acid receptors in human breast cancer cells. Cancer Res 52:2236-2242.

Ross RK, Paganini-Hill A, Wan PC and Pike MC (2000): Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. J Natl Cancer Inst 92:328-332.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM and Ockene J (2002): Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA 288:321-333.

Rowan BG, Weigel NL and O'Malley BW (2000): Phosphorylation of steroid receptor coactivator-1. Identification of the phosphorylation sites and phosphorylation through the mitogen- activated protein kinase pathway. J Biol Chem 275:4475-4483.

Russo J, Ao X, Grill C and Russo IH (1999): Pattern of distribution of cells positive for estrogen receptor alpha and progesterone receptor in relation to proliferating cells in the mammary gland. Breast Cancer Res Treat 53:217-227.

Rutherford T, Brown WD, Sapi E, Aschkenazi S, Munoz A and Mor G (2000): Absence of estrogen receptor-beta expression in metastatic ovarian cancer. Obstet Gynecol 96:417-421.

Saceda M, Lippman ME, Chambon P, Lindsey RL, Ponglikitmongkol M, Puente M and Martin MB (1988): Regulation of the estrogen receptor in MCF-7 cells by estradiol. Mol Endocrinol 2:1157-1162.

Said TK, Conneely OM, Medina D, O'Malley BW and Lydon JP (1997): Progesterone, in addition to estrogen, induces cyclin D1 expression in the murine mammary epithelial cell, in vivo. Endocrinology 138:3933-3939.

Saji S, Omoto Y, Shimizu C, Warner M, Hayashi Y, Horiguchi S, Watanabe T, Hayashi S, Gustafsson JA and Toi M (2002): Expression of estrogen receptor (ER) (beta)cx protein in ER(alpha)- positive breast cancer: specific correlation with progesterone receptor. Cancer Res 62:4849-4853.

Salmen T, Heikkinen AM, Mahonen A, Kroger H, Komulainen M, Saarikoski S, Honkanen R and Mäenpää PH (2000): The protective effect of hormone-replacement therapy on fracture risk is modulated by estrogen receptor alpha genotype in early postmenopausal women. J Bone Miner Res 15:2479-2486.

Santen RJ, Pinkerton J, McCartney C and Petroni GR (2001): Risk of breast cancer with progestins in combination with estrogen as hormone replacement therapy. J Clin Endocrinol Metab 86:16-23.

Saporita AJ, Zhang Q, Navai N, Dincer Z, Hahn J, Cai X and Wang Z (2003): Identification and characterization of a ligand-regulated nuclear export signal in androgen receptor. J Biol Chem.

Sartorius CA, Melville MY, Hovland AR, Tung L, Takimoto GS and Horwitz KB (1994): A third transactivation function (AF3) of human progesterone receptors located in the unique N-terminal segment of the B-isoform. Mol Endocrinol 8:1347-1360.

Sartorius CA, Shen T and Horwitz KB (2003): Progesterone receptors A and B differentially affect the growth of estrogen-dependent human breast tumor xenografts. Breast Cancer Res Treat 79:287-299.

Sasaki M, Dharia A, Oh BR, Tanaka Y, Fujimoto S and Dahiya R (2001): Progesterone receptor B gene inactivation and CpG hypermethylation in human uterine endometrial cancer. Cancer Res 61:97-102.

Saunders DE, Christensen C, Lawrence WD, Malviya VK, Malone JM, Williams JR and Deppe G (1992): Receptors for 1,25-dihydroxyvitamin D3 in gynecologic neoplasms. Gynecol Oncol 44:131-136.

Schairer C, Lubin J, Troisi R, Sturgeon S, Brinton L and Hoover R (2000): Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk. JAMA 283:485-491.

Schomberg DW, Couse JF, Mukherjee A, Lubahn DB, Sar M, Mayo KE and Korach KS (1999): Targeted disruption of the estrogen receptor-alpha gene in female mice: characterization of ovarian responses and phenotype in the adult. Endocrinology 140:2733-2744.

Shang Y and Brown M (2002): Molecular determinants for the tissue specificity of SERMs. Science 295:2465-2468.

Shang Y, Hu X, DiRenzo J, Lazar MA and Brown M (2000): Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843-852.

Shang Y, Myers M and Brown M (2002): Formation of the androgen receptor transcription complex. Mol Cell 9:601-610.

Shao D and Lazar MA (1999): Modulating nuclear receptor function: may the phos be with you. J Clin Invest 103:1617-1618.

Shaw JA, Udokang K, Mosquera JM, Chauhan H, Jones JL and Walker RA (2002): Oestrogen receptors alpha and beta differ in normal human breast and breast carcinomas. J Pathol 198:450-457.

Sheppard HM, Harries JC, Hussain S, Bevan C and Heery DM (2001): Analysis of the steroid receptor coactivator 1 (SRC1)-CREB binding protein interaction interface and its importance for the function of SRC1. Mol Cell Biol 21:39-50.

Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA and Greene GL (1998): The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell 95:927-937.

Shiozawa T, Itoh K, Horiuchi A, Konishi I, Fujii S and Nikaido T (2002): Down-regulation of estrogen receptor by the methylation of the estrogen receptor gene in endometrial carcinoma. Anticancer Res 22:139-143.

Shiozawa T, Shih HC, Miyamoto T, Feng YZ, Uchikawa J, Itoh K and Konishi I (2003): Cyclic changes in the expression of steroid receptor coactivators and corepressors in the normal human endometrium. J Clin Endocrinol Metab 88:871-878.

Shoker BS, Jarvis C, Clarke RB, Anderson E, Hewlett J, Davies MP, Sibson DR and Sloane JP (1999a): Estrogen receptor-positive proliferating cells in the normal and precancerous breast. Am J Pathol 155:1811-1815.

Shoker BS, Jarvis C, Sibson DR, Walker C and Sloane JP (1999b): Oestrogen receptor expression in the normal and pre-cancerous breast. J Pathol 188:237-244.

Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN, 3rd, Assaf AR, Jackson RD, Kotchen JM, Wassertheil-Smoller S and Wactawski-Wende J (2003): Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA 289:2651-2662.

Shyamala G, Yang X, Silberstein G, Barcellos-Hoff MH and Dale E (1998): Transgenic mice carrying an imbalance in the native ratio of A to B forms of progesterone receptor exhibit developmental abnormalities in mammary glands. Proc Natl Acad Sci U S A 95:696-701.

Simoncini T and Genazzani AR (2003): Non-genomic actions of sex steroid hormones. Eur J Endocrinol 148:281-292.

Sirchia SM, Ferguson AT, Sironi E, Subramanyan S, Orlandi R, Sukumar S and Sacchi N (2000): Evidence of epigenetic changes affecting the chromatin state of the retinoic acid receptor beta2 promoter in breast cancer cells. Oncogene 19:1556-1563.

Sjöberg I, Rylander E and von Schoultz B (1989): Menstrual variation of estrogen receptor content in vaginal tissue. Gynecol Obstet Invest 27:48-51.

Slayden OD, Nayak NR, Burton KA, Chwalisz K, Cameron ST, Critchley HO, Baird DT and Brenner RM (2001): Progesterone antagonists increase androgen receptor expression in the rhesus macaque and human endometrium. J Clin Endocrinol Metab 86:2668-2679.

Slotman BJ, Nauta JJ and Rao BR (1990): Survival of patients with ovarian cancer. Apart from stage and grade, tumor progesterone receptor content is a prognostic indicator. Cancer 66:740-744.

Smid-Koopman E, Blok LJ, Kuhne LC, Burger CW, Helmerhorst TJ, Brinkmann AO and Huikeshoven FJ (2003): Distinct functional differences of human progesterone receptors A and B on gene expression and growth regulation in two endometrial carcinoma cell lines. J Soc Gynecol Investig 10:49-57.

Smith CL, Nawaz Z and O'Malley BW (1997): Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11:657-666.

Smith RA, Lea RA, Curran JE, Weinstein SR and Griffiths LR (2003): Expression of glucocorticoid and progesterone nuclear receptor genes in archival breast cancer tissue. Breast Cancer Res 5:R9-12.

Snijders MP, de Goeij AF, Debets-Te Baerts MJ, Rousch MJ, Koudstaal J and Bosman FT (1992): Immunocytochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. J Reprod Fertil 94:363-371.

Soderqvist G, von Schoultz B, Tani E and Skoog L (1993): Estrogen and progesterone receptor content in breast epithelial cells from healthy women during the menstrual cycle. Am J Obstet Gynecol 168:874-879.

Speirs V, Malone C, Walton DS, Kerin MJ and Atkin SL (1999): Increased expression of estrogen receptor beta mRNA in tamoxifen-resistant breast cancer patients. Cancer Res 59:5421-5424.

Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ and O'Malley BW (1997): Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389:194-198.

Stjernholm Y, Sahlin L, Akerberg S, Elinder A, Eriksson HA, Malmstrom A and Ekman G (1996): Cervical ripening in humans: potential roles of estrogen, progesterone, and insulin-like growth factor-I. Am J Obstet Gynecol 174:1065-1071.

Stonelake PS, Baker PG, Gillespie WM, Dunn JA, Spooner D, Morrison JM, Bundred NJ, Oates GD, Lee MJ, Neoptolemos JP and et al. (1994): Steroid receptors, pS2 and cathepsin D in early clinically node-negative breast cancer. Eur J Cancer 30A:5-11.

Sullivan WP, Beito TG, Proper J, Krco CJ and Toft DO (1986): Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology 119:1549-1557.

Suzuki T, Sasano H, Kimura N, Tamura M, Fukaya T, Yajima A and Nagura H (1994): Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes. Hum Reprod 9:1589-1595.

Syvälä H, Vienonen A, Zhuang YH, Kivineva M, Ylikomi T and Tuohimaa P (1998): Evidence for enhanced ubiquitin-mediated proteolysis of the chicken progesterone receptor by progesterone. Life Sci 63:1505-1512.

Takama F, Kanuma T, Wang D, Kagami I and Mizunuma H (2001): Oestrogen receptor beta expression and depth of myometrial invasion in human endometrial cancer. Br J Cancer 84:545-549.

Takeshita A, Yen PM, Misiti S, Cardona GR, Liu Y and Chin WW (1996): Molecular cloning and properties of a full-length putative thyroid hormone receptor coactivator. Endocrinology 137:3594-3597.

Takeshita A, Cardona GR, Koibuchi N, Suen CS and Chin WW (1997): TRAM-1, A novel 160-kDa thyroid hormone receptor activator molecule, exhibits distinct properties from steroid receptor coactivator-1. J Biol Chem 272:27629-27634.

Takeyama K, Masuhiro Y, Fuse H, Endoh H, Murayama A, Kitanaka S, Suzawa M, Yanagisawa J and Kato S (1999): Selective interaction of vitamin D receptor with transcriptional coactivators by a vitamin D analog. Mol Cell Biol 19:1049-1055.

Tanabe Y, Nakamura T, Fujioka K and Doi O (1979): Production and secretion of sex steroid hormones by the testes, the ovary, and the adrenal glands of embryonic and young chickens (Gallus domesticus). Gen Comp Endocrinol 39:26-33.

Tanaka Y, Naruse I, Maekawa T, Masuya H, Shiroishi T and Ishii S (1997): Abnormal skeletal patterning in embryos lacking a single Cbp allele: a partial similarity with Rubinstein-Taybi syndrome. Proc Natl Acad Sci U S A 94:10215-10220.

Taylor AH and Al-Azzawi F (2000): Immunolocalisation of oestrogen receptor beta in human tissues. J Mol Endocrinol 24:145-155.

Thenot S, Charpin M, Bonnet S and Cavailles V (1999): Estrogen receptor cofactors expression in breast and endometrial human cancer cells. Mol Cell Endocrinol 156:85-93.

Tian S, Poukka H, Palvimo JJ and Janne OA (2002): Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. Biochem J 367:907-911.

Toma S, Isnardi L, Raffo P, Dastoli G, De Francisci E, Riccardi L, Palumbo R and Bollag W (1997): Effects of all-trans-retinoic acid and 13-cis-retinoic acid on breast- cancer cell lines: growth inhibition and apoptosis induction. Int J Cancer 70:619-627.

Tong D, Schuster E, Seifert M, Czerwenka K, Leodolte S and Zeillinger R (2002): Expression of estrogen receptor beta isoforms in human breast cancer tissues and cell lines. Breast Cancer Res Treat 71:249-255.

Tora L, Gronemeyer H, Turcotte B, Gaub MP and Chambon P (1988): The N-terminal region of the chicken progesterone receptor specifies target gene activation. Nature 333:185-188.

Tora L, White J, Brou C, Tasset D, Webster N, Scheer E and Chambon P (1989): The human estrogen receptor has two independent nonacidic transcriptional activation functions. Cell 59:477-487.

Torchia J, Rose DW, Inostroza J, Kamei Y, Westin S, Glass CK and Rosenfeld MG (1997): The transcriptional co-activator p/CIP binds CBP and mediates nuclear- receptor function. Nature 387:677-684.

Torgerson DJ and Bell-Syer SE (2001): Hormone replacement therapy and prevention of nonvertebral fractures: a meta-analysis of randomized trials. JAMA 285:2891-2897.

Tremblay A, Tremblay GB, Labrie F and Giguere V (1999): Ligand-independent recruitment of SRC-1 to estrogen receptor beta through phosphorylation of activation function AF-1. Mol Cell 3:513-519.

Trial WGftP (1996): Effects of hormone replacement therapy on endometrial histology in postmenopausal women. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. The Writing Group for the PEPI Trial. JAMA 275:370-375.

Tsibris JC, Porter KB, Jazayeri A, Tzimas G, Nau H, Huang H, Kuparadze K, Porter GW, O'Brien WF and Spellacy WN (1999): Human uterine leiomyomata express higher levels of peroxisome proliferator-activated receptor gamma, retinoid X receptor alpha, and all-trans retinoic acid than myometrium. Cancer Res 59:5737-5744.

Tuohimaa P, Renoir JM, Radanyi C, Mester J, Joab I, Buchou T and Baulieu EE (1984): Antibodies against highly purified B-subunit of the chick oviduct progesterone receptor. Biochem Biophys Res Commun 119:433-439.

Turcotte B, Meyer ME, Bellard M, Dretzen G, Gronemeyer H and Chambon P (1991): Control of transcription of the chicken progesterone receptor gene. In vitro and in vivo studies. J Biol Chem 266:2582-2589.

Tyagi RK, Amazit L, Lescop P, Milgrom E and Guiochon-Mantel A (1998): Mechanisms of progesterone receptor export from nuclei: role of nuclear localization signal, nuclear export signal, and ran guanosine triphosphate. Mol Endocrinol 12:1684-1695.

Tyagi RK, Lavrovsky Y, Ahn SC, Song CS, Chatterjee B and Roy AK (2000): Dynamics of intracellular movement and nucleocytoplasmic recycling of the ligand-activated androgen receptor in living cells. Mol Endocrinol 14:1162-1174.

Tzukerman MT, Esty A, Santiso-Mere D, Danielian P, Parker MG, Stein RB, Pike JW and McDonnell DP (1994): Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. Mol Endocrinol 8:21-30.

Umayahara Y, Kawamori R, Watada H, Imano E, Iwama N, Morishima T, Yamasaki Y, Kajimoto Y and Kamada T (1994): Estrogen regulation of the insulin-like growth factor I gene transcription involves an AP-1 enhancer. J Biol Chem 269:16433-16442.

Vegeto E, Shahbaz MM, Wen DX, Goldman ME, O'Malley BW and McDonnell DP (1993): Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol Endocrinol 7:1244-1255.

Viville B, Charnock-Jones DS, Sharkey AM, Wetzka B and Smith SK (1997): Distribution of the A and B forms of the progesterone receptor messenger ribonucleic acid and protein in uterine leiomyomata and adjacent myometrium. Hum Reprod 12:815-822.

Voegel JJ, Heine MJ, Zechel C, Chambon P and Gronemeyer H (1996): TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. EMBO J 15:3667-3675.

Wagner BL, Norris JD, Knotts TA, Weigel NL and McDonnell DP (1998): The nuclear corepressors NCoR and SMRT are key regulators of both ligand- and 8-bromo-cyclic AMP-dependent transcriptional activity of the human progesterone receptor. Mol Cell Biol 18:1369-1378.

Wang C, Fu M, Angeletti RH, Siconolfi-Baez L, Reutens AT, Albanese C, Lisanti MP, Katzenellenbogen BS, Kato S, Hopp T, Fuqua SA, Lopez GN, Kushner PJ and Pestell RG (2001a): Direct acetylation of the estrogen receptor alpha hinge region by p300 regulates transactivation and hormone sensitivity. J Biol Chem 276:18375-18383.

Wang H, Wu X, Englund K, Masironi B, Eriksson H and Sahlin L (2001b): Different expression of estrogen receptors alpha and beta in human myometrium and leiomyoma during the proliferative phase of the menstrual cycle and after GnRHa treatment. Gynecol Endocrinol 15:443-452.

Wang H, Stjernholm Y, Ekman G, Eriksson H and Sahlin L (2001c): Different regulation of oestrogen receptors alpha and beta in the human cervix at term pregnancy. Mol Hum Reprod 7:293-300.

Webb P, Lopez GN, Uht RM and Kushner PJ (1995): Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. Mol Endocrinol 9:443-456.

Webb P, Nguyen P and Kushner PJ (2003): Differential SERM effects on corepressor binding dictate ERalpha activity in vivo. J Biol Chem 278:6912-6920.

Webb P, Nguyen P, Shinsako J, Anderson C, Feng W, Nguyen MP, Chen D, Huang SM, Subramanian S, McKinerney E, Katzenellenbogen BS, Stallcup MR and Kushner PJ (1998): Estrogen receptor activation function 1 works by binding p160 coactivator proteins. Mol Endocrinol 12:1605-1618.

Webster NJ, Green S, Jin JR and Chambon P (1988): The hormone-binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. Cell 54:199-207.

Wei LL and Miner R (1994): Evidence for the existence of a third progesterone receptor protein in human breast cancer cell line T47D. Cancer Res 54:340-343.

Wei LL, Hawkins P, Baker C, Norris B, Sheridan PL and Quinn PG (1996): An amino-terminal truncated progesterone receptor isoform, PRc, enhances progestin-induced transcriptional activity. Mol Endocrinol 10:1379-1387.

Weihua Z, Saji S, Makinen S, Cheng G, Jensen EV, Warner M and Gustafsson JA (2000): Estrogen receptor (ER) beta, a modulator of ERalpha in the uterus. Proc Natl Acad Sci U S A 97:5936-5941.

Weinberger C, Hollenberg SM, Rosenfeld MG and Evans RM (1985): Domain structure of human glucocorticoid receptor and its relationship to the v-erb-A oncogene product. Nature 318:670-672.

Welsh J, Wietzke JA, Zinser GM, Smyczek S, Romu S, Tribble E, Welsh JC, Byrne B and Narvaez CJ (2002): Impact of the Vitamin D3 receptor on growth-regulatory pathways in mammary gland and breast cancer. J Steroid Biochem Mol Biol 83:85-92.

Westin S, Kurokawa R, Nolte RT, Wisely GB, McInerney EM, Rose DW, Milburn MV, Rosenfeld MG and Glass CK (1998): Interactions controlling the assembly of nuclear-receptor heterodimers and co-activators. Nature 395:199-202.

Whitfield GK, Jurutka PW, Haussler CA and Haussler MR (1999): Steroid hormone receptors: evolution, ligands, and molecular basis of biologic function. J Cell Biochem Suppl:110-122.

Widschwendter M, Berger J, Daxenbichler G, Muller-Holzner E, Widschwendter A, Mayr A, Marth C and Zeimet AG (1997): Loss of retinoic acid receptor beta expression in breast cancer and morphologically normal adjacent tissue but not in the normal breast tissue distant from the cancer. Cancer Res 57:4158-4161.

Widschwendter M, Berger J, Hermann M, Muller HM, Amberger A, Zeschnigk M, Widschwendter A, Abendstein B, Zeimet AG, Daxenbichler G and Marth C (2000): Methylation and silencing of the retinoic acid receptor-beta2 gene in breast cancer. J Natl Cancer Inst 92:826-832.

Wieser F, Schneeberger C, Hudelist G, Singer C, Kurz C, Nagele F, Gruber C, Huber JC and Tschugguel W (2002): Endometrial nuclear receptor co-factors SRC-1 and N-CoR are increased in human endometrium during menstruation. Mol Hum Reprod 8:644-650.

Wijayaratne AL and McDonnell DP (2001): The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 276:35684-35692.

Wijayaratne AL, Nagel SC, Paige LA, Christensen DJ, Norris JD, Fowlkes DM and McDonnell DP (1999): Comparative analyses of mechanistic differences among antiestrogens. Endocrinology 140:5828-5840.

Wilson CM and McPhaul MJ (1994): A and B forms of the androgen receptor are present in human genital skin fibroblasts. Proc Natl Acad Sci U S A 91:1234-1238.

Wolf H, Wandt H and Jonat W (1991): Immunohistochemical evidence of estrogen and progesterone receptors in the female lower urinary tract and comparison with the vagina. Gynecol Obstet Invest 32:227-231.

Wong CW, Komm B and Cheskis BJ (2001): Structure-function evaluation of ER alpha and beta interplay with SRC family coactivators. ER selective ligands. Biochemistry 40:6756-6765.

Wood JR, Likhite VS, Loven MA and Nardulli AM (2001): Allosteric modulation of estrogen receptor conformation by different estrogen response elements. Mol Endocrinol 15:1114-1126.

Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT, Parks DJ, Moore JT, Kliewer SA, Willson TM and Stimmel JB

(2002): Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARalpha. Nature 415:813-817.

Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C and O'Malley BW (2000): The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. Proc Natl Acad Sci U S A 97:6379-6384.

Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai MJ and O'Malley BW (1998): Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science 279:1922-1925.

Xu XC, Sneige N, Liu X, Nandagiri R, Lee JJ, Lukmanji F, Hortobagyi G, Lippman SM, Dhingra K and Lotan R (1997): Progressive decrease in nuclear retinoic acid receptor beta messenger RNA level during breast carcinogenesis. Cancer Res 57:4992-4996.

Xu XC, Mitchell MF, Silva E, Jetten A and Lotan R (1999): Decreased expression of retinoic acid receptors, transforming growth factor beta, involucrin, and cornifin in cervical intraepithelial neoplasia. Clin Cancer Res 5:1503-1508.

Yabushita H, Hirata M, Noguchi M and Nakanishi M (1996): Vitamin D receptor in endometrial carcinoma and the differentiation- inducing effect of 1,25-dihydroxyvitamin D3 on endometrial carcinoma cell lines. J Obstet Gynaecol Res 22:529-539.

Yamamoto Y, Wada O, Suzawa M, Yogiashi Y, Yano T, Kato S and Yanagisawa J (2001): The tamoxifen-responsive estrogen receptor alpha mutant D351Y shows reduced tamoxifen-dependent interaction with corepressor complexes. J Biol Chem 276:42684-42691.

Yan F, Gao X, Lonard DM and Nawaz Z (2003): Specific ubiquitin-conjugating enzymes promote degradation of specific nuclear receptor coactivators. Mol Endocrinol 17:1315-1331.

Yang X, Ferguson AT, Nass SJ, Phillips DL, Butash KA, Wang SM, Herman JG and Davidson NE (2000): Transcriptional activation of estrogen receptor alpha in human breast cancer cells by histone deacetylase inhibition. Cancer Res 60:6890-6894.

Yang XJ, Ogryzko VV, Nishikawa J, Howard BH and Nakatani Y (1996): A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature 382:319-324.

Yao TP, Oh SP, Fuchs M, Zhou ND, Ch'ng LE, Newsome D, Bronson RT, Li E, Livingston DM and Eckner R (1998): Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. Cell 93:361-372.

Yi P, Driscoll MD, Huang J, Bhagat S, Hilf R, Bambara RA and Muyan M (2002): The Effects of Estrogen-Responsive Element- and Ligand-Induced Structural Changes on the Recruitment of Cofactors and Transcriptional Responses by ERalpha and ERbeta. Mol Endocrinol 16:674-693.

Ylikomi T, Bocquel MT, Berry M, Gronemeyer H and Chambon P (1992): Cooperation of proto-signals for nuclear accumulation of estrogen and progesterone receptors. EMBO J 11:3681-3694.

Ylikomi T, Wurtz JM, Syvälä H, Passinen S, Pekki A, Haverinen M, Bläuer M, Tuohimaa P, Gronemeyer H, Meyer ME, Pornon A, Ji JW, Bocquel MT and Chambon P (1998): Reappraisal of the role of heat shock proteins as regulators of steroid receptor activity. Crit Rev Biochem Mol Biol 33:437-466.

Yoh SM, Chatterjee VK and Privalsky ML (1997): Thyroid hormone resistance syndrome manifests as an aberrant interaction between mutant T3 receptors and transcriptional corepressors. Mol Endocrinol 11:470-480.

Yoshida T, Eguchi H, Nakachi K, Tanimoto K, Higashi Y, Suemasu K, Iino Y, Morishita Y and Hayashi S (2000): Distinct mechanisms of loss of estrogen receptor alpha gene expression in human breast cancer: methylation of the gene and alteration of trans- acting factors. Carcinogenesis 21:2193-2201.

Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, Masushige S, Fukamizu A, Matsumoto T and Kato S (1997): Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet 16:391-396.

Yudt MR and Cidlowski JA (2002): The glucocorticoid receptor: coding a diversity of proteins and responses through a single gene. Mol Endocrinol 16:1719-1726.

Zhang X, Jeyakumar M, Petukhov S and Bagchi MK (1998): A nuclear receptor corepressor modulates transcriptional activity of antagonist-occupied steroid hormone receptor. Mol Endocrinol 12:513-524.

Zhou Y, Gross W, Hong SH and Privalsky ML (2001): The SMRT corepressor is a target of phosphorylation by protein kinase CK2 (casein kinase II). Mol Cell Biochem 220:1-13.

Zhuang YH, Saaristo R and Ylikomi T (2003): An in vitro long-term culture model for normal human mammary gland: expression and regulation of steroid receptors. Cell Tissue Res 311:217-226.

Zou A, Marschke KB, Arnold KE, Berger EM, Fitzgerald P, Mais DE and Allegretto EA (1999): Estrogen receptor beta activates the human retinoic acid receptor alpha-1 promoter in response to tamoxifen and other estrogen receptor antagonists, but not in response to estrogen. Mol Endocrinol 13:418-430.

ORIGINAL COMMUNICATIONS