HBGS

Helsinki Biomedical Graduate School

MOLECULAR DETERMINANTS OF ANDROGEN ACTION

Henrikki Santti

Institute of Biomedicine / Physiology

University of Helsinki

Finland

ACADEMIC DISSERTATION

Helsinki University Biomedical Dissertations No. 55

To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki, in the lecture hall 3, Biomedicum Helsinki, Haartmaninkatu 8, on August 27st , 2004, at 12 o'clock.

Helsinki 2004

Supervised by

Professor Olli A. Jänne

and

Professor Jorma J. Palvimo

Reviewed by

Professor Leo Dunkel

University of Helsinki

and

Docent Matti Poutanen

University of Turku

CONTENTS

Contents

ABSTRACT

Androgens are mandatory for normal sexual development and maintenance of spermatogenesis. Also, androgens are important in the development and treatment of pathological processes such prostate cancer. Effects of androgens, like other steroid hormones, are mediated by their intracellular receptors. The androgen receptor is activated by the ligand, relocalizes into nucleus, binds to DNA as a dimer, and activates transcription. In addition to the receptor and general transcription factors, a number of auxiliary proteins that modulate the androgen response have been isolated in recent years. Coregulators are proteins that either increase or decrease androgen receptor-dependent transcription without affecting basal level of transcription. One of the coregulators is PIASx that is capable of modulating androgen action similar to other members of protein inhibitor of activated STAT (PIAS) protein family. Recently, PIAS proteins have been shown to act as E3 ligases in sumoylation, a posttranslational modification that resembles ubiquitination.

This study aimed at delineating effects of ligands, receptor mutations, and the coregulator PIASx on androgen action. Serum androgen bioactivity was measured with an assay that is based on the interaction between N- and C-terminal regions of the androgen receptor. Serum androgen bioactivity was lower in men with prostate cancer than in men with benign prostatic hyperplasia ($P < 0.005$). Moreover, serum testosterone level alone overestimated serum androgen bioactivity, especially in men with non-aggressive prostate cancer. Mutations in the DNA-binding domain of androgen receptor inactivated the transactivation function of the receptor, but left the transrepression function relatively unchanged. Likewise, mutations in the DNA-binding domain did not alter the interaction with CBP/p300 coactivator. Androgen receptor coregulator PIASx, which is predominantly expressed in testis, was found to be present in Sertoli cells and germ cells. Another PIAS protein, PIAS1, was also expressed in Sertoli cells and germ cells. Highest levels of PIASx and PIAS1 were detected in pachytene spermatocytes and round spermatids, respectively. Thus, expression patterns of PIASx and PIAS1 genes are overlapping, but partially distinct. Proximal promoter region of the PIASx gene is GC-rich and sufficient for testis-specific expression *in vivo*. Sp transcription factors formed a major complex with the promoter and are candidate regulators of PIASx expression. Disruption of PIASx gene in mice resulted in a 23 % decrease in testis weight and reduced epididymal sperm count by 19 %. Also, the rate of apoptosis was increased in the testis of PIASx knockout mice. Mice defective of PIASx were fertile and sperm cells were qualitatively normal. Thus, PIASx is required for quantitatively normal spermatogenesis, and the consequences of the disruption of the PIASx gene are likely compensated by other PIAS proteins.

ORIGINAL PUBLICATIONS

The thesis is based on following original articles that are referred to in the text by their Roman numerals.

I Raivio T*, Santti H*, Schatzl G, Gsur A, Haidinger G, Palvimo JJ, Jänne OA, Madersbacher S (2003) Reduced circulating androgen bioactivity in patients with prostate cancer. Prostate 55: 194-198 *Equal contribution   Link

II Aarnisalo P, Santti H, Poukka H, Palvimo JJ, Jänne OA (1999) Transcription activating and repressing functions of the androgen receptor are differentially influenced by mutations in the deoxyribonucleic acid-binding domain. Endocrinology 140: 3097-3105   Link

III Yan W, Santti H, Jänne OA, Palvimo JJ, Toppari J (2003) Expression of the E3 SUMO-1 ligases PIASx and PIAS1 during spermatogenesis in the rat. Gene Expr Patterns 3: 301-308   Link

IV Santti H, Mikkonen L, Hirvonen-Santti S, Toppari J, Jänne OA, Palvimo JJ (2003) Identification of a short PIASx gene promoter that directs male germ cell-specific transcription in vivo. Biochem Biophys Res Commun 308: 139-147   Link

V Santti H, Anand A, Hirvonen-Santti S, Toppari J, Panhuysen M, Vauti F, Perera M, Corte G, Wurst W, Jänne OA, Palvimo JJ (2004) Disruption of murine PIASx gene results in reduced testis weight. Submitted

In addition, some unpublished data are presented.

Original publication II was also included in the thesis entitled "Cross-talk of androgen receptor with other pathways" by Piia Aarnisalo. The original publications are reproduced with permission of the copyright holders.

ABBREVIATIONS

AF1	activation function 1
AF2	activation function 2
AP-1	activator protein 1
AR	androgen receptor
ARE	androgen response element
bp	base pair
BPH	benign prostatic hyperplasia
CAIS	complete androgen insensitivity syndrome
CARM	coactivator-associated arginine methyltransferase
CBP	CREB-binding protein
DBD	DNA binding domain
DHEA	dehydroepiandrosterone
DHT	5α-dihydrotestosterone
dpc	days post coitum
DRIP	vitamin D receptor-interacting protein
ER	estrogen receptor
GR	glucocorticoid receptor
HAT	histone acetyltransferase
HDAC	histone deacetylase
LBD	ligand-binding domain
LBP	ligand-binding pocket
MAPK	mitogen-activated protein kinase
MAR	matrix attachment region
MR	mineralocorticoid receptor
N-CoR	nuclear hormone receptor corepressor
NF-κB	nuclear factor κB
NPC	nuclear pore complex
NR	nuclear receptor
PAIS	partial androgen insensitivity syndrome
PCAF	p300/CBP-associated factor
PIAS	protein inhibitor of activated STAT
PML	promyelocytic leukemia

PR	progesterone receptor
PRMT	protein arginine methyltransferase
RPII	RNA polymerase II
SHBG	sex-hormone-binding globulin
SP-RING	Siz/PIAS RING
SMRT	silencing mediator for retinoid and thyroid hormone receptors
SRC-1	steroid receptor coactivator-1
STAT	signal transducer and activator of transcription
SUMO	small ubiquitin-like modifier
SWI/SNF	Switch/sucrose non-fermenting
TAF	TBP-associated factor
TBP	TATA box-binding protein
TRAP	thyroid hormone receptor-associated protein

INTRODUCTION

Androgens are steroid hormones that are produced mainly by testis in males and in minor amounts by adrenal gland, peripheral tissues, and ovary in females. Androgens are important for functions such as regulation of male sexual development, production of male secondary sexual characteristics, and maintenance of spermatogenesis. Androgens have also anabolic effects that increase, for example, muscle strength and bone density. All these actions of androgens are mediated by the intracellular androgen receptor (AR). AR binds its ligand in cytosol, translocates to nucleus and binds to androgen response element (ARE) as a homodimer. Binding of AR to DNA may either increase or decrease the transcription of a target gene. Specificity of this regulation is achieved at multiple levels. In physiological hormone concentrations, androgens bind specifically to AR whose expression is restricted to certain cell-types. Androgens may be metabolized to more active forms by enzymes present in some androgen target-tissues. Activity of AR depends also on posttranslational modifications, which enables other signaling pathways to regulate androgen signaling. AREs are present in the regulatory regions of certain genes and therefore only these genes can be regulated by AR. Coregulator proteins are able to either increase or decrease transcription of a target gene in an AR-dependent manner. Coregulators may also have cell-specific expression patterns and may be selective to only a certain steroid hormone receptors, and thus, add a new level of regulation.

Androgens play an important role in the pathogenesis of relatively common diseases, such as benign prostatic hyperplasia (BPH) and prostate cancer. As anabolic substances, androgens can be used to treat catabolic states caused by aging, infection or cancer. Spermatogenesis, a process maintained by androgens, is also the target of hormonal male contraception. Better understanding of androgen action in normal processes and in the pathogenesis of androgen-dependent diseases should, therefore, open new options for prevention and treatment of these diseases, for example, by selective modification of androgen action.

REVIEW OF THE LITERATURE

1. ANDROGENS

1.1. Production of androgens

In men, androgens are produced mainly in the testis and to a lesser extent in the adrenal gland. Like for all steroid hormones, cholesterol is a precursor for synthesis of androgens. In the first rate-limiting step, cholesterol is converted to pregnenolone by P450scc enzyme in mitochondria. Subsequently, P450C17 catalyzes conversion of pregnenolone to 17α-hydroxypregnenolone and then to dehydroepiandrosterone (DHEA). In testis DHEA is further converted to androstenedione, and then to testosterone that acts locally within testis and is also released into blood (for review, Miller 2002). Testosterone is produced in minor amounts in women by ovaries, and both in men and in women by peripheral tissues through the conversion of blood-derived DHEA to testosterone (for review, Labrie et al. 2001). Testosterone is metabolized to 5α-dihydrotestosterone (DHT) in some peripheral tissues by two types of 5α-reductases. In adults 5α-reductase type I is expressed mainly in skin (sebaceous glands) and liver, whereas 5α-reductase type II is predominantly present in skin (hair follicles), liver, and prostate (for review, Andersson 2001, Steers 2001). Therefore, DHT is the major active androgen in skin and prostate, and the DHT in peripheral blood is derived mostly from these tissues.

1.2. Transport of androgens

Androgens are lipophilic molecules and transported in blood mostly as bound to proteins, and only a few percent remain unbound. Three plasma proteins significantly bind androgens: albumin, sex-hormone binding globulin (SHBG), and corticosteroid-binding globulin (CBG). The relative amount of testosterone that is bound to these proteins varies depending on its concentration. In spermatic vein, where the concentration is high, most of testosterone is bound to albumin that has a high binding capacity but a low binding affinity. However, at normal serum testosterone concentration of men, 50%, 44%, and 4% of testosterone is bound to albumin, SHBG, and CBG, respectively (Dunn et al. 1981). The level of free and non-SHBG bound testosterone tends to correlate well with the biological androgen effects, and it is generally regarded as the biological active fraction (van den Beld et al. 2000). Although SHBG receptors have been found on the plasma membrane of androgen-responsive cells, the importance of these

receptors in the import of androgens into cell is unclear, and it is assumed that testosterone rather enters the cell by free diffusion through the plasma membrane (for review, Hammond 2002).

1.3. Physiological androgens

The activity of androgens depends on their concentration within the cell and on their affinity for AR. Intracellular concentration of androgen is probably affected by its concentration in serum (Table 1), because the transport through plasma membrane is passive. Furthermore, intracellular concentration depends on the presence of androgen-metabolizing enzymes. For example, 5α-reductase type II enzyme converts 95% of the testosterone to DHT in prostatic cells (Taplin and Ho 2001). Likewise, DHEA can be converted to testosterone in peripheral cells (for review, Labrie et al. 2001). Androgens exhibit drastic differences in their ability to bind to AR. While adrenal androgens (DHEA and androstenedione) have a low affinity to AR, testosterone and DHT have a high affinity. Affinity of a given androgen for AR seems to correlate closely with its potential to activate AR. DHT has 5-10-times lower K_d than testosterone, and in reporter gene assays, DHT is 5-10-times more potent than testosterone (Deslypere et al. 1992). Thus, DHT is the most potent natural androgen formed in cells that contain 5α-reductase, whereas in cells devoid of 5α-reductase, testosterone is typically more important because of its higher concentration. Although adrenal androgens are of limited importance in men, in women they may cause for example hirsutism when produced in excessive amounts. Additionally, testosterone and DHT have been demonstrated to activate partially distinct genes in the prostate, indicating that the ligand regulates AR function through a complex allosteric manner (Avila et al. 1998).

Table 1. Reference ranges for androgen concentrations in serum of men and women (Helsinki University Central Hospital Laboratory).

Hormone	Men	Women
Androstenedione	1.4 – 7.0 nM	1.2 – 7.0 nM
Testosterone	10.0 – 38.0 nM	0.9 – 2.8 nM
DHT	1.0 – 10.0 nM	0.3 – 1.2 nM

1.4. Androgens in sexual differentiation

Gonad formation begins in human at about 5 weeks post coitum and in mouse at 10 days post coitum (dpc) by formation of genital ridge from mesonephros (for review, Clarkson and Harley 2002, Rey and Picard 1998, Swain and Lovell-Badge 1999). Both Müllerian ducts (progenitor of the oviducts, uterus and upper vagina) and Wolffian ducts (progenitor of the epididymis and vas deferens) form at the same time with gonad. At 11 dpc, SRY encoded in Y chromosome is expressed transiently triggering the development of Sertoli cells. The Sertoli cells, probably through activity of SOX9, orchestrate the development of testicular cords as well as differentiation of other cell types into Leydig cells, gonocytes, and peritubular myoid cells. The Sertoli cells start to secret anti-Müllerian hormone (AMH) that causes the regression of Müllerian ducts. At the same time, Leydig cell-derived testosterone induces transformation of the Wolffian duct to epididymis, vas deferens, and seminal vesicle. Development of external genitalia is also androgen-dependent. However, in contrast to internal genitalia, external genitalia express 5α-reductase type II that catalyzes conversion of testosterone to DHT. The dependence of external genital development on DHT is demonstrated by the ambiguous external genitalia at birth in patients with inactivating mutation in 5α-reductase type II (Imperato-McGinley et al. 1974, Walsh et al. 1974). Androgens are likely to play an important role in testis descent, which is commonly disturbed in newborn boys. Testis descent is divided to transabdominal and inguinoscrotal phases. The first phase appears to be mediated by the insulin-like peptide hormone INSL3 and insulin receptor family, while the second phase depends on normal androgen action (Klonisch et al. 2004, Nef et al. 2003). Taken together, the proper male sexual differentiation in man requires an adequate testosterone synthesis as well as intact 5α-reductase activity and AR function.

1.5. Androgens in spermatogenesis

Spermatogenesis is a complex process; it starts from mitotically dividing spermatogonia and ends up to mature haploid spermatozoa through two rounds of meiotic cell divisions. The importance of androgens for normal spermatogenesis has been demonstrated, for example, with ethane dimethane sulfonate (EDS) treatment of rats that destroys Leydig cells and reduces serum and intratesticular testosterone levels to undetectable (Bartlett et al. 1986). This treatment results in disruption of spermatogenesis, which may be overcome by simultaneous testosterone supplementation (Sharpe et al. 1988). Spermatogenesis has been thought to depend on high local

testosterone concentration. However, a recent study suggests that, at least in mouse, spermatogenesis is maintained even at low intratesticular testosterone concentration (Zhang et al. 2003). Localization of AR in testis has been controversial; in some studies (Vornberger et al. 1994, Zhou et al. 1996), immunostaining has been seen also in germ cells, but in most of the studies, AR has not been detected in germ cells (Bremner et al. 1994, Ruizeveld de Winter et al. 1991, Sar et al. 1990). In a recent study, Zhou et al. (2002) could detect AR by immunohistochemistry in Leydig cells, peritubular myoid cells, and in Sertoli cells in a stage-dependent manner. However, the germ cells were completely devoid of AR, suggesting that androgens have an indirect effect on germ cells through Sertoli cells. This hypothesis was further strengthened in a recent study where Sertoli cell-specific AR knockout mice were generated (De Gendt et al. 2004). These gene-targeted mice were infertile and did not produce spermatozoa, although the development of genital tract was otherwise normal. Functional AR was mandatory to complete meiosis, as indicated by a decrease in the number of spermatocytes and almost complete absence of round spermatids. Although this study did not formally rule out the peritubular myoid cells as mediators of androgen action, the data showed nevertheless that indirect effects of androgens on germ cells via the Sertoli cells is an absolute requirement for normal spermatogenesis.

1.6. Androgens in normal prostate gland

The prostate gland develops by an outgrowth of epithelial buds from urogenital sinus in mouse at 17.5 dpc and in human at around 11 weeks post coitum (for review, Marker et al. 2003, Sciavolino and Abate-Shen 1998). This development occurs in an androgen-dependent manner; mutations in AR may prevent the development of the prostate. Urogenital sinus expresses 5α-reductase type II, and therefore, the majority of testosterone in the urogenital sinus is converted to DHT. The development of the prostate regarding its dependency on DHT, however, seems to differ between mouse and human. Knockout mice, in which type II or both type I and type II 5α-reductases have been disrupted, are fertile and their prostates form normally (Mahendroo et al. 2001). These animals' prostate weight was, however, reduced compared to wild-type mice, suggesting that DHT acts as an amplifier of androgen action in mice. In contrast to mice, human patients with 5α-reductase deficiency have a rudimentary prostate, indicating that in man, the development of the prostate is much more dependent on DHT.

Finasteride, a 5α-reductase type II inhibitor, is an effective drug for the treatment of BPH, causing 25% decrease in the weight of the prostate (Edwards and Moore 2002). The effect of

finasteride treatment on the prostate is, however, not uniform. Prostate gland can be divided to two compartments: the stroma and the epithelium (Fig. 1). The stroma consists of smooth muscle cells and fibroblasts, whereas the epithelium is composed of basal, luminal and neuroendocrine cells. During finasteride treatment, 90% of luminal cells undergo apoptosis, whereas only 20% of basal cells die (Marks et al. 1997). AR is expressed both in the stroma and in the epithelium. In the epithelium, the basal cells contain little or no AR, whereas the luminal cells are clearly positive for AR (for review, Heinlein and Chang 2004, Litvinov et al. 2003). In contrast luminal epithelial cells, the prostatic neuroendocrine cells do not contain AR.
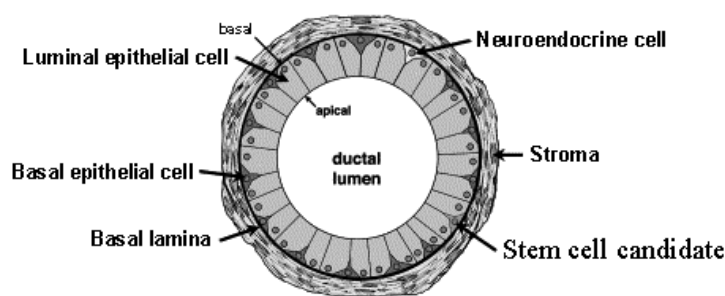
Figure 1. Cross-section of prostate duct. Adapted from Marker et al. 2003.

1.7. Non-genomic actions of androgens

Classically, the effects of androgens are thought to be a result of changes in gene transcription mediated by AR. Some of the effects occur, however, too quickly to be mediated by the classical AR, or they take place in the absence of the characterized AR or in the presence of inhibitors of transcription or translation. These effects are termed nongenomic effects, because they are not attributable to the classical AR-mediated pathway (Heinlein and Chang 2002a). Three kinds of mechanism for nongenomic actions have been described. Firstly, binding of a ligand to AR may promote interaction between the receptor and c-Src, which leads to stimulation of c-Src kinase activity and activation of mitogen-activated protein kinase (MAPK) signaling pathway (Kousteni et al. 2001, Migliaccio et al. 2000). Secondly, SHBG may bind to a putative SHBG plasma membrane receptor and then bind to testosterone, which leads to increase of intracellular cAMP concentration and activation of protein kinase A (PKA) (Rosner et al. 1999). Thirdly, testosterone may bind to an as yet unidentified membrane AR that would cause an increase in the intracellular calcium level, which could, in turn, activate several signaling cascades (reviewed by Heinlein and Chang 2002a). Such a membrane receptor has been cloned for progesterone, and

the membrane progesterone receptor appears to be involved in germ cell functions (Lösel and Wehling 2003). Additionally, MAPK and PKA activation may lead to changes in phosphorylation status of AR and AR coactivators. However, to date no membrane AR has been cloned and nor has SHBG receptor been isolated. Moreover, some of the nongenomic effects of androgens occur in the presence of unphysiologically high concentrations of testosterone. Therefore, the majority of androgenic effects occur by AR-mediated changes in the transcription of genes.

2. TRANSCRIPTION

2.1. Regulatory DNA elements

Gene transcription is regulated by non-coding DNA elements that harbor binding sites for transcription factors. Promoter is a DNA segment typically located upstream of transcription start site of the gene, and it can be divided into proximal and core promoter regions. While the proximal promoter contains binding sites for sequence-specific transcription factors, the core promoter (segment about 35 nt upstream and downstream of transcription start site) is the site of transcription initiation. Typical segments of core promoter include the TATA box, the initiator (Inr) element, downstream core promoter element (DPE), and TFIIB recognition element (BRE) (for review, Butler and Kadonaga 2002, Smale 2001). The TATA box is usually located 25-30 nt upstream of transcription start site and serves as a binding site for TATA box-binding protein (TBP). The Inr is the region were transcription initiates at single or multiple sites. General transcription factor TFIID uses the Inr and DPE elements cooperatively for binding to DNA especially on TATA-less promoters. BRE, on the other hand, is the segment where TFIIB binds to. However, core promoters typically do not contain all of the above-mentioned elements. Therefore, rather than being a passive place for the assembly of transcription factors, the composition of the core promoter may, for example, define which enhancer cooperate with the promoter (Ohtsuki et al. 1998). Only 32% of human promoters contain the TATA box and TATA-less promoters often regulate GC-rich housekeeping genes (Suzuki et al. 2001). GC-rich regions usually contain CpG dinucleotides that are mostly unmethylated and harbor GC boxes that serve as binding sites for members of the Sp transcription factor family (for review, Hapgood et al. 2001).

Not all regulatory sequences are located in the vicinity of transcription start site. Instead, enhancers and silencers may regulate gene expression from sites several kilobases upstream or downstream of transcription start site. Besides the distance from transcription start site, the

enhancers differ from the proximal promoter elements by their orientation requirement. Whereas the proximal promoter is in a fixed orientation, the enhancers are functional in both orientations. According to current view, enhancers can both increase the rate of transcription and, perhaps more importantly, affect chromatin structure enabling transcription to occur (for review, Hertel et al. 1997, Martin 2001). In the recruitment model, activators bind to the enhancer and then interact with the transcription machinery to stabilize binding of general transcription factors to the core promoter (Hertel et al. 1997). The effect of enhancers on transcription is nicely illustrated by the PSA gene. The PSA promoter governs elements for androgen-dependent regulation and transcriptional activity of the PSA gene is clearly increased by the enhancer (Schuur et al. 1996).

The function of an enhancer is also dependent on the neighboring sequences. For example, a transgene containing an enhancer may be transcriptional silent due to chromatin structure at the site of integration. The function of elements such as insulators and matrix attachment regions (MAR) is site-independent. Insulators are further divided to blockers and barriers (for review, Kuhn and Geyer 2003, Oki and Kamakaka 2002,). The main function of a blocker is to inhibit the activity of an enhancer. Typically, a blocker element is located downstream of an enhancer or at both ends of a gene. Thus, a blocker may regulate the usage of an enhancer or insulate one gene from the influence of *cis*-acting regulatory regions of other genes by blocking the enhancer-promoter communication (Cai and Levine 1995). The barrier activity of an insulator may, on the other hand, reduce the activity of nearby silencer or protect the gene from the repressive influence of surrounding heterochromatin. MAR sequences are AT-rich regions that may be able to bind to nuclear matrix, and they may be involved in the organization of nucleus (Cremer and Cremer 2001).

2.2. Chromatin

Most of genomic DNA in the nucleus is tightly packed. The basic structure of chromatin is a nucleosome that consists of 146 base pairs (bp) of DNA and two heterodimers of histones H3 and H4 as well as two heterodimers of histones H2A and H2B. With the help of linker histone H1, chromatin is packed further and can be detected as heterochromatin and euchromatin. In general, the more packed the DNA is, the more inactive it is in terms of gene transcription. Therefore, regulation of chromatin structure is an important means to regulate transcription.

Chromatin structure may be modified by altering the structure of histones. The N-terminal tails of histones protrude from the nucleosome and are important for nucleosome-nucleosome

contacts. Thus, a covalent modification of a histone tail, for example, by acetylation may disrupt the higher-order chromatin structure. Indeed, many transcriptional coactivator proteins possess histone acetyltransferase (HAT) activity and acetylate specific lysine residues in the histone tail (Brownell et al. 1996). HATs are often found in complexes with other proteins (for review, Ogryzko 2001). Acetylation is thought to result in a decreased affinity of a lysine residue of the histone for DNA and neighboring nucleosomes that, in turn, may permit the entry of transcription machinery. Histone acetylation tends to increase transcription, although it is not sufficient for transcription to occur. On the other hand, several transcriptional corepressors have histone deacetylase (HDAC) activity, which maintains histones in an unacetylated state.

Chromatin structure can also be modified by chromatin remodelers, such as Switch/sucrose non-fermenting (SWI/SNF), imitation switch (ISWI), Mi-2, and INO80 complexes, which do not catalyze covalent modifications in histones (for review, Becker and Hörz 2002). Instead, chromatin remodelers have ATPase activity and are able to utilize the energy released in ATP hydrolysis to alter nucleosome conformations by sliding of nucleosomes along the DNA permitting the basal transcription machinery to access DNA (Kassabov et al. 2003). Interestingly, only a few members of SWI/SNF complex are necessary for remodeling to occur *in vitro* (Phelan et al. 1999). The other members can mediate contacts with transcription factors, such as BAF250 with glucocorticoid receptor (GR) (Nie et al. 2000).

2.3. Subnuclear structures

Nucleus is not a homogenous structure; rather, it is composed of several compartments that are not surrounded by a membrane (for review, Spector 2001). Chromosomes form their own compartment, the so called chromosome territory in which chromatin resides in heterochromatin and euchromatin forms. While the compact heterochromatin is located near the nuclear lamina, the less compact euchromatin tends to form loops that protrude into interchromatin compartment (Visser et al. 2000). Chromosome territory forms a barrier that restricts the movement of high-molecular-weight molecules, suggesting that individual proteins can access into heterochromatin, whereas large protein complexes reside in interchromatin compartment (Cremer and Cremer 2001, Lukacs et al. 2000). Localization of a gene into either heterochromatin or euchromatin is not fixed; rather, an active gene may be relocalized into heterochromatin loop (Francastel et al. 1999). It is currently not known whether this localization of inactive gene into heterochromatin is a cause for or a consequence of the lack of active transcription (Carmo-Fonseca 2002).

Interchromatin compartment contains numerous subcompartments. Besides several thousands of transcription sites, interchromatin harbors structures such as nuclear speckles, cleavage bodies, and promyelocytic leukemia (PML) bodies. PML protein plays a central role in PML body formation, since PML bodies do not form without PML protein. Also, sumoylation of PML protein is required for the PML body formation (Zhong et al. 2000a). Several transcription-related factors have been detected in PML bodies, implicating PML bodies in transcription (for review, Zhong et al. 2000b). Three different models have been proposed to explain the functions of PML bodies in transcription (Zhong et al. 2000b). Firstly, PML bodies may regulate the concentration of transcription factors in interchromatin compartment by sequestering them. Secondly, transcription factors may be subjected to modifications in PML bodies, such as sumoylation, which may alter their transcriptional activity. Thirdly, PML bodies may serve as compartments where otherwise active transcription factors, such as Daxx, are inactive (Li et al. 2000). Transcription factors gain entry from cytoplasm to the interchromatin compartment through nuclear pore complex (NPC). Although small molecules can pass through NPC freely, the transport of proteins is thought to be active (for review, Fahrenkrog and Aebi 2003). In conclusion, the transcriptional activity of transcription factors can be regulated by affecting their nuclear transport and intranuclear localization.

2.4. General transcription machinery

Transcription requires six general transcription factors and RNA polymerase II (RPII) to occur *in vitro* (for review, Hampsey 1998, Lee and Young 2000). The first step is the recognition of the promoter by TBP which is a part of the TFIID complex harboring several TBP-associated factors (TAF). Interestingly, TBP alone is able to bind to a TATA-box containing promoter, but TAFs of TFIID complex are required for binding to a TATA-less promoter (Burke and Kadonaga 1997). The binding of TBP bends DNA and forms a platform for other factors. This binding is further stabilized by TFIIB that binds to TFIID and core promoter elements. Binding of TFIIB, on the other hand, is re-enforced by TFIIA, which may also block transcriptional repressors (Ge and Roeder 1994). TFIIB recruits the complex of RPII and TFIIF, which is likely to block nonspecific DNA binding and facilitate promoter melting. In addition, closely linked TFIIE and TFIIH participate in promoter opening, and the kinase complex of TFIIH phosphorylates the carboxy-terminal domain of RPII, causing RPII to change the initiator complex to the elongation complex in order to start elongation (Lee and Young 2000).

2.5. Transcription during spermatogenesis

Spermatogenesis is a multi-step process that begins with mitotic cell-division of diploid spermatogonia and ends up to haploid spermatozoa that have a highly specialized structure (Fig. 2). In addition, meiotic recombination occurs between the sister chromosomes. In spermatids, histones are first replaced by transition proteins and subsequently by protamines. These changes put special requirements for transcription during spermatogenesis, and transcription in male germ cells differs indeed from that in somatic cells in multiple ways (for review, Grootegoed et al. 2000, Kleene 2001, Sassone-Corsi 2002). Due to chromatin reorganizations, such as meiotic recombination and replacement of histones, transcription tends to occur mainly in spermatogonia and in pachytene spermatocytes and round spermatids. However, the temporally restricted transcription is compensated by the abundance of general transcription factors (Schmidt and Schibler 1995). Moreover, some general transcription factors such as TFIIA and $TAF_{II}55$ have testis-specific isoforms $TFIIA\tau$ and $TAF_{II}Q$, respectively (Ozer et al. 2000, Wang et al. 2001). A specific feature of spermatogenesis is the inactivation of the X and Y chromosomes that pair with each other and are stored in a sex-vesicle as heterochromatin. Therefore, for example, the inactivation of the phosphoglycerate kinase 1 (Pgk1) gene, located in X chromosome, is compensated for by the expression of the autosomal intronless Pgk2 gene, which is not expressed in other cell types (McCarrey and Thomas 1987). Several testis-specific genes lack the TATA box and TBP-like proteins are likely to utilized in transcription of TATA-less promoters during spermatogenesis (Sassone-Corsi 2002). Disruption of the testis-specific TBP-related factor 2 (Trf2) indeed results in a complete arrest of spermiogenesis, indicating that Trf2 controls transcription of genes that are important for normal spermatogenesis (Martianov et al. 2001, Zhang et al. 2001). Transcription and translation appear to be loosely coupled during spermiogenesis due to changes in chromatin structure. Histones are replaced by protamines during the last steps of spermatogenesis, after which transcription ceases. Therefore, the protamine genes are transcribed during the early elongating steps, whereas the translation occurs later on and is regulated by the element in 3' untranslated region of the protamine mRNA (Fajardo et al. 1997). Delayed translation may even be a more general phenomenon during spermatogenesis (Kleene 2001).
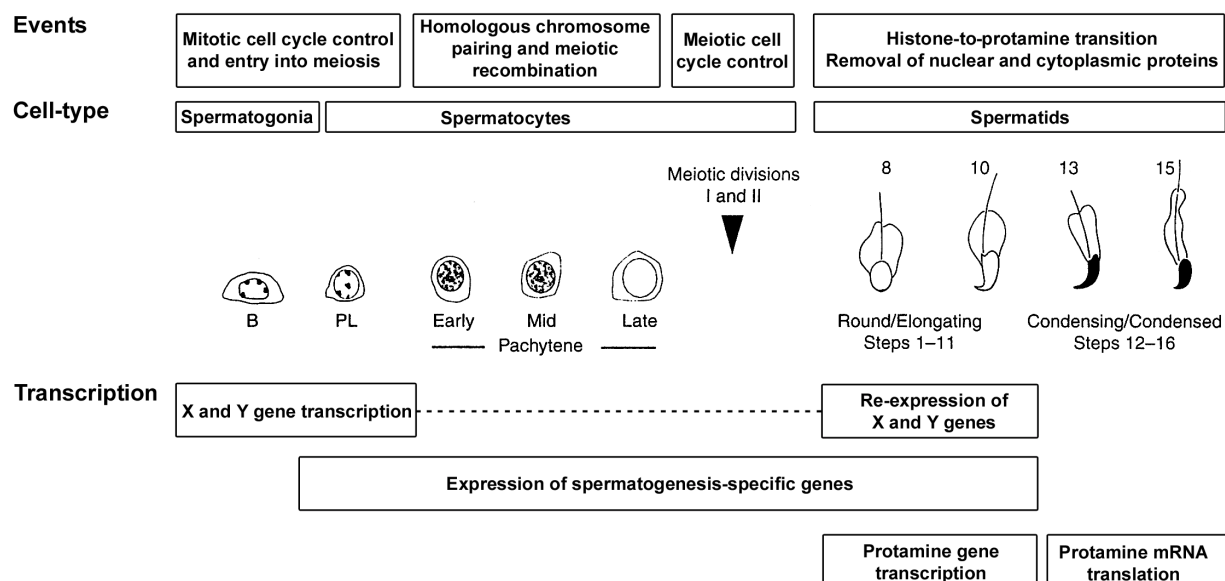
Figure 2. Cellular events and transcription in specific cell types during spermatogenesis. Adapted from Grootegoed et al. 2000. B, type B spermatogonia; PL, preleptotene spermatocyte; 8, 10, 13,15, steps of spermiogenesis.

3. NUCLEAR RECEPTOR SUPERFAMILY

3.1. Members

Nuclear receptors (NR) is one the most abundant class of transcriptional regulators; the human genome contains 48 genes encoding NRs (Robinson-Rechavi et al. 2001). However, due to alternative splicing, the number of NR proteins is much higher (Robinson-Rechavi et al. 2001). The majority of NRs are ligand-regulated, sequence-specific transcription factors that share similarities with respect to their ligands, structures, and functions. Ligands that activate NRs are small lipophilic substances, such as testosterone in the case of AR. Until recently, many of the NRs lacked an identified physiological ligand and were therefore called orphan receptors. The newly-identified ligands in many cases are metabolic substances produced by the cell and recognize a receptor within the same cell. Thus, these new ligands seem to participate in intracrine rather than endocrine regulation. NR can be phylogenetically divided into six groups (Table 2). This phylogenetical grouping also parallels their function. For example, all steroid receptors belong to group III, their hormone response element is usually a palindrome, and they bind to DNA as homodimers. By contrast, members of the groups I and IV bind to DNA as a heterodimer with RXR.

Table 2. Phylogenetic classification of mammalian nuclear receptors on the basis of the sequence similarity of DNA-binding domain, hinge region and ligand-binding domain (Laudet 1997).

Class	Representative member(s)	Ligand	Response element	Binding
Class I	Thyroid hormone receptor	Thyroid hormone	Palindrome, DR-4, inverted palindrome	Heterodimer (with RXR)
Class II	Retinoid X receptor	9-*cis*-retinoic acid	Palindrome, DR-1	Homodimer
Class III	Androgen receptor	Androgens	Palindrome	Homodimer
	Estrogen receptor α and β	Estrogens	Palindrome	Homodimer
	Glucocorticoid receptor	Glucocorticoids	Palindrome	Homodimer
	Progesterone receptor	Progestins	Palindrome	Homodimer
Class IV	NGF-induced clone B	Unknown	Palindrome, DR-5	Monomer, homodimer, heterodimer
Class V	Steroidogenic factor 1	Oxysterols	Hemisite	Monomer
Class VI	Germ cell nuclear factor	Unknown	DR-0	Homodimer
Class 0	Small heterodimeric partner	Unknown		Heterodimer

3.2. DNA-binding domain

A typical nuclear receptor protein can be structurally divided into three functional domains that each have their unique functions (Fig. 3). Among these domains, the DNA-binding domain (DBD) is the most conserved one. DBD harbors two zinc-fingers that are encoded by two different exons. Zinc-finger is formed around one zinc ion coordinated by four conserved cysteine residues (Luisi et al. 1991, Schwabe et al. 1993). DBD contains two to three α-helices: The first is located one after the third conserved cysteine, the second one is formed by the carboxy-terminal part of the second zinc-finger, and the third one, present only in RXR, resides in the carboxy-terminal end of the DBD. The first α-helix located in the first zinc-finger makes contact with the major groove of DNA (Luisi et al. 1991, Schwabe et al. 1993). Mutational studies have revealed a so-called P box that harbors amino acids that discriminate between response elements. These amino acids are conserved among GR, progesterone receptor (PR), mineralocorticoid receptor (MR), and AR which all recognize the same consensus sequence. Moreover, these residues are different in estrogen receptor (ER) that recognizes different consensus sequence. Indeed, mutation in the P box of GR may result in recognition of estrogen response element (Danielsen et al. 1989, Mader et al. 1989, Umesono and Evans 1989).

21
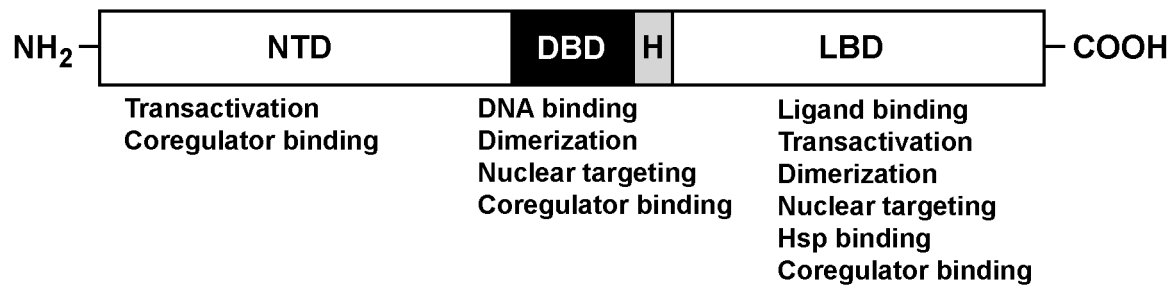
Figure 3. Domain structure of NRs. NTD, N-terminal domain; DBD, DNA-binding domain; H, hinge; LBD, ligand-binding domain; Hsp, heat-shock protein.

NRs have two different core DNA recognition motifs. While GR, PR, MR, and AR recognize the sequence AGAACA, the rest of the receptors recognize AGG/TTCA (for review, Glass 1994). A response element can consist of one core recognition motif, but more often it comprises two core motifs that form either a palindrome, a direct repeat or an inverted palindrome. Moreover, the spacing between the two core motifs is critical. Steroid receptor response elements often are palindromes with a spacing of three nucleotides. Thus, both GR and AR can recognize the same consensus response element 5'-AGAACAnnnTGTTCT-3'. However, many AR elements found in natural promoters differ from the consensus sequence. For example, the probasin gene, which is an androgen-regulated prostate-specific gene, contains two AREs of which ARE1 resembles consensus response element, whereas ARE2 does not resemble the consensus site (Kasper et al. 1994, Rennie et al. 1993). ARE2 is recognized by AR, but not by GR, and the binding requires C-terminal residues of the AR DBD. The core motifs of ARE2 are in a direct repeat orientation, suggesting that at least some of the AREs are direct repeats rather than palindromes (Schoenmakers et al. 1999, Schoenmakers et al. 2000).

Binding of NRs to their response elements occurs either as monomers, homodimers, or heterodimers with RXR. Receptors that bind DNA as monomers are orphan receptors and their binding depends on the third α-helix that contacts with the minor groove of DNA assisting in high-affinity binding (Meinke and Sigler 1999). Steroid receptors bind to DNA as homodimers and the receptor monomers contact each other through residues in both ligand-binding domain (LBD) and in the second zinc-finger. The RXR is of special importance for NRs that bind DNA as heterodimer with, since the action of these NRs can be regulated by availability of RXR and by the presence of its ligand 9-*cis*-retinoic acid (Kliewer et al. 1992, Zhang et al. 1992). Interestingly, two members of the NR superfamily belonging to class 0, DAX1 and small heterodimer partner (SHP), are devoid of the DBD (Seol et al. 1996a, Zanaria et al. 1994). Both

DAX1 and SHP can heterodimerize with other NRs and inhibit transactivational activity of their partners (Seol et al. 1996a, Zanaria et al. 1994).

3.3. Ligand-binding domain

Crystal structures of several NR LBDs have been solved (Bourguet et al. 1995, Renaud et al. 1995, Wagner et al. 1995), and these studies have shown that a ligand-binding domain (LBD) of NR is typically formed from 12 α-helices and one β-turn. A hydrophobic cavity called ligand-binding pocket (LBP) that can accommodate the ligand is in the core of the LBD. These studies have highlighted the role of helix 12 in ligand binding. Binding of the ligand induces a series of conformational changes which lead to swinging of helix 12 to close the LBP. Besides forming a roof for the LBP, helix 12 together with helices 3 and 4 forms a surface. Simultaneous movement of other helices results in a tighter contact between LBP and ligand. Brzozowski et al. (1997) solved the structure of ER LBD in the presence of both agonist and raloxifen, which is antagonist against the ligand-dependent activation function 2 (AF2). The group demonstrated that helix 12 is not correctly positioned in the antagonist-bound LBD due to larger raloxifen. Recently, many of the orphan receptors have shown to possess a ligand thus rising a possibility that a ligand could be identified for all of the orphan receptors. A crystal structure of the orphan receptor Nurr1, however, showed that the receptor does not contain a cavity that is large enough to accommodate ligand, suggesting that true orphan nuclear receptors do exist (Wang et al. 2003).

In addition to ligand binding, LBD is crucial for protein-protein contacts, which are formed between coactivators, between receptor monomers, and within the same receptor monomer. Helix 12 together with helices 3 and 4 form a surface that is termed as AF2 (Lees et al. 1990). This surface is important for interaction between the receptors and coactivators through the LXXLL (L = leucine, X = any amino acid) motif located in the AF2 (Darimont et al. 1998, Nolte et al. 1998, Shiau et al. 1998). The AF2 surface in the raloxifen-bound ER is different from that of the agonist-bound, and coactivators are unable to bind to it, thus explaining the antagonistic-effect of raloxifen. Also corepressors are able to interact with LBD in the presence of antagonist, and the binding of the corepressor antagonizes coactivator binding, for example, silencing mediator for retinoid and thyroid hormone receptors (SMRT) blocks the coactivator binding site of PPARα (Xu et al. 2002). Like DBD, also LBD participates in receptor-dimer formation as illustrated by ER LBD in which hydrophobic region of helix 10 is the key mediator, but also helices 7, 8, and 9 participate in the dimer formation (Tanenbaum et al. 1998). Residues among

the dimer surfaces are conserved, suggesting that also other NRs use the same residues for dimerization.

3.4. N-terminal domain

The least conserved functional domain of NRs is the N-terminal domain (NTD). The size of NTD varies from 23 amino acids in vitamin D receptor to 602 residues in mineralocorticoid receptor, and the sequence of NTD is unconserved. NTD harbors a ligand-independent activation function 1 (AF1) that is, in the case of AR, masked by the C-terminus of the apo (empty)-receptor (Simental et al. 1991). A truncated receptor that lacks LBD, but has functional NTD and DBD, is constitutively active (Miesfeld et al. 1987). NTD is poorly structured compared to DBD and LBD in that studies have not detected secondary structures in NTD (Dahlman-Wright et al. 1995, Wärnmark et al. 2001). However, secondary structures may be formed upon binding to TBP or DNA (Bain et al. 2000, Wärnmark et al. 2001). Individual hydrophobic residues of NTD, in contrast to individual acidic residues, appear to be important for transcriptional activity of the GR (Almlöf et al. 1995, Almlöf et al. 1997). In view of this, Wärnmark et al. (2003) have proposed that coactivator binding to AF1 may involve an initial electrostatic contact, which is followed by an α-helix formation and a hydrophobic interaction.

3.5. Covalent modifications of nuclear receptors

NRs are subjected to multiple posttranslational modifications, such as phosphorylation. Phosphorylation is known to occur in NTD, DBD, and LBD, but especially in steroid receptors, the majority of phosphorylated residues lie in the NTD (Rochette-Egly 2003). NRs are phosphorylated by various kinases, including cyclin-dependent kinases, MAPK and Akt, and phosphorylation may enable cross-talk between NR and other signaling pathways (Chen et al. 2000a, Lin et al. 2001, Yeh et al. 1999). Phosphorylation of a NR may either increase or decrease its transcriptional activity. Phosphorylation of NTD may facilitate recruitment of coactivators, and thus, has a positive effect on transcription (Tremblay et al. 1999). On the other hand, phosphorylation of ER DBD prevents dimerization and DNA binding, having a negative effect on the receptor activity (Chen et al. 1999a). In the case of GR, phosphorylation of AF1 has been shown to promote ligand-dependent degradation of the receptor and to result in termination of glucocorticoid signaling (Wallace and Cidlowski 2001).

NRs such as GR and AR are also targets of sumoylation in which a small ubiquitin-like modifier (SUMO) protein is attached to a specific lysine residue of the receptor (Le Drean et al. 2002, Poukka et al. 2000, Tian et al. 2002). Sumoylation of AR is ligand-dependent, and disruption of sumoylated lysines increases the transcriptional activity of the receptor (Poukka et al. 2000). Studies that have addressed the effects of sumoylation on GR function have been inconsistent. While Tian et al. (2002) showed that mutation of sumoylated residues in GR results in an increased transcriptional activity, Le Drean et al. (2002) could detect a similar increase when GR was cotransfected with SUMO.

Lysine residues of proteins may be modified by ubiquitination and acetylation in addition to sumoylation. Treatment of AR-expressing cell line with MG-132, which is a compound that inhibits proteasomal degradation, increases the amount of AR, suggesting that polyubiquitination may modify androgen action by regulating the levels of AR (Sheflin et al. 2000). Moreover, polyubiquitination of AR may be dependent on phosphorylation of AR, since Akt-pathway enhances both phosphorylation and polyubiquitination of AR (Lin et al. 2002). Polyubiquitination of AR may be important for efficient transcription of androgen-regulated genes, such as the PSA gene, since inhibition of proteasomal activity retained AR on the PSA promoter and reduced transcription of the PSA gene, although the level AR is increased (Kang et al. 2002). Effect of ubiquitination on AR activity depends on whether AR is polyubiquitinated or monoubiquitinated. Monoubiquitination of AR increases its transcriptional activity. Accordingly, AR coactivator, the tumor susceptibility gene product TSG101, increases monoubiquitination of AR (Burgdorf et al. 2004). Transcriptional activity of AR may be increased also by acetylation of a specific lysine residue by coactivators p300 and p300/CBP-associated factor PCAF (Fu et al. 2000). Acetylation-defective AR possessed a normal transrepression function, but an abrogated transactivation function probably due to increased binding of corepressors to AR (Fu et al. 2002).

3.6. Coactivators

Coactivators are proteins that increase the rate of signal-specific but not basal transcription (for review, McKenna et al. 1999, McKenna and O'Malley 2002, Näär et al. 2001, Robyr et al. 2000, Xu et al. 1999). Their presence was initially postulated on the basis of transcriptional interference between NRs, as exemplified by competition between ER and PR for a limiting transcription factor (Meyer et al. 1989). Functions of coactivators can be divided into three classes. Firstly, some of the coactivators are likely to mediate and enhance interactions between

NRs and general transcription machinery, although NRs are, to certain extent, capable for these interactions without coactivators. Secondly, many of the coactivators can catalyze covalent modifications of histones by either acetylation or methylation, and thereby, alter the chromatin structure. Also, chromatin structure is modified by some of the coactivators in an ATP-dependent manner. Thirdly, coactivators may recruit other coactivators and facilitate the assembly of coactivator complexes.

Mediators

CREB-binding protein (CBP) and p300 are highly similar to each other but are encoded by different genes. CBP was first identified to be associated with cAMP response element-binding protein (CREB), whereas p300 was found to interact with viral E1A protein (Chrivia et al. 1993, Eckner et al. 1994). CBP/p300 has been shown to interact with a number of transcription factors, such activator protein 1 (AP-1) and nuclear factor κB (NF-κB), as well as components of basal transcription machinery including TBP and TFIIB (for review, Chan and La Thangue et al. 2001, Shikama et al. 1997). CBP/p300 is also involved in coactivator complex assembly, since it is capable of interacting with other coactivators such as steroid receptor coactivator-1 (SRC-1), p300/CBP/cointegrator-associated protein (p/CIP) and PCAF (Chen et al. 1997, Ogryzko et al. 1996, Spencer et al. 1997, Yao et al. 1996). Functional mapping of CBP/p300 has revealed a HAT domain in the center of the protein. CBP/p300 is able to catalyze acetylation not only of histones *in vitro* but also of other transcription factors such as p53 (Gu et al. 1997, Ogryzko et al. 1996). Disruption of either CBP or p300 gene causes an embryonically lethal phenotype in homozygote mice, and even some of the heterozygotes die during embryogenesis (Yao et al. 1998). This kind of haploinsufficiency suggests that an adequate amount of CBP/p300 is critical for normal development. These knockout mice have similar defects in growth and neural tube closure, indicating that CBP and p300 are involved in similar processes. However, CBP +/- mice have defects in hematopoietic differentiation, whereas p300 +/- mice are normal in this respect, suggesting that functions of CBP and p300 overlap only partially (Kung et al. 2000). Mutations of CBP have been associated with Rubinstein-Taybi syndrome that is characterized with similar defects as CBP +/- mice (Petrij et al. 1995).

Thyroid hormone receptor-associated protein (TRAP) and vitamin D receptor-interacting protein (DRIP) complexes were separately identified as complexes that interact with thyroid hormone receptor and vitamin D receptor, respectively, and contain more than 25 different proteins (Fondell et al. 1996, Rachez et al. 1999). These complexes are also similar to the

Mediator complex (Boyer et al. 1999). Several NRs have been shown to associate with a member of TRAP/DRIP complex called TRAP220 (Hittelman et al. 1999, Yuan et al. 1998, Zhu et al. 1997). The interaction is ligand-dependent and is likely to occur between the two LXXLL boxes located in the TRAP220 and the AF2 region of the NR (Ren et al. 2000, Yuan et al. 1998). GR AF1 has been shown to be capable of interaction with a different member of TRAP/DRIP complex, TRAP170 (Hittelman et al. 1999). Interestingly, transcription factors other than NRs such as p53 and E1A also interact with the TRAP/DRIP complex (for review, Ito and Roeder 2001). However, they use TRAP80 and TRAP 150β subunits. Importantly, other subunits of the TRAP/DRIP complex, such as human homolog of yeast SOH1, are able to bind to TAFs and RPII and therefore act as mediators between NRs and general transcription machinery (Gu et al. 1999). TRAP220 knockout mice die at 10.5 dpc and have defects in growth and development of heart (Ito et al. 2000, Zhu et al. 2000). In TRAP220 +/- mice, there are also signs of haploinsufficiency, since the mice have hypothyroidism due to decreased expression TSHβ gene (Ito et al. 2000).

Two recent studies have addressed the interplay between CBP/p300 and TRAP/DRIP/Mediator. Acevedo and Kraus (2003) demonstrated that there is transcriptional synergism in ERα-dependent transcription between CBP/p300 and TRAP/DRIP. Interestingly, this phenomenon was observed only on chromatin templates. They also found that Mediator had a distinct role in reinitiation of transcription. Wallberg and collegues (2003) have highlighted the role of PGC-1α in this synergism. PGC-1α, which interacts with both p300 and Mediator, stimulates both histone acetylation and formation of preinitiation complex.

Histone acetyltransferases

The p160 family is a coactivator group that consists of three members. SRC-1/NcoA-1 was the first identified nuclear receptor coactivator and was originally found as an interaction partner for ligand-bound PR, ER, and thyroid hormone receptor (Kamei et al. 1996, Onate et al. 1995, Takeshita et al. 1996). The second member of the p160 family, SRC-2/TIF2/GRIP1/NcoA-2, was found to associate with LBD of GR and ER (Hong et al. 1996, Torchia et al. 1997, Voegel et al. 1996). The third member of the group, SRC-3/p/CIP/ACTR/AIB1/TRAM1/RAC3, was originally identified as a gene amplified in breast cancer and was subsequently shown to be a NR coactivator (Anzick et al. 1997, Chen et al. 1997, Li et al. 1997a, Suen et al. 1998). Thus, all three members of the p160 family are coactivators of multiple NRs. They act as coactivators also for other transcription factors such as AP-1 and NF-κB (Lee at al. 1998, Na et al. 1998).

Members of the p160 family share similar organization of functional domains. In the N-terminal part of the protein is located the bHLH/PAS domain that is, however, not required for coactivation of NRs (Onate et al. 1995). In the central region of p160 family members reside three LXXLL motifs that mediate the interaction with NRs (Heery et al. 1997, Vogel et al. 1998). Two activation domains, AD1 and AD2, are located in the C-terminal part of SRCs. AD1 mediates the interaction with CBP/p300, whereas AD2 interacts with the histone methyltransferase coactivator-associated arginine methyltransferase 1/protein arginine methyltransferase 1 (CARM1/PRMT1). An important HAT domain resides also in C-terminal part of SRCs. The HAT activity of the SRC is, however, weak and is not mandatory for the coactivation function *in vitro*. More likely, histones are modified by other factors such as CBP/p300, PCAF, or CARM1/PRMT1.

Members of the p160 family are, in general, widely expressed in various tissues (for review, Xu and Li 2003). However, there are differences in certain cell types. For example, in Sertoli cells, SRC-2 is highly expressed, whereas only low levels of SRC-1 are present and SRC-3 is not detected at all (Gehin et al. 2002, Mark et al. 2004). Gene disruption experiments in mice have demonstrated variable degree of functional redundancy among the members of the p160 family. SRC-1 knockout mice are viable and fertile (Xu et al. 1998). However, they are partially resistant to steroid and thyroid hormones and have defects in neuronal development (Nishihara et al. 2003, Weiss et al. 1999, Xu et al. 1998). On the other hand, disruption of SRC-2 results in a reduced fertility in male mice due to apoptosis in germ cells and detachment of germ cells from Sertoli cells (Gehin et al. 2002). However, the failure is likely to be caused by Sertoli cells, because SRC-2 is expressed only in Sertoli cells and not in germ cells. Female SRC-2 knockouts have also reduced fertility due to defects in placenta (Gehin et al. 2002). In addition, SRC-2 -/- mice are resistant to obesity, have higher lipolysis in white fat and produce higher amounts of energy in brown fat (Picard et al. 2002) SRC-3 knockout mice have impaired estrogen action (Xu et al. 2000). The levels of estrogen in serum of female mice are lower than those in wild-type littermates, and this is paralleled by a delay in pubertal development, vaginal opening, and mammary gland development. SRC-3 -/- mice have also a reduced growth probably due to diminished IGF-1 levels (Wang et al. 2000, Xu et al. 2000).

PCAF/GCN5 is a member of the PCAF complex that consists of over ten subunits and has been shown to be mandatory for NR activation (Korzus et al. 1998, Ogryzko et al. 1998). In the C-terminal part of PCAF resides a bromodomain that recognizes acetylated lysine residues (Dhalluin et al. 1999, Kanno et al. 2004, Ornaghi et al. 1999). The N-terminal part of PCAF, on the other hand, harbors a HAT domain. Indeed, PCAF has been shown to interact with histone

H3 and is capable of acetylating H3 (Clements et al. 1999, Kuo et al. 1996, Rojas et al. 1999). PCAF interacts with NRs as well as with SRC-1 and CBP/p300 (Blanco et al. 1998, Korzus et al. 1998). Therefore, PCAF regulates transcription by acetylating histones and mediating interactions of other coactivators. Interestingly, a recent study demonstrated that there is specificity in the recruitment of PCAF and other coactivators (Li et al. 2003). PR recruited SRC-1 and CBP, which lead to acetylation of H4, whereas GR recruited SRC-2 and PCAF, which resulted in acetylation of H3.

Histone methyltransferases

In addition to acetylation, histones are also subjected to methylation. Two well studied methylases, CARM1 and PRMT1, belong to the PRMT family are capable of methylating H3 and H4, respectively (Schurter et al. 2001, Strahl et al. 2001). Both CARM1 and PRMT1 function as secondary coactivators of NRs, meaning that they require another coactivator to be functional (Chen et al. 1999b, Koh et al. 2001). Indeed, members of the p160 family seem to be required for CARM1 to be functional; CARM1 and PRMT1 interact with SRC-2 through the AD2 domain of SRC-2 (Chen et al. 1999b, Koh et al. 2001). CARM1 acts synergistically with SRC-2, CBP/p300, and PCAF (Chen et al. 2000b, Lee et al. 2002). This phenomenon is more drastic at low levels of NR, which mimics the situation *in vivo* (Chen et al. 2000b, Lee et al. 2002). Interestingly, CARM1 is able to catalyze methylation of not only histones, but also of CBP (Chevillard-Briet et al. 2002, Xu et al. 2001). Methylation of CBP is required for normal transcriptional activation by SRC-2 and NRs (Chevillard-Briet et al. 2002). Interestingly, methylation of CBP inhibits its coactivation function in CREB-dependent transcription (Xu et al. 2001). Recently, a CARM1 knockout mice have been generated (Yadav et al. 2003). These mice are small in size, die perinatally and exhibit defects in CBP and H3 methylation.

ATP-dependent chromatin remodelers

The SWI/SNF complex that is capable of modifying chromatin structure noncovalently appears to enhance GR, ER, and RAR mediated transcriptional activation (Chiba et al. 1994, Muchardt and Yaniv 1993). NRs display specificity regarding the different chromatin remodeling complexes; for example, PR recruits ISWI rather than SWI/SNF (Di Croce et al. 1999). Also, NRs tend to associate with the brahma-related gene 1 (BRG1) subunit of SWI/SNF rather than the brahma (BRM) subunit (Kadam and Emerson 2003). Relationship between acetylation and

ATP-dependent remodeling has been addressed by studying their interactions and temporal requirement. Belandia et al. showed that the BAF57 subunit of SWI/SNF is capable of interacting with p160 coactivators and that the coactivation function of p160 is dependent on BAF57 (Belandia et al. 2002). Dilworth et al. (2000), on the other hand, examined the temporal pattern of coactivator recruitment by RARα/RXRα. They proposed a model (Fig. 4) in which NR is able bind to DNA weakly and this binding is tightened by ATP-dependent chromatin remodelers. In the next step, NRs recruit coactivators with acetylation activity. In the last step, general transcription factors are recruited to the promoter with the help of TRAP/DRIP complexes.
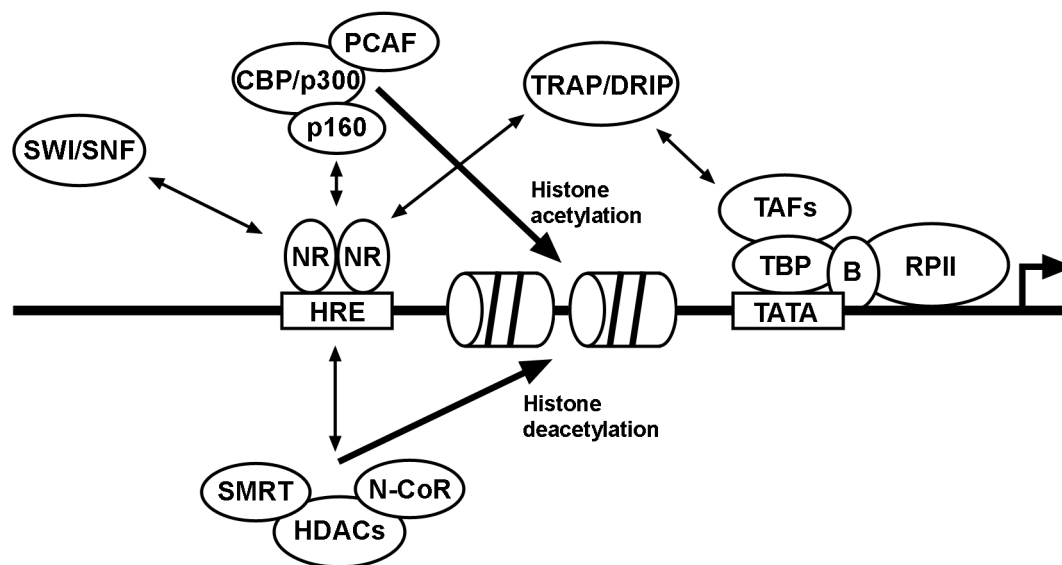
Figure 4. A model for nuclear receptor (NR)-dependent transcriptional activation. HRE, hormone response element; TATA, TATA box; TBP, TATA box-binding protein; TAFs, TBP-associated factors; B, transcription factor B; RPII, RNA polymerase II. NR-dependent transcription is modulated by complexes containing chromatin remodeling (SWI/SNF), histone acetylation (p160/CBP/p300/PCAF), histone deacetylation (SMRT/N-CoR/HDACs), mediator (TRAP/DRIP) activities.

3.7. Corepressors

Corepressors decrease the ability of NRs to activate transcription. Two well studied corepressors, nuclear hormone receptor-corepressor (N-CoR) and SMRT, were originally identified by their ability to interact with unliganded NRs (Chen and Evans 1995, Hörlein et al. 1995). N-CoR and SMRT are encoded by related genes and have similarities in their functional organization. N-CoR and SMRT interact with NRs trough their C-terminal part that contains two to three interaction domains (Li et al. 1997b, Seol et al. 1996b). Association of corepressors to NRs is selective and depends on a particular NR and homo/heterodimer (for review, Privalsky 2004). The interaction between N-CoR/SMRT and NRs is dependent on the CORNR box (L/I-X-X-I/V-

I motif) of the corepressor and on the surface formed by helices 3/5/6 of the NR LBD (Hu and Lazar 1999, Nagy et al. 1999, Perissi et al. 1999, Xu et al. 2002). Interestingly, helix 12 plays an important role also in corepressor interaction; in the apo-NR, helix 12 is in a position where a corepressor is able to bind, whereas in the holo (agonist-bound)-NR it occludes the corepressor binding (Schulman et al. 1996, Xu et al. 2002, Zhang et al. 1999). An important feature of N-CoR and SMRT is their ability to recruit proteins that have histone deacetylase activity such as HDAC3, and thus convert the chromatin to an inactive form (Fischle et al. 2002, Guenther et al. 2001, Guenther et al. 2002, Wen et al. 2000a). Although N-CoR and SMRT are similar, their tendency to bind to different NRs and gene knockout experiments suggest nonredundant roles for the two corepressors (Jepsen et al. 2000). Interestingly, SMRT is subjected to phosphorylation, thus enabling a cross-talk with other signaling pathways (Hong et al. 1998, Hong et al. 2000).

3.8. Miscellaneous coregulators

Coregulators are proteins whose effects on NR activity are not as clear as those of coactivators and corepressors, and the effects may vary depending on the cell type or NR. RIP140 was originally identified as a protein that interacts with ER in agonist-dependent manner (Cavailles et al. 1994, Cavailles et al. 1995). In the early studies, low levels of RIP140 were shown to activate ER-dependent transcription (Cavailles et al. 1994, Cavailles et al. 1995). A similar enhancement of ER activity was also seen in yeast (Joyeux et al. 1997). However, subsequent studies have demonstrated that RIP140 usually functions as a corepressor (Lee and Wei 1999, Subramaniam et al. 1998, Treuter et al. 1998). RIP140 interacts with the AF2 region of NRs in agonist-dependent manner and appears to compete with SRC-1 for the same binding site, thus functioning as agonist-dependent corepressor (Heery et al. 1997, L'Horset et al. 1996, Treuter et al. 1998, Subramaniam et al. 1999). In addition, RIP140 is capable of recruiting HDAC and CtBP (Wei et al. 2000, Wei et al. 2001). Interestingly, the interaction between RIP140 and CtBP can be reversed by CBP/p300-dependent acetylation of RIP140 (Vo et al. 2001). Gene knockout experiments have also revealed that it is required for normal fertility of female mice, since its disruption causes defect in ovulation (White et al. 2000).

ARA proteins is a heterogeneous group of proteins that were initially identified as AR coactivators by Chang's group, and in general, have thereafter been shown to coactivate also other steroid hormone receptors (for review, Heinlein and Chang 2002b). ARA70 interacts with AR and stimulates its activity weakly (Gao et al. 1999, Yeh and Chang 1996). The possible interaction of ARA70 with other NRs is discrepant (Alen et al. 1999, Yeh and Chang 1996).

ARA70 appears to change the ligand-specificity of AR; ARA70 increased the activation of AR by both dihydrotestosterone and estradiol (Miyamoto et al. 1998, Yeh et al. 1998). ARA24 is an AR coactivator that interacts with the NTD of AR (Hsiao et al. 1999). This interaction may involve the polyglutamine repeat of the NTD, since an increase in the length of the CAG-repeat tends to diminish the interaction. ARA24 is a homologue of RanGTPase which is involved in nuclear import and export, thus enabling regulate the nuclear trafficking of AR (Görlich and Kutay 1999). AR coactivator ARA54 contains a RING finger domain, suggesting that it might act as a E3 ligase in ubiquitination (Kang et al. 1999). Indeed, Ito et al. (2001) demonstrated that ARA54 is an ubiquitin E3 ligase capable of catalyzing autoubiquitination. In addition to ARA54, two other coactivators, namely SNURF and E6-AP, are ubiquitin E3 ligases (Häkli et al. 2004, Moilanen et al. 1998, Nawaz et al. 1999). E6-AP is a protein that associates with Angelman syndrome, and its disruption hampers the androgen-dependent growth of the prostate (Kishino et al. 1993, Smith et al. 2002). However, it is likely that Angelman syndrome is caused by a defect in ubiquitin ligase function rather than the coactivator function (Nawaz et al. 1999). Ubiquitination activity of a coactivator might have several consequences. For example, degradation of corepressors might activate transcription and degradation of preinitiation complex might facilitate reinitiation of transcription. Also, monoubiquitination of histones might convert the chromatin structure to a more accessible state (for review, Muratani and Tansey 2003).
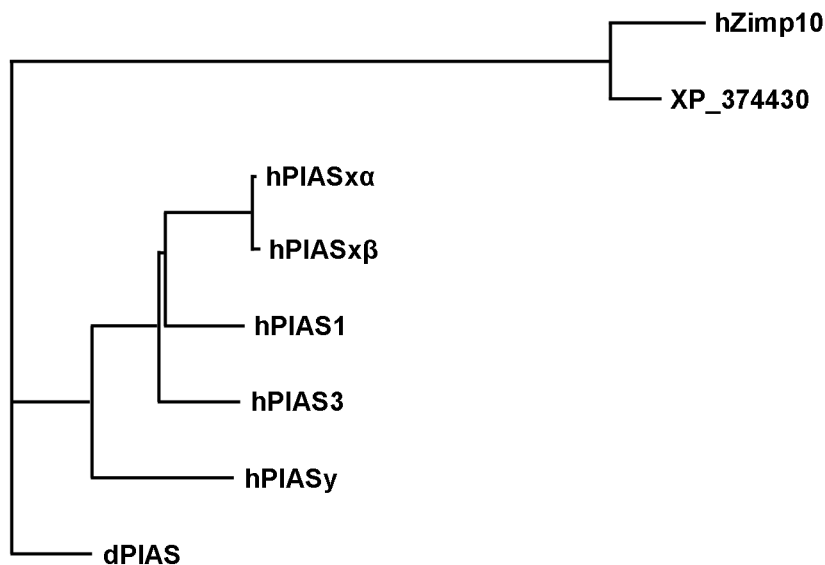
4. PIAS PROTEINS

4.1. Discovery

The name of the PIAS protein family derives from the first member, PIAS3 that was identified as a protein inhibitor of activated signal transducer and activator of transcription (STAT) 3 (Chung et al. 1997). Also, PIAS1 was originally identified by its association with STAT1 (Liu et al. 1998). Simultaneously with PIAS1, Liu et al. (1998) also cloned the cDNAs for PIASxα, PIASxβ, and PIASy. The first functional clues for the α and β forms of PIASx, which are formed through utilization of alternatively spliced exons in the 3' end of the gene, were from unrelated studies. While PIASxβ/Miz1 was found by its association with homeobox gene Msx2, PIASxα /ARIP3 was identified as an AR coregulator (Moilanen et al. 1999, Wu et al. 1997). Recently, a PIAS-like gene hZimp10 has been isolated and, like PIASxα, it is an AR coactivator (Sharma et al. 2003). In addition to the thus far identified members, chromosomal location 7p13 harbors a putative PIAS-like gene XP_374430 that resembles hZimp10 (Fig. 5). In addition to PIASx, also

PIAS1 and PIAS3 may have alternative forms; Gu/RNA helicase II-binding protein differs from PIAS1 in that it lacks the first nine amino acids, and PIAS3L/KchAP has an additional 39 amino acids inserted in the middle of PIAS3 sequence (Valdez et al. 1997, Wible et al. 1998). The presence of PIAS proteins is not restricted to mammals; *Drosophila melanogaster* has one PIAS-like protein, dPIAS/Zimp, and *Saccharomyces cerevisiae* has two PIAS-like proteins Siz1 and Siz2 (Mohr and Boswell 1999, Strunnikov et al. 2001).


Figure 5. The phylogenetic tree of the PIAS proteins. The phylogenetic tree of PIAS protein reference sequences was constructed with the neighbor-joining method. An additional hypothetical protein (XP_374430) is also included. Distance from the left depicts the dissimilarity from dPIAS.

4.2. Structure

PIAS proteins are structurally related and share similar functional domains (Fig. 6). In the N-terminal part of PIAS proteins resides a SAP (SAF-A/B, Acinus and PIAS) domain. It is a 35-amino acid long motif that is anticipated to form two α-helices (Aravind and Koonin 2000, Kipp et al. 2000). SAP domain is found in many chromatin-associated proteins, such as scaffold attachment factor-A/B (SAF) and Acinus, and it is mandatory for DNA binding of SAF-A (Göhring et al. 1997, Romig et al. 1992, Sahara et al. 1999). SAF-A binds to AT-rich chromosomal regions called MARs (Romig et al. 1992). PIAS-like proteins hZimp10 and XP_374430 do not contain the SAP domain. In the center of PIAS proteins is the Siz/PIAS RING (SP-RING) motif that is present also in PIAS-like proteins (Hochstrasser 2001, Jackson 2001). The SP-RING motif resembles a RING motif that is often found in ubiquitin E3 ligases. Compared to the classical RING motif, the SP-RING motif lacks two cysteine residues,

suggesting that the SP-RING motif might bind only one zinc ion, but still form a RING finger-like structure (Schmidt and Müller 2003). PIAS proteins also contain LXXLL motifs found in many coactivators that interact with LBD of NRs. However, in the case of PIASy, LXXLL motif is not required for interaction with AR, although it is required for the transrepression function (Gross et al. 2001). Disruption of LXXLL motifs of PIASxα similarly has only a minor effect for its ability to regulate the transcriptional activity of AR and GR (Kotaja et al. 2002a).
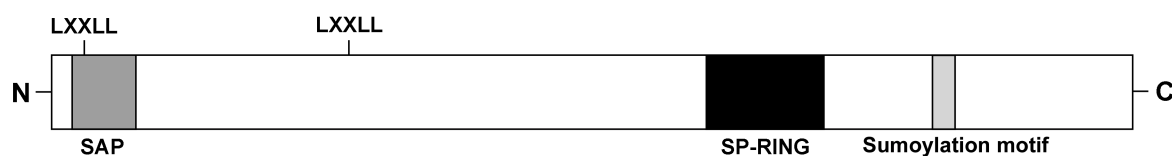
Figure 6. Structural motifs of PIASx protein. Grey and black boxes depict SAP and SP-RING motifs, respectively. LXXLL motifs are also shown.
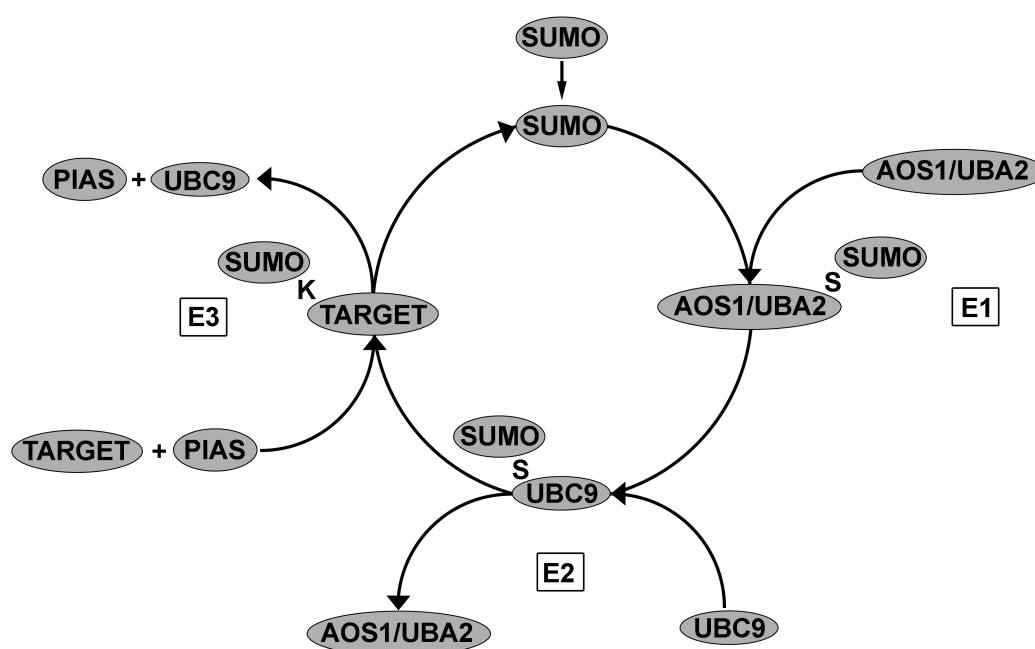
4.3. PIAS proteins as SUMO E3 ligases

Sumoylation pathway

Sumoylation is a covalent posttranslational modification that closely resembles ubiquitination. Although SUMO and ubiquitin proteins share only 18% identity in amino acid sequence, the three-dimensional structure of SUMO-1 is remarkably similar to that of ubiquitin and consist of a $\beta\beta\alpha\beta\beta\alpha\beta$ fold (Bayer et al. 1998). While mammals harbor only one ubiquitin gene, there are three mammalian SUMO genes that encode SUMO-1, SUMO-2, and SUMO-3 (Kamitani et al. 1998). SUMO proteins are small; SUMO-1 consists of 101 amino acids. SUMO-1 shares 48% sequence similarity with SUMO-2 that is almost identical with SUMO-3. A major difference between SUMO-1 and SUMO-2/3 is that SUMO-2/3 can form SUMO-polymers, whereas SUMO-1 cannot (Tatham et al. 2001). Moreover, the majority of SUMO-2/3 appears to be in free form, while SUMO-1 is mostly conjugated to other proteins (Saitoh and Hinchey 2000).

Sumoylation of the target protein is a multistep process (Fig. 7) in which SUMO protein is covalently attached to a specific lysine residue of the target protein. Usually, the target lysine is part of the ΨKXE (a large hydrophobic residue, a lysine residue, any residue, a glutamate residue) consensus motif (Bernier-Villamor et al. 2002). The first enzyme involved in sumoylation is the E1 activating enzyme that is actually a dimer of two proteins Aos1 and Uba2 (Johnson et al. 1997). Interestingly, Aos1 and Uba2 have regions that are similar to those of the ubiquitin E1 activating enzyme Uba1. During the activation process, a thioester bond is formed

between the terminal glycine residue of SUMO and the cysteine residue of Aos1/Uba2. In the second step, SUMO is transferred to the E2 conjugating enzyme Ubc9, and again a thioester bond is formed between SUMO and Ubc9. Initially, Ubc9 was thought to be an E2 conjugating enzyme in ubiquitination. However, Johnson and Blobel (1997) showed that Ubc9 acts as an E2 enzyme in sumoylation rather than in ubiquitination. SUMO-1 and Ubc9 have charged surfaces that match each other well (Liu et al. 1999). Ubc9 is also capable of interacting with the sumoylation consensus motif of the target protein and thereby, participates in the recognition of sumoylation targets (Bernier-Villamor et al. 2002). Sumoylation of the target proteins occurs *in vitro* in the presence of Aos1/Uba2 and Ubc9 and thus, for a while, SUMO E3 ligases were not thought to exist at all (Saitoh et al. 1998). Sumoylation is a reversible process, and there are currently four confirmed and two putative isopeptidases that are capable of desumoylating substrates (for review, Melchior et al. 2003). All of the SUMO proteases belong to the Ulp1 cysteine protease family. The SUMO proteases are also likely to differ from each other in terms of their substrate specificity (Melchior et al. 2003).

Figure 7. Sumoylation of a target protein. SUMO protein is activated by E1 enzyme AOS1/UBA2, conjugated by E2 enzyme UBC9, and ligated by E3 enzyme such as a PIAS protein.

E3 ligases

The first characterized SUMO E3 ligases were Siz1 and Siz2 that are *Saccharomyces cerevisiae* homologs of the PIAS proteins (Johnson and Gupta 2001). Thereafter, all known PIAS proteins have been shown to possess SUMO E3 ligase activity (for review, Melchior et al. 2003). For example, PIASxα is capable of facilitating sumoylation of AR, c-Jun, STAT1, and Smad4 (Kotaja et al. 2002b, Lee and Chang 2003, Nishida and Yasuda 2002, Rogers et al. 2003). Different PIAS proteins can enhance sumoylation of the same target protein, for example, PIAS1, PIASxα, PIASy, and hZimp10 participate in sumoylation of AR (Gross et al. 2004, Kotaja et al. 2002b, Nishida and Yasuda 2002, Sharma et al. 2003). SUMO E3 ligase activity is not restricted to the PIAS protein family. In addition PIAS proteins, Ran-GTP binding protein 2 (RanBP2) and polycomb group protein Pc2 have recently been shown to act as SUMO E3 ligases (Kagey et al. 2003, Pichler et al. 2002). Interestingly, RanBP2 and Pc2 localize to different subnuclear structures than the PIAS proteins, which in part localize to PML bodies. RanBP2 is detected in NPC and Pc2 is observed in PcG bodies, suggesting that thus far identified SUMO E3 ligases may have distinct functions due to their different localization. RanBP2 and Pc2 also lack the SP-RING motif that is required for enhancement of sumoylation by PIAS proteins (Kotaja et al. 2002b)

4.4. PIAS proteins and subnuclear compartmentalization

As discussed earlier, sumoylation has a well established role in the formation of PML bodies (for review, Wilson and Rangasamy 2001). The first link between subnuclear compartmentalization and the PIAS proteins was revealed in a study on PIASy. Sachdev and collegues (2001) identified PIASy as a SUMO E3 ligase that interacts with LEF1 and facilitates its sumoylation. LEF1 is a transcription factor involved in Wnt signaling. Importantly, sumoylated LEF1 colocalizes with PIASy to nuclear dots that partially overlap with PML bodies, and localization to PML bodies was accompanied with a reduced activity of LEF1. Similar to PIASy, other PIAS proteins have been shown to localize to nuclear dots, although there appears to be member-to-member variation in the localization pattern (Kotaja et al. 2002b).

4.5. PIAS proteins in transcription

Many of the interaction partners of the PIAS proteins are transcription factors (for review, Schmidt and Müller 2003). However, the effects of the PIAS proteins on transcription appear to

be complex and vary depending on target protein, cell line, and promoter context (Kotaja et al. 2000). The complexity is likely to arise, at least in part, from sumoylation of both the transcription factor and its coregulators (Kotaja et al. 2002c). For example, sumoylation of a coactivator may increase its ability to interact with a steroid receptor, whereas subsequent sumoylation of the steroid receptor may attenuate the receptor (Kotaja et al. 2002c). Dual effects on transcription may be due to the fact that PIAS proteins interact not only with coactivators, but also with corepressors such as HDACs (Tussie-Luna et al. 2002). PIAS-dependent repression of steroid receptor activity may occur through usage of HDACs, for example, PIASy can recruit HDAC1 and HDAC2, and represses transcriptional activity of AR independent of sumoylation (Gross et al. 2004).

Transcriptional synergy is a phenomenon where two transcription factors work together and have a more than an additive effect on transcription (Iniguez-Lluhi and Pearce 2000). Interestingly, the region associated for a synergistic effect, the so-called synergy control motif, contains the sumoylation consensus sequence, and indeed is the major sumoylation site in GR and AR (Le Drean et al. 2002, Poukka et al. 2000, Tian et al. 2002). The disruption of synergy control motif augments transcriptional synergy at promoters containing multiple glucocorticoid response elements (Iniguez-Lluhi and Pearce 2000). In line with this, sumoylation of the lysine residue in the synergy control motif leads to inhibition of synergy (Holmstrom et al. 2003). Therefore, the PIAS proteins could affect transcription by disrupting the transcriptional synergy.

Another feature of the PIAS proteins potentially involved in transcriptional regulation is their ability to bind to MAR through the SAP domain (Sachdev et al. 2001, Tan et al. 2002). As exemplified by the *Drosophila* PIAS homolog, PIAS proteins have been implicated in the maintenance of proper chromosome structure during interphase (Hari et al. 2001). Therefore, PIAS proteins could be important for transcriptional regulation by controlling the chromosome structure in general or by interacting with proteins that bind to MAR. At least the latter possibility seems to be true, since a MAR-binding protein, SATB2, was recently shown to be sumoylated in PIAS1-facilitated manner (Dobreva et al. 2003). Sumoylation of SATB2 results in diminished gene activation and relocalization of the protein to nuclear periphery.

4.6. PIAS proteins in cytokine signaling

Cytokines are polypeptides that function via cognate receptors on the plasma membrane. The plasma membrane receptors, in turn, activate the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway that results in the binding of STAT to DNA and activation of

transcription. Inhibitors of the JAK-STAT pathway fall into three groups: Src homology 2 domain-containing protein-tyrosine phosphatase (SHP), suppressor of cytokine signaling (SOCS), and PIAS (for review, Wormald and Hilton 2004). It was initially thought that PIAS proteins regulate negatively the transcriptional activity of STATs by preventing their DNA binding. While this may be true for interaction of PIAS1 with STAT1 and PIAS3 with STAT3, it fails to explain the inhibitory effect of PIASy and PIASx on STAT1 and STAT4, respectively (Arora et al. 2003, Chung et al. 1997, Liu et al. 1998, Liu et al. 2001). The inhibitory effects of PIAS proteins on STATs can be, at least in part, attributed to their role in sumoylation. Indeed, PIAS1, PIAS3, and PIASx have been shown to act as SUMO E3 ligases towards STAT1 (Rogers et al. 2003, Ungureanu et al. 2003). Moreover, mutation of the major sumoylation site may results in increased activity of STAT1 proving support for the importance of PIAS-facilitated sumoylation in cytokine signaling (Ungureanu et al. 2003). Sumoylation of STATs may, however, be restricted to STAT1, since other STATs do not possess consensus sumoylation sequences (Ungureanu et al. 2003).

4.7. PIAS proteins and nuclear receptor function

First clues about the role of PIAS proteins in NR-mediated transcriptional regulation were gained when PIASxα and PIAS1 were identified as coregulators of AR (Moilanen et al. 1999, Tan et al. 2000). Thereafter, all PIAS proteins have been shown to regulate steroid receptor-dependent transcription as assessed by reporter gene assays (Table 3). Effects of PIAS proteins on steroid receptor function are somewhat puzzling for several reasons. First, many PIAS proteins are able to modulate the function of same receptor. Second, a single PIAS protein changes the activity of several different receptors. Third, the effect of a given PIAS protein depends on the cell-type and promoter context. Therefore, it is likely that the *in vivo* effects depend on the presence of receptor(s), PIAS protein(s), and other coregulators. Moreover, the effects are also likely to be gene-specific.

TABLE 3. Summary of effects of PIAS proteins on steroid receptor-regulated transcription.

Receptor	PIAS protein	Effect(s)	Reference(s)
AR	PIAS1	Activation	Gross et al. 2001, Kotaja et al. 2000, Tan et al. 2000
	PIAS3	Activation, unchanged	Gross et al. 2001, Jimenez-Lara et al. 2002, Kotaja et al. 2000
	PIASxα	Activation, unchanged, inhibition	Kotaja et al. 2000, Moilanen et al. 1999
	PIASxβ	Activation	Kotaja et al. 2000
	PIASy	Inhibition	Gross et al. 2001, Gross et al. 2004
	hZimp10	Activation	Sharma et al. 2003
ERα	PIAS1	Activation	Kotaja et al. 2000
	PIAS3	Activation	Kotaja et al. 2000
	PIASxα	Activation	Kotaja et al. 2000
	PIASxβ	Activation	Kotaja et al. 2000
ERβ	PIAS1	Activation	Kotaja et al. 2000
	PIAS3	Activation	Kotaja et al. 2000
	PIASxα	Unchanged	Kotaja et al. 2000
	PIASxβ	Activation	Kotaja et al. 2000
GR	PIAS1	Activation, unchanged	Kotaja et al. 2000, Tan et al. 2000
	PIAS3	Activation, inhibition	Jimenez-Lara et al. 2002, Kotaja et al. 2000
	PIASxα	Activation, inhibition	Kotaja et al. 2000
	PIASxβ	Activation, unchanged	Kotaja et al. 2000
MR	PIAS1	Inhibition	Pascual-Le Tallec et al. 2003
PR	PIAS1	Activation, inhibition	Kotaja et al. 2000, Tan et al. 2000
	PIAS3	Activation, unchanged	Jimenez-Lara et al. 2002, Kotaja et al. 2000
	PIASxα	Activation	Kotaja et al. 2000
	PIASxβ	Activation	Kotaja et al. 2000

5. ANDROGEN RECEPTOR

5.1. The androgen receptor gene

The cDNA encoding AR was cloned in 1988 by several groups (Chang et al. 1988, Lubahn et al. 1988, Tan et al. 1988, Trapman et al. 1988). The AR gene is located on X chromosome at Xq11-12. It has eight exons and spans 180 kb. Thus far, there is no evidence for alternatively spliced forms of the AR transcript (Hirata et al. 2003). However, two alternative transcription start sites are likely to exist, but they differ from each other by only 10 nucleotides and encode the same protein (Faber et al. 1993).

5.2. Structure of the androgen receptor

Structure of AR is typical for NRs and comprises the following regions: NTD, DBD, hinge region, and LBD. Although alternatively spliced forms of AR do not exist, two different isoforms of AR may be present. An N-terminally truncated 87-kDa form of AR was originally detected in genital skin fibroblasts and was shown to represent about 10% of total amount of AR (Wilson and McPhaul 1994). Two subsequent studies have examined possible functional differences between the two isoforms, but the results are inconsistent (Gao and McPhaul 1998, Liegibel et al. 2003). Moreover, a recent study by Gregory et al. (2001a) demonstrated that the 87-kDa isoform is likely to result from *in vitro* proteolysis and not to exist *in vivo*.

The NTD of AR is the least conserved part of the receptor compared to other NRs. There are two stretches of repetitive sequences in this region of AR: polyglutamine (CAG) and polyglycine (GGC) repeat. The roles of these repeats in physiological function of AR are currently unknown, but the polyglutamine repeat has been implicated in several diseases, such as prostate cancer and Kennedy's disease. Another unique feature of the AR NTD is the AF1. Compared to other NRs, the AF1 of AR is strong, whereas the AF2 is relatively weak (Simental et al. 1991). In general, AF2 of NRs functions as the binding site for coactivators, but this function may be less important in AR (He et al. 2001). The N-terminal part of AR is capable of interacting with the C-terminal part of the receptor in an agonist-dependent manner (Ikonen et al. 1997, Langley et al. 1995). The N/C interaction is mediated predominantly by an LXXLL-like motif, FXXLF, of the NTD and the AF2 region of the AR LBD (He et al. 2000). The interaction between N-terminal and C-terminal parts of AR is important, because mutations found in the AR LBD of patients with androgen insensitivity syndromes may blunt the N/C interaction and receptor activity without affecting ligand or DNA binding (Thompson et al. 2001). N/C interaction can be further strengthened by coregulators such as CBP, GRIP1, and PIASxα (Ikonen et al. 1997, Moilanen et al. 1999, Thompson et al. 2001). Therefore, whereas other NR use their AF2 as the interaction interface for coactivators, the AR AF2 acts mostly as a docking surface for the N-terminal part of the receptor.

5.3. Androgen insensitivity syndromes

Almost 500 germline mutations for the AR gene causing an androgen insensitivity syndrome are known in the literature and they are listed in a database dedicated for AR mutations (www.mcgill.ca/androgendb, Gottlieb et al. 2004). There are several reasons for the existence of

such a high number of mutations. Firstly, the AR gene is located on X chromosome and in an XY individual only one copy of AR gene is present. Secondly, mutations of the AR gene are not lethal, and individuals survive to adulthood. Thirdly, the phenotype of an AR mutation is often easily detectable, for example, due to ambiguous genitalia or primary amenorrhea. Phenotypes of AR mutations can be grouped into three classes: complete, partial, and mild androgen insensitivity. Complete androgen insensitivity syndrome (CAIS) is characterized by normal female external genitals, the absence of uterus and the presence of testes. This kind of phenotype is usually detected in early childhood due to the presence of labioscrotal or inguinal testes (Ahmed et al. 2000). However, sometimes the phenotype is not evident until around puberty, when menstruation should occur. In partial androgen insensitivity syndrome (PAIS), external genitalia are ambiguous and therefore PAIS is evident at the time of birth. Mild androgen insensitivity syndrome (MAIS) is not easily detected, and its phenotype may be, for example, infertility (Yong et al. 2003). The majority of AR mutations are single point mutations, and they cluster in the DBD and the LBD. Interestingly, the same mutation can lead to different phenotypes as illustrated by the phenotype for M780I mutation that varies from a normal female to a male with ambiguous genitalia, suggesting that other factors modify the phenotype (Gottlieb et al. 2001). A coactivator defect is a possible cause of androgen insensitivity. Interestingly, there is a case-report of a patient who has normal AR despite clinical signs of androgen insensitivity (Adachi et al. 2000). Mutations of AR coactivators were, however, not studied in the report.

5.4. Kennedy's disease

Kennedy's disease or spinal and bulbar muscular atrophy (SBMA) is an X-linked motorneuron disease. In addition to neurological defects, the patients may also exhibit signs of androgen insensitivity (Dejager et al. 2002). Kennedy's disease is caused by an expansion of the polyglutamine repeat located in the AR NTD (La Spada et al. 1991). Kennedy's disease shares many similar features with other polyglutamine repeat diseases such as neuronal cell death. Cytotoxicity in trinucleotide-repeat diseases is often caused by accumulation of undegradable protein. Transfection of an extended-polyglutamine form of AR indeed generates a degraded 75-kDa mutant form of AR that accumulates to nucleus and is resistant to proteolysis (for review, Lee and Chang 2003). Moreover, AR with an expanded polyglutamine repeat has been shown to form aggregates and hampers axonal traffic (Piccioni et al. 2002, Szebenyj et al. 2003). Cytotoxicity caused by the SBMA mutant AR is ligand-dependent, since castration prevented the

development of the disease in a mouse model (Katsuno et al. 2002). Similar results were achieved in this mouse model by using an gonadotropin-releasing hormone receptor agonist, leuproline, which reduced testosterone production of testis (Katsuno et al. 2003). In contrast, flutamide, a pure AR antagonist, did not have any therapeutic effect, possibly due to its ability to promote nuclear translocation of AR (Katsuno et al. 2003).

5.5. Male breast cancer

Male breast cancer is a rare disease; in Finland only 15 new cases were found in the year 2001 (Finnish Cancer Registry, www.cancerregistry.fi, last updated on 30 January 2004). AR has been implicated in male breast cancer, since germline mutations R607Q and R608K were found in men with breast cancer (Lobaccaro et al. 1993). However, other studies have failed to detect germline or somatic mutations of AR in male breast cancer (Hiort et al. 1996, Syrjäkoski et al. 2003). Although AR mutations are rare in men with breast cancer, a deficient androgen action may represent a risk factor for breast cancer in men with PAIS. Androgen insensitivity results in normal or elevated levels of serum testosterone that, in turn, is aromatized to estrogens (Quigley et al. 1995). Therefore, in men with PAIS, the protective effects of androgens on breast cancer development may be diminished due deficient androgen action, and also elevated estrogen levels may increase the likelihood of breast cancer (Hiort et al. 1996).

6. PROSTATE CANCER

Prostate cancer is the most common cancer of men in many industrialized countries; 3533 new cases were detected in Finland in 2001. Prostate cancer is one of the major causes of cancer mortality, as 774 deaths were attributed to prostate cancer in the same year, making it the second most common death-causing cancer in Finland (Finnish Cancer Registry, www.cancerregistry.fi, last updated on 30 January 2004). A number of risk factors for prostate cancer are known including positive family history, high-fat diet, sexually-transmitted disease, and ethnicity (for review, Bosland 2000). Several findings point out that androgens are important for prostate cancer; the growth and maintenance of prostate is dependent on androgens, and administration of androgens to rats results in an increase in prostate cancer (Noble 1977). Moreover, androgen deprivation therapy by surgical or pharmacological means is an effective treatment for recurrent prostate cancer (Huggins and Hodges 1941). However, after the initial suppression of growth, prostate cancer gains the ability to grow even in an androgen-deprived environment. Two

general mechanisms have been suggested to explain this phenomenon. According to the first explanation, prostate cancer cells might acquire the ability to grow by using stimulatory signaling pathways other than androgen action. Alternatively, prostate cancer cells may rely on a modified androgen action.

6.1. Androgens in cancer etiology

The importance of serum androgen levels for prostate cancer development has been studied in various study settings. One setting has been to compare serum hormone levels between higher risk and lower risk populations. The results from these studies have, however, been conflicting. Studies comparing African-American men to European-American men as well as Caucasian-Dutch men to Japanese men have found higher total testosterone levels in serum in high risk than in low risk groups (de Jong et al. 1991, Ross et al. 1986). In contrast, studies comparing Japanese men to American whites and blacks as well as Asian-Americans to European-Americans have revealed lower testosterone levels in the high risk population (Ross et al. 1992, Wu et al. 1995). These studies were performed in adult men and, therefore, do not address the effects of earlier periods, such as pregnancy, on prostate cancer risk. Interestingly, black women have 47% higher serum testosterone concentrations than white women during pregnancy, raising a possibility that exposure of developing prostate gland to higher androgen amounts during pregnancy may later result in an increased risk of prostate cancer (Henderson et al. 1988).

Another common study approach has been to perform case-control studies in both prospective and cross-sectional settings (for review, Bosland 2000, Debes and Tindall 2002, Hsing et al. 2002, Ntais et al. 2003a, Taplin and Ho 2001). Several prospective studies have evaluated the relationship between serum testosterone and DHT levels; most studies have failed to detect statistical correlation between serum testosterone levels and prostate cancer risk. For example, no association was found in serum testosterone, androstenedione and SHBG concentrations between control and prostate cancer groups during 24 years of follow-up period in a Finnish study (Heikkilä et al. 1999). However, Gann et al. found a strong positive correlation between increased prostate cancer risk and increased adjusted testosterone levels, whereas there was a negative correlation between SHBG concentration and prostate cancer risk (Gann et al. 1996). Two other studies have also found that there may be a correlation between the testosterone/DHT ratio and prostate cancer risk (Hsing and Comstock 1993, Nomura et al. 1988). In many studies, the failure to detect association between androgen levels and prostate

cancer risk may be due to problems in hormone measurements or that the study does not have enough statistical power due to small differences in androgen levels between the groups.

6.2. Germline mutations in etilogy

Enzymes that are involved in androgen metabolism are plausible candidate genes for prostate cancer. Role of CYP17 in prostate cancer risk was evaluated in a recent meta-analysis and the conclusion was that CYP17 is unlikely to have a great impact on prostate cancer risk (Ntais et al. 2003b). Aromatase, which catalyzes conversion of androgens to estrogens, has been found to harbor two alleles that associate with an increased prostate cancer risk. Latil et al. (2001) found that 171-bp and 187-bp alleles (produced by variation in the length of TTTA repeat in intron 4) correlated statistically significantly with increased prostate cancer. However, functional consequences of this variation have not been studied. Also, mutations in 5α-reductase type II have been examined in several studies. The results have, however, been inconsistent. Ntais et al. (2003c) performed a meta-analysis on these studies and concluded that A49T and TA repeat length polymorphism may have an effect on prostate cancer risk.

Although AR is a candidate gene for prostate cancer, surprisingly few germline mutations have been found to be associated with an increased prostate cancer risk. The most studied mutation R726L has been detected both in Finnish and American prostate cancer patients, but it seems to be present in only a few percent of the patients (Gruber et al. 2003, Koivisto et al. 2004, Mononen et al. 2000). This mutation may change the function of AR so that it is activated by estrogens (Elo et al. 1995). However, R726L may show slightly reduced activity in response to androgens (Thompson et al. 2001). Transcriptional activity of AR seems to correlate negatively with the length of CAG repeat (Chamberlain et al. 1994, Tut et al. 1997). Therefore, number of studies examining the association between the length of CAG-repeat and the prostate cancer have been carried out (for review, Hsing et al. 2002, Nelson and Witte 2002, Ntais et al. 2003a). These studies have failed to demonstrate an association between short CAG-repeat and increased prostate cancer risk. However, the short CAG repeat tends to accumulate in populations with an increased risk.

6.3. Somatic mutations in cancer etiology

While only a few germline mutations of AR have been detected in prostate cancer, somatic mutations are numerous. In general, these mutations have been detected in recurrent cancers,

while the mutations are rare in untreated cancers (Marcelli et al. 2000). This may be, in part, due to genetic instability of cancer cells, but the selection by androgen deprivation is likely to be important as well. The influence of somatic mutations on AR function found in recurrent cancer cells can be classified to four groups: increased sensitivity to androgens, increased promiscuity to steroids, altered cross-talk with other signaling pathways, and by-pass of receptor dependence.

Increased sensitivity to androgens can be achieved by increased AR level or by gain-of-function mutations, such as V715M, H784Y, and the so-called LNCaP mutation T877A. These mutations modify the function of AR in such manner that adrenal androgens are able to activate AR, leading to growth of tumor after castration (Fenton et al. 1997, Tan et al. 1997). Due to the presence of gain-of-function mutations in recurrent prostate cancers, a few clinical trials have evaluated the potential benefit of using both castration and anti-androgen therapy. Unfortunately, maximal androgen blockade (MAB) therapy has not brought about any significant benefit compared to castration alone (Prostate Cancer Trialists' Collaborative Group 1995). Sensitivity of AR towards androgens is governed by the law of mass action and may therefore be augmented through an increase in the amount of the receptor. In line with this notion, amplification of the AR gene seems to be a common finding as ~30% of hormone-refractory tumors contained the amplification (Koivisto et al. 1995, Visakorpi et al. 1995). A recent study using expression-profiling in prostate cancer xenocrafts showed that AR was the only gene that was consistently upregulated in antiandrogen-resistant tumors (Chen et al. 2004). Amplification of the AR gene is one explanation for the increased expression of AR in antiandrogen-resistant xenocrafts. However, it seems not to happen in all xenocraft models (Laitinen et al. 2002). Other explanations for androgen hypersensitivity may be autoupregulation of AR mRNA through exonic AREs, or increased stability of AR protein (Grad et at. 1999, Gregory et al. 2001b).

Mutations of the AR gene may modify the function of the receptor in a way that makes the receptor more promiscuous for ligand binding as exemplified by the T877A mutation, which is activated by adrenal androgens and is also responsive to the non-steroidal antiandrogen flutamide (Taplin et al. 1999). The receptor harboring the T877A mutation, however, is not generally responsive to antiandrogens, since bicalutamide does not activate this AR mutant (Taplin et al. 1999). Activation of AR by antiandrogen may, therefore, explain the failure of MAB therapies. Certain mutations may convert AR to be activated by other steroid hormones such as corticosteroids. A representative of such a receptor is the L701H mutation with increased affinity for cortisol and cortisone, and the affinity for glucocorticoids is further increased by the concomitant T877A mutation (Zhao et al. 1999).

Various signaling pathways have been postulated to activate AR in an androgen-independent manner and are thus potential ways for the cancer cell to escape androgen dependency. Peptide growth factors, such as insulin-like growth factor 1 (IGF-I), keratinocyte growth factor (KGF), and epidermal growth factor (EGF), have been reported to activate AR even in the absence of androgens (Culig et al. 1994). Interestingly, the activation can be reversed by bicalutamide (Culig et al. 1994). Another factor implicated in ligand-independent activation of AR is HER-2/neu – a receptor tyrosine kinase that is overexpressed in prostate cancer xenocrafts in castrated mice (Craft et al. 1999). HER-2/neu-mediated activation differs from that elicited by the peptide growth factors, because in the case of HER-2/neu, AR activation cannot be blocked by bicalutamide (Craft et al. 1999). HER-2/neu activation has been suggested to be mediated by MAPK or Akt pathways (Wen et al. 2000b, Yeh et al. 1999). Akt is a protein kinase that regulates cell-cycle and possesses anti-apoptotic activity by regulating proapoptotic proteins such as procaspase-9 and Bad. Furthermore, the tumor suppressor gene PTEN, which is often inactivated in prostate cancer, is an upstream negative regulator of Akt (Li et al. 1997c). Thus, the Akt pathway may offer a way for by-passing the AR-dependent pathway to promote the growth of cancer cell. However, the ligand-binding capability of AR has been shown to be mandatory for growth of LNCaP cells, suggesting that AR is an important factor also in advanced prostate cancer (Chen et al. 2004).

6.4. Coregulators in prostate cancer

AR coregulators are potentially important for the progression of prostate cancer. Therefore, many of these coregulators have been studied at both mRNA and protein level. The results gained so far have been contradictory. SRC-1 and SRC-2 were initially found to be overexpressed in prostate cancer specimen as judged by immunohistochemistry (Gregory et al. 2001c). However, other studies have detected either unchanged or decreased levels of SRC-1 mRNA in prostate cancer (Fujimoto et al. 2001, Li et al. 2002, Linja et al. 2004). While Li et al.(2002) demonstrated by *in situ* hybridization that both ARA24 and PIAS1 were upregulated in prostate cancer, Linja et al. (2004) detected by RT-PCR-based method unchanged and decreased expression of ARA24 and PIAS1, respectively. These contradictory results obtained thus far may be due to the use of different techniques and targets. Indeed, Gregory et al. (2004) have found that EGF increases SRC-2 protein but not mRNA levels. Naturally, there can be differences within prostate cancer patients and specimen. For example, Linja et al. (2004) found that although SRC-1 mRNA was generally downregulated in hormone-refractory prostate

carcinomas, the highest individual levels were detected in the treated group. Therefore, prostate cancer may contain subtypes that differ from each other with respect to their coregulator content. Although an interesting possibility, coregulator mutations have thus far not been found.

AIMS OF THE STUDY

The aim of this study was to gain better insight into androgen action under physiological and pathological conditions. The specific objectives of the work were:

- To examine serum androgen bioactivity in patients with prostate cancer

- To characterize the functional consequences of mutations in the DNA-binding domain of AR found in androgen insensitivity patients

- To study expression of AR coregulator PIASx in adult testis and during testicular development

- To investigate regulation of PIASx gene transcription

- To study the physiological role of the PIASx gene by gene disruption in mice

MATERIALS AND METHODS

For more detailed descriptions of materials and methods, the reader is referred to the original publications.

1. Study subjects and study design (I)

The study population consisted of Caucasian men with lower urinary tract symptoms from area near Vienna. The prostate cancer status of the men was determined by digital rectal examination and PSA, or by histological examination of tissues derived from prostate biopsy or transurethral resection of prostate.

The study population was divided into prostate cancer (cases) and BPH (controls) groups on the basis of their cancer status. The prostate cancer group consisted of 101 men, and the BPH group was composed 103 age-matched men. The serum samples were taken at the time of diagnosis before any treatments, and thus, the study setting was cross-sectional. The study protocol was approved by the ethics committee of the University of Vienna, and all patients gave written informed consent.

2. Hormone measurements (I, V)

Murine serum LH and FSH levels were measured by immunofluorometric assays and intratesticular testosterone concentration was determined after diethyl ether extracting the gonadal homogenates followed by testosterone measurement with standard radioimmunoassay after reconstitution of the extract to phosphate-buffered saline. Testosterone concentration in human serum was determined by radioimmunoassay and SHBG levels were measured with fluoroimmunoassay.

Serum androgen bioactivity was measured by recombinant cell bioassay as described by Raivio et al. (2001). Briefly, the assay is based on androgen-dependent interaction between N- and C-terminal parts of AR expressed as separate polypeptides in recipient cells. Additionally, PIASxα is included in the assay to facilitate the interaction. The assay is responsive to DHT, testosterone, and androstenedione, but not to DHEA. Moreover, the assay also takes into account the presence of antiandrogenic substances and androgen binding proteins.

3. Statistical analyses (I, II, V)

The statistical difference between groups was analyzed by two-tailed *t* test unless otherwise stated. In publication number II, differences between prostate cancer and BPH groups were assessed with Mann-Whitney *U*-test and with analysis of variance in the case of subgroup analysis. The correlation between serum androgen bioactivity and testosterone levels was investigated using Pearson's correlation coefficient. $P < 0.05$ was considered as statistically significant.

4. Recombinant DNA techniques (II, III, IV, V)

Plasmids, probes, and transgene constructs were prepared using standard recombinant DNA techniques.

5. Cell culture, transfections, and reporter gene assays (II, IV)

CV-1, COS-1, and PC-3 cells were from American Type Culture Collection (Manassas, VA). Murine Sertoli cell line MSC-1 (Peschon et al. 1992) was obtained from Dr. Ilpo Huhtaniemi, (University of Turku, Finland) and murine spermatogonial cell line GC-1spg (Hofmann et al. 1992) was obtained from Dr. Jorma Keski-Oja (University of Helsinki, Finland). Transfections were performed by using FuGene6 reagent according to manufacturer's instructions. In brief, cells were plated 24 h before adding the DNA. Eighteen hours after transfection, the cells received fresh medium containing 2% charcoal-stripped fetal bovine serum with or without testosterone. Forty-eight hours after transfection, the cells were harvested and luciferase, chloramphenicol acetyltransferase, and β-galactosidase activities were measured. Transfections were performed with triplicate dishes, and the results were repeated in at least two independent experiments. Promoters of probasin, p75, collagenase, interleukin-6, and PIASx genes as well as artificial promoters CMV-ARE$_2$ and ARE$_4$-tk were used to drive the reporter gene.

6. Mutagenesis (II)

Mutant ARs were contructed by a PCR-based targeted mutagenesis, and the mutations were verified by sequencing.

7. DNA-binding studies (II, IV)

In electrophoretic mobility shift assays, testis- and liver-derived nuclear extracts, whole cell extracts as well as *in vitro*-translated proteins were incubated with a ^{32}P-labeled probe. After the incubation at room temperature, protein-DNA complexes were resolved by electrophoresis on 4% polyacrylamide gel under nondenaturing conditions. Cold probes and antibodies were used in binding reactions to address specificity and to identify unknown complexes.

8. Immunoblotting (II, III)

Protein samples were resolved by polyacrylamide gel electrophoresis under denaturing conditions (SDS-PAGE). Proteins were electrophoretically transferred onto polyvinylidene fluoride or nitrocellulose membrane and blocked. For detection of specific proteins, the following antibodies were used: anti-AR (residues 14-32 of rAR), anti-PIASx/ARIP3 (residues 443-548 of rPIASxα), and anti-actin. The immunocomplexes were visualized with a horseradish peroxidase-conjugated secondary antibody and chemiluminesence-based detection system.

9. *In vitro* protein-binding assays (II)

GST pull-down experiments were conducted by using either purified GST alone or GST-CBP-NT adsorbed to glutathione Sepharose and [^{35}S]methionine-labeled rAR variants produced by translation *in vitro*.

For immunoprecipitations, aliquots from tissue supernatants (500 μg protein) were diluted to 1 ml with washing buffer (50 mM Tris-HCl, pH 7.8, 140 mM NaCl, 5 mM EDTA and 0.02% Nonidet P-40), centrifuged at 16,000 x g for 15 min at 4ºC. The supernatants were transferred to new tubes and 30 μl of protein G Sepharose (Amersham Biosciences) was added. The tubes were rotated for 30 min at 4ºC and centrifuged at 13,000 x g for 5 min at 4ºC. The supernatants were transferred to new tubes and 30 μl of anti-FLAG M2 affinity gel (Sigma-Aldrich) was added. The tubes were rotated for 2 h at 4ºC and then centrifuged at 16,000 x g for 5 s. Agarose beads were washed three times with 1 ml of washing buffer and once with 1 ml of TBS (10 mM Tris-HCl, pH 8.0, and 100 mM NaCl) and 50 μl of SDS-PAGE sample buffer (100 mM Tris-HCl, 20% glycerol, 2% SDS, 0.1 M dithiothreitol, and 0.01% bromophenol blue) was added before boiling of the samples at 95ºC for four min. Immunoblotting was performed with 15-μl samples.

10. Tissue samples (III, IV, V)

All animal experiments were approved by the committee on ethics of animal experimentation of University of Helsinki or University of Turku. Sprague–Dawley male rats as well as FVB/N and C57BL/6 mice were used. The animals were sacrificed by CO_2 anesthesia and cervical dislocation, and the tissues were snap-frozen in liquid nitrogen and stored at -70°C until used. Seminiferous tubule segments at stages II–VI, VII–VIII, IX–XII and XIII–I of the epithelial cycle were isolated under a stereomicroscope by a transillumination-assisted microdissection technique. The tubular segments were snap-frozen in liquid nitrogen and stored at -70°C.

11. RNA blotting (III, V)

Total RNA was isolated using a single-step method and was size-fractionated in 1% denaturing agarose gel and transferred onto nylon membrane. Prehybridization and hybridization were performed with ULTRAhyb hybridization solution according to the manufacturer's instructions. After hybridization with ^{32}P-labeled RNA probe, the blots were stripped and subsequently hybridized with ^{32}P-labeled 28S rRNA cDNA.

12. *In situ* hybridization (III)

Tissues were fixed in paraformaldehyde and embedded in paraffin. ^{35}S-Labelled probes recognizing PIASx or PIAS1 were hybridized on the slides for overnight, and the probes were visualized with emulsion autoradiography.

13. Immunohistochemistry (III)

Tissues were fixed in paraformaldehyde, embedded in paraffin, and sectioned. PIASx protein was detected with of α-PIASx/ARIP3 (residues 443-548 of rPIASxα) primary antibody. Positive cells were visualized using Vectastain Elite-kit according to the manufacturer's instructions. Preimmune serum or rabbit IgG was used as primary antibody in immunohistochemistry to control the specificity of the staining.

14. Sequence analysis (IV)

Sequence of the proximal promoter of the murine PIASx gene was analyzed for potential transcription factor binding sites with Match program using Transfac 5.0 Public database (www.gene-regulation.com).

15. Primer extension (IV)

Transcription start site of PIASx gene was determined by using two Cy5-labeled antisense primers located near the translation start site. Primers were annealed with murine testis RNA and then extended by avian myeloblastosis virus reverse transcriptase. The primer extension products were subjected to electrophoresis under denaturing conditions on a 6% polyacrylamide gel parallel to the sequencing reaction and analyzed with ALFexpress DNA sequencer.

16. Production of transgenic mice (IV, V)

For studying the expression of PIASx, two promoter fragments, -4199/+76 and -168/+76, were cloned into the SmaI/HindIII site of the SDKlacZpA-derived vector that contains a Kozak consensus sequence fused in-frame to the lacZ coding region followed by an SV40 polyadenylation signal. The transgene was injected into fertilized mouse oocytes of the FVB/N strain.

PIASx knockout mouse was derived from a gene trap experiment (Hansen et al. 2003). Briefly, ES cells were electroporated with pT1βgeo gene-trap vector that contains En-2 splice acceptor upstream of β-galactosidase and neomycin resistance genes. Gene-trap integration occurred in the second intron of the PIASx gene. Mice were backcrossed with C57BL/6 mice for several generations and thus their genetic background is C57BL/6.

In order to create a PIASxα-overexpressing mouse line, the sequence encoding FLAG-epitope and rat PIASxα cDNA was amplified by PCR using the primers 5'-TCTAGAACCATGGACTACAAAGACG-3' and 5'-TCTAGATACAAATTATGTTTTATT TTGCA-3' and the product was cloned into the XbaI site of the pEF-BOS (Mizushima and Nagata 1990). The gene construct is driven by the human elongation factor 1α promoter, which has been shown to target the transgene to murine spermatogonia and spermatocytes (Furuchi et al. 1996, Meng et al. 2000). The expression vector fragment was injected into fertilized mouse oocytes of the FVB/N strain to create three lines of FLAG-rPIASxα mice. Identification of transgenic mice was performed from tail biopsy derived genomic DNA by PCR using primers 5'-

CATTCTCAAGCCTCAGACAGTGGTTC-3' and 5'-GACAGGAGATGACGGTGA ATGAGG-3'.

17. β-Galactosidase assays (IV)

For measurement of β-galactosidase activity, the tissues were homogenized and heat-treated to inactivate endogenous β-galactosidase activity. The β-galactosidase activity was measured from the samples with a chemiluminesence based assay (Galacto-Light) and the activity was related to protein concentrations measured by Bradford's method.

To localize the β-galactosidase activity, the testes were dissected out, decapsulated, fixed, and then stained for β-galactosidase activity for overnight. After staining, the testes were dehydrated, embedded in paraffin, sectioned, and counterstained with eosin.

18. Sperm analyses (V)

Spermatozoa from cauda epididymides were stained in a solution containing 0.02% acridine orange and staining was examined using a fluorescence microscope. Spermatozoa with normal double-stranded DNA structure displayed green fluorescent color, whereas yellow to red marked abnormal single-stranded DNA structure.

Cauda epididymal sperm count was measured by piercing the cauda epididymis with a needle and allowing sperm cells to disperse to Brinster's BMOC-3 medium. Subsequently, sperm counts were determined using a hemocytometer. Sperm count is expressed as the number of sperm per epididymis.

19. TUNEL-assay (V)

Testes were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. Sections were stained with DeadEnd colorimetric TUNEL system according to the manufacturer's instructions to detect the fragmentation of DNA which is a hallmark of apoptotic cell.

20. Microarray experiment (V)

Total RNA from testes of three mutant and three wild-type mice were labeled and hybridized on Mouse (Development) Oligo Array slides (Agilent Technologies). After background subtraction, the ratios were calculated by dividing average intensity of each spot in mutant hybridization by the average intensity of the corresponding spot in the wild-type hybridization. For transcripts

with two or three high quality measurements from separate slides, mean of log transformed ratios was calculated.

21. Real-time quantitative RT-PCR (V)

cDNA was synthesized from testicular total RNA with avian myeloblastosis virus reverse transcriptase. PCRs were performed with the Lightcycler system using FastStart DNA master SYBR green I mix. Standard curve for PCR test was constructed using serial 1:5 dilutions of normal murine testis cDNA. The concentrations of samples, performed as triplicates, were determined with the help of standard curve using fit-points method. The mean of the two repeated PCR values was used in the statistical analyses. Results were normalized to porphobilinogen deaminase by dividing the individual RT-PCR values by mean of three repeated PBGD test values of the respective sample.

RESULTS AND DISCUSSION

1. Serum androgen bioactivity is reduced in patients with prostate cancer (I)

Androgens are important for normal development of the prostate gland, and androgen deprivation therapy is initially an effective treatment for advanced prostate cancer. Roles of androgens in the development of prostate cancer have, however, remained elusive. In this study, we applied a recombinant cell bioassay, developed in our laboratory, to study the role of androgens in prostate cancer in a cross-sectional study setting. The study population consisted of Caucasian men with lower urinary tract symptoms. The men with histologically verified prostate cancer were assigned to cancer group, whereas the men in whom prostate cancer was excluded were assigned to BPH group.

The main aim of this study was to compare serum androgen bioactivity between the men with prostate cancer and the men with BPH. The serum samples were taken at the time of diagnosis. Although serum testosterone, SHBG, or free androgen index (testosterone/SHBG*100) did not significantly differ between the groups, there was a significant difference in serum androgen bioactivity. Serum androgen bioactivity was significantly lower in the cancer group compared to the BPH group. Although the overall difference in serum androgen bioactivity was relatively small, the difference may be more drastic in certain subgroups of prostate cancer.

Prostate cancer is not a homogenous disease entity: some prostate cancers remain local, while others are aggressive and metastasize. The most well-known classification system of prostate cancer is the Gleason score that grades the prostate cancer from two to ten according to the ductal structure that is preserved in the tumor (Gleason and Mellinger 1974). A low Gleason score corresponds to a non-aggressive disease, while a high Gleason score is usually associated with an aggressive disease. We pooled together the grades from two to five and those from eight to ten, and kept grades six and seven separate according to the recommendation by Gleason (Gleason 1992). When compared to the BPH group, serum androgen bioactivity displayed variations depending on the subgroup. The least aggressive group showed a tendency for lower activity, and in the most aggressive group, serum androgen bioactivity was significantly lower than in the BPH group. However, we could not detect any significant differences in serum testosterone levels between the BPH and the prostate cancer subgroups.

Due to the difference between serum androgen bioactivity and testosterone levels, we studied their correlation with each other. Serum testosterone levels and androgen bioactivity

correlated significantly in both groups. However, the correlation was stronger in the BPH group than in the prostate cancer group. We also studied the androgen bioactivity-to-testosterone ratio among the groups and, indeed, the prostate cancer group had significantly lower ratio, indicating that the measurement of serum testosterone levels overestimates androgenic activity in the serum. There were also differences among the prostate cancer subgroups, in that the androgen bioactivity-to-testosterone ratio was lower than expected in both the lowest and the highest Gleason score groups. The observed differences in the serum androgen bioactivity-to-testosterone ratio are likely to reflect differences between the subgroups. While the low ratio may be a characteristic feature of a non-aggressive cancer, in the high Gleason score group, it may reflect increased tumor volume.

Several reasons can be proposed to cause the mismatch between serum androgen bioactivity and testosterone concentration. This phenomenon was particularly prominent in four men with prostate cancer, who had high serum testosterone concentration and low androgen bioactivity. Higher than expected androgen-to-testosterone ratio could be due to the presence of other androgenic substance in addition to testosterone. Although endogenous DHT levels do not correlate with androgen bioactivity, exogenous DHT does increase serum androgen bioactivity (Raivio et al. 2002). However, the men that were enrolled into this study did not receive any androgen supplementation. The lower than expected ratio could also due to antiandrogenic substances in serum. The serum samples were taken before any treatment for prostate cancer and antiandrogenic medication cannot be the reason for the difference. Environmental substances with antiandrogenic activity also represent an unlikely explanation, since both the cases and the controls were from the same geographical area. In this study we could not exclude the usage of herbal products as a possible interfering . Antibodies against testosterone or AR could also disturb the measurement of androgen bioactivity. In conclusion, regardless of the mechanism, the measurement of serum testosterone level tends to overestimate androgen bioactivity of serum in the patients with prostate cancer.

2. Effects of AR DBD mutations on androgen action (II)

Currently, hundreds of AR mutations have been found both in prostate cancer and in androgen insensitivity syndrome. The mutations are usually single amino acid substitutions and tend to concentrate to DBD and LBD of the AR. While the observed mutations in human offer a unique model to verify the importance of the residues *in vivo*, the molecular mechanism that are behind

the functional deficiencies are poorly understood. Therefore, we generated a series of mutations that are observed in human and examined the defects in molecular functions of the variant ARs.

The generated mutations are shown in Fig. 8. In man, G551V, A579, and R590Q mutations cause PAIS, whereas mutation of cysteine 562 to tyrosine or phenylalanine cause CAIS (Alléra et al. 1995, Poujol et al. 1997, Wooster et al. 1992, Zoppi et al. 1992). Mutation of K563 has not yet been found in humans, but in the context of GR, this mutation converts transrepressive function of GR to transactivation (Meyer et al. 1997, Starr et al. 1996).
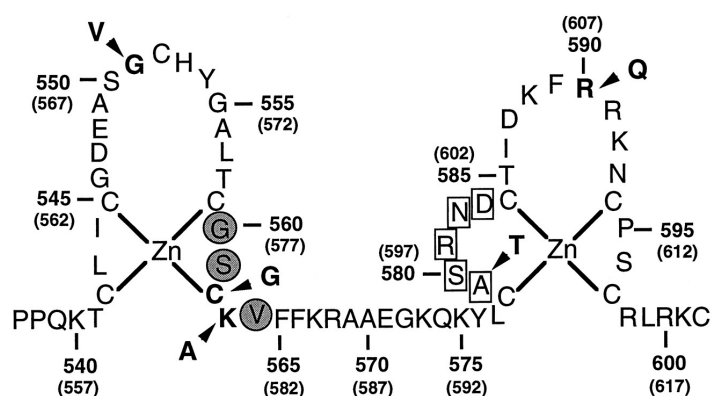
Figure 8. Location of the mutated residues of the AR DBD studied in this work. Numbers refer to rat AR according to Tan et al. 1988. In brackets are the numbering corresponding to human AR according to Lubahn et al. 1988. Grey circles and open boxes refer to the P box and the D box, respectively.

We first studied DNA-binding ability of the mutant ARs. As shown in Table 4, the majority of the mutants had reduced DNA-binding capability, and the C562G mutant did not bind to DNA at all. The C562G mutant's inability to bind DNA was predictable, since C562 is one the four cysteine residues of the first zinc-finger that coordinate binding of the zinc ion, and thus, the structure of the first zinc-finger is likely to be disturbed. In addition to *in vitro* DNA-binding, we studied DNA-binding of the variant ARs in intact cells by using a promoter interference assay. The results obtained *in vivo* were in line with those obtained *in vitro*.

Next, we studied the transactivation functions of the mutant ARs. First, we used an artificial promoter that contains four AREs in front of the thymidine kinase promoter. As shown in Table 4, G551V and K563A mutants had reduced transactivation capability, and C562G mutant did not activate transcription at all. Interestingly, A579T and R590Q mutants activated transcription stronger than the wild-type receptor. The results observed with the artificial promoter were similar to the data with the natural probasin promoter which contains two AREs, with the exception of K563A mutant that was weaker than with the artificial promoter. Transactivation functions of A579T and R590Q mutants were also studied with a shorter version of the probasin promoter that contains only one ARE. Although at high testosterone concentrations, the mutants

were as active as the wild-type receptor, at lower levels of testosterone their transactivation function was clearly weaker. In the case of A579T mutation, a similar phenomenon has been observed in a more recent study (Lundberg Giwercman et al. 2000). Moreover, a patient carrying R590Q mutation has been successfully treated with androgen supplementation (Weideman et al. 1998). The explanation for the difference between the two forms of the probasin promoter may lie in the difference between the two AREs in the promoter. The upstream ARE-1 resembles a classical ARE and is activated also by GR, whereas the downstream ARE-2 is a non-classical ARE and seems to be specifically activated by AR (Rennie et al. 1993, Schoenmakers et al. 2000). Schoenmakers and colleagues (2000) studied extensively the interaction between the probasin ARE-2 and the AR, and they concluded that the receptor is likely to bind to ARE-2 in a head-to-head orientation opposite to the classical head-to-tail orientation. Also, Geserick et al. (2003) have recently demonstrated that the A579T mutation impairs AR function on an AR-specific element of the Pem gene, whereas the transactivation function of the mutant was either unimpaired or even enhanced in the case of a classical ARE.

In addition to transactivation, we also studied transrepression functions of the mutant ARs. The mutations caused only mild impairment in the transrepression activity of the NF-κB pathway, and in the case of AP-1 signaling, transrepression was more or less normal (Table 4). Therefore, we conclude that, while the DBD mutations hamper transactivation function of AR, transrepression activities of AR that do not require DNA-binding are relatively unaffected. Studies on the dimerization-deficient GR mutant mice have clearly demonstrated the importance of GR-mediated transrepression (Reichardt et al. 1998). These mutant mice have abolished transactivation function and unaffected transrepression function, whereas in GR knockout mice both functions are affected. The dimerization-deficient GR mutant mice are viable in contrast to GR knockout mice that die shortly after birth, suggesting that GR-mediated transrepression, but not transactivation, is essential for mice.

TABLE 4. Effects of AR DBD mutations on DNA binding, transactivation and transrepression.

Mutation	Phenotype	DNA binding	Transactivation			Transrepression	
			ARE$_4$-tk	PB(2xARE)	PB(1xARE)	NF-κB	AP-1
Wild-type	Normal	++	++	++	++	++	++
G551V	PAIS	+	+	+	n.d.	+	++
C562G	CAIS	-	-	-	n.d.	+	++
K563A	n.d.	+	+	-	n.d.	+	+
A579T	PAIS	n.d.	+++	+++	+	++	++
R590Q	PAIS	+	+++	+++	+	++	++

+++, enhanced function; ++, normal function; +, deficient function; -, lack of function; n.d., not determined

We also investigated the interaction between AR mutants and the coactivator CBP. However, we were unable to detect any differences between the mutant and wild-type ARs. Geserick et al. (2003) have also studied the relationship between AR activity and AR coactivators that are known to interact with the DBD. The SUMO E2 conjugase Ubc9 and the SUMO E3 ligase PIASxα downregulated AR activity on AR-specific response elements, but not in classical response elements. Thus, altered interaction with Ubc9 or PIASxα may contribute to the impaired function of the A579T mutant on AR-specific elements. A very recent study suggests, however, that sumoylation may be an important regulator of transcriptional synergy on classical rather than AR-specific response elements (Callewaert et al. 2004)

3. Regulation of PIASx gene expression (III, IV)

PIASxα was originally identified as an AR coregulator that is predominantly expressed in testis (Moilanen et al. 1999). Importantly, testes are responsible for androgen production in male, and spermatogenesis is dependent on sufficient androgen action. To understand better such a specific expression, we studied the expression pattern and regulation of the PIASx gene. Spermatogenesis is a multi-step differentiation process in which each cell-type is characterized by unique events. Therefore, it is mandatory to know the expression of a gene at a cellular level. We examined the expression pattern of the PIASx gene by using *in situ* hybridization in adult rats. While PIASx mRNA was absent in Leydig cells, we detected PIASx transcript in Sertoli cells and throughout the germinal epithelium. However, the expression pattern was uneven in the seminiferous epithelium: PIASx mRNA was more abundant in pachytene spermatocytes than in other cell-types. The expression of PIASx mRNA as assessed by *in situ* hybridizations appeared to be stage-specific, which was further proven by northern blot analysis of stage-specific RNAs. Indeed, intensity was at its highest at stages IX-XII, which is concordant with the abundance of PIASx mRNA in late spermatocytes. We also applied methoxyacetic acid (MAA) treatment that destroys late spermatocytes in rats. We observed that the amount of PIASx mRNA first decreased when pachytene spermatocytes were depleted, which was followed by increased mRNA levels due to reappearance of pachytene spermatocytes as well as disappearance of round spermatids and later on elongated spermatids. Thus, the expression pattern of PIASx indicates that it is involved also in processes other than androgen action in Sertoli cells, and that PIASx is likely to play a role in spermatogenesis, especially in pachytene spermatocytes.

To understand better the regulation of PIASx gene transcription, we isolated the putative promoter region 4.2 kb upstream of the 5' untranslated region of the PIASx gene. Sequence

analysis revealed that the promoter is very GC-rich, a feature typical of a housekeeping gene. Also, several putative binding sites for transcription factors such as Sp1 were identified. We used reporter gene assays to verify that the isolated DNA indeed contains a functional promoter. Transcriptional activity of the PIASx promoter was the highest in a Sertoli cell-derived cell-line (MSC-1), whereas promoter activity was lowest in COS-1, a cell-line originating from kidney. Furthermore, in all studied cell lines the promoter fragment +168/-76 exhibited activity comparable to the longest promoter fragment. Thus, we concluded that promoter fragment +168/-76 is likely to correspond to the proximal promoter of the PIASx gene. The activity of PIASx promoter fragments failed, however, to display a similar degree of specificity that was observed at tissue level. Therefore, we used the +4199/-76 and +168/-76 promoter fragments to target the expression of β-galactosidase in mice. The two promoters retained testis-specific expression, and their activities were similar. The cellular localization of the β-galactosidase reporter gene product was, however, different from that of the native PIASx mRNA. While PIASx mRNA was evident throughout the seminiferous epithelium with the highest levels in pachytene spermatocytes, β-galactosidase activity was absent in spermatogonia and early spermatocytes, but it was clearly detectable in spermatids. This disparity may arise from the lack of regulatory elements needed for expression in spermatogonia and early spermatocytes. On the other hand, translation of the transgene or stability of the β-galactosidase protein may be different from that of PIASx protein. Importantly, however, the expression of the transgene was evident in the pachytene spermatocytes, the primary site of PIASx mRNA accumulation.

We used electrophoretic mobility shift assay to study the binding of transcription factors to the proximal promoter. Nuclear extracts derived from rat testis formed one major complex with two probes encompassing upstream and downstream regions of the proximal promoter. Oligo-nucleotide competition and antibody supershift assays showed that the major complex is formed by several proteins: Sp1, Sp2, and Sp3 that belong to the Sp family and are known to be expressed in the testis. The binding of at least Sp1 and Sp3 is likely to be physiologically relevant, since Sp1 is expressed in Sertoli cells and germ cells up to pachytene spermatocytes, and Sp3 is present in primary spermatocytes and early spermatids (Shell et al. 2002, Wilkerson et al. 2002). In addition to acting as a transcription factor, Sp1 could potentially influence PIASx expression by regulating the methylation status of the PIASx promoter (Brandeis et al. 1994, Macleod et al. 1994). Testicular nuclear extract also formed a minor complex with 5' end of the proximal promoter containing a putative binding site for the Ets family of transcription factors. However, this complex was not competed out with an Ets consensus binding site-containing oligonucleotide, indicating that the protein is not a classical member of the Ets transcription

factor family. A similar Ets-like-binding site has been found also in two other testis-specific genes, namely phosphoglycerate kinase 2 and β1,4-galactosyltransferase-I. However, the protein binding to the element has not been characterized (Charron et al. 1999, Goto et al. 1993).

In conclusion, the proximal promoter of the PIASx gene is GC-rich, and the members of the Sp transcription factor family and possibly also an Ets-like transcription factor are able to bind to the promoter. The proximal promoter includes the DNA-elements that are required for germ cell-specific expression, and therefore, this promoter sequence could be used to target transgene expression specifically to germ cells.

4. Role of PIASx in the spermatogenesis (III, V)

Expression pattern of PIASx mRNA in the testis suggests that PIASx plays a role in spermatogenesis. However, transcription in germ cells is not as tightly coupled to translation as in somatic cells. For example, the protamine genes are transcribed in round spermatids, but translation does not occur until in elongated spermatids. Therefore, we performed immunohistochemical staining with anti-PIASx/ARIP3 antibody that recognizes both α and β forms of the PIASx protein. We detected PIASx immunostaining in Sertoli cells and in germ cells up to stage XII pachytene spermatocytes. Similar to *in situ* hybridization analysis, we did not detect any PIASx in Leydig cells. Moreover, there were differences in the intensity of immunostaining in germ cells; the highest levels of PIASx protein were detected in pachytene spermatocytes. Pachytene is next to the last phase in spermatogenesis before meiotic cell divisions. It is the longest phase of spermatogenesis, extending from stage XIV to stage XII in rat. The most important process taking place in the pachynema is the homologous recombination. During pachytene, sister chromatids are completely paired or synapsed by a special structure called synaptonemal complex (for review, Cohen and Pollard 2001). Interestingly, Ubc9 has been shown to localize to the synaptonemal complex and to interact with core proteins Cor1 and Syn1 of the synaptonemal complex (Kovalenko et al. 1996, Tarsounas et al. 1997). Moreover, sumoylation is likely to play an important role in homologous recombination occurring in somatic cell in response to DNA damage (for review, Müller et al. 2004). It is thus tempting to speculate that sumoylation and PIAS proteins have functions related to homologous recombination in germ cells.

To study the functional role of the PIASx gene in spermatogenesis, we generated transgenic mice that overexpress Flag-tagged rPIASxα/ARIP3 under the control of elongation factor 1 alpha promoter that has been shown to direct expression of the transgene into spermatogonia and

spermatocytes. We produced three independent transgenic lines that all expressed Flag-tagged PIASxα protein (Fig. 9A). When compared to endogenous PIASx protein, the expression levels of PIAS was up to 3.9-fold higher in line B19 (Fig. 9B). The mice of all three independent lines were, however, fertile and their spermatogenesis appeared to be grossly normal. The lack of phenotype in these mice may in part be due to relatively modest overexpression of PIASx, or alternatively, the level of endogenous PIASx is already so high that no gain-of-function can be achieved by increasing the amount of PIASxα. Therefore, we proceeded to study a loss-of-function model of the PIASx gene.
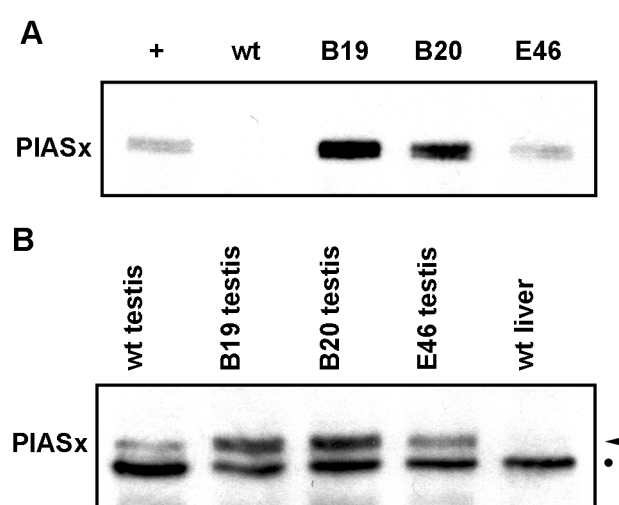
Figure 9. Detection of epitope-tagged PIASx transgene product in the testes of three transgenic mouse lines. A, Protein extracts from testes of wild-type (wt) or three different transgenic lines B19, B20, and E46 were immunoprecipitated with anti-FLAG M2 affinity gel matrix and immunoblotted with polyclonal rabbit anti-PIASx/ARIP3 antibody. Lane + corresponds to COS-1-produced FLAG-tagged PIASxα used as a positive control. B, A representative immunoblot of whole testis extracts from wt and the transgenic lines B19, B20, and E46. Arrowhead, band corresponding to PIASx protein; dot, unspecific band due to secondary antibody.

PIASx knockout mice originated from a gene-trap experiment in which an embryonic stem cell clone with a disruption in the PIASx gene was identified. In this clone, a gene-trap vector is integrated to second intron of the PIASx gene, resulting in disruption of the PIASx gene. Although PIASx mutant mice were viable and fertile, we nevertheless studied the reproductive functions of the male mice. Whereas the overall weight of the PIASx knockout mice was normal, their testis weight was significantly reduced, and the reduced gonadal size was accompanied with a smaller diameter of seminiferous tubules and fewer mature spermatozoa in the epididymis. However, normal DNA structure and normal motility of spermatozoa of the PIASx -/- mice imply that the quality of the sperm cells is unaffected. Thus, PIASx is quantitatively rather than qualitatively required for normal spermatogenesis.

5. PIASx and apoptosis (V)

Apoptosis during spermatogenesis is an important determinant for the number of germ cells that achieve maturity. It has been estimated that 75% of germ cells never mature due to apoptotic cell death occurring predominantly during spermatogonial development (for review, Sinha Hikim et al. 2003). Decreased levels of gonadotropins and intratesticular testosterone are known to increase the rate of apoptosis. Moreover, in certain knockout mouse models, such as ubiquitin E3 ligase Siah1a -/- mice, spermatogenesis is arrested at a specific stage, which is paralleled with a dramatic stage-specific increase in the number of apoptotic cells (Dickins et al. 2002). Therefore, we studied the number of apoptotic cells in the testes of PIASx -/- mice by TUNEL-staining. We found that the number of TUNEL-positive cells was increased significantly, albeit not dramatically, in PIASx knockouts. However, the apoptotic cells did not appear to accumulate in any specific stage or step. We also measured the levels of serum gonadotropins and intratesticular testosterone. The levels did not, however, differ significantly between the wild-type and mutant mice, suggesting that the increased rate of apoptosis is not due to the hormonal status of the knockout mice. Interestingly, several sumoylated proteins such as p53 and Smads are involved in apoptosis, and sumoylation of the latter proteins is facilitated by PIAS proteins. However, the roles of individual PIAS proteins in the regulation of p53 and Smad activity are currently elusive.

6. Regulation of gene transcription by PIASx (V)

PIAS proteins and sumoylation are heavily implicated in the regulation of transcription. Therefore, we used DNA microarray analysis to examine changes in gene expression at the level of transcriptome in testis of wild-type and PIASx knockout animals. We identified several upregulated and downregulated transcripts that corresponded to genes that have an intron-exon structure and more or less well-characterized functions. We also grouped the differentially regulated genes on the basis of their function, but we were unable to detect any significant enrichment of genes in to a particular functional group. We further examined the expression of the genes with a putative testicular function by using RT-PCR, and could confirm the decreased expression of a growth factor, erv1-like (Gfer) in testes of PIASx knockout mice. Gfer is a sulphydryl oxidase that is expressed in spermatogonia and primary spermatocytes, and it is known to regulate the growth of other tissues, such as liver (Adams et al. 1998, Hagiya et al. 1994, Klissenbauer et al. 2002). It is, therefore, possible that Gfer has a growth-related function

also during the early spermatogenesis. In addition to protein coding mRNAs, we identified 29 downregulated and 2 upregulated ESTs that are likely to belong to so-called regulatory noncoding RNAs. Although the physiological importance of such noncoding RNAs is poorly understood, they have been shown to participate in the regulation of transcription and translation (for review, Mattick 2003, Szymański and Barciszewski 2002). Interestingly, steroid receptor coactivator SRA is an RNA rather than a protein, but currently no other RNA-based coactivators have been reported (Lanz et al. 1999).

Since PIASxα was originally identified as an AR coregulator and is known to facilitate sumoylation of AR, we also examined the consequence of PIASx disruption on AR-mediated transcription. As a target gene, we chose the Pem homeobox gene that is a Sertoli cell-specific and androgen-regulated gene (Rao et al. 2003). Pem mRNA level, as assessed by real-time quantitative RT-PCR, was decreased by 42% in PIASx knockout testes, indicating that PIASx has an impact on androgen receptor-mediated transcriptional regulation.

7. Functional redundancy among PIAS proteins (III, V)

Several lines of evidence suggest that PIAS proteins are functionally redundant. First, several PIAS proteins are capable of facilitating sumoylation of the same protein, such as AR. Second, PIASx, PIAS1, and PIASy are highly expressed in the testis (Gross et al. 2001, Moilanen et al. 1999, Tan et al. 2000). On the other hand, PIAS proteins have clearly different effects on AR-dependent reporter gene activity in transfection assays (Table 3). In addition to PIASx, we studied the expression pattern of PIAS1 in testis. Like PIASx, also PIAS1 mRNA was detected throughout the seminiferous epithelium. In contrast to PIASx, however, PIAS1 mRNA was most abundant in postmeiotic spermatids. This difference was also evident in the developmental appearance of PIASx and PIAS1 mRNAs. PIASx mRNA was clearly detectable at day 20 corresponding to development of pachytene spermatocytes, whereas PIAS1 emerged at day 30 when round spermatids are formed. Tan et al. (2000) have also shown that PIASy is expressed equally in spermatocytes and spermatids in murine testis. The expression patterns of PIAS1, PIASx, and PIASy thus overlap, although they are partially distinct. We also compared expression levels of PIAS mRNA in PIASx -/- and PIASx +/+ mice and found a slight increase and decrease in the mRNA levels of PIAS3 and PIAS1, respectively. However, both PIAS1 and PIASy are already expressed at high levels in the testis, and it is, therefore, possible that no increase in mRNA level is needed to compensate for the loss of PIASx. Thus, the relatively mild

phenotype of the PIASx knockout mice may be, at least in part, due to a functional redundancy among the PIAS proteins.

CONCLUSIONS

This study aimed at deciphering molecular determinants of androgen action at the level of bioactive ligand, receptor, and coregulatory proteins to understand better how androgen action is convoyed in both physiological and pathological conditions. The main conclusions of this study are as follows:

- The serum androgen bioactivity is lower in prostate cancer patients than in patients with BPH, and it is overestimated solely on the basis of their serum testosterone levels, especially in patients with low Gleason score. These results suggest that the reduced androgen bioactivity-to-testosterone is a characteristic feature of less aggressive prostate cancer.

- Mutations in the AR DBD alter the transactivation ability of the receptor, but the transrepression functions are only mildly affected, indicating that the varying phenotype of androgen insensitivity syndrome is a result of differentially affected AR functions.

- The PIASx gene encoding an AR coregulator is expressed during spermatogenesis in both AR target Sertoli cells and germ cells, especially in pachytene spermatocytes. This suggests that the functions of PIASx are not restricted to its AR coregulator function and that PIASx may participate in structural changes of chromosome in pachytene spermatocytes.

- Transcription of the PIASx gene is regulated by a short GC-rich promoter region, and members of the Sp family are candidate *trans*-acting factors binding to the promoter. The proximal promoter of the PIASx gene includes the DNA elements that are required for male germ cell-specific transcription, which allows the highly specific expression of a transgene in male germ cells.

- Disruption of the PIASx gene in mice results in a reduced testis weight and epididymal sperm count despite the maintenance of normal fertility. These results indicate that the PIASx gene is important for quantitatively rather than qualitatively normal spermatogenesis.

ACKNOWLEDGEMENTS

Helsinki, August 2004

REFERENCES

Acevedo ML, Kraus WL (2003) Mediator and p300/CBP-steroid receptor coactivator complexes have distinct roles, but function synergistically, during estrogen receptor alpha-dependent transcription with chromatin templates. Mol Cell Biol 23:335-348

Adachi M, Takayanagi R, Tomura A, Imasaki K, Kato S, Goto K, Yanase T, Ikuyama S, Nawata H (2000) Androgen-insensitivity syndrome as a possible coactivator disease. N Engl J Med 343:856-862

Adams GA, Maestri M, Squiers EC, Alfrey EJ, Starzl TE, Dafoe DC (1998) Augmenter of liver regeneration enhances the success rate of fetal pancreas transplantation in rodents. Transplantation 65:32-36

Ahmed SF, Cheng A, Dovey L, Hawkins JR, Martin H, Rowland J, Shimura N, Tait AD, Hughes IA (2000) Phenotypic features, androgen receptor binding, and mutational analysis in 278 clinical cases reported as androgen insensitivity syndrome. J Clin Endocrinol Metab 85:658-665

Alen P, Claessens F, Schoenmakers E, Swinnen JV, Verhoeven G, Rombauts W, Peeters B (1999) Interaction of the putative androgen receptor-specific coactivator ARA70/ELE1alpha with multiple steroid receptors and identification of an internally deleted ELE1beta isoform. Mol Endocrinol 13:117-128

Alléra A, Herbst MA, Griffin JE, Wilson JD, Schweikert H-U, McPhaul MJ (1995) Mutations in the androgen receptor coding sequence are infrequent in patients with isolated hypospadias. J Clin Endocrinol Metab 80:2697-2699

Almlöf T, Wright AP, Gustafsson JÅ (1995) Role of acidic and phosphorylated residues in gene activation by the glucocorticoid receptor. J Biol Chem 270:17535-17540

Almlöf T, Gustafsson JÅ, Wright AP (1997) Role of hydrophobic amino acid clusters in the transactivation activity of the human glucocorticoid receptor. Mol Cell Biol 17:934-945

Andersson S (2001) Steroidogenic enzymes in skin. Eur J Dermatol 11:293-295

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

Aravind L, Koonin EV (2000) SAP - a putative DNA-binding motif involved in chromosomal organization. Trends Biochem Sci 25:112-114

Arora T, Liu B, He H, Kim J, Murphy TL, Murphy KM, Modlin RL, Shuai K (2003) PIASx is a transcriptional co-repressor of signal transducer and activator of transcription 4. J Biol Chem 278:21327-21330

Avila DM, Fuqua SA, George FW, McPhaul MJ (1998) Identification of genes expressed in the rat prostate that are modulated differently by castration and Finasteride treatment. J Endocrinol 159:403-411

Bain DL, Franden MA, McManaman JL, Takimoto GS, Horwitz KB (2000) The N-terminal region of the human progesterone A-receptor. Structural analysis and the influence of the DNA binding domain. J Biol Chem 275:7313-7320

Bartlett JM, Kerr JB, Sharpe RM (1986) The effect of selective destruction and regeneration of rat Leydig cells on the intratesticular distribution of testosterone and morphology of the seminiferous epithelium. J Androl 7:240-253

Bayer P, Arndt A, Metzger S, Mahajan R, Melchior F, Jaenicke R, Becker J (1998) Structure determination of the small ubiquitin-related modifier SUMO-1. J Mol Biol 280:275-286

Becker PB, Hörz W (2002) ATP-dependent nucleosome remodeling. Annu Rev Biochem 71:247-273

Belandia B, Orford RL, Hurst HC, Parker MG (2002) Targeting of SWI/SNF chromatin remodelling complexes to estrogen-responsive genes. EMBO J 21:4094-4103

References

van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW (2000) Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. J Clin Endocrinol Metab 85:3276-3282

Bernier-Villamor V, Sampson DA, Matunis MJ, Lima CD (2002) Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. Cell 108:345-356

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

Bosland MC (2000) The role of steroid hormones in prostate carcinogenesis. J Natl Cancer Inst Monogr 27:39-66

Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha. Nature 375:377-382

Boyer TG, Martin ME, Lees E, Ricciardi RP, Berk AJ (1999) Mammalian Srb/Mediator complex is targeted by adenovirus E1A protein. Nature 399:276-279

Brandeis M, Frank D, Keshet I, Siegfried Z, Mendelsohn M, Nemes A, Temper V, Razin A, Cedar H (1994) Sp1 elements protect a CpG island from de novo methylation. Nature 371:435-438

Bremner WJ, Millar MR, Sharpe RM, Saunders PT (1994) Immunohistochemical localization of androgen receptors in the rat testis: evidence for stage-dependent expression and regulation by androgens. Endocrinology 135:1227-1234

Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, Allis CD (1996) Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell 84:843-851

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

Burgdorf S, Leister P, Scheidtmann KH (2004) TSG101 Interacts with Apoptosis-antagonizing Transcription Factor and Enhances Androgen Receptor-mediated Transcription by Promoting Its Monoubiquitination. J Biol Chem 279:17524-17534

Burke TW, Kadonaga JT (1997) Drosophila TFIID binds to a conserved downstream basal promoter element that is present in many TATA-box-deficient promoters. Genes Dev 10:711-724

Butler JE, Kadonaga JT (2002) The RNA polymerase II core promoter: a key component in the regulation of gene expression. Genes Dev 16:2583-2592

Cai H, Levine M (1995) Modulation of enhancer-promoter interactions by insulators in the Drosophila embryo. Nature 376:533-536

Callewaert L, Verrijdt G, Haelens A, Claessens F (2004) Differential effect of SUMOylation of the androgen receptor in the control of cooperativity on selective versus canonical response elements. Mol Endocrinol, in press

Carmo-Fonseca M (2002) The contribution of nuclear compartmentalization to gene regulation. Cell 108:513-521

Cavailles V, Dauvois S, Danielian PS, Parker MG (1994) Interaction of proteins with transcriptionally active estrogen receptors. Proc Natl Acad Sci USA 91:10009-10013

Cavailles V, Dauvois S, L'Horset F, Lopez G, Hoare S, Kushner PJ, Parker MG (1995) Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. EMBO J 14:3741-3751

Chamberlain NL, Driver ED, Miesfeld RL (1994) The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 22:3181-3186

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

Chang CS, Kokontis J, Liao ST (1988) Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 240:324-326

Charron M, Shaper NL, Rajput B, Shaper JH (1999) A novel 14-base-pair regulatory element is essential for in vivo expression of murine beta4-galactosyltransferase-I in late pachytene spermatocytes and round spermatids. Mol Cell Biol 19:5823-5832

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

Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, 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 D, Pace PE, Coombes RC, Ali S (1999a) Phosphorylation of human estrogen receptor alpha by protein kinase A regulates dimerization. Mol Cell Biol 19:1002-1015

Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR (1999b) Regulation of transcription by a protein methyltransferase. Science 284:2174-2177

Chen D, Riedl T, Washbrook E, Pace PE, Coombes RC, Egly JM, Ali S (2000a) Activation of estrogen receptor alpha by S118 phosphorylation involves a ligand-dependent interaction with TFIIH and participation of CDK7. Mol Cell 6:127-137

Chen D, Huang SM, Stallcup MR (2000b) Synergistic, p160 coactivator-dependent enhancement of estrogen receptor function by CARM1 and p300. J Biol Chem 275:40810-40816

Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG, Sawyers CL (2004) Molecular determinants of resistance to antiandrogen therapy. Nat Med 10:33-39

Chevillard-Briet M, Trouche D, Vandel L (2002) Control of CBP co-activating activity by arginine methylation. EMBO J 21:5457-5466

Chiba H, Muramatsu M, Nomoto A, 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

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

Chung CD, Liao J, Liu B, Rao X, Jay P, Berta P, Shuai K (1997) Specific inhibition of Stat3 signal transduction by PIAS3. Science 278:1803-1805

Clarkson MJ, Harley VR (2002) Sex with two SOX on: SRY and SOX9 in testis development. Trends Endocrinol Metab 13:106-111

Clements A, Rojas JR, Trievel RC, Wang L, Berger SL, Marmorstein R (1999) Crystal structure of the histone acetyltransferase domain of the human PCAF transcriptional regulator bound to coenzyme A. EMBO J 18:3521-3532

Cohen PE, Pollard JW (2001) Regulation of meiotic recombination and prophase I progression in mammals. Bioessays 23:996-1009

Craft N, Shostak Y, Carey M, Sawyers CL (1999) A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the HER-2/neu tyrosine kinase. Nat Med 5:280-285

Cremer T, Cremer C (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2:292-301

References

Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H (1994) Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 54:5474-5478

Dahlman-Wright K, Baumann H, McEwan IJ, Almlöf T, Wright AP, Gustafsson JÅ, Härd T (1995) Structural characterization of a minimal functional transactivation domain from the human glucocorticoid receptor. Proc Natl Acad Sci USA 92:1699-1703

Danielsen M, Hinck L, Ringold GM (1989) Two amino acids within the knuckle of the first zinc finger specify DNA response element activation by the glucocorticoid receptor. Cell 57:1131-1138

Darimont BD, Wagner RL, Apriletti JW, Stallcup MR, Kushner PJ, Baxter JD, Fletterick RJ, Yamamoto KR (1998) Structure and specificity of nuclear receptor-coactivator interactions. Genes Dev 12:3343-3356

De Gendt K, Swinnen JV, Saunders PT, Schoonjans L, Dewerchin M, Devos A, Tan K, Atanassova N, Claessens F, Lecureuil C, Heyns W, Carmeliet P, Guillou F, Sharpe RM, Verhoeven G (2004) A Sertoli cell-selective knockout of the androgen receptor causes spermatogenic arrest in meiosis. Proc Natl Acad Sci USA 101:1327-1332

Debes JD, Tindall DJ (2002) The role of androgens and the androgen receptor in prostate cancer. Cancer Lett 187:1-7

Dejager S, Bry-Gauillard H, Bruckert E, Eymard B, Salachas F, LeGuern E, Tardieu S, Chadarevian R, Giral P, Turpin G (2002) A comprehensive endocrine description of Kennedy's disease revealing androgen insensitivity linked to CAG repeat length. J Clin Endocrinol Metab 87:3893-3901

Deslypere JP, Young M, Wilson JD, McPhaul MJ (1992) Testosterone and 5 alpha-dihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTV-CAT reporter gene. Mol Cell Endocrinol 88:15-22

Dhalluin C, Carlson JE, Zeng L, He C, Aggarwal AK, Zhou MM (1999) Structure and ligand of a histone acetyltransferase bromodomain. Nature 399:491-496

Di Croce L, Koop R, Venditti P, Westphal HM, Nightingale KP, Corona DF, Becker PB, Beato M (1999) Two-step synergism between the progesterone receptor and the DNA-binding domain of nuclear factor 1 on MMTV minichromosomes. Mol Cell 4:45-54

Dickins RA, Frew IJ, House CM, O'Bryan MK, Holloway AJ, Haviv I, Traficante N, de Kretser DM, Bowtell DD (2002) The ubiquitin ligase component Siah1a is required for completion of meiosis I in male mice. Mol Cell Biol 22:2294-2303

Dilworth FJ, Fromental-Ramain C, Yamamoto K, 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

Dobreva G, Dambacher J, Grosschedl R (2003) SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin mu gene expression. Genes Dev 17:3048-3061

Dunn JF, Nisula BC, Rodbard D (1981) Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 53:58-68

Eckner R, Ewen ME, Newsome D, Gerdes M, DeCaprio JA, Lawrence JB, 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

Edwards JE, Moore RA (2002) Finasteride in the treatment of clinical benign prostatic hyperplasia: a systematic review of randomised trials. BMC Urol 2:14

Elo JP, Kvist L, Leinonen K, Isomaa V, Henttu P, Lukkarinen O, Vihko P (1995) Mutated human androgen receptor gene detected in a prostatic cancer patient is also activated by estradiol. J Clin Endocrinol Metab 80:3494-3500

Faber PW, van Rooij HC, Schipper HJ, Brinkmann AO, Trapman J (1993) Two different, overlapping pathways of transcription initiation are active on the TATA-less human androgen receptor promoter. The role of Sp1. J Biol Chem 268:9296-9301

Fahrenkrog B, Aebi U (2003) The nuclear pore complex: nucleocytoplasmic transport and beyond. Nat Rev Mol Cell Biol 4:757-766

Fajardo MA, Haugen HS, Clegg CH, Braun RE (1997) Separate elements in the 3' untranslated region of the mouse protamine 1 mRNA regulate translational repression and activation during murine spermatogenesis. Dev Biol 191:42-52

Fenton MA, Shuster TD, Fertig AM, Taplin ME, Kolvenbag G, Bubley GJ, Balk SP (1997) Functional characterization of mutant androgen receptors from androgen-independent prostate cancer. Clin Cancer Res 3:1383-1388

Fischle W, Dequiedt F, Hendzel MJ, Guenther MG, Lazar MA, Voelter W, Verdin E (2002) Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell 9:45-57

Fondell JD, Ge H, Roeder RG (1996) Ligand induction of a transcriptionally active thyroid hormone receptor coactivator complex. Proc Natl Acad Sci USA 93:8329-8333

Francastel C, Walters MC, Groudine M, Martin DI (1999) A functional enhancer suppresses silencing of a transgene and prevents its localization close to centrometric heterochromatin. Cell 99:259-269

Fu M, Wang C, Reutens AT, Wang J, Angeletti RH, Siconolfi-Baez L, Ogryzko V, Avantaggiati ML, 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, Rao M, Wang C, Sakamaki T, Wang J, Di Vizio D, Zhang X, Albanese C, Balk S, Chang C, Fan S, Rosen E, Palvimo JJ, Jänne OA, Muratoglu S, Avantaggiati ML, Pestell RG (2002) Acetylation of androgen receptor enhances coactivator binding and promotes prostate cancer cell growth. Mol Cell Biol 23:8563-8575

Fujimoto N, Mizokami A, Harada S, Matsumoto T (2001) Different expression of androgen receptor coactivators in human prostate. Urology 58:289-294

Furuchi T, Masuko K, Nishimune Y, Obinata M, Matsui Y (1996) Inhibition of testicular germ cell apoptosis and differentiation in mice misexpressing Bcl-2 in spermatogonia. Development 122:1703-1709

Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ (1996) Prospective study of sex hormone levels and risk of prostate cancer. J Natl Cancer Inst 88:1118-1126

Gao T, McPhaul MJ (1998) Functional activities of the A and B forms of the human androgen receptor in response to androgen receptor agonists and antagonists. Mol Endocrinol 12:654-663

Gao T, Brantley K, Bolu E, McPhaul MJ (1999) RFG (ARA70, ELE1) interacts with the human androgen receptor in a ligand-dependent fashion, but functions only weakly as a coactivator in cotransfection assays. Mol Endocrinol 13:1645-1656

Ge H, Roeder RG (1994) The high mobility group protein HMG1 can reversibly inhibit class II gene transcription by interaction with the TATA-binding protein. J Biol Chem 269:17136-17140

Gehin M, Mark M, Dennefeld C, Dierich A, Gronemeyer H, 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

Geserick C, Meyer HA, Barbulescu K, Haendler B (2003) Differential modulation of androgen receptor action by deoxyribonucleic acid response elements. Mol Endocrinol 17:1738-1750

Glass CK (1994) Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. Endocr Rev 15:391-407

References

Gleason DF, Mellinger GT (1974) Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111:58-64

Gleason DF (1992) Histologic grading of prostate cancer: a perspective. Hum Pathol 23:273-279

Goto M, Masamune Y, Nakanishi Y (1993) A factor stimulating transcription of the testis-specific Pgk-2 gene recognizes a sequence similar to the binding site for a transcription inhibitor of the somatic-type Pgk-1 gene. Nucleic Acids Res 21:209-214

Gottlieb B, Beitel LK, Trifiro MA (2001) Variable expressivity and mutation databases: The androgen receptor gene mutations database. Hum Mutat 17:382-388

Gottlieb B, Beitel LK, Wu JH, Trifiro M (2004) The androgen receptor gene mutations database (ARDB): 2004 update. Hum Mutat 23:527-533

Grad JM, Dai JL, Wu S, Burnstein KL (1999) Multiple androgen response elements and a Myc consensus site in the androgen receptor (AR) coding region are involved in androgen-mediated up-regulation of AR messenger RNA. Mol Endocrinol 13:1896-1911

Gregory CW, He B, Wilson EM (2001a) The putative androgen receptor-A form results from in vitro proteolysis. J Mol Endocrinol 27:309-319

Gregory CW, Johnson RT Jr, Mohler JL, French FS, Wilson EM (2001b) Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res 61:2892-2898

Gregory CW, He B, Johnson RT, Ford OH, Mohler JL, French FS, Wilson EM (2001c) A mechanism for androgen receptor-mediated prostate cancer recurrence after androgen deprivation therapy. Cancer Res 61:4315-4319

Gregory CW, Fei X, Ponguta LA, He B, Bill HM, French FS, Wilson EM (2004) Epidermal growth factor increases coactivation of the androgen receptor in recurrent prostate cancer. J Biol Chem 279:7119-7130

Grootegoed JA, Siep M, Baarends WM (2000) Molecular and cellular mechanisms in spermatogenesis. Baillieres Best Pract Res Clin Endocrinol Metab 14:331-343

Gross M, Liu B, Tan J, French FS, Carey M, Shuai K (2001) Distinct effects of PIAS proteins on androgen-mediated gene activation in prostate cancer cells. Oncogene 20:3880-3887

Gross M, Yang R, Top I, Gasper C, Shuai K (2004) PIASy-mediated repression of the androgen receptor is independent of sumoylation. Oncogene 23:3059-3066

Gruber SB, Chen H, Tomsho LP, Lee N, Perrone EE, Cooney KA (2003) R726L androgen receptor mutation is uncommon in prostate cancer families in the united states. Prostate 54:306-309

Gu W, Shi XL, Roeder RG (1997) Synergistic activation of transcription by CBP and p53. Nature 387:819-823

Gu W, Malik S, Ito M, Yuan CX, Fondell JD, Zhang X, Martinez E, Qin J, Roeder RG (1999) A novel human SRB/MED-containing cofactor complex, SMCC, involved in transcription regulation. Mol Cell 3:97-108

Guenther MG, Barak O, Lazar MA (2001) The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. Mol Cell Biol 21:6091-6101

Guenther MG, Yu J, Kao GD, Yen TJ, Lazar MA (2002) Assembly of the SMRT-histone deacetylase 3 repression complex requires the TCP-1 ring complex. Genes Dev 16:3130-3135

Göhring F, Schwab BL, Nicotera P, Leist M, Fackelmayer FO (1997) The novel SAR-binding domain of scaffold attachment factor A (SAF-A) is a target in apoptotic nuclear breakdown. EMBO J 16:7361-7371

Görlich D, Kutay U (1999) Transport between the cell nucleus and the cytoplasm. Annu Rev Cell Dev Biol 15:607-660

Hagiya M, Francavilla A, Polimeno L, Ihara I, Sakai H, Seki T, Shimonishi M, Porter KA, Starzl TE (1994) Cloning and sequence analysis of the rat augmenter of liver regeneration (ALR) gene: expression of biologically active recombinant ALR and demonstration of tissue distribution. Proc Natl Acad Sci USA 91:8142-8146

Hammond GL (2002) Access of reproductive steroids to target tissues. Obstet Gynecol Clin North Am 29:411-423

Hampsey M (1998) Molecular genetics of the RNA polymerase II general transcriptional machinery. Microbiol Mol Biol Rev 62:465-503

Hansen J, Floss T, Van Sloun P, Fuchtbauer EM, Vauti F, Arnold HH, Schnutgen F, Wurst W, von Melchner H, Ruiz P (2003) A large-scale, gene-driven mutagenesis approach for the functional analysis of the mouse genome. Proc Natl Acad Sci USA 100:9918-9922

Hapgood JP, Riedemann J, Scherer SD (2001) Regulation of gene expression by GC-rich DNA cis-elements. Cell Biol Int 25:17-31

Hari KL, Cook KR, Karpen GH (2001) The Drosophila Su(var)2-10 locus regulates chromosome structure and function and encodes a member of the PIAS protein family. Genes Dev 15:1334-1348

He B, Kemppainen JA, Wilson EM (2000) FXXLF and WXXLF sequences mediate the NH2-terminal interaction with the ligand binding domain of the androgen receptor. J Biol Chem 275:22986-22994

He B, Bowen NT, Minges JT, Wilson EM (2001) Androgen-induced NH2- and COOH-terminal Interaction Inhibits p160 coactivator recruitment by activation function 2. J Biol Chem 276:42293-42301

Heery DM, Kalkhoven E, Hoare S, Parker MG (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387:733-736

Heikkilä R, Aho K, Heliövaara M, Hakama M, Marniemi J, Reunanen A, Knekt P (1999) Serum testosterone and sex hormone-binding globulin concentrations and the risk of prostate carcinoma: a longitudinal study. Cancer 86:312-315

Heinlein CA, Chang C (2002a) The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. Mol Endocrinol 16:2181-2187

Heinlein CA, Chang C (2002b) Androgen receptor (AR) coregulators: an overview. Endocr Rev 23:175-200

Heinlein CA, Chang C (2004) Androgen receptor in prostate cancer. Endocr Rev 25:276-308

Henderson BE, Bernstein L, Ross RK, Depue RH, Judd HL (1988) The early in utero oestrogen and testosterone environment of blacks and whites: potential effects on male offspring. Br J Cancer 57:216-218

Hertel KJ, Lynch KW, Maniatis T (1997) Common themes in the function of transcription and splicing enhancers. Curr Opin Cell Biol 9:350-357

Hiort O, Naber SP, Lehners A, Muletta-Feurer S, Sinnecker GH, Zollner A, Komminoth P (1996) The role of androgen receptor gene mutations in male breast carcinoma. J Clin Endocrinol Metab 81:3404-3407

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

Hittelman AB, Burakov D, Iniguez-Lluhi JA, Freedman LP, Garabedian MJ (1999) Differential regulation of glucocorticoid receptor transcriptional activation via AF-1-associated proteins. EMBO J 18:5380-5388

Hochstrasser M (2001) SP-RING for SUMO: new functions bloom for a ubiquitin-like protein. Cell 107:5-8

Hofmann MC, Narisawa S, Hess RA, Millan JL (1992) Immortalization of germ cells and somatic testicular cells using the SV40 large T antigen. Exp Cell Res 201:417-435

References

Holmstrom S, Van Antwerp ME, Iniguez-Lluhi JA (2003) Direct and distinguishable inhibitory roles for SUMO isoforms in the control of transcriptional synergy. Proc Natl Acad Sci USA 100:15758-15763

Hong H, Kohli K, Trivedi A, Johnson DL, Stallcup MR (1996) GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. Proc Natl Acad Sci USA 93:4948-4952

Hong SH, Wong CW, Privalsky ML (1998) Signaling by tyrosine kinases negatively regulates the interaction between transcription factors and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) corepressor. Mol Endocrinol 12:1161-1171

Hong SH, 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

Hsiao PW, Lin DL, Nakao R, Chang C (1999) The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. J Biol Chem 274:20229-20234

Hsing AW, Comstock GW (1993) Serological precursors of cancer: serum hormones and risk of subsequent prostate cancer. Cancer Epidemiol Biomarkers Prev 2:27-32

Hsing AW, Reichardt JK, Stanczyk FZ (2002) Hormones and prostate cancer: current perspectives and future directions. Prostate 52:213-235

Hu X, Lazar MA (1999) The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. Nature 402:93-96

Huggins C and Hodges CV (1941) The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1:293-297

Häkli M, Lorick KL, Weissman AM, Jänne OA, Palvimo JJ (2004) Transcriptional coregulator SNURF (RNF4) possesses ubiquitin E3 ligase activity. FEBS Lett 560:56-62

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

Ikonen T, Palvimo JJ, Jänne OA (1997) Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. J Biol Chem 272:29821-29828

Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE (1974) Steroid 5alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism. Science 186:1213-1215

Iniguez-Lluhi JA, Pearce D (2000) A common motif within the negative regulatory regions of multiple factors inhibits their transcriptional synergy. Mol Cell Biol 20:6040-6050

Ito M, Yuan CX, Okano HJ, Darnell RB, Roeder RG (2000) Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action. Mol Cell 5:683-693

Ito M, Roeder RG (2001) The TRAP/SMCC/Mediator complex and thyroid hormone receptor function. Trends Endocrinol Metab 12:127-134

Ito K, Adachi S, Iwakami R, Yasuda H, Muto Y, Seki N, Okano Y (2001) N-Terminally extended human ubiquitin-conjugating enzymes (E2s) mediate the ubiquitination of RING-finger proteins, ARA54 and RNF8. Eur J Biochem 268:2725-2732

Jackson PK (2001) A new RING for SUMO: wrestling transcriptional responses into nuclear bodies with PIAS family E3 SUMO ligases. Genes Dev 15:3053-3058

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, Rosenfeld MG (2000) Combinatorial roles of the nuclear receptor corepressor in transcription and development. Cell 102:753-763

Jimenez-Lara AM, Heine MJ, Gronemeyer H (2002) PIAS3 (protein inhibitor of activated STAT-3) modulates the transcriptional activation mediated by the nuclear receptor coactivator TIF2. FEBS Lett 526:142-146

Johnson ES, Schwienhorst I, Dohmen RJ, Blobel G (1997) The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. EMBO J 16:5509-5519

Johnson ES, Blobel G (1997) Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. J Biol Chem 272:26799-26802

Johnson ES, Gupta AA (2001) An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell 106:735-744

de Jong FH, Oishi K, Hayes RB, Bogdanowicz JF, Raatgever JW, van der Maas PJ, Yoshida O, Schroeder FH (1991) Peripheral hormone levels in controls and patients with prostatic cancer or benign prostatic hyperplasia: results from the Dutch-Japanese case-control study. Cancer Res 51:3445-3450

Joyeux A, Cavailles V, Balaguer P, Nicolas JC (1997) RIP 140 enhances nuclear receptor-dependent transcription in vivo in yeast. Mol Endocrinol 11:193-202

Kadam S, Emerson BM (2003) Transcriptional specificity of human SWI/SNF BRG1 and BRM chromatin remodeling complexes. Mol Cell 11:377-389

Kagey MH, Melhuish TA, Wotton D (2003) The polycomb protein Pc2 is a SUMO E3. Cell 113:127-137

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

Kamitani T, Kito K, Nguyen HP, Fukuda-Kamitani T, Yeh ET (1998) Characterization of a second member of the sentrin family of ubiquitin-like proteins. J Biol Chem 273:11349-11353

Kang HY, Yeh S, Fujimoto N, Chang C (1999) Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. J Biol Chem 274:8570-8576

Kang Z, Pirskanen A, Jänne OA, Palvimo JJ (2002) Involvement of proteasome in the dynamic assembly of the androgen receptor transcription complex. J Biol Chem 277:48366-48371

Kanno T, Kanno Y, Siegel RM, Jang MK, Lenardo MJ, Ozato K (2004) Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. Mol Cell 13:33-43

Kasper S, Rennie PS, Bruchovsky N, Sheppard PC, Cheng H, Lin L, Shiu RP, Snoek R, Matusik RJ (1994) Cooperative binding of androgen receptors to two DNA sequences is required for androgen induction of the probasin gene. J Biol Chem 269:31763-31769

Kassabov SR, Zhang B, Persinger J, Bartholomew B (2003) SWI/SNF unwraps, slides, and rewraps the nucleosome. Mol Cell 11:391-403

Katsuno M, Adachi H, Kume A, Li M, Nakagomi Y, Niwa H, Sang C, Kobayashi Y, Doyu M, Sobue G (2002) Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron 35:843-854

Katsuno M, Adachi H, Doyu M, Minamiyama M, Sang C, Kobayashi Y, Inukai A, Sobue G (2003) Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. Nat Med 9:768-773

References

Kipp M, Gohring F, Ostendorp T, van Drunen CM, van Driel R, Przybylski M, Fackelmayer FO (2000) SAF-Box, a conserved protein domain that specifically recognizes scaffold attachment region DNA. Mol Cell Biol 20:7480-7489

Kishino T, Lalande M, Wagstaff J (1993) UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet 15:70-73

Kleene KC (2001) A possible meiotic function of the peculiar patterns of gene expression in mammalian spermatogenic cells. Mech Dev 106:3-23

Kliewer SA, Umesono K, Mangelsdorf DJ, Evans RM (1992) Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. Nature 355:446-449

Klissenbauer M, Winters S, Heinlein UA, Lisowsky T (2002) Accumulation of the mitochondrial form of the sulphydryl oxidase Erv1p/Alrp during the early stages of spermatogenesis. J Exp Biol 205:1979-1986

Klonisch T, Fowler PA, Hombach-Klonisch S (2004) Molecular and genetic regulation of testis descent and external genitalia development. Dev Biol 270:1-18

Koh SS, Chen D, Lee YH, Stallcup MR (2001) Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. J Biol Chem 276:1089-1098

Koivisto P, Hyytinen E, Palmberg C, Tammela T, Visakorpi T, Isola J, Kallioniemi OP (1995) Analysis of genetic changes underlying local recurrence of prostate carcinoma during androgen deprivation therapy. Am J Pathol 147:1608-1614

Koivisto PA, Hyytinen ER, Matikainen M, Tammela TL, Ikonen T, Schleutker J (2004) Germline mutation analysis of the androgen receptor gene in Finnish patients with prostate cancer. J Urol 171:431-433

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

Kotaja N, Aittomaki S, Silvennoinen O, Palvimo JJ, Jänne OA (2000) ARIP3 (androgen receptor-interacting protein 3) and other PIAS (protein inhibitor of activated STAT) proteins differ in their ability to modulate steroid receptor-dependent transcriptional activation. Mol Endocrinol 14:1986-2000

Kotaja N, Vihinen M, Palvimo JJ, Jänne OA (2002a) Androgen receptor-interacting protein 3 and other PIAS proteins cooperate with glucocorticoid receptor-interacting protein 1 in steroid receptor-dependent signaling. J Biol Chem 277:17781-17788

Kotaja N, Karvonen U, Jänne OA, Palvimo JJ (2002b) PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol Cell Biol 22:5222-5234

Kotaja N, Karvonen U, Jänne OA, Palvimo JJ (2002c) The nuclear receptor interaction domain of GRIP1 is modulated by covalent attachment of SUMO-1. J Biol Chem 277:30283-30288

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

Kovalenko OV, Plug AW, Haaf T, Gonda DK, Ashley T, Ward DC, Radding CM, Golub EI (1996) Mammalian ubiquitin-conjugating enzyme Ubc9 interacts with Rad51 recombination protein and localizes in synaptonemal complexes. Proc Natl Acad Sci USA 93:2958-2963

Kuhn EJ, Geyer PK (2003) Genomic insulators: connecting properties to mechanism. Curr Opin Cell Biol 15:259-265

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

Kuo MH, Brownell JE, Sobel RE, Ranalli TA, Cook RG, Edmondson DG, Roth SY, Allis CD (1996) Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. Nature 383:269-272

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352:77-79

Labrie F, Luu-The V, Labrie C, Simard J (2001) DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. Front Neuroendocrinol 22:185-212

Laitinen S, Karhu R, Sawyers CL, Vessella RL, Visakorpi T (2002) Chromosomal aberrations in prostate cancer xenografts detected by comparative genomic hybridization. Genes Chromosomes Cancer 35:66-73

Langley E, Zhou ZX, Wilson EM (1995) Evidence for an anti-parallel orientation of the ligand-activated human androgen receptor dimer. J Biol Chem 270:29983-29990

Lanz RB, McKenna NJ, Onate SA, Albrecht U, Wong J, Tsai SY, Tsai MJ, O'Malley BW (1999) A steroid receptor coactivator, SRA, functions as an RNA and is present in an SRC-1 complex. Cell 97:17-27

Latil AG, Azzouzi R, Cancel GS, Guillaume EC, Cochan-Priollet B, Berthon PL, Cussenot O (2001) Prostate carcinoma risk and allelic variants of genes involved in androgen biosynthesis and metabolism pathways. Cancer 92:1130-1137

Laudet V (1997) Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. J Mol Endocrinol 19:207-226

Le Drean Y, Mincheneau N, Le Goff P, Michel D (2002) Potentiation of glucocorticoid receptor transcriptional activity by sumoylation. Endocrinology 143:3482-3489

Lee SK, Kim HJ, Na SY, Kim TS, Choi HS, Im SY, Lee JW (1998) Steroid receptor coactivator-1 coactivates activating protein-1-mediated transactivations through interaction with the c-Jun and c-Fos subunits. J Biol Chem 273:16651-16654

Lee CH, Wei LN (1999) Characterization of receptor-interacting protein 140 in retinoid receptor activities. J Biol Chem 274:31320-31326

Lee TI, Young RA (2000) Transcription of eukaryotic protein-coding genes. Annu Rev Genet 34:77-137

Lee YH, Koh SS, Zhang X, Cheng X, Stallcup MR (2002) Synergy among nuclear receptor coactivators: selective requirement for protein methyltransferase and acetyltransferase activities. Mol Cell Biol 22:3621-3632

Lee DK, Chang C (2003) Endocrine mechanisms of disease: Expression and degradation of androgen receptor: mechanism and clinical implication. J Clin Endocrinol Metab 88:4043-4054

Lee PS, Chang C, Liu D, Derynck R (2003) Sumoylation of Smad4, the common Smad mediator of transforming growth factor-beta family signaling. J Biol Chem 278:27853-27863

Lees JA, Fawell SE, White R, Parker MG (1990) A 22-amino-acid peptide restores DNA-binding activity to dimerization-defective mutants of the estrogen receptor. Mol Cell Biol 10:5529-5531

L'Horset F, Dauvois S, Heery DM, Cavailles V, Parker MG (1996) RIP-140 interacts with multiple nuclear receptors by means of two distinct sites. Mol Cell Biol 16:6029-6036

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

Li H, Leo C, Schroen DJ, Chen JD (1997b) Characterization of receptor interaction and transcriptional repression by the corepressor SMRT. Mol Endocrinol 11:2025-2037

References

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997c) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275:1943-1947

Li H, Leo C, Zhu J, Wu X, O'Neil J, Park EJ, Chen JD (2000) Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. Mol Cell Biol 20:1784-1796

Li P, Yu X, Ge K, Melamed J, Roeder RG, Wang Z (2002) Heterogeneous expression and functions of androgen receptor co-factors in primary prostate cancer. Am J Pathol 161:1467-1474

Li X, Wong J, Tsai SY, Tsai MJ, O'Malley BW (2003) Progesterone and glucocorticoid receptors recruit distinct coactivator complexes and promote distinct patterns of local chromatin modification. Mol Cell Biol 23:3763-3773

Liegibel UM, Sommer U, Boercsoek I, Hilscher U, Bierhaus A, Schweikert HU, Nawroth P, Kasperk C (2003) Androgen receptor isoforms AR-A and AR-B display functional differences in cultured human bone cells and genital skin fibroblasts. Steroids 68:1179-1187

Lin HK, Yeh S, Kang HY, Chang C (2001) Akt suppresses androgen-induced apoptosis by phosphorylating and inhibiting androgen receptor. Proc Natl Acad Sci USA 98:7200-7205

Lin HK, Wang L, Hu YC, Altuwaijri S, Chang C (2002) Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. EMBO J 21:4037-4048

Linja MJ, Porkka KP, Kang Z, Savinainen KJ, Jänne OA, Tammela TL, Vessella RL, Palvimo JJ, Visakorpi T (2004) Expression of androgen receptor coregulators in prostate cancer. Clin Cancer Res 10:1032-1040

Litvinov IV, De Marzo AM, Isaacs JT (2003) Is the Achilles' heel for prostate cancer therapy a gain of function in androgen receptor signaling? J Clin Endocrinol Metab 88:2972-2982

Liu B, Liao J, Rao X, Kushner SA, Chung CD, Chang DD, Shuai K (1998) Inhibition of Stat1-mediated gene activation by PIAS1. Proc Natl Acad Sci USA 95:10626-10631

Liu Q, Jin C, Liao X, Shen Z, Chen DJ, Chen Y (1999) The binding interface between an E2 (UBC9) and a ubiquitin homologue (UBL1). J Biol Chem 274:16979-16987

Liu B, Gross M, ten Hoeve J, Shuai K (2001) A transcriptional corepressor of Stat1 with an essential LXXLL signature motif. Proc Natl Acad Sci USA 98:3203-3207

Lobaccaro JM, Lumbroso S, Belon C, Galtier-Dereure F, Bringer J, Lesimple T, Namer M, Cutuli BF, Pujol H, Sultan C (1993) Androgen receptor gene mutation in male breast cancer. Hum Mol Genet 2:1799-1802

Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM (1988) Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 240:327-330

Luisi BF, Xu WX, Otwinowski Z, Freedman LP, Yamamoto KR, Sigler PB (1991) Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. Nature 352:497-505

Lukacs GL, Haggie P, Seksek O, Lechardeur D, Freedman N, Verkman AS (2000) Size-dependent DNA mobility in cytoplasm and nucleus. J Biol Chem 275:1625-1629

Lundberg Giwercman Y, Nikoshkov A, Lindsten K, Bystrom B, Pousette A, Knudtzon J, Alm J, Wedell A (2000) Response to treatment in patients with partial androgen insensitivity due to mutations in the DNA-binding domain of the androgen receptor. Horm Res 53:83-88

Lösel R, Wehling M (2003) Nongenomic actions of steroid hormones. Nat Rev Mol Cell Biol 4:46-56

Macleod D, Charlton J, Mullins J, Bird AP (1994) Sp1 sites in the mouse aprt gene promoter are required to prevent methylation of the CpG island. Genes Dev 8:2282-2292

Mader S, Kumar V, de Verneuil H, Chambon P (1989) Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid-responsive element. Nature 338:271-274

Mahendroo MS, Cala KM, Hess DL, Russell DW (2001) Unexpected virilization in male mice lacking steroid 5 alpha-reductase enzymes. Endocrinology 142:4652-4662

Marcelli M, Ittmann M, Mariani S, Sutherland R, Nigam R, Murthy L, Zhao Y, DiConcini D, Puxeddu E, Esen A, Eastham J, Weigel NL, Lamb DJ (2000) Androgen receptor mutations in prostate cancer. Cancer Res 60:944-949

Mark M, Yoshida-Komiya H, Gehin M, Liao L, Tsai MJ, O'Malley BW, Chambon P, Xu J (2004) Partially redundant functions of SRC-1 and TIF2 in postnatal survival and male reproduction. Proc Natl Acad Sci USA 101:4453-4458

Marker PC, Donjacour AA, Dahiya R, Cunha GR (2003) Hormonal, cellular, and molecular control of prostatic development. Dev Biol 253:165-174

Marks LS, Partin AW, Gormley GJ, Dorey FJ, Shery ED, Garris JB, Subong EN, Stoner E, deKernion JB (1997) Prostate tissue composition and response to finasteride in men with symptomatic benign prostatic hyperplasia. J Urol 157:2171-2178

Martianov I, Fimia GM, Dierich A, Parvinen M, Sassone-Corsi P, Davidson I (2001) Late arrest of spermiogenesis and germ cell apoptosis in mice lacking the TBP-like TLF/TRF2 gene. Mol Cell 7:509-515

Martin DI (2001) Transcriptional enhancers--on/off gene regulation as an adaptation to silencing in higher eukaryotic nuclei. Trends Genet 17:444-448

Mattick JS (2003) Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. Bioessays 25:930-939

McCarrey JR, Thomas K (1987) Human testis-specific PGK gene lacks introns and possesses characteristics of a processed gene. Nature 326:501-505

McKenna NJ, Lanz RB, O'Malley BW (1999) Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321-344

McKenna NJ, O'Malley BW (2002) Minireview: nuclear receptor coactivators--an update. Endocrinology 143:2461-2465

Meinke G, Sigler PB (1999) DNA-binding mechanism of the monomeric orphan nuclear receptor NGFI-B. Nat Struct Biol 6:471-477

Melchior F, Schergaut M, Pichler A (2003) SUMO: ligases, isopeptidases and nuclear pores. Trends Biochem Sci 28:612-618

Meng X, Lindahl M, Hyvönen ME, Parvinen M, de Rooij DG, Hess MW, Raatikainen-Ahokas A, Sainio K, Rauvala H, Lakso M, Pichel JG, Westphal H, Saarma M, Sariola H (2000) Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. Science 287:1489-1493

Meyer ME, Gronemeyer H, Turcotte B, Bocquel MT, Tasset D, Chambon P (1989) Steroid hormone receptors compete for factors that mediate their enhancer function. Cell 57:433-442

Meyer T, Starr DB, Carlstedt-Duke J (1997) The rat glucocorticoid receptor mutant K461A differentiates between two different mechanisms of transrepression. J Biol Chem 272:21090-21095

Miesfeld R, Godowski PJ, Maler BA, Yamamoto KR (1987) Glucocorticoid receptor mutants that define a small region sufficient for enhancer activation. Science 236:423-427

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

References

Miller WL (2002) Disorders of androgen biosynthesis. Semin Reprod Med 20:205-216

Miyamoto H, Yeh S, Wilding G, Chang C (1998) Promotion of agonist activity of antiandrogens by the androgen receptor coactivator, ARA70, in human prostate cancer DU145 cells. Proc Natl Acad Sci USA 95:7379-7384

Mizushima S, Nagata S (1990) pEF-BOS, a powerful mammalian expression vector. Nucleic Acids Res 18:5322

Mohr SE, Boswell RE (1999) Zimp encodes a homologue of mouse Miz1 and PIAS3 and is an essential gene in Drosophila melanogaster. Gene 229:109-116

Moilanen AM, Poukka H, Karvonen U, Häkli M, Jänne OA, Palvimo JJ (1998) Identification of a novel RING finger protein as a coregulator in steroid receptor-mediated gene transcription. Mol Cell Biol 18:5128-5139

Moilanen AM, Karvonen U, Poukka H, Yan W, Toppari J, Jänne OA, Palvimo JJ (1999) A testis-specific androgen receptor coregulator that belongs to a novel family of nuclear proteins. J Biol Chem 274:3700-3704

Mononen N, Syrjäkoski K, Matikainen M, Tammela TL, Schleutker J, Kallioniemi OP, Trapman J, Koivisto PA (2000) Two percent of Finnish prostate cancer patients have a germ-line mutation in the hormone-binding domain of the androgen receptor gene. Cancer Res 60:6479-6481

Muchardt C, 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

Muratani M, Tansey WP (2003) How the ubiquitin-proteasome system controls transcription. Nat Rev Mol Cell Biol 4:192-201

Müller S, Ledl A, Schmidt D (2004) SUMO: a regulator of gene expression and genome integrity. Oncogene 23:1998-2008

Na SY, Lee SK, Han SJ, Choi HS, Im SY, Lee JW (1998) Steroid receptor coactivator-1 interacts with the p50 subunit and coactivates nuclear factor kappaB-mediated transactivations. J Biol Chem 273:10831-10834

Nagy L, Kao HY, Love JD, Li C, Banayo E, Gooch JT, Krishna V, Chatterjee K, Evans RM, Schwabe JW (1999) Mechanism of corepressor binding and release from nuclear hormone receptors. Genes Dev 13:3209-3216

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

Nef S, Verma-Kurvari S, Merenmies J, Vassalli JD, Efstratiadis A, Accili D, Parada LF (2003) Testis determination requires insulin receptor family function in mice. Nature 426:291-295

Nelson KA, Witte JS (2002) Androgen receptor CAG repeats and prostate cancer. Am J Epidemiol 155:883-890

Nie Z, Xue Y, Yang D, Zhou S, Deroo BJ, Archer TK, Wang W (2000) A specificity and targeting subunit of a human SWI/SNF family-related chromatin-remodeling complex. Mol Cell Biol 20:8879-8888

Nishida T, Yasuda H (2002) PIAS1 and PIASxalpha function as SUMO-E3 ligases toward androgen receptor and repress androgen receptor-dependent transcription. J Biol Chem 277:41311-41317

Nishihara E, Yoshida-Komiya H, Chan CS, Liao L, Davis RL, O'Malley BW, Xu J (2003) SRC-1 null mice exhibit moderate motor dysfunction and delayed development of cerebellar Purkinje cells. J Neurosci 23:213-222

Noble RL (1977) The development of prostatic adenocarcinoma in Nb rats following prolonged sex hormone administration. Cancer Res 37:1929-1933

Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV (1998) Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. Nature 395:137-143

Nomura A, Heilbrun LK, Stemmermann GN, Judd HL (1988) Prediagnostic serum hormones and the risk of prostate cancer. Cancer Res 48:3515-3517

Ntais C, Polycarpou A, Tsatsoulis A (2003a) Molecular epidemiology of prostate cancer: androgens and polymorphisms in androgen-related genes. Eur J Endocrinol 149:469-477

Ntais C, Polycarpou A, Ioannidis JP (2003b) Association of the CYP17 gene polymorphism with the risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 12:120-126

Ntais C, Polycarpou A, Ioannidis JP (2003c) SRD5A2 gene polymorphisms and the risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 12:618-624

Näär AM, Lemon BD, Tjian R (2001) Transcriptional coactivator complexes. Annu Rev Biochem 70:475-501

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

Ogryzko VV, Kotani T, Zhang X, Schiltz RL, Howard T, Yang XJ, Howard BH, Qin J, Nakatani Y (1998) Histone-like TAFs within the PCAF histone acetylase complex. Cell 94:35-44

Ogryzko VV (2001) Mammalian histone acetyltransferases and their complexes. Cell Mol Life Sci 58:683-692

Ohtsuki S, Levine M, Cai HN (1998) Different core promoters possess distinct regulatory activities in the Drosophila embryo. Genes Dev 12:547-556

Oki M, Kamakaka RT (2002) Blockers and barriers to transcription: competing activities? Curr Opin Cell Biol 14:299-304

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

Ornaghi P, Ballario P, Lena AM, Gonzalez A, Filetici P (1999) The bromodomain of Gcn5p interacts in vitro with specific residues in the N terminus of histone H4. J Mol Biol 287:1-7

Ozer J, Moore PA, Lieberman PM (2000) A testis-specific transcription factor IIA (TFIIAtau) stimulates TATA-binding protein-DNA binding and transcription activation. J Biol Chem 275:122-128

Pascual-Le Tallec L, Kirsh O, Lecomte MC, Viengchareun S, Zennaro MC, Dejean A, Lombes M (2003) Protein inhibitor of activated signal transducer and activator of transcription 1 interacts with the N-terminal domain of mineralocorticoid receptor and represses its transcriptional activity: implication of small ubiquitin-related modifier 1 modification. Mol Endocrinol 17:2529-2542

Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Krones A, Rose DW, Lambert MH, Milburn MV, Glass CK, Rosenfeld MG (1999) Molecular determinants of nuclear receptor-corepressor interaction. Genes Dev 13:3198-3208

Peschon JJ, Behringer RR, Cate RL, Harwood KA, Idzerda RL, Brinster RL, Palmiter RD (1992) Directed expression of an oncogene to Sertoli cells in transgenic mice using mullerian inhibiting substance regulatory sequences. Mol Endocrinol 6:1403-1411

Petrij F, Giles RH, Dauwerse HG, Saris JJ, Hennekam RC, Masuno M, Tommerup N, van Ommen GJ, Goodman RH, Peters DJ, Breuning MH (1995) Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. Nature 376:348-351

Phelan ML, Sif S, Narlikar GJ, Kingston RE (1999) Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol Cell 3:247-253

Picard F, Gehin M, Annicotte J, Rocchi S, Champy MF, O'Malley BW, Chambon P, Auwerx J (2002) SRC-1 and TIF2 control energy balance between white and brown adipose tissues. Cell 111:931-941

References

Piccioni F, Pinton P, Simeoni S, Pozzi P, Fascio U, Vismara G, Martini L, Rizzuto R, Poletti A (2002) Androgen receptor with elongated polyglutamine tract forms aggregates that alter axonal trafficking and mitochondrial distribution in motor neuronal processes. FASEB J 16:1418-1420

Pichler A, Gast A, Seeler JS, Dejean A, Melchior F (2002) The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell 108:109-120

Poujol N, Lobaccaro J-M, Chiche L, Lumbroso S, Sultan C (1997) Functional and structural analysis of R607Q and R608K androgen receptor substitution associated with male breast cancer. Mol Cell Endocrinol 130:43-51

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

Privalsky ML (2004) The role of corepressors in transcriptional regulation by nuclear hormone receptors. Annu Rev Physiol 66:315-360

Prostate Cancer Trialists' Collaborative Group (1995) Maximum androgen blockade in advanced prostate cancer: an overview of 22 randomised trials with 3283 deaths in 5710 patients. Lancet 346:265-269

Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS (1995) Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16:271-321

Rachez C, Lemon BD, Suldan Z, Bromleigh V, Gamble M, Naar AM, Erdjument-Bromage H, Tempst P, Freedman LP (1999) Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. Nature 398:824-828

Raivio T, Palvimo JJ, Dunkel L, Wickman S, Jänne OA (2001) Novel assay for determination of androgen bioactivity in human serum. J Clin Endocrinol Metab 86:1539-1544

Raivio T, Tapanainen JS, Kunelius P, Jänne OA (2002) Serum androgen bioactivity during 5alpha-dihydrotestosterone treatment in elderly men. J Androl 23:919-921

Rao MK, Wayne CM, Meistrich ML, Wilkinson MF (2003) Pem homeobox gene promoter sequences that direct transcription in a Sertoli cell-specific, stage-specific, and androgen-dependent manner in the testis in vivo. Mol Endocrinol 17:223-233

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

Ren Y, Behre E, Ren Z, Zhang J, Wang Q, Fondell JD (2000) Specific structural motifs determine TRAP220 interactions with nuclear hormone receptors. Mol Cell Biol 20:5433-5446

Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, Moras D (1995) Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. Nature 378:681-689

Rennie PS, Bruchovsky N, Leco KJ, Sheppard PC, McQueen SA, Cheng H, Snoek R, Hamel A, Bock ME, MacDonald BS, Nickel BE, Chang C, Liao S, Cattini PA, Matusik R (1993) Characterization of two cis-acting DNA elements involved in the androgen regulation of the probasin gene. Mol Endocrinol 7:23-36

Rey R, Picard JY (1998) Embryology and endocrinology of genital development. Baillieres Clin Endocrinol Metab 12:17-33

Robinson-Rechavi M, Carpentier AS, Duffraisse M, Laudet V (2001) How many nuclear hormone receptors are there in the human genome? Trends Genet 17:554-556

Robyr D, Wolffe AP, Wahli W (2000) Nuclear hormone receptor coregulators in action: diversity for shared tasks. Mol Endocrinol 14:329-347

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

Rogers RS, Horvath CM, Matunis MJ (2003) SUMO modification of STAT1 and its role in PIAS-mediated inhibition of gene activation. J Biol Chem 278:30091-30097

Rojas JR, Trievel RC, Zhou J, Mo Y, Li X, Berger SL, Allis CD, Marmorstein R (1999) Structure of Tetrahymena GCN5 bound to coenzyme A and a histone H3 peptide. Nature 401:93-98

Romig H, Fackelmayer FO, Renz A, Ramsperger U, Richter A (1992) Characterization of SAF-A, a novel nuclear DNA binding protein from HeLa cells with high affinity for nuclear matrix/scaffold attachment DNA elements. EMBO J 11:3431-3440

Rosner W, Hryb DJ, Khan MS, Nakhla AM, Romas NA (1999) Androgen and estrogen signaling at the cell membrane via G-proteins and cyclic adenosine monophosphate. Steroids 64:100-106

Ross R, Bernstein L, Judd H, Hanisch R, Pike M, Henderson B (1986) Serum testosterone levels in healthy young black and white men. J Natl Cancer Inst 76:45-48

Ross RK, Bernstein L, Lobo RA, Shimizu H, Stanczyk FZ, Pike MC, Henderson BE (1992) 5-alpha-reductase activity and risk of prostate cancer among Japanese and US white and black males. Lancet 339:887-889

Ruizeveld de Winter JA, Trapman J, Vermey M, Mulder E, Zegers ND, van der Kwast TH (1991) Androgen receptor expression in human tissues: an immunohistochemical study. J Histochem Cytochem 39:927-936

Sachdev S, Bruhn L, Sieber H, Pichler A, Melchior F, Grosschedl R (2001) PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. Genes Dev 15:3088-3103

Sahara S, Aoto M, Eguchi Y, Imamoto N, Yoneda Y, Tsujimoto Y (1999) Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. Nature 401:168-173

Saitoh H, Sparrow DB, Shiomi T, Pu RT, Nishimoto T, Mohun TJ, Dasso M (1998) Ubc9p and the conjugation of SUMO-1 to RanGAP1 and RanBP2. Curr Biol 8:121-124

Saitoh H, Hinchey J (2000) Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. J Biol Chem 275:6252-6258

Sar M, Lubahn DB, French FS, Wilson EM (1990) Immunohistochemical localization of the androgen receptor in rat and human tissues. Endocrinology 127:3180-3186

Sassone-Corsi P (2002) Unique chromatin remodeling and transcriptional regulation in spermatogenesis. Science 296:2176-2178

Schmidt EE, Schibler U (1995) High accumulation of components of the RNA polymerase II transcription machinery in rodent spermatids. Development 121:2373-2383

Schmidt D, Müller S (2003) PIAS/SUMO: new partners in transcriptional regulation. Cell Mol Life Sci 60:2561-2574

Schoenmakers E, Alen P, Verrijdt G, Peeters B, Verhoeven G, Rombauts W, Claessens F (1999) Differential DNA binding by the androgen and glucocorticoid receptors involves the second Zn-finger and a C-terminal extension of the DNA-binding domains. Biochem J 341:515-521

Schoenmakers E, Verrijdt G, Peeters B, Verhoeven G, Rombauts W, Claessens F (2000) Differences in DNA binding characteristics of the androgen and glucocorticoid receptors can determine hormone-specific responses. J Biol Chem 275:12290-12297

Schulman IG, Juguilon H, Evans RM (1996) Activation and repression by nuclear hormone receptors: hormone modulates an equilibrium between active and repressive states. Mol Cell Biol 16:3807-3813

Schurter BT, Koh SS, Chen D, Bunick GJ, Harp JM, Hanson BL, Henschen-Edman A, Mackay DR, Stallcup MR, Aswad DW (2001) Methylation of histone H3 by coactivator-associated arginine methyltransferase 1. Biochemistry 40:5747-5756

References

Schuur ER, Henderson GA, Kmetec LA, Miller JD, Lamparski HG, Henderson DR (1996) Prostate-specific antigen expression is regulated by an upstream enhancer. J Biol Chem 271:7043-7051

Schwabe JW, Chapman L, Finch JT, Rhodes D (1993) The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements. Cell 75:567-578

Sciavolino PJ, Abate-Shen C (1998) Molecular biology of prostate development and prostate cancer. Ann Med 30:357-368

Seol W, Choi HS, Moore DD (1996a) An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. Science 272:1336-1339

Seol W, Mahon MJ, Lee YK, Moore DD (1996b) Two receptor interacting domains in the nuclear hormone receptor corepressor RIP13/N-CoR. Mol Endocrinol 10:1646-1655

Sharma M, Li X, Wang Y, Zarnegar M, Huang CY, Palvimo JJ, Lim B, Sun Z (2003) hZimp10 is an androgen receptor co-activator and forms a complex with SUMO-1 at replication foci. EMBO J 22:6101-6114

Sharpe RM, Donachie K, Cooper I (1988) Re-evaluation of the intratesticular level of testosterone required for quantitative maintenance of spermatogenesis in the rat. J Endocrinol 117:19-26

Sheflin L, Keegan B, Zhang W, Spaulding SW (2000) Inhibiting proteasomes in human HepG2 and LNCaP cells increases endogenous androgen receptor levels. Biochem Biophys Res Commun 276:144-150

Shell SA, Fix C, Olejniczak D, Gram-Humphrey N, Walker WH (2002) Regulation of cyclic adenosine 3',5'-monophosphate response element binding protein (CREB) expression by Sp1 in the mammalian testis. Biol Reprod 66:659-666

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

Shikama N, Lyon J, La Thangue NB (1997) The p300/CBP family: integrating signals with transcription factors and chromatin. Trends Cell Biol 7:230-236

Simental JA, Sar M, Lane MV, French FS, Wilson EM (1991) Transcriptional activation and nuclear targeting signals of the human androgen receptor. J Biol Chem 266:510-518

Sinha Hikim AP, Lue Y, Diaz-Romero M, Yen PH, Wang C, Swerdloff RS (2003) Deciphering the pathways of germ cell apoptosis in the testis. J Steroid Biochem Mol Biol 85:175-182

Smale ST (2001) Core promoters: active contributors to combinatorial gene regulation. Genes Dev 15:2503-2508

Smith CL, DeVera DG, Lamb DJ, Nawaz Z, Jiang YH, Beaudet AL, O'Malley BW (2002) Genetic ablation of the steroid receptor coactivator-ubiquitin ligase, E6-AP, results in tissue-selective steroid hormone resistance and defects in reproduction. Mol Cell Biol 22:525-535

Spector DL (2001) Nuclear domains. J Cell Sci 114:2891-2893

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

Starr DB, Matsui W, Thomas JR, Yamamoto KR (1996) Intracellular receptors use a common mechanism to interpret signaling information at response elements. Genes Dev 10:1271-1283

Steers WD (2001) 5alpha-reductase activity in the prostate. Urology 58:17-24

Strahl BD, Briggs SD, Brame CJ, Caldwell JA, Koh SS, Ma H, Cook RG, Shabanowitz J, Hunt DF, Stallcup MR, Allis CD (2001) Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. Curr Biol 11:996-1000

Strunnikov AV, Aravind L, Koonin EV (2001) Saccharomyces cerevisiae SMT4 encodes an evolutionarily conserved protease with a role in chromosome condensation regulation. Genetics 158:95-107

Subramaniam N, Cairns W, Okret S (1998) Glucocorticoids repress transcription from a negative glucocorticoid response element recognized by two homeodomain-containing proteins, Pbx and Oct-1. J Biol Chem 273:23567-23574

Subramaniam N, Treuter E, Okret S (1999) Receptor interacting protein RIP140 inhibits both positive and negative gene regulation by glucocorticoids. J Biol Chem 274:18121-18127

Suen CS, Berrodin TJ, Mastroeni R, Cheskis BJ, Lyttle CR, Frail DE (1998) A transcriptional coactivator, steroid receptor coactivator-3, selectively augments steroid receptor transcriptional activity. J Biol Chem 273:27645-27653

Suzuki Y, Tsunoda T, Sese J, Taira H, Mizushima-Sugano J, Hata H, Ota T, Isogai T, Tanaka T, Nakamura Y, Suyama A, Sakaki Y, Morishita S, Okubo K, Sugano S (2001) Identification and characterization of the potential promoter regions of 1031 kinds of human genes. Genome Res 11:677-684

Swain A, Lovell-Badge R (1999) Mammalian sex determination: a molecular drama. Genes Dev 13:755-767

Syrjäkoski K, Hyytinen ER, Kuukasjarvi T, Auvinen A, Kallioniemi OP, Kainu T, Koivisto PA (2003) Androgen receptor gene alterations in Finnish male breast cancer. Breast Cancer Res Treat 77:167-170

Szebenyi G, Morfini GA, Babcock A, Gould M, Selkoe K, Stenoien DL, Young M, Faber PW, MacDonald ME, McPhaul MJ, Brady ST (2003) Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. Neuron 40:41-52

Szymański M, Barciszewski J (2002) Beyond the proteome: non-coding regulatory RNAs. Genome Biol 3:reviews0005.1-0005.8

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

Tan JA, Joseph DR, Quarmby VE, Lubahn DB, Sar M, French FS, Wilson EM (1988) The rat androgen receptor: primary structure, autoregulation of its messenger ribonucleic acid, and immunocytochemical localization of the receptor protein. Mol Endocrinol 2:1276-1285

Tan J, Sharief Y, Hamil KG, Gregory CW, Zang DY, Sar M, Gumerlock PH, deVere White RW, Pretlow TG, Harris SE, Wilson EM, Mohler JL, French FS (1997) Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. Mol Endocrinol 11:450-459

Tan J, Hall SH, Hamil KG, Grossman G, Petrusz P, Liao J, Shuai K, French FS (2000) Protein inhibitor of activated STAT-1 (signal transducer and activator of transcription-1) is a nuclear receptor coregulator expressed in human testis. Mol Endocrinol 14:14-26

Tan JA, Hall SH, Hamil KG, Grossman G, Petrusz P, French FS (2002) Protein inhibitors of activated STAT resemble scaffold attachment factors and function as interacting nuclear receptor coregulators. J Biol Chem 277:16993-17001

Tanenbaum DM, Wang Y, Williams SP, Sigler PB (1998) Crystallographic comparison of the estrogen and progesterone receptor's ligand binding domains. Proc Natl Acad Sci USA 95:5998-6003

Taplin ME, Bubley GJ, Ko YJ, Small EJ, Upton M, Rajeshkumar B, Balk SP (1999) Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res 59:2511-2515

Taplin ME, Ho SM (2001) Clinical review 134: The endocrinology of prostate cancer. J Clin Endocrinol Metab 86:3467-3477

Tarsounas M, Pearlman RE, Gasser PJ, Park MS, Moens PB (1997) Protein-protein interactions in the synaptonemal complex. Mol Biol Cell 8:1405-1414

References

Tatham MH, Jaffray E, Vaughan OA, Desterro JM, Botting CH, Naismith JH, Hay RT (2001) Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. J Biol Chem 276:35368-35374

Thompson J, Saatcioglu F, Jänne OA, Palvimo JJ (2001) Disrupted amino- and carboxyl-terminal interactions of the androgen receptor are linked to androgen insensitivity. Mol Endocrinol 15:923-935

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

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

Trapman J, Klaassen P, Kuiper GG, van der Korput JA, Faber PW, van Rooij HC, Geurts van Kessel A, Voorhorst MM, Mulder E, Brinkmann AO (1988) Cloning, structure and expression of a cDNA encoding the human androgen receptor. Biochem Biophys Res Commun 153:241-248

Tremblay A, Tremblay GB, Labrie F, 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

Treuter E, Albrektsen T, Johansson L, Leers J, Gustafsson JÅ (1998) A regulatory role for RIP140 in nuclear receptor activation. Mol Endocrinol 12:864-881

Tussie-Luna MI, Bayarsaihan D, Seto E, Ruddle FH, Roy AL (2002) Physical and functional interactions of histone deacetylase 3 with TFII-I family proteins and PIASxbeta. Proc Natl Acad Sci USA 99:12807-12812

Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 82:3777-3782

Umesono K, Evans RM (1989) Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell 57:1139-1146

Ungureanu D, Vanhatupa S, Kotaja N, Yang J, Aittomaki S, Jänne OA, Palvimo JJ, Silvennoinen O (2003) PIAS proteins promote SUMO-1 conjugation to STAT1. Blood 102:3311-3313

Valdez BC, Henning D, Perlaky L, Busch RK, Busch H (1997) Cloning and characterization of Gu/RH-II binding protein. Biochem Biophys Res Commun 234:335-340

Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi OP (1995) In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 9:401-406

Visser AE, Jaunin F, Fakan S, Aten JA (2000) High resolution analysis of interphase chromosome domains. J Cell Sci 113:2585-2593

Vo N, Fjeld C, Goodman RH (2001) Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP. Mol Cell Biol 21:6181-6188

Voegel JJ, Heine MJ, Zechel C, Chambon P, 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

Voegel JJ, Heine MJ, Tini M, Vivat V, Chambon P, Gronemeyer H (1998) The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. EMBO J 17:507-519

Vornberger W, Prins G, Musto NA, Suarez-Quian CA (1994) Androgen receptor distribution in rat testis: new implications for androgen regulation of spermatogenesis. Endocrinology 134:2307-2316

Wagner RL, Apriletti JW, McGrath ME, West BL, Baxter JD, Fletterick RJ (1995) A structural role for hormone in the thyroid hormone receptor. Nature 378:690-697

Wallace AD, Cidlowski JA (2001) Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem 276:42714-42721

Wallberg AE, Yamamura S, Malik S, Spiegelman BM, Roeder RG (2003) Coordination of p300-mediated chromatin remodeling and TRAP/mediator function through coactivator PGC-1alpha. Mol Cell 12:1137-1149

Walsh PC, Madden JD, Harrod MJ, Goldstein JL, MacDonald PC, Wilson JD (1974) Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. N Engl J Med 291:944-949

Wang Z, Rose DW, Hermanson O, Liu F, Herman T, Wu W, Szeto D, Gleiberman A, Krones A, Pratt K, Rosenfeld R, Glass CK, Rosenfeld MG (2000) Regulation of somatic growth by the p160 coactivator p/CIP. Proc Natl Acad Sci USA 97:13549-13554

Wang PJ, McCarrey JR, Yang F, Page DC (2001) An abundance of X-linked genes expressed in spermatogonia. Nat Genet 27:422-426

Wang Z, Benoit G, Liu J, Prasad S, Aarnisalo P, Liu X, Xu H, Walker NP, Perlmann T (2003) Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. Nature 423:555-560

Wei LN, Hu X, Chandra D, Seto E, Farooqui M (2000) Receptor-interacting protein 140 directly recruits histone deacetylases for gene silencing. J Biol Chem 275:40782-40787

Wei LN, Farooqui M, Hu X (2001) Ligand-dependent formation of retinoid receptors, receptor-interacting protein 140 (RIP140), and histone deacetylase complex is mediated by a novel receptor-interacting motif of RIP140. J Biol Chem 276:16107-16112

Weidemann W, Peters B, Romalo G, Spindler K-D, Schweikert U-H (1998) Response to androgen treatment in a patient with partial androgen insensitivity and a mutation in the deoxyribonucleic acid-binding domain of the androgen receptor. J Clin Endocrinol Metab 83:1173-1176

Weiss RE, Xu J, Ning G, Pohlenz J, O'Malley BW, Refetoff S (1999) Mice deficient in the steroid receptor co-activator 1 (SRC-1) are resistant to thyroid hormone. EMBO J 18:1900-1904

Wen YD, Perissi V, Staszewski LM, Yang WM, Krones A, Glass CK, Rosenfeld MG, Seto E (2000a) The histone deacetylase-3 complex contains nuclear receptor corepressors. Proc Natl Acad Sci USA 97:7202-7207

Wen Y, Hu MC, Makino K, Spohn B, Bartholomeusz G, Yan DH, Hung MC (2000b) HER-2/neu promotes androgen-independent survival and growth of prostate cancer cells through the Akt pathway. Cancer Res 60:6841-6845

White R, Leonardsson G, Rosewell I, Ann Jacobs M, Milligan S, Parker M (2000) The nuclear receptor co-repressor nrip1 (RIP140) is essential for female fertility. Nat Med 6:1368-1374

Wible BA, Yang Q, Kuryshev YA, Accili EA, Brown AM (1998) Cloning and expression of a novel K+ channel regulatory protein, KChAP. J Biol Chem 273:11745-11751

Wilkerson DC, Wolfe SA, Grimes SR (2002) Sp1 and Sp3 activate the testis-specific histone H1t promoter through the H1t/GC-box. J Cell Biochem 86:716-725

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

Wilson VG, Rangasamy D (2001) Intracellular targeting of proteins by sumoylation. Exp Cell Res 271:57-65

References

Wooster R, Mangion J, Eeles R, Smith S, Dowsett M, Averill D, Barrett-Lee P, Easton DF, Ponder BAJ, Stratton MR (1992) A germline mutation in the androgen receptor gene in two brothers with breast cancer and Reifenstein syndrome. Nat Genet 2:132-134

Wormald S, Hilton DJ (2004) Inhibitors of cytokine signal transduction. J Biol Chem 279:821-824

Wu AH, Whittemore AS, Kolonel LN, John EM, Gallagher RP, West DW, Hankin J, Teh CZ, Dreon DM, Paffenbarger RS Jr (1995) Serum androgens and sex hormone-binding globulins in relation to lifestyle factors in older African-American, white, and Asian men in the United States and Canada. Cancer Epidemiol Biomarkers Prev 4:735-741

Wu L, Wu H, Ma L, Sangiorgi F, Wu N, Bell JR, Lyons GE, Maxson R (1997) Miz1, a novel zinc finger transcription factor that interacts with Msx2 and enhances its affinity for DNA. Mech Dev 65:3-17

Wärnmark A, Wikstrom A, Wright AP, Gustafsson JÅ, Härd T (2001) The N-terminal regions of estrogen receptor alpha and beta are unstructured in vitro and show different TBP binding properties. J Biol Chem 276:45939-45944

Wärnmark A, Treuter E, Wright AP, Gustafsson JÅ (2003) Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. Mol Endocrinol 17:1901-1909

Xu J, Qiu Y, DeMayo FJ, Tsai SY, Tsai MJ, 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 L, Glass CK, Rosenfeld MG (1999) Coactivator and corepressor complexes in nuclear receptor function. Curr Opin Genet Dev 9:140-147

Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, 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 USA 97:6379-6384

Xu W, Chen H, Du K, Asahara H, Tini M, Emerson BM, Montminy M, Evans RM (2001) A transcriptional switch mediated by cofactor methylation. Science 294:2507-2511

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

Xu J, Li Q (2003) Review of the in vivo functions of the p160 steroid receptor coactivator family. Mol Endocrinol 17:1681-1692

Yadav N, Lee J, Kim J, Shen J, Hu MC, Aldaz CM, Bedford MT (2003) Specific protein methylation defects and gene expression perturbations in coactivator-associated arginine methyltransferase 1-deficient mice. Proc Natl Acad Sci USA 100:6464-6468

Yao TP, Ku G, Zhou N, Scully R, Livingston DM (1996) The nuclear hormone receptor coactivator SRC-1 is a specific target of p300. Proc Natl Acad Sci USA 93:10626-10631

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

Yeh S, Chang C (1996) Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 93:5517-5521

Yeh S, Miyamoto H, Shima H, Chang C (1998) From estrogen to androgen receptor: a new pathway for sex hormones in prostate. Proc Natl Acad Sci USA 95:5527-5532

Yeh S, Lin HK, Kang HY, Thin TH, Lin MF, Chang C (1999) From HER2/Neu signal cascade to androgen receptor and its coactivators: a novel pathway by induction of androgen target genes through MAP kinase in prostate cancer cells. Proc Natl Acad Sci USA 96:5458-5463

Yong EL, Loy CJ, Sim KS (2003) Androgen receptor gene and male infertility. Hum Reprod Update 9:1-7

Yuan CX, Ito M, Fondell JD, Fu ZY, Roeder RG (1998) The TRAP220 component of a thyroid hormone receptor-associated protein (TRAP) coactivator complex interacts directly with nuclear receptors in a ligand-dependent fashion. Proc Natl Acad Sci USA 95:7939-7944

Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, Moser C, Walker AP, McCabe ER, Meitinger T, Monaco AP, Sassone-Corsi P, Camerino G (1994) An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 372:635-641

Zhang XK, Lehmann J, Hoffmann B, Dawson MI, Cameron J, Graupner G, Hermann T, Tran P, Pfahl M (1992) Homodimer formation of retinoid X receptor induced by 9-cis retinoic acid. Nature 358:587-591

Zhang J, Hu X, Lazar MA (1999) A novel role for helix 12 of retinoid X receptor in regulating repression. Mol Cell Biol 19:6448-6457

Zhang D, Penttilä TL, Morris PL, Teichmann M, Roeder RG (2001) Spermiogenesis deficiency in mice lacking the Trf2 gene. Science 292:1153-1155

Zhang FP, Pakarainen T, Poutanen M, Toppari J, Huhtaniemi I (2003) The low gonadotropin-independent constitutive production of testicular testosterone is sufficient to maintain spermatogenesis. Proc Natl Acad Sci USA 100:13692-13697

Zhao XY, Boyle B, Krishnan AV, Navone NM, Peehl DM, Feldman D (1999) Two mutations identified in the androgen receptor of the new human prostate cancer cell line MDA PCa 2a. J Urol 162:2192-2199

Zhong S, Müller S, Ronchetti S, Freemont PS, Dejean A, Pandolfi PP (2000a) Role of SUMO-1-modified PML in nuclear body formation. Blood 95:2748-2752

Zhong S, Salomoni P, Pandolfi PP (2000b) The transcriptional role of PML and the nuclear body. Nat Cell Biol 2:E85-E90

Zhou X, Kudo A, Kawakami H, Hirano H (1996) Immunohistochemical localization of androgen receptor in mouse testicular germ cells during fetal and postnatal development. Anat Rec 245:509-518

Zhou Q, Nie R, Prins GS, Saunders PT, Katzenellenbogen BS, Hess RA (2002) Localization of androgen and estrogen receptors in adult male mouse reproductive tract. J Androl 23:870-881

Zhu Y, Qi C, Jain S, Rao MS, Reddy JK (1997) Isolation and characterization of PBP, a protein that interacts with peroxisome proliferator-activated receptor. J Biol Chem 272:25500-25506

Zhu Y, Qi C, Jia Y, Nye JS, Rao MS, Reddy JK (2000) Deletion of PBP/PPARBP, the gene for nuclear receptor coactivator peroxisome proliferator-activated receptor-binding protein, results in embryonic lethality. J Biol Chem 275:14779-14782

Zoppi S, Marcelli M, Deslypere J-P, Griffin JE, Wilson JD, McPhaul MJ (1992) Amino acid substitutions in the DNA-binding domain of the human androgen receptor are a frequent cause receptor-binding positive androgen resistance. Mol Endocrinol 6:409-415