PERGAMON

Journal of Steroid Biochemistry and Molecular Biology 69 (1999) 307–313

The Journal of
Steroid Biochemistry
&
Molecular Biology

Mechanisms of androgen receptor activation and function ☆

A.O. Brinkmann[a,*], L.J. Blok[a], P.E. de Ruiter[a], P. Doesburg[b], K. Steketee[b], C.A. Berrevoets[a], J. Trapman[b]

[a]Department of Endocrinology & Reproduction, Erasmus University Rotterdam, Rotterdam, The Netherlands
[b]Department of Pathology, Erasmus University Rotterdam, Rotterdam, The Netherlands

Abstract

Androgens play a crucial role in several stages of male development and in the maintenance of the male phenotype. Androgens act in their target cells via an interaction with the androgen receptor, resulting in direct regulation of gene expression. The androgen receptor is a phosphoprotein and modulation of the phosphorylation status of the receptor influences ligand-binding and consequently transcription activation of androgen responsive genes. Androgen binding induces a conformational change in the ligand-binding domain, accompanied by additional receptor phosphorylation. Subsequently the liganded androgen receptor interacts with specific androgen response elements in the regulatory regions of androgen target genes, resulting in stimulation of gene expression. Anti-androgens induce a different conformational change of the ligand-binding domain, which does not or only partially result in stimulation of transactivation. Interestingly, different anti-androgens can induce different inactive conformations of the androgen receptor ligand-binding domain. Recent evidence strongly supports a ligand dependent functional interaction between the ligand-binding domain and the NH_2-terminal transactivating domain of the androgen receptor. Two regions in the NH_2-terminal domain are involved in this interaction, whereas in the ligand-binding domain the AF-2 AD core region is involved. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Androgens play a crucial role in several stages of male development. They act via an interaction with the androgen receptor, a ligand dependent transcription factor which belongs to the superfamily of nuclear receptors. This superfamily includes the nuclear receptors for the other steroid hormones, for the retinoids, for the thyroid hormones, and a still growing number of orphan receptors [1,2]. In the last decade, since the cloning of the human androgen receptor cDNA, our insights in the mechanism of androgen action have been increased tremendously. Only one androgen receptor cDNA has been identified and cloned, despite the two different ligands [3–6]. The tissue specific actions of testosterone and 5α-dihydrotestosterone, mediated by the same androgen receptor, suggest a ligand specific recruitment of transcription intermediary factors (TIFs). However, experimental evidence for ligand specific TIFs for the androgen receptor has not been provided as yet. The androgen receptor protein displays a large homology in the DNA-binding domain and in the ligand-binding domain with the other members of the steroid hormone receptor subfamily (e.g. receptors for glucocorticoids, estradiol, progesterone and mineralocorticoids) [5,7–10].

The aim of the present review is to present some aspects in androgen action unravelled recently. In this overview information will be given on the functional domain structure of the human androgen receptor with emphasis on the recent findings of functional interactions between the NH_2-terminal domain and the ligand-binding domain. Post-translational modifications (phosphorylation) of the androgen receptor protein in relation to function will be discussed next. Throughout the text the numbering of the different

☆ Proceedings of Xth International Congress on Hormonal Steroids, Quebec, Canada, 17–21 June 1998.

* Corresponding author. Tel.: +31-10-408-7966; fax: +31-10-408-9461.

E-mail address: brinkmann@endov.fgg.eur.nl (A.O. Brinkmann)

0960-0760/99/$ - see front matter © 1999 Elsevier Science Ltd. All rights reserved.
PII: S0960-0760(99)00049-7

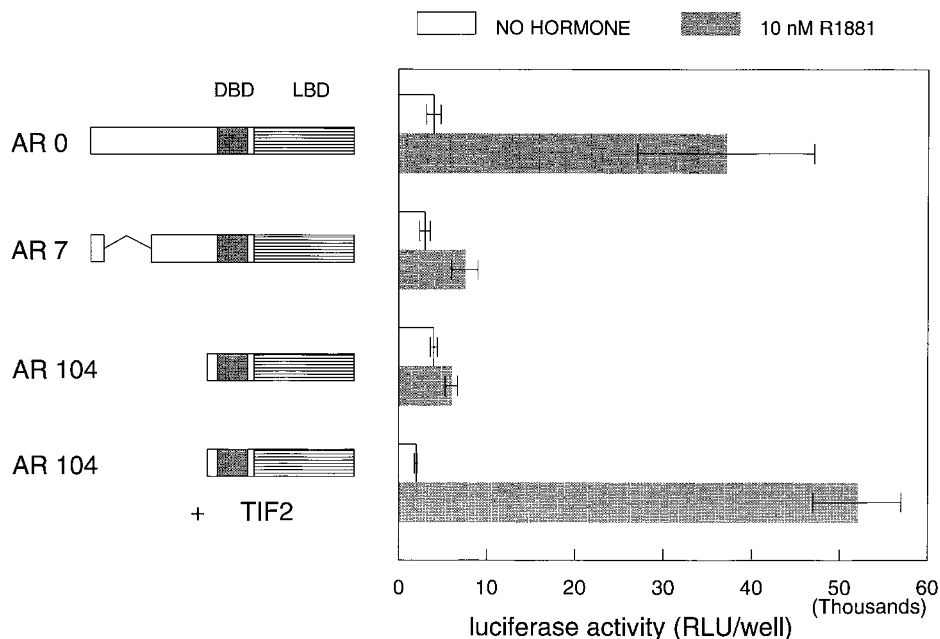

Fig. 1. Effect of TIF2 on the activity of the transcription activation function (AF-2) in the ligand-binding domain of the androgen receptor. CHO cells were transfected either with the wild type androgen receptor (AR0), or with a COOH-terminal construct lacking the complete NH_2-terminal domain (AR104). AR104 activity was tested in the presence (+TIF2) or absence of TIF2. For comparison also a NH_2-terminal domain mutant lacking AF-1 (AR7) was transfected. MMTV-LUC was used as a reporter gene. Cells were incubated with vehicle (open bars) or 10 nM R1881 (gray bars). Each bar represents the mean (±SEM) luciferase activity of three experiments.

codons is based on a total number of 910 amino acid residues of the androgen receptor protein [11]. This number differs from the amino acid content published by others [3,4,6]. These differences are caused by the variation in length of the polyglutamine and polyglycine stretches in the NH_2-terminal domain of the receptor.

2. Conformational changes induced by androgens and anti-androgens

Binding of androgens by the androgen receptor results in two conformational changes of the receptor [12,13]. Initially, a fragment of 35 kDa, spanning the complete ligand-binding domain and part of the hinge region, is protected by the ligand in a partial protease degradation assay, but after prolonged incubation times a second conformational change occurs resulting in protection of a smaller fragment of 29 kDa. In the presence of several anti-androgens (e.g. cyproterone acetate, hydroxyflutamide and bicalutamide) only the 35 kDa fragment is protected against proteolytic degradation, and no smaller fragments are detectable upon longer incubations. Obviously, the 35 kDa fragment is correlated with an inactive conformation, whereas the second conformational change, only inducible by agonists and considered as the necessary step for transcription activation, is lacking upon binding of anti-androgens. Further analyses with specific antibodies against different epitopes in the 35 kDa and 29 kDa fragments revealed that only the most COOH-terminal end of the androgen receptor protein is represented in the 29 kDa fragment [13].

3. Transactivation function in the ligand-binding domain

Deletion and mutation studies, as well as mutations found in patients with either the androgen insensitivity syndrome or prostate cancer have given some insight into which amino acid residues are important for ligand-binding [14–16]. The overall picture is that large deletions (>10 amino acid residues) severely affect hormone binding, but deletion of the complete ligand-binding domain results in a constitutive active molecule [14,17].

In the ligand-binding domain of the human androgen receptor a transcription activation function (designated as AF-2) has been identified, although it is very weak in comparison with that found in other steroid receptors (e.g. estrogen and glucocorticoid receptors) [18–20]. The AF-2 domain in the androgen receptor can be activated in a hormone dependent way and is strongly enhanced in a promoter dependent way by the co-activators TIF2 and GRIP1 [18–20] (Fig. 1). The boundaries of the AF-2 domain in the androgen receptor ligand-binding domain have not been deter-

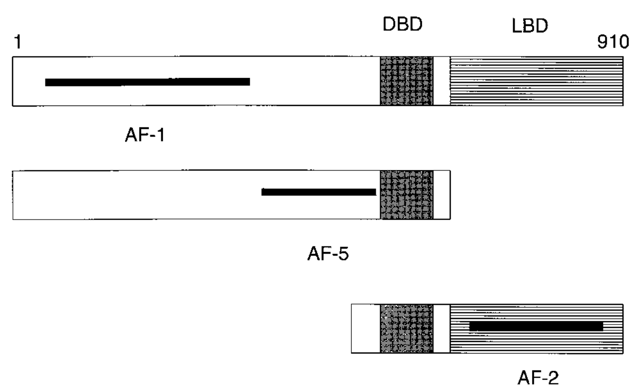

Fig. 2. Overview of the regions in the NH_2-terminal domain and in the COOH-terminal domain of the androgen receptor responsible for the transactivating capacity of either the wild type androgen receptor (AF-1), or the ligand-binding domain truncated constitutive active mutant (AF-5) or the NH_2-terminal domain truncated mutant (AF-2).

mined as yet, but it contains the core region as defined in the ligand-binding domains of several members of the ligand dependent nuclear receptor family. This AF-2 activation domain (AD) core region contains the conserved sequence 884-Glu-Met-Met-Ala-Glu-888. Mutations in this region can result in a decrease in activation function without affecting the ligand-binding capability. This indicates that the amino acid residues of the AF-2 AD core region are not directly involved in ligand-binding, but are part of, or are determining the interaction surface. Recent studies on mutations in this region and the interaction of co-activators confirm this presumption [20,21]. Interestingly, mutations have not been reported in the AF-2 AD core region of either individuals with the androgen insensitivity syndrome or prostate cancer patients, which most likely implicates that none of the individual amino acids in the AF-2 AD core region is essential in the full length androgen receptor.

4. Transactivation functions in the NH_2-terminal domain

The boundaries of the NH_2-terminal transactivation domain in the androgen receptor (designated as AF-1) are not exactly defined, but generally speaking it appears that the region between amino residues 51–211 is essential for transactivation activity in the full length receptor [14]. This region is not involved in the transactivation capacity of the COOH-terminal truncated androgen receptor, which displays constitutive activity [17]. The most important activating region in the constitutive receptor molecule is located in the

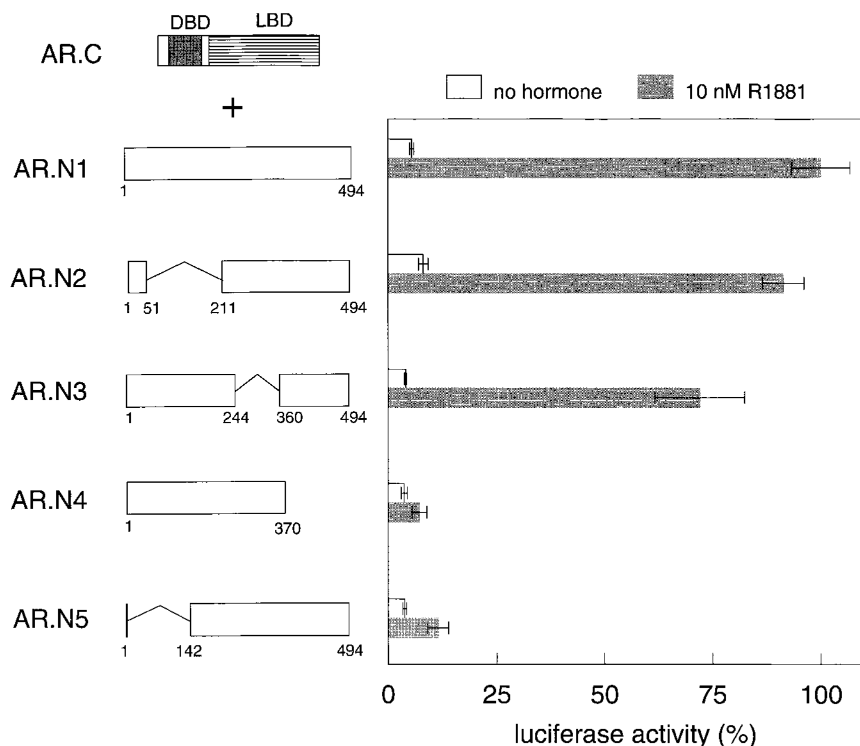

Fig. 3. Transcriptional activities of androgen receptor NH_2-terminal domain deletion mutants (AR.N1–AR.N5) cotransfected with a ligand-binding domain construct (AR.C) in CHO cells. Transcriptional activities were determined by cotransfection of the NH_2-terminal constructs together with a MMTV-LUC reporter plasmid. Cells were incubated either with vehicle (open bars) or with 10 nM R1881 (closed bars). Each bar represent the mean (±SEM) luciferase activity of three experiments.

NH_2-terminal domain between residues 370 and 494. This region is designated as AF-5.

So, the androgen receptor can use different transactivation domains (AF-1 and AF-5, respectively, in the NH_2-terminal domain and AF-2 AD in the COOH-terminal domain) depending on the 'form' of the receptor protein (Fig. 2). Two AF functions are ligand dependent (AF-1 and AF-2), whereas AF-5 operates in a ligand independent way. The ligand dependency of AF-1 in the full length androgen receptor and the switch to AF-5 in the COOH-terminal truncated androgen receptor strongly suggests a functional inhibitory action of the ligand-binding domain on AF-1 in the full length androgen receptor in the absence of ligand and on AF-5 both in the presence and absence of ligand. The AF-2 function is strongly dependent on the presence of ligand and androgen receptor coactivators.

5. Functional interaction of the NH_2-terminal domain and the COOH-terminal domain

In the previous section evidence is presented for a possible interaction between the ligand-binding domain and the AF functions in the NH_2-terminal domain. Investigating this NH_2-terminal domain–COOH-terminal domain (N/C) interaction in more detail reveals that only certain regions in the NH_2-terminal domain are involved in the interaction [20,22–24]. Interesting is that the AF-1 core region is not involved in this interaction; amino acid residues 3–36 as well as amino acid residues 370–494 are necessary for a proper functional interaction (Fig. 3). In the COOH-terminal domain, the AF-2 AD core region (amino acid residues: 884-Glu-Met-Met-Ala-Glu-888) is involved in the interaction as was established by substituting a highly conserved amino acid residue (Glu-888) by a glutamine residue. The same mutation also affects the functional interaction of the androgen receptor ligand-binding domain with TIF2, suggesting that both the NH_2-terminal domain and TIF2 are recognising the same interaction surface of the ligand-binding domain upon hormone binding [20].

6. Hormone independent phosphorylation and function

The newly synthesized androgen receptor migrates as a 110 kDa protein during SDS–PAGE, and becomes phosphorylated within 10 min upon synthesis, resulting in an additional protein band at 112 kDa [25,26]. This rapid post-translational modification is important for the acquisition of the hormone binding properties of the androgen receptor [27]. Evidence for this phosphorylation function was obtained from experiments in which dephosphorylation of the 112 kDa isoform was associated with a decreased hormone binding capacity, which could not be explained by an altered Kd value or decreased androgen receptor protein levels (Table 1). Dephosphorylation of 112 kDa isoform of the endogenous androgen receptor in the prostate cancer cell line LNCaP or the transiently expressed androgen receptor in COS-1 cells was accomplished via activation of the protein kinase A pathway, by stimulation of adenylyl cyclase by forskolin [27]. In order to establish which amino acid residues in the androgen receptor were phosphorylated in control and forskolin treated cells, trypsin digested androgen receptors were subjected to reverse-phase high pressure liquid chromatography and Edman degradation was performed on the isolated phosphorylated peptides. It was observed that serine residues 506, 641 and 653 were potentially phosphorylated in control cells, whereas strong evidence was obtained that phosphorylation of serines 641 and 653 was significantly reduced after forskolin treatment (Fig. 4). The forskolin induced dephosphorylation also had consequences for androgen induced transcription regulation in these cells as was illustrated by the decreased upregulation of PSA mRNA and the diminished down-regulation of the β1-subunit of Na,K-ATPase [27].

Table 1
Binding characteristics of androgen receptors in LNCaP cells cultured for 24 h in the absence (control) or presence of 20 μM of forskolin. Binding of R1881 was determined by Scatchard analysis

	Control	Forskolin
Kd (nM)	0.99 ± 0.4	0.72 ± 0.2
Bmax (fmol/mg P)	842 ± 97	459 ± 130

The mechanism by which forskolin induced the dephosphorylation of the androgen receptor is at present unknown. The swift nature of this process (within 10 min) suggests that androgen receptor dephosphorylation is an active process which involves activation of phosphatases rather than inhibition of kinases. Indeed, the activity of some phosphatases is known to be regulated by stimulation of protein kinase A. For example, the nuclear protein phosphatase-1 (PP-1N) is activated by protein kinase A-induced phosphorylation of NIPP-1 (nuclear inhibitor of protein phosphatase-1) [28–30]. Therefore a possible cascade of events could be the following (Fig. 5): forskolin activates adenylyl cyclase, which in turn activates protein kinase A. The protein kinase A either activates directly a protein phosphatase by a phosphorylation mechanism or inactivates a protein phosphatase inhibitor by phosphorylation, resulting also in activation of protein phosphatase. This activated phosphatase ultimately

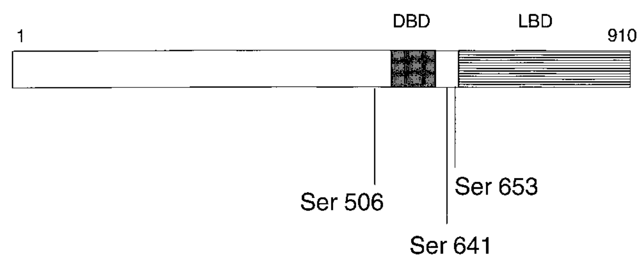

Fig. 4. Potential serine phosphorylation sites in the androgen receptor protein, which most likely become phosphorylated in the newly synthesized androgen receptor. The casein kinase II consensus sites Ser641 and Ser653 are dephosphorylated by activation of the protein kinase A (PKA) pathway. A possible mechanism for this dephosphorylation event is presented in Fig. 5. The mitogen-activated protein kinase (MAPK) consensus site Ser506 remains phosporylated upon PKA stimulation.

dephosphorylates the androgen receptor. Dephosphorylation of a protein upon stimulation of protein kinase A is not new: the retinoblastoma gene product (Rb) is dephosphorylated as a result of stimulation of the protein kinase A pathway [31].

7. Hormone dependent phosphorylation and function

A second important phosphorylation step of the androgen receptor occurs upon hormone binding resulting in a third isoform migrating at 114 kDa during SDS–PAGE [26,32]. All three isoforms (e.g. 110 kDa, 112 kDa and 114 kDa) exist in several androgen responsive cell lines in the presence of androgens and migrate as as triplet. The presence of the triplet correlates very well with DNA-binding by the androgen receptor: mutations of certain amino acid residues in the DNA-binding domain which severely affect the DNA-binding properties of the androgen receptor display simultaneously a defective hormone induced phosphorylation [26]. Recently the absence of the androgen receptor triplet in genital skin fibroblasts from a patient with the androgen insensitivity syndrome has been used as indicator for an androgen receptor defect in DNA-binding [32]. In the androgen receptor gene of this patient a mutation was found in the splice acceptor site of intron 2, resulting in a defective splicing of the androgen receptor mRNA. The mature transcript contained an additional 69 nucleotides between exon 2 and exon 3 sequences. The translation of this altered splice product results in a protein with an insertion of 23 amino acid residues between the first and the second zinc cluster of the DNA-binding domain. Additional protein analysis experiments revealed that in genital skin fibroblasts of the index patient the extended protein was expressed in large quantities. Tight nuclear binding of this mutated receptor protein could not be observed, corresponding with the absence of the triplet isoforms normally seen for wild type androgen receptors. Only a doublet of 110–112 kDa was expressed, indicating a defective DNA dependent phosphorylation of the human androgen receptor.

The experiments described above indicate that post-translational modification (e.g. phosphorylation) of the androgen receptor protein might be important at two different steps of receptor activation: (1) acquisition of ligand-binding capabilities and (2) during transformation to the DNA-binding /transcription activation form.

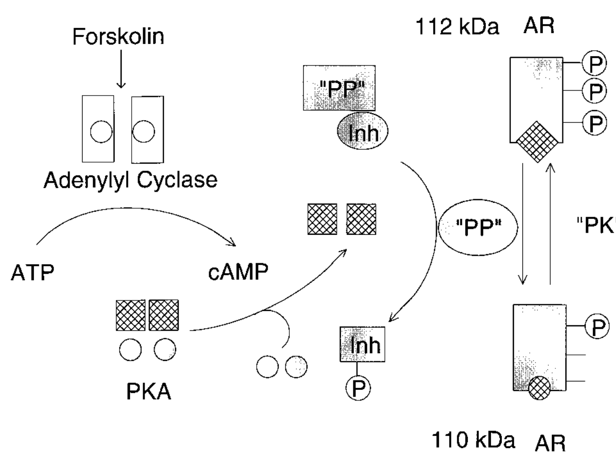

Fig. 5. Possible cascade of events during forskolin induced dephosphorylation of the androgen receptor in LNCaP cells. The newly synthesized androgen receptor (110 kDa AR) is phosphorylated by a protein kinase ('PK') resulting in the acquisition of ligand-binding capabilities and the formation of the 112 kDa form. Forskolin activates adenylyl cyclase, which in turn activates protein kinase A (PKA). Protein kinase A inactivates by phosphorylation a protein phosphatase inhibitor (Inh), resulting in activation of a protein phosphatase ('PP'), which ultimately dephosphorylates the 112 kDa androgen receptor form.

8. Conclusions

Androgen action is mediated by the androgen receptor, a ligand dependent transcription factor, belonging to the superfamily of nuclear receptors. The two most important androgens are testosterone and 5α-dihydrotestosterone and their tissue specific actions are mediated by the same androgen receptor protein.

Binding of androgens by the androgen receptor results in two consecutive conformational changes, which are different from those induced by anti-androgens.

The androgen receptor can use different transactivation domains (AF-1 and AF-5, respectively, in the NH_2-terminal domain and AF-2 AD in the COOH-terminal domain) depending on the 'form' of the receptor protein. The AF-2 function is strongly dependent on the presence of nuclear receptor coactivators.

Two AF functions are ligand dependent (AF-1 and AF-2), whereas AF-5 operates in a ligand independent way. The ligand dependency of AF-1 in the full length androgen receptor and the switch to AF-5 in the COOH-terminal truncated androgen receptor strongly suggests a functional inhibitory action of the ligand-binding domain on AF-1 in the absence of ligand and on AF-5 in the presence of ligand. In vivo experiments favour a ligand dependent functional interaction between the AF-2 AD core region in the ligand-binding domain with the NH_2-terminal domain. This interaction might be either direct or indirect, requiring additional factors, and results in androgen receptor driven transcription activation.

The androgen receptor protein can undergo two post-translational modifications during receptor activation. Firstly upon synthesis the protein is rapidly phosphorylated to acquire hormone binding capacities and secondly upon hormone binding an additional phosphorylation occurs during transformation to the DNA-binding transcription activation form.

Acknowledgements

The authors thank Drs Gronemeyer and Chambon for the generous gift of the expression plasmid for TIF2. This research was supported by the Dutch Cancer Society and the Netherlands Organization for Scientific Research through GB-MW.

References

[1] R. Evans, The steroid and thyroid hormone receptor superfamily, Science 240 (1988) 889–894.

[2] V. Laudet, C. Hänni, J. Coll, F. Catzeflis, D. Stéhelin, Evolution of the nuclear receptor gene superfamily, EMBO Journal 11 (1992) 1003–1013.

[3] C. Chang, J. Kokontis, S. Liao, Molecular cloning of human and rat complementary DNA encoding androgen receptors, Science 240 (1988) 324–326.

[4] D.B. Lubahn, D.R. Joseph, P.M. Sullivan, H.F. Willard, F.S. French, E.M. Wilson, Cloning of human androgen receptor complementary DNA and localization to the X chromosome, Science 240 (1988) 327–330.

[5] J. Trapman, P. Klaassen, G.G.J.M. Kuiper, J.A.G.M. van der Korput, P.W. Faber, H.C.J. van Rooij, A. Geurts van Kessel, M.M. Voorhorst, E. Mulder, A.O. Brinkmann, Cloning, structure and expression of a cDNA encoding the human androgen receptor, Biochemical and Biophysical Research Communications 153 (1988) 241–248.

[6] W.D. Tilley, M. Marcelli, J.D. Wilson, M.J. McPhaul, Characterization and expression of a cDNA encoding the human androgen receptor, Proceedings of the National Academy of Sciences of the United States of America 86 (1989) 327–331.

[7] S.M. Hollenberg, C. Weinberger, E.S. Ong, G. Cerelli, A. Oro, R. Lebo, E.B. Thompson, M.B. Rosenfeld, R.M. Evans, Primary structure and expression of a functional human glucocorticoid receptor cDNA, Nature 318 (1985) 635–641.

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

[9] M. Misrahi, M. Atger, L. d'Auriol, H. Loosfelt, C. Meriel, F. Fridlansky, A. Guiochon-Mantel, F. Galibert, E. Milgrom, Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA, Biochemical and Biophysical Research Communications 143 (1987) 740–748.

[10] J.L. Arriza, C. Weinberger, G. Cerelli, T.M. Glaser, B.L. Handelin, D.E. Housman, R.M. Evans, Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor, Science 237 (1987) 268–275.

[11] A.O. Brinkmann, P.W. Faber, H.C.J. van Rooy, G.G.J.M. Kuiper, C. Ris, P. Klaassen, J.A.G.M. van der Korput, M.M. Voorhorst, J.H. van Laar, E. Mulder, J. Trapman, The human androgen receptor: domain structure, genomic organization and regulation of expression, Journal of Steroid Biochemistry and Molecular Biology 34 (1989) 307–310.

[12] C.W. Kuil, E. Mulder, Mechanism of antiandrogen action: conformational changes of the receptor, Molecular and Cellular Endocrinology 102 (1994) R1–R5.

[13] C.W. Kuil, C.A. Berrevoets, E. Mulder, Ligand-induced conformational alterations of the androgen receptor analyzed by limited trypsinization—studies on the mechanism of antiandrogen action, Journal of Biological Chemistry 270 (1995) 27,569–27,576.

[14] G. Jenster, J.A.G.M. van der Korput, C. van Vroonhoven, ThH van der Kwast, J. Trapman, A.O. Brinkmann, Domains of the human androgen receptor involved in steroid binding, transcriptional activation and subcellular localization, Molecular Endocrinology 5 (1991) 1396–1404.

[15] C.A. Quigley, A. De Bellis, K.B. Marschke, M.K. El-Awady, E.M. Wilson, F.S. French, Androgen receptor defects: historical, clinical and molecular perspectives, Endocrine Reviews 16 (1995) 271–321.

[16] B. Gottlieb, H. Lehvaslaiho, L.K. Beitel, R. Lumbroso, L. Pinsky, M. Trifiro, The androgen receptor gene mutations database, Nucleic Acids Research 26 (1998) 151–154.

[17] G. Jenster, J.A.G.M. van der Korput, J. Trapman, A.O. Brinkmann, Identification of two transcription activation units in the N-terminal domain of the human androgen receptor, Journal of Biological Chemistry 270 (1995) 7341–7346.

[18] J.J. Voegel, M.J. Heine, C. Zechel, P. Chambon, H. Gronemeyer, TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors, EMBO Journal 15 (1996) 3667–3675.

[19] H. Hong, K. Kohli, A. Trivedi, D.L. Johnson, M. Stallcup, RGRIP-1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors, Proceedings of the National Academy of Sciences of the United States of America 93 (1996) 4948–4952.

[20] C.A. Berrevoets, P. Doesburg, K. Steketee, J. Trapman, A.O. Brinkmann, Functional interactions of the AF-2 activation domain core region of the human androgen receptor with the amino-terminal domain and with the transcriptional coactivator TIF2 (transcriptional intermediary factor 2), Molecular Endocrinology 12 (1998) 1172–1183.

[21] W. Feng, R.C.J. Ribeiro, R.L. Wagner, H. Nguyen, J.W. Apriletti, R.J. Fletterick, J.D. Baxter, P.J. Kushner, B.L. West, Hormone-dependent coactivator binding to a hydrophobic cleft on nuclear receptors, Science 280 (1998) 1747–1749.

[22] E. Langley, Z-X. Zhou, E.M. Wilson, Evidence for an anti-parallel orientation of the ligand-activated human androgen recep-

tor dimer, Journal of Biological Chemistry 270 (1995) 29,983–29,990.

[23] P. Doesburg, C.W. Kuil, C.A. Berrevoets, K. Steketee, P.W. Faber, E. Mulder, A.O. Brinkmann, J. Trapman, Functional in vivo interaction between the amino-terminal, transactivation domain and the ligand-binding domain of the androgen receptor, Biochemistry 36 (1997) 1052–1064.

[24] E. Langley, J.A. Kemppainen, E.M. Wilson, Intermolecular NH_2-/carboxy-terminal interactions in androgen receptor dimerization revealed by mutations that cause androgen insensitivity, Journal of Biological Chemistry 273 (1998) 92–101.

[25] G.G.J.M. Kuiper, P.E. de Ruiter, J.A. Grootegoed, A.O. Brinkmann, Synthesis and post-translational modification of the androgen receptor in LNCaP cells, Molecular and Cellular Endocrinology 80 (1991) 65–73.

[26] G. Jenster, P.E. de Ruiter, H.A.G.M. van der Korput, G.G.J.M. Kuiper, J. Trapman, A.O. Brinkmann, Changes in abundance of androgen receptor isotypes: effects of ligand treatment, glutamine-stretch variation and mutation of putative phosphorylation sites, Biochemistry 33 (1994) 14,064–14,072.

[27] L.J. Blok, P.E. de Ruiter, A.O. Brinkmann, Forskolin-induced dephosphorylation of the androgen receptor impairs ligand-binding, Biochemistry 37 (1998) 3850–3857.

[28] M. Beullens, A. Van Eynde, M. Bollen, W. Stalmans, Inactivation of nuclear inhibitory polypeptides of protein phosphatase-1 (NIPP-1) by protein kinase A, Journal of Biological Chemistry 268 (1993) 13,172–13,177.

[29] A. Van Eynde, M. Beullens, W. Stalmans, M. Bollen, Full activation of a nuclear species of protein phosphatase-1 by phosphorylation with protein kinase A and casein kinase-2, Biochemical Journal 297 (1994) 447–449.

[30] A. Van Eynde, S. Wera, M. Beullens, S. Torrekens, F. van Leuven, W. Stalmans, M. Bollen, Molecular cloning of NIPP-1, a nuclear inhibitor of protein phosphatase-1, reveals homology with polypeptides involved in RNA processing, Journal of Biological Chemistry 270 (1995) 28,068–28,074.

[31] J. Christoffersen, E.B. Smeland, T. Stokke, K. Tasken, K.B. Andersson, H.K. Blomhoff, Retinoblastoma protein is rapidly dephosphorylated by elevated cyclic adenosine monophosphate levels in human B-lymphoid cells, Cancer Research 54 (1994) 2245–2250.

[32] H.T. Brüggenwirth, A.L.M. Boehmer, S. Ramnarain, M.C.T. Verleun-Mooijman, D.P.E. Satijn, J. Trapman, J.A. Grootegoed, A.O. Brinkmann, Molecular analysis of the androgen receptor gene in a family with receptor-positive partial androgen insensitivity: An unusual type of intronic mutation, American Journal of Human Genetics 61 (1997) 1067–1077.