Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7866–7870, October 1990
Medical Sciences

Aberrant splicing of androgen receptor mRNA results in synthesis of a nonfunctional receptor protein in a patient with androgen insensitivity

(testicular feminization/steroid receptor/point mutation/splice donor site/male sexual differentiation)

C. RIS-STALPERS*†, G. G. J. M. KUIPER*, P. W. FABER‡, H. U. SCHWEIKERT§, H. C. J. VAN ROOIJ‡, N. D. ZEGERS¶, M. B. HODGINS‖, H. J. DEGENHART**, J. TRAPMAN‡, AND A. O. BRINKMANN*

Departments of *Biochemistry II, ‡Pathology, and **Pediatrics, Erasmus University, Rotterdam, The Netherlands; §Department of Internal Medicine, University of Bonn, Bonn, Federal Republic of Germany; ¶Department of Immunology, Medical Biological Laboratory–Organization for Applied Scientific Research, Rijswijk, The Netherlands; and ‖Department of Dermatology, Glasgow University, Glasgow, United Kingdom

Communicated by Josef Fried, June 29, 1990

ABSTRACT **Androgen insensitivity is a disorder in which the correct androgen response in an androgen target cell is impaired. The clinical symptoms of this X chromosome-linked syndrome are presumed to be caused by mutations in the androgen receptor gene. We report a G → T mutation in the splice donor site of intron 4 of the androgen receptor gene of a 46,XY subject lacking detectable androgen binding to the receptor and with the complete form of androgen insensitivity. This point mutation completely abolishes normal RNA splicing at the exon 4/intron 4 boundary and results in the activation of a cryptic splice donor site in exon 4, which leads to the deletion of 123 nucleotides from the mRNA. Translation of the mutant mRNA results in an androgen receptor protein ≈5 kDa smaller than the wild type. This mutated androgen receptor protein was unable to bind androgens and unable to activate transcription of an androgen-regulated reporter gene construct. This mutation in the human androgen receptor gene demonstrates the importance of an intact steroid-binding domain for proper androgen receptor functioning *in vivo*.**

Androgens play an essential role in the control of male sexual differentiation and development and in the maintenance of normal male reproductive function (1). Androgen action is mediated by the low-abundance intracellular androgen receptor protein, a member of the superfamily of ligand-responsive transcription regulators that includes the retinoic acid receptors, the thyroid hormone receptors, and the other steroid hormone receptors (2–4).

The human androgen receptor is composed of 910 amino acids, as deduced from the cDNA sequence (5–9). The corresponding gene is located on the X chromosome and has a length of >90 kilobases (kb) (5, 10, 11). The information for the protein-coding region is separated over eight exons. The sequence encoding the N-terminal domain is present in one large exon (exon 1) (8). The DNA-binding domain is encoded by exons 2 and 3, and the information for the steroid-binding domain is distributed over five exons (exons 4–8) (10). The positions of the exon/intron boundaries are conserved among progesterone, estrogen, and androgen receptor genes (10, 12, 13).

A number of aberrations of male sexual differentiation and development are associated with defects in the androgen receptor protein (1, 14). These defects can vary from a complete female phenotype in a 46,XY individual [complete androgen insensitivity syndrome (AIS)] to partial disorders of male sexual differentiation (partial AIS). Both the complete and the partial form of AIS can manifest at the protein level in either the absence or the presence of androgen binding. In the latter case, qualitative defects in androgen binding have been reported (1, 14). Therefore, naturally occurring mutations in the androgen receptor are a potentially interesting source for the investigation of receptor structure–function relationships. In addition, the variation in clinical syndromes provides the opportunity to correlate a mutation in the androgen receptor structure with the impairment of a specific physiological function of the androgen receptor.

Here we report a point mutation in the splice donor site of intron 4 of the androgen receptor gene of a patient with complete AIS and describe the consequences for androgen receptor properties.

MATERIALS AND METHODS

Index Patient. Clinical and biochemical data concerning patient 20.1 (age 17) showed a 46,XY karyotype but a female habitus with unambiguously female external genitalia. Serum concentrations of testosterone, dihydrotestosterone, and follitropin were within the normal range for men. Serum lutropin levels were 5 times higher than normal. Androgen receptor binding was assessed in genital skin fibroblast monolayers, cultured from a skin biopsy of the labia majora (15). Androgen binding could not be detected (maximal binding in genital skin fibroblasts of controls, >18 fmol/mg of protein). These data led to the diagnosis of complete AIS with no detectable androgen binding to receptors.

Cell Culture. Genital skin fibroblasts were cultured in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, nonessential amino acids, and antibiotics. COS-1 cells (simian virus 40-transformed monkey kidney fibroblasts) were grown in Dulbecco's modified Eagle's medium supplemented with 5% dextran/charcoal-treated fetal bovine serum and antibiotics.

RNA Preparation. Total cellular RNA was isolated by the guanidinium isothiocyanate method (16). cDNA was synthesized using 4 μg of total RNA, 100 ng of oligodeoxynucleotide primer (E8: 5′-AAGGCACTGCAGAGGAGTA-3′), 10 units of avian myeloblastosis virus reverse transcriptase (Promega), and 10 units of RNase inhibitor (RNasin; Promega). Synthesis was done according to the standard protocol (Promega).

DNA Amplification and Sequencing. Amplification by the polymerase chain reaction (PCR; ref. 17) took place in 100-μl reaction mixtures containing 1 μg of genomic DNA or 2% of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AIS, androgen insensitivity syndrome; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; MMTV, mouse mammary tumor virus; LTR, long terminal repeat.

†To whom reprint requests should be addressed at: Department of Biochemistry II, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

the cDNA-synthesis reaction mixture. PCR mixtures contained 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.2 μmol of each dNTP, 17 μg of bovine serum albumin, 2 units of *Thermus aquaticus* (*Taq*) DNA polymerase (Amersham), and 600 ng of each oligonucleotide. Amplification was performed during 24 cycles; each cycle included denaturation for 1 min at 92°C, primer annealing for 2 min at 60°C, and primer extension for 1–5 min at 70°C. For Southern blotting, samples were electrophoresed in 2% agarose, transferred to nitrocellulose, and hybridized as described previously (probe C in ref. 10). For sequence analysis, amplified fragments were made blunt-ended and inserted into the *Sma* I site of M13mp18 (18) prior to sequencing by the dideoxy chain-termination method (19). The following oligonucleotides were used (mismatches are indicated by lowercase letters): I3, ATTCAAGTCTCTCTTCCTTC; I4, GCGTTCACTAAATATGATCC; E1, ggatCCACATGCGTTTGGAGACTGC; E4, CAGAAGCTtACAGTGTCACACA; E5, CGAAGTAGAGgATCCTGGAGTT; E8, AAGGCACTGCAGAGGAGTA.

Construction of the Expression Vectors. A human androgen receptor cDNA expression vector (pAR_0) was constructed using the simian virus 40 early promoter and the rabbit β-globin polyadenylylation signal (20). The $pAR_{\Delta 674-714}$ expression vector (pAR_Δ) was generated by exchanging the 898-base-pair *Kpn* I–*Eco*RI fragment of pAR_0 with the mutant 775-bp *Kpn* I–*Eco*RI fragment obtained by amplification of cDNA with oligonucleotides flanking the *Kpn* I site in exon 1 and the *Eco*RI site in exon 6 (5′-GACTTCACCGCACCTGATG-3′ and 5′-TGCTGAAGAGTAGCAGTGCT-3′).

Transfection. COS cells were transfected by the calcium phosphate precipitation method (21). For immunoblotting studies, 5×10^6 COS cells were transfected with 40 μg of either pAR_0 or pAR_Δ and 40 μg of pTZ (Pharmacia) carrier plasmid. For binding studies, 10^7 COS cells were transfected with 80 μg of either pAR_0 or pAR_Δ and 80 μg carrier plasmid. For transcription studies, 5×10^5 cells were transfected with 2.5 μg of either pAR_0 or pAR_Δ, 2.5 μg of MMTV–CAT reporter gene [bacterial chloramphenicol acetyltransferase (CAT) gene under control of the mouse mammary tumor virus (MMTV) long terminal repeat (LTR)], and 2.5 μg of pCH110 (β-galactosidase reporter plasmid; Pharmacia). Carrier DNA (pTZ) was added to give a total of 10 μg of DNA per dish. pSV2cat (2.5 μg) was used in a control experiment. Each experiment was carried out in duplicate.

Western Blot Analysis. COS cells were lysed in 40 mM Tris·HCl, pH 7.0/1 mM EDTA/4% (vol/vol) glycerol/10 mM dithiothreitol/2% SDS, and 1 volume of the protein fraction of the whole cell lysate was precipitated with 5 volumes of methanol. SDS/PAGE (0.1 mg of protein per lane), Western blotting, and immunostaining with antibody Sp061 (diluted 1:1000) were done as described (22).

Hormone Binding Assay. Transfected COS cells were cultured for 3 days in steroid-depleted medium and then incubated for 1 hr at 37°C with 0.1–10 nM [^{3}H]R1881 (17β-hydroxy-17α-methyl-4,9,11-estratrien-3-one) in the presence of a 500-fold molar excess of triamcinolone acetonide. Nonspecific binding was determined in parallel incubations with an additional 100-fold molar excess of nonradioactive R1881. Separation of bound and unbound steroid was achieved by the oil microassay method (23).

β-Galactosidase and CAT Assays. β-Galactosidase was assayed (24) by incubation of 5 μl of cell extract with 10 μl of 1 nM 4-methylumbelliferyl β-D-galactopyranoside (Koch Light) for 30 min at 37°C. The reaction was terminated by adding 200 μl of 1 M $NaHCO_3$ and fluorescence was determined at 365 and 448 nm. The CAT assay was essentially as described (25). After correction for transfection efficiency (β-galactosidase assay), CAT activity was quantitated (26).

RESULTS

Southern blotting with specific androgen receptor cDNA probes showed that genomic DNA from genital skin fibroblasts of patient 20.1 contained the complete coding region of the gene (data not shown). To investigate whether a point mutation or a small gene deletion might have caused the absence of hormone binding, exons 4–8, which encode the steroid-binding domain, and exons 2 and 3, which encode the DNA-binding domain, were amplified from genomic DNA and sequenced (17). Sequences were found to be identical with the previously published wild-type structure with only one exception: a G → T mutation at position 1 in the splice donor site of intron 4 (Fig. 1). This mutation was detected in each of four independent clones produced by two separate PCR amplifications. RNA was isolated from genital skin fibroblasts of patient 20.1 and first-strand cDNA was prepared using an oligonucleotide primer corresponding to an exon 8 sequence. The resulting cDNA was amplified using exon 4- and 5-specific primers. Amplified fragments were analyzed by size fractionation in a 2% agarose gel and hybridization with a cDNA probe specific for the steroid-binding domain of the human androgen receptor. This resulted in the detection of only one fragment, which, however, was shorter than the corresponding fragment from the wild-type receptor. Amplification of a cDNA fragment spanning exons 2–8 also resulted in one amplification product with a similar length difference, implying total abolishment of normal RNA splicing and the effective use of only one alternative splice site (Fig. 2*a*).

Sequence analysis of the mutant fragment revealed the use of a cryptic splice donor site, CAG/GTGTAG at position 2020/2021 (10) in exon 4 of the human androgen receptor gene. The use of this cryptic splice site results in the deletion of 123 nucleotides from the mRNA (Fig. 2*b*).

Translation of the deleted mRNA would result in an in-frame deletion of 41 amino acids (residues 674–714; ref. 10) in the steroid-binding domain of the androgen receptor protein. To investigate whether the deleted mRNA could be translated, androgen receptor expression vectors were constructed. Expression vectors containing either the wild-type sequence (pAR_0) or the mutated sequence ($pAR_{\Delta 674-714}$) were transiently expressed in COS-1 cells. Western immunoblotting using a polyclonal antibody (SP061; ref. 22) directed against the human androgen receptor showed the presence of

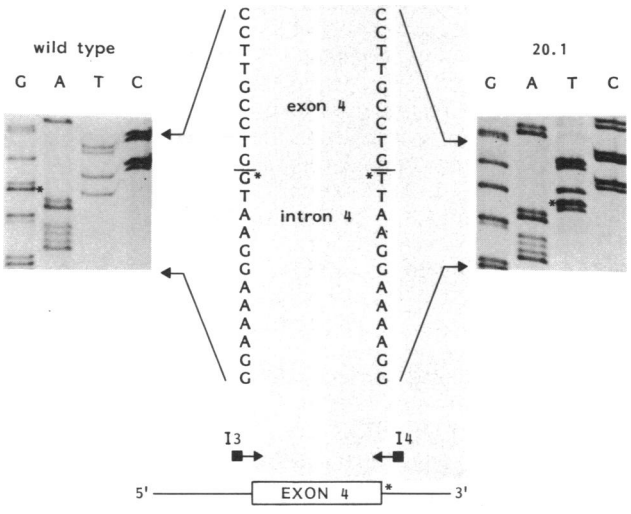

FIG. 1. Sequence comparison of the exon 4/intron 4 boundaries of the wild-type (*Left*) and mutant 20.1 (*Right*) androgen receptor genes. Asterisks indicate the single base substitution (G → T) in the splice donor site. Genomic DNA was amplified using oligonucleotide primers I3 and I4 as indicated.

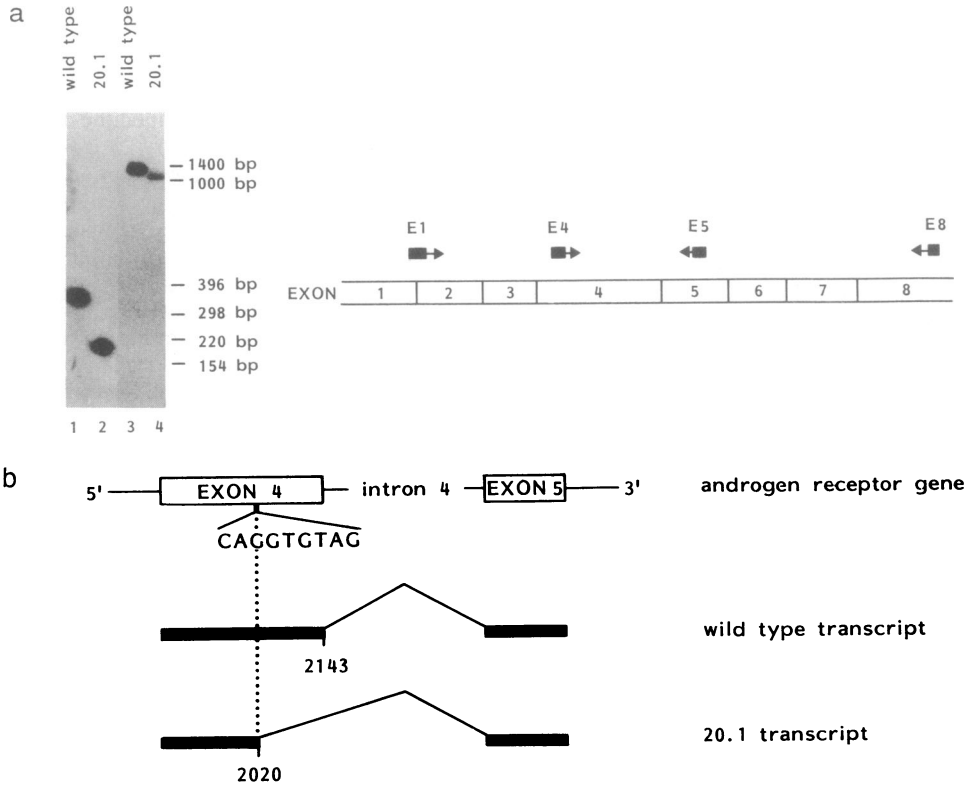

FIG. 2. (*a*) Size comparison of amplified androgen receptor cDNA. Oligonucleotides E4 and E5 (lanes 1 and 2) or E1 and E8 (lanes 3 and 4) were used after cDNA synthesis with oligonucleotide E8. RNA was isolated from genital skin fibroblasts of patient 20.1 (lanes 2 and 4) and from control cells (lanes 1 and 3). Marker sizes and the relative positions of the oligonucleotide primers are indicated. (*b*) Sequencing of the E4–E5 amplification product elucidated the position of the cryptic splice donor site in exon 4 of the androgen receptor gene, resulting in the deletion of 123 nucleotides from the mRNA of patient 20.1.

comparable amounts of the pAR_0 protein (calculated molecular mass, 98,845 Da) and the $pAR_{\Delta 674-714}$ protein (94,334 Da) (Fig. 3). The protein bands around 50 kDa and 70 kDa are probably due to proteolytic breakdown. A protein product of 50 kDa originating from an alternative initiation site of translation lacks ≈400 amino acid residues from the N-terminal domain and would in that case have lost the epitope for Sp061 recognition.

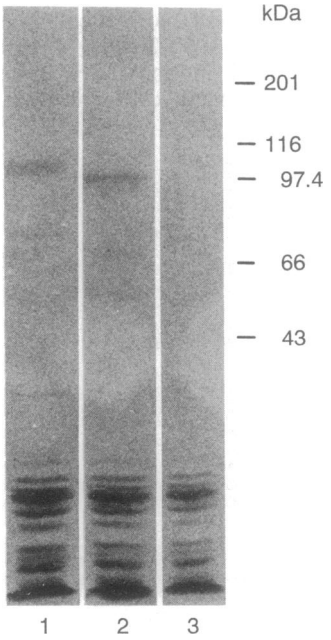

FIG. 3. Western blot analysis of protein products of pAR_0 (wild-type sequence; lane 1), $pAR_{\Delta 674-714}$ (mutant sequence; lane 2) and pSV2cat (lane 3) expressed in COS cells and analyzed by SDS/7.5% PAGE. The androgen receptor was visualized by immunostaining with the polyclonal antibody Sp061.

No androgen receptor expression was found after mock transfection (Fig. 3). Immunoblot detection of the normal or mutant androgen receptor in genital skin fibroblasts was not possible, probably due to the low concentration of androgen receptor protein in these cells.

In genital skin fibroblasts of the patient, no binding of androgens to androgen receptors was detected, and the clinical syndrome of this patient indicates the inability of the receptor to regulate transcription of androgen target genes. To investigate whether the mutation described above could

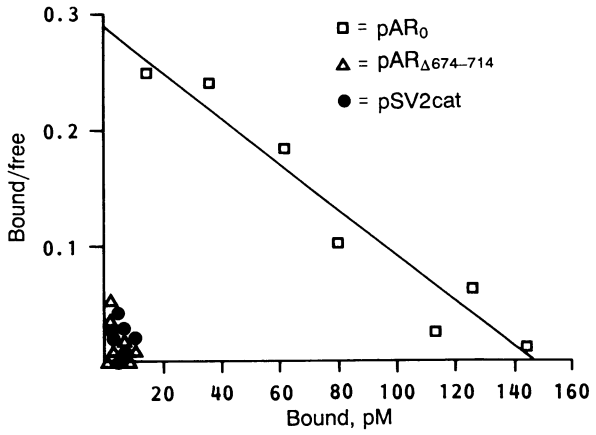

FIG. 4. Scatchard plot analysis of androgen ([^{3}H]R1881) binding in COS cells transfected with pAR_0, $pAR_{\Delta 674-714}$, or pSV2cat.

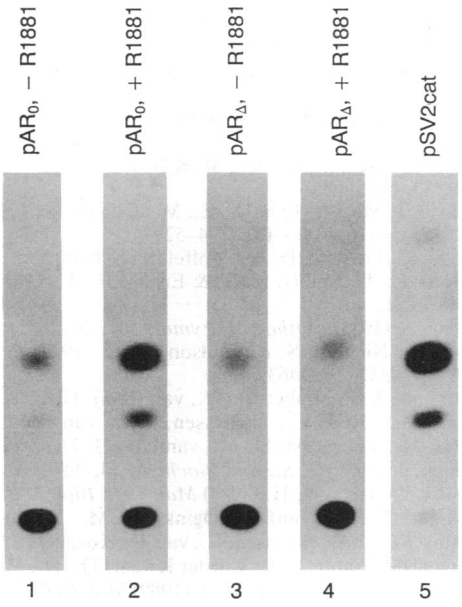

FIG. 5. Regulation of MMTV–CAT expression in COS cells cotransfected with pAR_0 (lanes 1 and 2) or $pAR_{\Delta 674-714}$ (lanes 3 and 4). Lane 5 shows the pSV2cat control. The cells were cultured in the absence (lanes 1, 3, and 5) or presence (lanes 2 and 4) of 0.1 nM R1881. Autoradiograms display the conversion of [^{14}C]chloramphenicol to acetylated products.

be the cause of absence of hormone binding and transcription activation, androgen binding and MMTV-LTR-driven CAT expression were determined in COS cells transfected with either pAR_0 or $pAR_{\Delta 674-714}$. pSV2cat was used as a control. Specific binding of the synthetic androgen [^{3}H]R1881 could not be demonstrated for the $pAR_{\Delta 674-714}$ protein, whereas the pAR_0 protein showed a maximum binding capacity of 730 fmol/mg of protein and a dissociation constant of 0.5 nM (Fig. 4).

In the presence of the synthetic androgen R1881, the wild-type androgen receptor protein expressed in COS cells activated the expression of the MMTV–CAT reporter gene, but the $pAR_{\Delta 674-714}$ deletion mutant did not (Fig. 5). The deletion mutant was also unable to activate transcription in the absence of R1881, indicating that the mutant receptor protein is not constitutively active. When the CAT activity induced by pAR_0 in the presence of R1881 was set to 100%, the relative CAT activity in the absence of R1881 was 19%. The relative CAT activity induced by $pAR_{\Delta 674-714}$ in either the presence or the absence of R1881 was 14%. The low CAT activity observed in the case of transfection with $pAR_{\Delta 674-714}$ and in the absence of hormone in the case of pAR_0 was considered background activity stemming from the MMTV–CAT construct. COS cells transfected with MMTV–CAT alone also show this low basal CAT activity (20).

DISCUSSION

In this study a point mutation in a splice donor site of the androgen receptor gene was characterized in detail. It is well documented (27) that an effective splice donor site resembles the consensus sequence $\begin{matrix}C\\A\end{matrix}AG/GU\begin{matrix}A\\G\end{matrix}AGU$ (Fig. 6*a*). Within this consensus splice sequence, the G at intron position 1 is obligatory. Mutation of this G leads to abolishment of normal splicing and to aberrant splicing products due to the activation of one or more cryptic splice sites (30–32). A G → T mutation at intron position 1 can, in an *in vitro* model, lead to an upstream shift of the cleavage site of 1 nucleotide (33). The G → T mutation reported here also generates a possible splice site 1 nucleotide upstream from the original junction, but this site is not activated in the mutant *in vivo*.

Recognition by means of hybrid formation of the splice donor site with nucleotides 4–11 of the U1 small nuclear RNA is one of the key steps in the splicing mechanism (28, 29). The most frequently formed hybrids comprise only 5–7 bp. For the human androgen receptor gene, the splice donor sequence normally used at the exon 4/intron 4 boundary (CTG/GTAAGG) is able to form 7 bp with U1 RNA. Obviously, in the wild-type situation this splice site is preferred to the cryptic splice site (CAG/GTGTAG) although the latter has six possible base-pairing positions with U1 RNA (including G·U pairing) and conforms to the splice consensus rule (Fig. 6).

Only a few naturally occurring mutations involving human steroid hormone receptors have been described. A recently published mutation involves a single nucleotide change leading to an amino acid change in the steroid-binding domain of the androgen receptor of a complete AIS patient with evidence of X chromosome linkage. The mutated protein has a decreased affinity for ligand but the effect on androgen target-gene activation has not been investigated (34). A deletion of part of the androgen receptor gene of a complete AIS patient also has been published (35). The effect of this deletion on receptor synthesis or function has not been established.

A steroid receptor that lacks the steroid-binding domain may show constitutive transcriptional activity; this has been demonstrated for progesterone, glucocorticoid, and estrogen receptors (2–4). The same holds for the androgen receptor. When the complete steroid-binding domain is deleted the receptor has a constitutive transcriptional activity that is about 30% of the activity induced by the wild-type androgen receptor (unpublished data).

The lack of androgen binding found in genital skin fibroblasts of patient 20.1 is the result of the deletion of 41 amino acids (residues 674–714) in the steroid-binding domain of the androgen receptor. Residues 674–714 of the receptor are located in a region that displays a high degree of sequence conservation in the family of steroid hormone receptors (5, 7). The importance for steroid binding and transcription regulation of a region similar to the one deleted in the

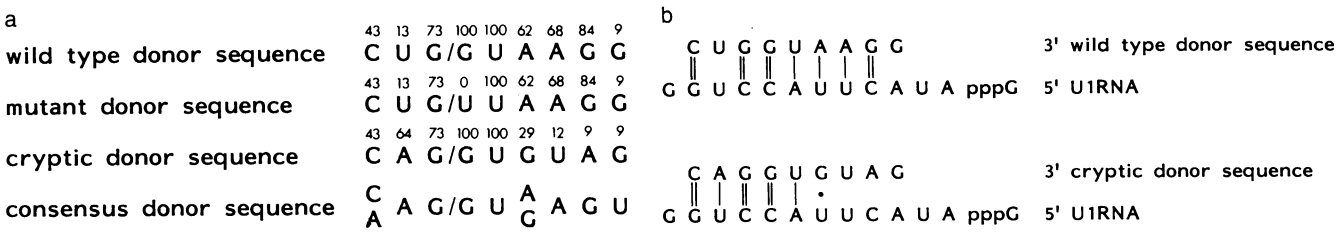

FIG. 6. (*a*) Comparison of the wild-type splice donor site of intron 4, the mutant splice site, and the cryptic splice donor site with the consensus donor sequence according to Mount (27). The percentage of occurrence is indicated. (*b*) Possible base-pairing of nucleotides 4–11 of U1 small nuclear RNA with the wild-type or the cryptic splice donor site according to previous models (28, 29). G·C pairing is indicated by double bars, A·U pairing by single bars, and G·U pairing by dots.

androgen receptor of AIS patient 20.1 has been established for other steroid hormone receptors (2–4), where *in vitro* generated deletions in this region abolish hormone binding and decrease the ability of the protein to activate transcription. For the glucocorticoid receptor, it has been postulated that the 90-kDa heat shock protein is able to associate with this part of the steroid-binding domain (36). This region of the glucocorticoid receptor also harbors a nuclear localization signal (37) and a domain that has a potent effect on transcription (38).

Whether there is a parallel between the glucocorticoid receptor and the androgen receptor regarding the function of this region is still unresolved. The deletion mutant described here does not enable us to answer this question, because it probably changes the folding of the receptor in such a way that the structure of the ligand-binding pocket is destroyed. Further research on the effect of deletions and single amino acid mutations of the androgen receptor on hormone binding and the regulation of androgen target-gene expression will provide more insight into the mechanism of androgen receptor action.

We thank Dr. Anton Grootegoed for helpful discussions and for reading the manuscript. This investigation was supported by the Netherlands Organization for Scientific Research and by the Netherlands Cancer Foundation.

1. Griffin, J. E. & Wilson, J. D. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), pp. 1919–1944.
2. Green, S. & Chambon, P. (1988) *Trends Genet.* **4,** 309–314.
3. Evans, R. M. (1988) *Science* **240,** 889–895.
4. Beato, M. (1989) *Cell* **56,** 335–344.
5. Trapman, J., Klaassen, P., Kuiper, G. G. J. M., van der Korput, J. A. G. M., Faber, P. W., van Rooij, H. C. J., Geurts van Kessel, A., Voorhorst, M. M., Mulder, E. & Brinkmann, A. O. (1988) *Biochem. Biophys. Res. Commun.* **153,** 241–248.
6. Chang, C., Kokontis, J. & Liao, S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7211–7215.
7. Lubahn, D. B., Joseph, D. R., Sar, M., Tan, J., Higgs, H., Larson, R. E., French, F. S. & Wilson, E. M. (1988) *Mol. Endocrinol.* **2,** 1265–1275.
8. Faber, P. W., Kuiper, G. G. J. M., van Rooij, H. C. J., van der Korput, J. A. G. M., Brinkmann, A. O. & Trapman, J. (1989) *Mol. Cell. Endocrinol.* **61,** 257–262.
9. Tilley, W. D., Marcelli, M., Wilson, J. D. & McPhaul, M. J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 327–331.
10. Kuiper, G. G. J. M., Faber, P. W., van Rooij, H. C. J., van der Korput, J. A. G. M., Ris-Stalpers, C., Klaassen, P., Trapman, J. & Brinkmann, A. O. (1989) *J. Mol. Endocrinol.* **2,** R1–R4.
11. Brown, C. J., Goss, S. J., Lubahn, D. B., Joseph, D. R., Wilson, E. M., French, F. S. & Willard, H. F. (1989) *Am. J. Hum. Genet.* **44,** 264–269.
12. Huckaby, C. S., Conneely, O. M., Beattie, W. G., Dobson, A. D. W., Tsai, M. J. & O'Malley, B. W. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8380–8384.
13. Ponglikitmongkol, M., Green, S. & Chambon, P. (1988) *EMBO J.* **7,** 3385–3388.
14. Pinsky, L. & Kaufman, M. (1987) *Adv. Hum. Genet.* **16,** 299–472.
15. Schweikert, H. U., Schlüter, M. & Romalo, G. (1989) *J. Clin. Invest.* **83,** 662–668.
16. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. (1977) *Biochemistry* **18,** 5294–5299.
17. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
18. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
19. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
20. Brinkmann, A. O., Faber, P. W., van Rooij, H. C. J., Kuiper, G. G. J. M., Ris, C., Klaassen, P., van der Korput, J. A. G. M., Voorhorst, M. M., van Laar, J. H., Mulder, E. & Trapman, J. (1989) *J. Steroid Biochem.* **34,** 307–310.
21. Chen, C. & Okayama, H. (1987) *Mol. Cell. Biol.* **7,** 2745–2752.
22. Van Laar, J. H., Voorhorst-Ogink, M. M., Zegers, N. D., Boersma, W. J. A., Claassen, E., van der Korput, J. A. G. M., Ruizeveld de Winter, J. A., van der Kwast, Th. H., Mulder, E., Trapman, J. & Brinkmann, A. O. (1989) *Mol. Cell. Endocrinol.* **67,** 29–38.
23. McLaughlin, W. H., Milius, R. A., Gill, L. M., Adelstein, S. J. & Bloomer, W. D. (1984) *J. Steroid Biochem.* **20,** 1129–1133.
24. Maddocks, J. L. & Greenan, M. J. (1975) *J. Clin. Pathol.* **28,** 686–687.
25. Gorman, C. M., Moffat, L. F. & Horward, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
26. Seed, B. & Sheen, J. Y. (1988) *Gene* **67,** 271–277.
27. Mount, S. M. (1982) *Nucleic Acids Res.* **10,** 459–472.
28. Lerner, M. R., Boyle, J. A., Mount, S. M., Woling, S. L. & Steitz, J. A. (1980) *Nature (London)* **283,** 220–224.
29. Rogers, J. & Wall, R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1877–1879.
30. Treisman, R., Proudfoot, N. J., Shander, M. & Maniatis, T. (1982) *Cell* **29,** 903–911.
31. Treisman, R., Orkin, S. H. & Maniatis, T. (1983) *Nature (London)* **302,** 591–596.
32. Wieringa, B., Meyer, F., Reiser, J. & Weissmann, C. (1983) *Nature (London)* **301,** 38–43.
33. Aebi, M., Hornig, H. & Weissmann, C. (1987) *Cell* **50,** 237–246.
34. Lubahn, D. B., Brown, T. R., Simental, J. A., Higgs, H. N., Migeon, C. J., Wilson, E. M. & French, F. S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9534–9538.
35. Brown, T. R., Lubahn, D. B., Wilson, E. M., Joseph, D. R., French, F. S. & Migeon, C. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8151–8155.
36. Pratt, W. B., Jolly, D. J., Pratt, D. V., Hollenberg, S. M., Giguere, V., Cadepond, M., Schweizer-Groyer, G., Catelli, M.-G., Evans, R. M. & Baulieu, E.-E. (1988) *J. Biol. Chem.* **263,** 267–273.
37. Picard, D. & Yamamoto, K. R. (1987) *EMBO J.* **6,** 3333–3340.
38. Evans, R. M. (1989) in *The Steroid/Thyroid Hormone Receptor Family and Gene Regulation*, eds. Carlstedt-Duke, J., Eriksson, H. & Gustafsson, J.-A. (Birkhäuser, Basel), pp. 11–28.