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A and B forms of the androgen receptor are present in human genital skin fibroblasts

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ABSTRACT **Two forms of the androgen receptor (AR) protein (apparent molecular masses, ≈110 kDa and ≈87 kDa) are present in human genital skin fibroblasts. The 87-kDa isoform (AR-A) contains an intact C terminus but lacks the normal N terminus found in the 110-kDa isoform (AR-B). AR-A is the same size as the mutant form of AR produced in fibroblasts from an androgen-resistant individual (R776) by initiation of AR synthesis at the internal Met-188 residue of AR-B. The ratio of AR-B to AR-A in fibroblasts derived from normal individuals is ≈10:1. The AR isoforms detected in these experiments resemble the A and B forms of the human progesterone receptor, which also are encoded by a single gene and differ by the absence or presence of an N-terminal segment. The A and B forms of the human progesterone receptor differ in their ability to activate target genes and are regulated differently in various types of cells. The identification of similar forms of human AR raises the possibility that AR-A and AR-B also subserve different functions.**

Androgen action is mediated by a high-affinity androgen receptor (AR) protein that is expressed in target tissues. Genetic defects that impair the function of this molecule in 46,XY males can cause a spectrum of phenotypic abnormalities, ranging from subjects with a complete female phenotype (complete testicular feminization) to men with infertility or minor degrees of undervirilization (1). In common with other members of the thyroid/steroid/retinoic acid receptor superfamily of ligand-activated transcription regulation factors, the AR protein contains an N-terminal segment that is involved in transactivation, a central DNA-binding domain, and a C-terminal hormone-binding domain (Fig. 1*A*). Within this receptor family, diversity is generated in at least two ways: in some instances receptors that respond to the same ligand are encoded by different genes (e.g., retinoic acid and thyroid hormone receptors). In other cases, isoforms of the same receptor are derived from a single gene. In some instances, both mechanisms appear to operate. The different receptors and isoforms are believed to subserve different functions (for review, see refs. 3–6).

The AR, glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and progesterone receptor (PR) proteins make up the GR-like family of nuclear steroid receptors, characterized by high sequence homology and the ability to interact with the same steroid hormone response element consensus sequence (5). Each of the GR-like receptors is encoded by a unique single copy gene. Two naturally occurring hormone binding forms of PR have been identified (for review, see ref. 6). The PR-A isoform is an N-terminally truncated version of the full-length PR-B isoform. Several lines of evidence suggest that the PR-A and PR-B isoforms serve specific functions. Tora *et al.* (7) demonstrated promoter and cell-specific differences in the ability of PR-A and PR-B isoforms of the chicken to activate transcription of target genes, and two groups have shown that human PR-A and PR-B functions can be different, depending on cell and promoter context (8, 9). Furthermore, PR-A can act under different conditions as an activator of gene transcription, a dominant repressor of activation by PR-B, or an inhibitor of GR-, AR-, or MR-mediated gene transcription (9, 28).

The normal human AR, detected by immunoblot analysis with antibodies that recognize the N terminus, appears as an incompletely resolved doublet band migrating in SDS/PAGE with an apparent molecular mass of 110 kDa (10). This AR doublet is also found in the human prostate tumor cell line LNCaP and is believed to reflect posttranslational phosphorylation of the 110-kDa AR gene product (11). In a previous study (12), we identified a mutation in the N-terminal region of the AR gene carried by a 46,XY phenotypic female (R776) with the syndrome of complete testicular feminization; this mutation introduces a premature termination codon in place of the normal AR codon for amino acid 60 and results in reduced levels of qualitatively abnormal AR in cultured genital skin fibroblasts. COS cells transfected with cDNA encoding the R776 mutation produce an 87-kDa form of AR that lacks the normal N terminus. Although the mutant AR can activate an androgen-responsive reporter gene coexpressed in CV1 cells, the level of activation appears markedly reduced when compared to the effect of normal full-length AR in this system. The 87-kDa AR isoform is recognized in immunoblots by antibodies directed at an epitope [internal A (Int A), amino acids 200–220] within the N-terminal segment of human AR (Fig. 1*B*). These findings suggested that synthesis of the mutant 87-kDa AR protein is initiated at the first internal methionine residue (Met-188, Fig. 1*C*).

In this report we show that normal genital skin fibroblasts contain both the full-length 110-kDa AR protein (termed AR-B) and an 87-kDa N-terminally truncated AR isoform (termed AR-A). Thus, a second member of the GR-like steroid receptor family exists in more than one hormone-binding form. The similarity between the AR isoforms and the more extensively studied PR isoforms raises the possibility that the A and B forms of AR may play distinct roles in the regulation of gene expression by androgens.

METHODS

Cell Culture and Extraction. Genital skin fibroblasts were grown to confluence, and soluble whole-cell extracts were prepared and stored as described (10) except that cell pellets were homogenized in 4 vol of high salt buffer [pH 8.5; 50 mM Tris/1.25 mM EDTA/12.5 mM dithiothreitol/12.5% (vol/vol) glycerol/62.5 mM NaF/12.5 mM sodium molybdate/

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Abbreviations: Int A, internal A; AR, androgen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor.

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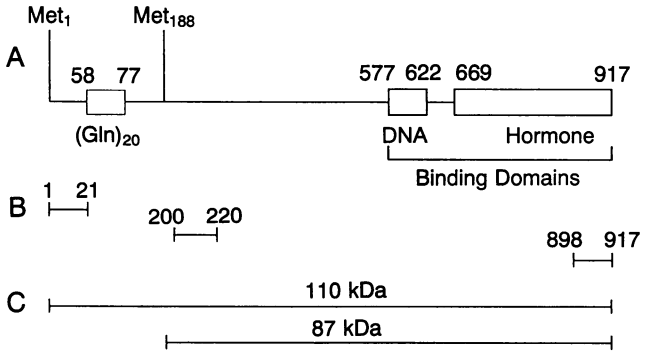

FIG. 1. (*A*) Schematic representation of the human AR showing the position of the glutamine (Gln) homopolymeric region, the normal initiation methionine (Met-1), the first internal methionine residue (Met-188), and the DNA- and hormone-binding domains. Numbers represent the amino acid coordinates predicted from the nucleotide sequence of AR cDNA. The coordinates used throughout correspond to those of Tilley *et al.* (2). (*B*) Synthetic peptide antigens used to elicit antibodies that recognize N-terminal (amino acids 1–21), Int A (amino acids 200–220), or C-terminal (amino acids 898–917) regions of the AR protein. (*C*) The apparent molecular mass of AR proteins initiated at Met-1 (110 kDa) or Met-188 (87 kDa).

0.625 mM bacitracin/0.025 mM leupeptin/0.75 mM phenylmethylsulfonyl fluoride/0.625 M NaCl] and diluted 1:1 with 2× SDS/PAGE loading buffer [200 mM dithiothreitol/4% (wt/vol) SDS/20% glycerol/0.008% bromophenol blue/160 mM Tris·HCl, pH 6.9] prior to electrophoresis. The presence of additional protease inhibitors [20 mM benzamidine/pepstatin A (1 μg/ml)/aprotinin (77 μg/ml)/0.1 mM leupeptin] in the standard homogenization buffer did not change the results obtained with 704 fibroblasts in a pilot experiment.

SDS/PAGE and Immunoblot Analysis. Western blots were prepared as described (10) except that SDS/PAGE was performed in "low bis" gels containing 7.5% (wt/vol) acrylamide, 0.05% *N,N'*-methylenebisacrylamide, and 0.1% SDS. Electrophoresis was continued until a prestained 49.5-kDa protein molecular mass marker (Bio-Rad) was within 1 cm of the bottom of the gel. Under these conditions, the two forms of full-length AR, which migrate as an incompletely resolved 110-kDa doublet in standard 7.5% gels, are clearly resolved into two bands, well-separated from the 87-kDa form of AR, which appears to migrate as a single species. After transfer to nitrocellulose and incubation with anti-peptide antibodies, immunoreactive proteins were labeled with ^{125}I-labeled anti-rabbit IgG and visualized by autoradiography. Blocking experiments were performed as before with antibodies preincubated with excess peptide antigen (13). Relative levels of immunoreactive AR were estimated by densitometric analysis (10).

Molecular sizes were estimated by comparison with radiolabeled standard markers electrophoresed in the same gel (Rainbow molecular weight markers, Amersham). Polyclonal antibodies were obtained from rabbits U402, U407, and R489 that had been immunized, respectively, with the N-terminal, Int A, and C-terminal synthetic peptides, illustrated in Fig. 1*B*, and were purified on peptide affinity columns (13).

Immunoadsorption of AR Proteins. Antibody–Sepharose conjugates were prepared by incubating affinity-purified anti-C-terminal, anti-N-terminal, or anti-Int A antibodies (0.4–0.6 mg of protein) with 0.1 g of CNBr-activated Sepharose, preswollen as directed by the manufacturer (Pharmacia LKB), in 2 ml of coupling buffer (0.25 M Na_2CO_3/0.5 M NaCl, pH 8.5) overnight at 4°C on an end-over-end rotator. Control CNBr-Sepharose was incubated without antibodies. The remaining active groups were blocked by incubation for 4 h at 4°C with 0.2 M glycine (pH 8). Control Sepharose and antibody-Sepharose conjugates were washed twice with coupling buffer followed by 0.3 M KCl/0.1 M sodium acetate, pH 4, and resuspended in 2 ml of PBS (0.14 M NaCl/2.7 mM KCl/1.5 mM KH_2PO_4/8 mM Na_2HPO_4). Pellets obtained from 0.5-ml aliquots were incubated for 4 h on an end-over-end rotator at room temperature with 150-μl samples of high salt fibroblast extracts. The supernatant fraction containing free AR was separated by centrifugation for 5 min at 16,000 ×*g*, recentrifuged, and diluted 1:1 with 2× SDS/PAGE loading buffer. The precipitate fraction containing immunoadsorbed AR was washed with three 1-ml portions of Tris-buffered saline (20 mM Tris·HCl/0.14 M NaCl, pH 7.6) containing 0.1% Triton X-100 and with one 1-ml portion of PBS, resuspended in 300 μl of 1× SDS/PAGE loading buffer, heated for 3 min in boiling water, and clarified by centrifugation prior to electrophoresis.

RESULTS

An 87-kDa Form of AR Is Present in Genital Skin Fibroblasts from Patient R776 and from a Normal Individual (Strain 704). Genital skin fibroblasts from normal individuals express full-length AR protein, which appears as a 110-kDa doublet in immunoblots prepared with antibodies that recognize either the N-terminal sequence of amino acids 1–21 (10) or the Int A sequence of 200–220 amino acids (strain 704, Fig. 2*A*) encoded by the normal AR gene. Anti-Int A antibodies also recognize an 87-kDa protein present in 704 fibroblasts. A similar protein band is identified by anti-Int A antibodies in extracts from fibroblasts (strain R776) that carry a mutant AR gene (12) and produce an N-terminally truncated form of the AR protein. As shown in Fig. 2*B*, both 110-kDa and 87-kDa AR isoforms are absent from genital skin fibroblasts (strain 1009) derived from an androgen-resistant subject with complete deletion of the coding portion of the AR gene (14). This result indicates that both AR isoforms are encoded by the same gene.

The 87-kDa AR Isoform Is Present in Normal Genital Skin Fibroblasts. A survey of immunoreactive AR proteins in fibroblast strains showed that both 110-kDa and 87-kDa forms are detectable in samples from seven unrelated normal

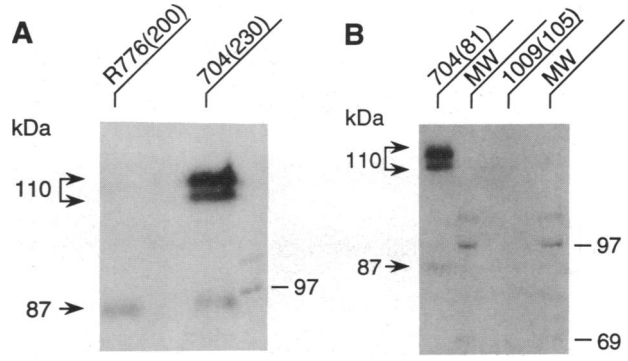

FIG. 2. Detection of 87-kDa AR-A and 110-kDa AR-B isoforms by immunoblot analysis with anti-Int A antibodies. (*A*) An 87-kDa form of AR is expressed by fibroblasts cultured from an androgen-resistant subject (strain R776) carrying a mutant AR gene (12). Fibroblasts from a control subject (strain 704) with the normal AR gene produce both 87-kDa and 110-kDa AR isoforms. (*B*) Neither form of AR protein is detected in fibroblasts from an individual (strain 1009; subject 8812 in ref. 14) who lacks the entire coding region of the AR gene, whereas both forms were detected in control fibroblasts (strain 704). Extracts of genital skin fibroblasts were fractionated by SDS/PAGE and analyzed on an immunoblot as described in text. Numbers in parenthesis indicate the amount of protein (μg) applied to each lane. Positions and sizes of labeled molecular mass markers (lane MW) are shown on the right of each radiogram. The positions of the AR isoform bands are indicated by arrows on the left.

individuals (Fig. 3 *Upper Left*). The 87-kDa isoform is 7–15% of the total AR recognized by anti-Int A antibodies in these samples. The position of 87-kDa AR bands appears similar in all cases. Polymorphism in the length of the glutamine homopolymeric tract encoded by normal AR genes (see below) may account for variation in the positions of AR doublet bands located in the 110-kDa region of both immunoblots.

The 87-kDa AR isoform is not recognized by anti-N-terminal antibodies (Fig. 3 *Lower Left*). Furthermore, the reaction of 87-kDa and 110-kDa AR isoforms with these anti-peptide antibodies is specific and can be blocked by preincubating antibodies with excess peptide antigen (Fig. 3 *Upper Right* and *Lower Right*).

Changes in the Size of a Polyglutamine Tract in the N-Terminal AR Region Influence the Mobility of 110-kDa AR on SDS/PAGE but Have No Effect on the Mobility of 87-kDa AR. A polymorphic tandem CAG/CAA repeat in exon 1 of the AR gene encodes a homopolymeric glutamine repeat (Fig. 1*A*) that varies from 11 to 31 glutamine residues in normal subjects (15). Although the R776 mutant AR gene contains 21 CAG/CAA repeats, the polyglutamine tract is not present in the 87-kDa isoform initiated at Met-188 in R776 fibroblasts. AR genes carried by individuals with X chromosome-linked spinal and bulbar muscular atrophy (Kennedy syndrome) encode homopolymeric tracts with >40 glutamine residues (16). Immunoblot analysis of fibroblast strains from two such patients (strain 814, Fig. 4; strain 703, data not shown) reveals a decrease in the mobility of the full-length AR doublet compared to a normal control (strain 704), which has 20 CAG/CAA repeats but no change in mobility of the 87-kDa AR isoform. Mobility of the 87-kDa AR isoform from an androgen-resistant subject (strain 980) whose AR gene contains 15 CAG/CAA repeats also is normal, although the full-length AR doublet from this individual moves faster on SDS/PAGE. These results demonstrate that variation in the number of glutamine repeats influences mobility of the 110-kDa AR isoform but does not alter the mobility of the 87-kDa AR isoform, in keeping with the prediction that the shorter protein is initiated at a site downstream from the polyglutamine tract.

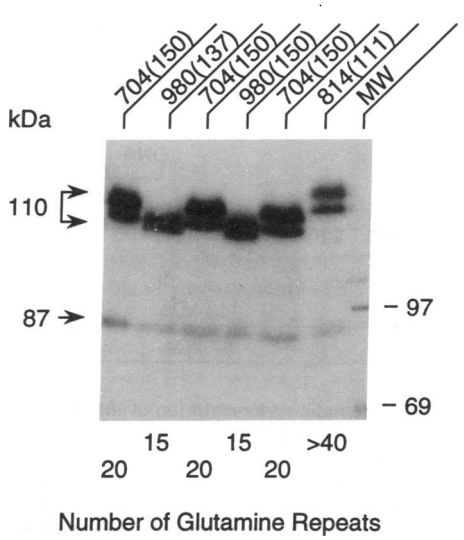

FIG. 4. Length of the polymorphic homopolymeric glutamine (Gln) tract influences the mobility of the 110-kDa AR-B isoform but has no effect on the 87-kDa AR-A isoform. Genital skin fibroblasts were derived from individuals whose AR genes contain different numbers of the CAG/CAA repeats in AR exon 1 that encode a homopolymeric Gln tract. The number of CAG/CAA repeats was estimated from nucleotide sequence analysis. Immunoblot analysis of fibroblast extracts was performed with anti-Int A antibodies as described in text. Numbers in parentheses indicate the amount of protein (μg) applied to each lane. Positions and sizes of labeled molecular mass markers (lane MW) are shown on the right. The positions of the AR isoform bands are indicated by arrows on the left.

The Immunoreactive AR C Terminus Is Present in both 110-kDa and 87-kDa Forms of AR. AR levels in genital skin fibroblasts are too low to be detected by immunoblot analysis with the anti-C-terminal antibodies available to us. For this reason, we used anti-C-terminal antibodies linked to Sepharose to determine whether the 87-kDa AR isoform contains the normal AR C terminus. This experiment also included Sepharose linked to anti-N-terminal antibodies, Sepharose linked to anti-Int A antibodies, and Sepharose without linked

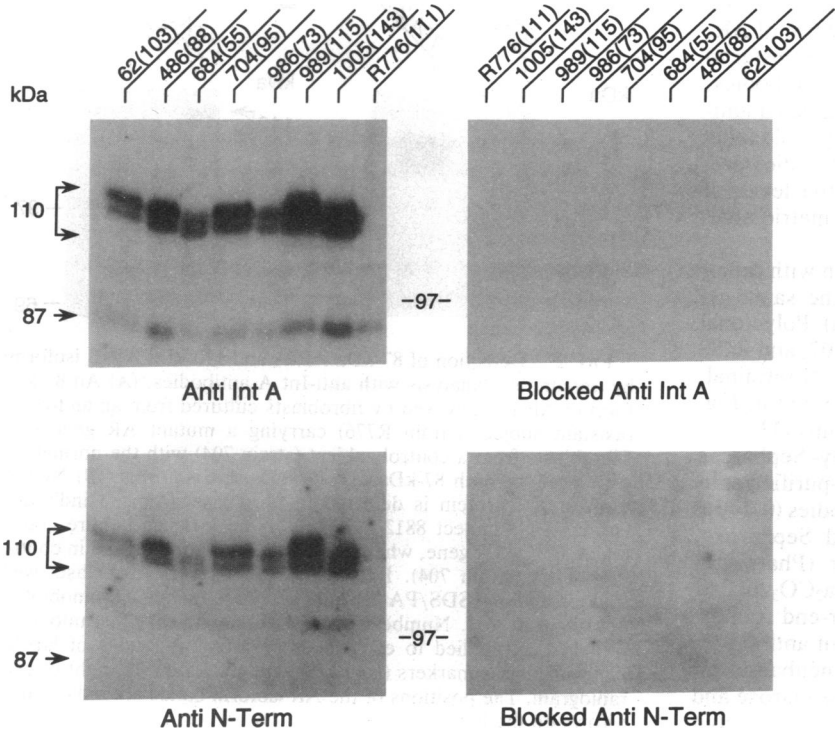

FIG. 3. AR-A (87-kDa) isoform is detected by anti-Int A antibodies in fibroblast strains from normal individuals but not by anti-N-terminal (N-term) antibodies. Fibroblasts derived from the seven unrelated normal individuals identified by numbers at the top or from R776, an androgen-resistant patient (described in ref. 12) who expresses an N-terminally truncated 87-kDa form of AR, were extracted, fractionated by SDS/PAGE, and analyzed on an immunoblot with anti-Int A antibodies and exposed for 18 h (*Upper*) or analyzed on an immunoblot with anti-N-terminal (N-term) antibodies and exposed for 64 h (*Lower*). Doubling the exposure time revealed no additional immunoreactive bands (data not shown). Antibodies were preincubated with PBS (*Left*) or were blocked by preincubation with the appropriate peptide antigen (*Right*). Each sample represents 10^6 cells. Numbers in parentheses indicate the amount of protein (μg) applied to each lane. The position and size of a labeled molecular mass marker electrophoresed in the same gels are shown in the center. The positions of the AR isoform bands are indicated by arrows on the left.

antibodies. R776 fibroblast extracts containing 87-kDa AR (Fig. 5 *Upper*) or 704 fibroblast extracts containing both 87-kDa and 110-kDa AR isoforms (Fig. 5 *Lower*) were incubated with Sepharose–antibody conjugates to immunoabsorb reactive AR proteins or with unlinked Sepharose to determine the amount of nonspecific absorption. Insoluble Sepharose–antibody–AR protein complexes were separated from free AR proteins by centrifugation. The supernatant and precipitate fractions were subjected to immunoblot analysis with anti-Int A antibodies that recognize both AR isoforms. The 87-kDa and 110-kDa AR bands shown in Fig. 5 indicate the amount of free and immunoabsorbed AR in each treated sample compared to the amount of each isoform present in the original unfractionated sample.

Both AR isoforms remain in the supernatant after treatment with unlinked Sepharose as shown in Fig. 5, lanes Control S. The faint 87-kDa and 110-kDa bands visible in lanes Control P probably represent nonspecific trapping of AR proteins in the precipitate. The 110-kDa AR is recognized by anti-N-terminal antibodies linked to Sepharose and the majority of this isoform appears in the precipitate (Fig. 5 *Lower*, lane Anti-N-Term P). As expected from previous results (12), the 87-kDa isoform does not react with anti-N-terminal antibodies and remains in the supernatant. Several faint bands are present in the precipitate obtained with anti-N-terminal antibodies linked to Sepharose and are also produced in the absence of fibroblast extracts (data not shown), presumably reflecting nonspecific reactivity of the anti-N-terminal antibodies used in this experiment.

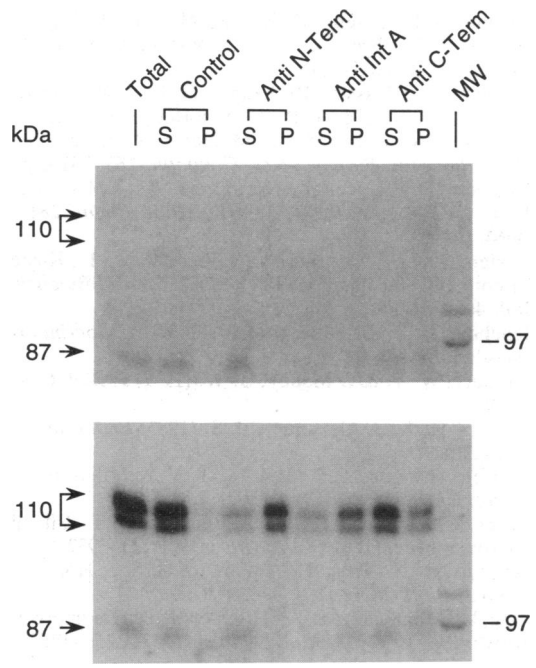

FIG. 5. AR C terminus is present in both 110-kDa AR-B and 87-kDa AR-A isoforms. Extracts from fibroblast strains that produce an N-terminally truncated 87-kDa form of AR (strain R776) (*Upper*) or both 87-kDa and 110-kDa AR isoforms (strain 704) (*Lower*) were diluted with SDS/PAGE loading buffer (lane Total), incubated with Sepharose without linked antibodies (lanes Control), or incubated with Sepharose linked to antibodies directed at the N terminus (lanes Anti-N Term), C terminus (lanes Anti-C-Term), or the sequence of amino acids 200–220 (lanes Anti-Int A) of human AR. AR proteins immunoabsorbed by antibodies linked to Sepharose were separated from unreacted AR proteins by centrifugation. Free AR proteins remained in the supernatant (lanes S) fraction. Immunoabsorbed AR proteins were precipitated with the antibody–Sepharose complex. The precipitate (lanes P) fraction was resuspended in 1× SDS/PAGE loading buffer and heated to release AR proteins prior to electrophoresis. Equivalent amounts of each sample were fractionated by SDS/PAGE and analyzed on an immunoblot with anti-Int A antibodies. The position and size of a labeled molecular mass (lane MW) marker are shown on the right. The positions of the AR isoform bands are indicated by arrows on the left.

Anti-Int A antibodies recognize both 87-kDa and 110-kDa ARs and immunoabsorption with anti-Int A antibodies linked to Sepharose shifts the majority of both isoforms to the precipitate fraction. Attempts to increase the amount of AR immunoabsorbed by adding more antibody–Sepharose conjugate to the reaction mixture were not informative because of the concomitant increase in nonspecific absorption by unlinked Sepharose in the control sample.

The 87-kDa AR band from R776 fibroblasts is present in both precipitate and supernatant fractions obtained with anti-C-terminal antibodies linked to Sepharose (Fig. 5 *Upper*). The same results are seen with both 87-kDa and 110-kDa AR isoforms produced by 704 fibroblasts (Fig. 5 *Lower*). As previously observed, anti-C-terminal antibodies do not react with AR proteins as efficiently as the anti-N-terminal antibodies or anti-Int A antibodies (12, 13). However, the observation that the 87-kDa AR isoform is recognized by anti-C-terminal antibodies in this experiment indicates that the immunoreactive C terminus of normal AR protein is present in the 87-kDa AR molecule and provides further evidence that the truncated AR protein present in normal genital skin fibroblasts is the same 87-kDa isoform produced by mutant R776 cells.

DISCUSSION

Two forms of the AR are present in normal human fibroblasts. The predominant AR isoform (AR-B; apparent molecular mass, ≈110 kDa) is predicted by the complete coding sequence of the human AR cDNA (2, 17–19). It migrates as a doublet and reacts with antibodies directed at an epitope (Int A, amino acids 200–220) within the N-terminal segment of the molecule and with antibodies directed against either the N terminus or C terminus of the AR open-reading frame. The second form (AR-A; apparent molecular mass, ≈87 kDa) was first detected in our study (12) of the mutant AR gene from a patient with androgen resistance (R776). AR-A is recognized by anti-Int A antibodies and anti-C-terminal antibodies but not by anti-N-terminal antibodies. It is present at similar levels in R776 fibroblasts and in fibroblast strains from normal individuals. These findings suggest that AR-A is produced in genital skin fibroblasts by initiation of protein synthesis at the internal Met-188 residue of AR-B. This mechanism has also been proposed to account for a similar form of AR produced by *in vitro* translation of *in vitro*-synthesized AR mRNA (17).

The two forms of human AR protein identified in this report show a striking resemblance in structure to the A and B isoforms of PR (for review, see ref. 6). PR-A is an N-terminally truncated variant of PR-B that may arise by translation initiation at an internal methionine codon of the PR mRNA (20) or by translation of a specific PR mRNA (21). The ratio of PR-A to PR-B in different species varies widely. For example, PR-A is the predominant isoform in rat uterus (22), equivalent amounts of PR-A and PR-B are found in chicken oviduct (23) and human breast cells (24), and PR-B may be the only isoform in rabbit uterus (25).

Several studies indicate that PR-A and PR-B isoforms are functionally and structurally distinct (7–9, 28). Depending on the promoter and cell context, the two forms of PR can influence gene transcription differently. For example, Kastner *et al.* (8) found that A and B isoforms of human PR activate transcription from an artificial promoter composed of a progesterone/glucocorticoid response element fused to the thymidine kinase promoter similarly, that PR-B is more effective than PR-A in activating the mouse mammary tumor

virus promoter, and that PR-A, but not PR-B, activates transcription from the ovalbumin promoter. Furthermore, progesterone-dependent activation of the mouse mammary tumor virus promoter is regulated differently in different types of cells transfected with expression vectors encoding either PR-A or PR-B. Only PR-B is effective in CV1 cells, PR-A is less effective than PR-B in HeLa cells, and both isoforms function efficiently in the HepG2 cell line (9).

Functional differences between the A and B forms of human PR also extend to their activation by antiprogestins. Human PR-A is inactive in reporter gene assays when complexed to the antiprogestin mifepristone (RU 486), whereas PR-B is able to activate transcription in the presence of mifepristone (26–28). In some contexts, PR-A can inhibit receptor-mediated gene transcription by PR-B (9, 28) and interfere with gene activation by GR, MR, and AR (9). Neither stoichiometric amounts of PR-A nor the presence of a functional DNA-binding domain in the PR-A isoform are required for these inhibitory actions, suggesting the effects are mediated via sequestration of cell-specific cofactors.

The functional roles of the A and B isoforms of human AR remain to be elucidated. Both the genetic evidence of patient R776 and the abnormally low level of transactivation observed in CV1 cells cotransfected with the mouse mammary tumor virus–chloramphenicol acetyltransferase reporter gene and cDNA encoding the R776 mutant AR protein (12) indicate that AR-A by itself is not capable of mediating all androgen action. However, we do not know whether this truncated AR isoform can activate some reporter genes, whether it can function in other types of cells, whether it reacts with antiandrogens, or whether it can influence the ability of the full-length AR-B isoform to mediate gene transcription. Furthermore, it is not known whether the level of AR-A expression in some tissues or at different developmental stages may be higher than the roughly 10% of total AR expression observed in normal human genital skin fibroblasts. Additional studies are required to establish the functional significance and pattern of AR isoform expression.

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1. Wilson, J. D. (1992) *Biol. Reprod.* **46**, 168–173.
2. Tilley, W. D., Marcelli, M., Wilson, J. D. & McPhaul, M. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 327–331.
3. Evans, R. M. (1988) *Science* **240**, 889–895.
4. Carson-Jurica, M. A., Schrader, W. T. & O'Malley, B. W. (1990) *Endocr. Rev.* **11**, 201–220.
5. Wahli, W. & Martinez, E. (1991) *FASEB J.* **5**, 2243–2249.
6. Horwitz, K. B. (1992) *Endocr. Rev.* **13**, 146–163.
7. Tora, L., Gronemeyer, H., Turcotte, B., Gaub, M.-P. & Chambon, P. (1988) *Nature (London)* **333**, 185–188.
8. Kastner, P., Krust, A., Turcotte, B., Stropp, U., Tora, L., Gronemeyer, H. & Chambon, P. (1990) *EMBO J.* **9**, 1603–1614.
9. Vegeto, E., Shahbaz, M. M., Wen, D. X., Goldman, M. E., O'Malley, B. W. & McDonnell, D. P. (1993) *Mol. Endocrinol.* **7**, 1244–1255.
10. Wilson, C. M., Griffin, J. E., Wilson, J. D., Marcelli, M., Zoppi, S. & McPhaul, M. J. (1992) *J. Clin. Endocrinol. Metab.* **75**, 1474–1478.
11. Kuiper, G. G. J. M., deRuiter, P. E. & Brinkmann, A. O. (1992) *J. Steroid Biochem. Mol. Biol.* **41**, 697–700.
12. Zoppi, S., Wilson, C. M., Harbison, M. D., Griffin, J. E., Wilson, J. D., McPhaul, M. J. & Marcelli, M. (1993) *J. Clin. Invest.* **91**, 1105–1112.
13. Husmann, D. A., Wilson, C. M., McPhaul, M. J., Tilley, W. D. & Wilson, J. D. (1990) *Endocrinology* **126**, 2359–2368.
14. Trifiro, M., Gottlieb, B., Pinsky, L., Kaufman, M., Prior, L., Belsham, D. D., Wrogemann, K., Brown, C. J., Willard, H. F., Trapman, J., Brinkmann, A. O., Chang, C., Liao, S., Sergovich, F. & Jung, J. (1991) *Mol. Cell. Endocrinol.* **75**, 37–47.
15. Edwards, A., Hammond, H. A., Jin, L., Caskey, C. T. & Chakraborty, R. (1992) *Genomics* **12**, 241–253.
16. La Spada, A. R., Wilson, E. M., Lubahn, D. B., Harding, A. E. & Fischbeck, K. H. (1991) *Nature (London)* **352**, 77–79.
17. Chang, C., Kokontis, J. & Liao, S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7211–7215.
18. Lubahn, D. B., Joseph, D. R., Sar, M., Tan, J., Higgs, H. N., Larson, R. E., French, F. S. & Wilson, E. M. (1988) *Mol. Endocrinol.* **2**, 1265–1275.
19. Trapman, J., Klaassen, P., Kuiper, G. G. J. M., van der Korput, J. A. G. M., Faber, P. W., van Rooij, H. C. J., van Kessel, A. G., Voorhorst, M. M., Mulder, E. & Brinkmann, A. O. (1988) *Biochem. Biophys. Res. Commun.* **153**, 241–248.
20. Conneely, O. M., Kettelberger, D. M., Tsai, M.-J., Schrader, W. T. & O'Malley, B. W. (1989) *J. Biol. Chem.* **264**, 14062–14064.
21. Gronemeyer, H., Meyer, M.-E., Bocquel, M.-T., Kastner, P., Turcotte, B. & Chambon, P. (1991) *J. Steroid Biochem. Mol. Biol.* **40**, 271–278.
22. Ilenchuk, T. T. & Walters, M. R. (1987) *Endocrinology* **120**, 1449–1456.
23. Schrader, W. T. & O'Malley, B. W. (1972) *J. Biol. Chem.* **247**, 51–59.
24. Horwitz, K. B. & Alexander, P. S. (1983) *Endocrinology* **113**, 2195–2201.
25. Loosfelt, H., Logeat, F., Hai, M. T. V. & Milgrom, E. (1984) *J. Biol. Chem.* **259**, 14196–14202.
26. Meyer, M.-E., Pornon, A., Ji, J., Bocquel, M.-T., Chambon, P. & Gronemeyer, H. (1990) *EMBO J.* **9**, 3923–3932.
27. Sartorius, C. A., Tung, L., Takimoto, G. S. & Horwitz, K. B. (1993) *J. Biol. Chem.* **268**, 9262–9266.
28. Tung, L., Mohamed, M. K., Hoeffler, J. P., Takimoto, G. S. & Horwitz, K. B. (1993) *Mol. Endocrinol.* **7**, 1256–1265.