Vol. 153, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1988 Pages 241-248

CLONING, STRUCTURE AND EXPRESSION OF A cDNA ENCODING THE HUMAN ANDROGEN RECEPTOR

J. Trapman[1], P. Klaassen[1], G.G.J.M. Kuiper[2], J.A.G.M. van der Korput[1], P.W. Faber[1], H.C.J. van Rooij[1], A. Geurts van Kessel[3], M.M. Voorhorst[2], E. Mulder[2] and A.O. Brinkmann[2]

Department of Pathology[1], Department of Biochemistry II[2] and Department of Cell Biology and Genetics[3], Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam The Netherlands

Received April 14, 1988

A cDNA clone has been isolated from a library prepared of mRNA of human breast cancer T47D cells with an oligonucleotide probe homologous to part of the region encoding the DNA-binding domain of steroid receptors. The clone has a size of 1505 bp and sequence analysis revealed an open reading frame of 1356 bp. The deduced amino acid sequence displays two highly conserved regions identified as the putative DNA-binding and hormone binding domains respectively of steroid receptors. Expression of this cDNA clone in COS cells produces a nuclear protein with all the binding characteristics of the human androgen receptor (hAR). The gene encoding the cDNA is assigned to the human X-chromosome. High levels of three hybridizing mRNA species of 11, 8.5 and 4.7 kb respectively are found in the human prostate cancer cell line (LNCaP), which contains elevated levels of hAR. The present data provide evidence that we have isolated a cDNA that encodes a major part of the human androgen receptor.

© 1988 Academic Press, Inc.

The elucidation of the primary structure of several human steroid receptors after molecular cloning of their corresponding cDNA's has increased considerably our knowledge of the functional properties of these gene regulatory proteins [1-5]. Structural comparison revealed that steroid receptors are members of a supergene family of ligand responsive transcriptional regulators which also includes thyroid hormone receptors and the retinoic acid receptor [6-11]. Among the steroid hormone receptors the androgen receptor is the least well characterized. All steroid receptor molecules identified so far are composed of a putative DNA-binding domain and a ligand binding domain at the C-terminal end [1-5; 12-14]. The DNA-binding region is the most conserved part and we have used the homology in this region for screening a cDNA-library for cDNA's encoding the human androgen receptor, assuming that the androgen receptor belongs to the same nuclear receptor multigene family. In the present study the cloning, structure and expression of a cDNA encoding a major part of the human androgen receptor is described.

0006-291X/88 $1.50
Copyright © 1988 by Academic Press, Inc.
All rights of reproduction in any form reserved.

MATERIALS AND METHODS

cDNA library screening

A λgt10 cDNA library prepared from mRNA of human breast cancer T47-D cells was kindly provided by Drs. E. Milgrom and M. Atger (Paris). An oligonucleotide corresponding to the most homologous part of the human progesterone, estrogen and glucocorticoid receptor DNA-binding domain [1-4] (3'-GGACGCTTTCGAC-GTTTCGGAAGAAGTTTTCTTGTTACCTTCCTGT-5') was synthesized, ^{32}P-end labeled and used for screening of the cDNA library. Duplicate nitrocellulose filters (Millipore, Molsheim, France) were hybridized overnight at 42°C in 6xSSC, 10xDenhardt solution containing 0.1 % SDS and 100 μg/ml salmon sperm DNA. Filters were washed in 3xSSC, 0.1% SDS (2x20 min) at 42°C and 1xSSC (1x20 min) at room temperature. After drying, filters were exposed for 16 to 64 h to a Kodak X-AR5 film at -70°C using intensifyer screens. Positive plaques were isolated by two cycles of purification.

Characterization of cDNA

EcoRI restriction fragments of positive clones were isolated, subcloned into pUC9, and a restriction map was prepared using standard methods [15]. Appropriate restriction fragments were immobilized on nitrocellulose filters and hybridized to the ^{32}P-end labeled oligonucleotide probe to map more precisely the hybridizing region. For sequencing by the dideoxy chain termination method [16] fragments were subcloned into M13mp 18/19.

Northern blot analysis

Total cellular RNA was isolated by the guanidinium thiocyanate method [17]. PolyA$^+$ RNA was prepared by oligo- dT cellulose chromatography. RNA was denatured by glyoxal treatment, separated by electrophoresis on a 1% agarose gel and transferred to a nylon membrane (GeneScreen; NEN, Boston) using the methods described by the manufacturer. Filters were hybridized with cDNA probes labeled as indicated [18]. Hybridization under stringent conditions (42°C in 50% formamide) and washing of the filters were as described by NEN. After washing, filters were exposed to X-ray film as described above.

Southern blot analysis and chromosome mapping

Somatic cell hybrids were generated by fusion of human cells with the hamster A3 cell line as described [19]. DNA was isolated from control and hybrid cells and analyzed by Southern blotting. Hybridization of nitrocellulose filter with ^{32}P-labeled cDNA probes was carried out overnight at 65°C in 6xSSC, 10xDenhardt solution containing 0.1% SDS and 100 μg salmon sperm DNA. Filters were washed at 65°C with several changes of SSC dilutions down to 0.3xSSC and exposed to X-ray film as described above. Chromosome analysis of the hybrid cells was carried out using reverse banding with acridine orange after heat denaturation.

Transfections and hormone binding analysis

The 1.4 kb Asp718-EcoRI fragment of the cDNA clone was inserted into the SmaI-EcoRI site of the eukaryotic expression vector pSV328A [20]. The pSV-AR construct was transiently expressed in COS cells using the DEAE-dextran method and 2 μg/ml of the expression vector. Cells were maintained for 3 days in Dulbecco's modification of Eagle's minimal essential medium supplemented with 5% fetal calf serum and antibiotics. For analysis of cytoplasmic binding cells were maintained during the third day on steroid depleted (charcoal stripped) medium. Cytosol was prepared in hypotonic buffer (40 mM Tris HCl (pH:7.4), 1 mM EDTA, 10% glycerol, 10 mM dithiotreitol, 10 mM molybdate and 0.6 mM phenylmethylsulfonyl fluoride) and relative binding affinities were determined with an established procedure [21]. For this purpose incubations were performed for 1 h at 4°C with 5 nM [3H]-5α-dihydrotestosterone (DHT). For analysis of nuclear binding the cells were incubated for 1 h at 37°C with 10 nM [3H]-R1881 (17α-methyl-3H-methyltrienolone) ± 100 fold molar excess of nonradioactive hormone either in the presence or absence of a 500-fold molar excess of triamcinolone acetonide. The nuclear extracts were analyzed for specific androgen binding sites on 5-20% sucrose gradients as described previously [22]. Scatchard plot analysis was performed after total cell uptake of [3H]-R1881 and separation of bound from unbound steroid with an oil-micro assay [23].

RESULTS AND DISCUSSION

A T47-D cDNA library in λgt10 ($2x10^5$ independent clones) was screened under low-stringency conditions with an oligonucleotide probe corresponding to a part of the DNA-binding domain of steroid receptors (see Materials and Methods). After several rounds of screening and purification one clone (designated 7A2M27) was isolated which specifically hybridized with the oligonucleotide probe, even under stringent conditions. The insert was subcloned and a partial restriction map was determined (Fig. 1). The digestion pattern indicated that clone 7A2M27 represented a cDNA, which was different from all presently known steroid receptor cDNAs. The cDNA had a size of 1505 bp and contained one internal EcoRI site. Nucleotide sequence analysis of clone 7A2M27 revealed an open reading frame of 1356 bp with a 149 bp stretch of non-coding sequences at the 3'end (Figures 1 and 2). The deduced sequence of 452 amino acids is shown in Figure 2. Two regions can be distinguished which show a high degree of homology with the structure of steroid receptors particularly the human progesterone and glucocorticoid receptors [1,4]. First a region rich in cystein, lysine and arginine residues (amino acids 90-154) which showed 83% and 80% homology with the DNA-binding domain of the human progesterone and glucocorticoid receptors respectively. All ten highly conserved cystein residues present in the DNA-binding domain of the various receptors were also present in 7A2M27 (Figure 3A, arrows). Interestingly, a histidine residue (at position 124) present in the putative second DNA-binding finger of all steroid receptors is absent in 7A2M27. A similar finding has also been reported for the vitamin D3 receptor [24,25]. Therefore the importance of a histidine residue for the secondary structure of the second DNA-binding finger, as repeatedly stated, has to be questioned [26].

Further structural analysis revealed that the 3'-end of the open reading frame (amino acids 203-452) encodes a domain with a homology of 55% and 50% with the corresponding region (steroid binding region) of the progesterone and glucocorticoid receptor respectively (Figure 3B). Homology with other receptors is much lower in this region (data not shown). These findings provide strong evidence that 7A2M27 is a member of the steroid receptor multigene family. The cDNA isolated, however, lacks the 5'-non-coding leader, and parts of the 5'-coding and 3'-non-coding sequences.

Fig. 1. Partial restriction map of cDNA clone 7A2M27. The horizontal bar shows the region corresponding to the oligonucleotide probe. The box represents the open reading frame. The hatched area is the region corresponding to a putative DNA binding domain. Restriction sites are A (Asp 718), E (EcoRI), H (HindIII), S (SstI), P (PstI) and St (StuI).

```
                                10                                  20                                  30
GGCGGCGGCGGCGGCGAGGCGGGAGCTGTAGCCCCCTACGGCTACACTCGGCCCCCTCAGGGGCTGGCGGGCCAGGAAAGCGACTTCACC   90
GlyGlyGlyGlyGlyGluAlaGlyAlaValAlaProTyrGlyTyrThrArgProProGlnGlyLeuAlaGlyGlnGluSerAspPheThr

                                40                                  50                                  60
GCACCTGATGTGTGGTACCCTGGCGGCATGGTGAGCAGAGTGCCCTATCCCAGTCCCACTTGTGTCAAAAGCGAAATGGGCCCCTGGATG  180
AlaProAspValTrpTyrProGlyGlyMetValSerArgValProTyrProSerProThrCysValLysSerGluMetGlyProTrpMet

                                70                                  80                                  90
GATAGCTACTCCGGACCTTACGGGGACATGCGTTTGGAGACTGCCAGGGACCATGTTTTGCCCATTGACTATTACTTTCCACCCCAGAAG  270
AspSerTyrSerGlyProTyrGlyAspMetArgLeuGluThrAlaArgAspHisValLeuProIleAspTyrTyrPheProProGlnLys

                               100                                 110                                 120
ACCTGCCTGATCTGTGGAGATAAGGCTTCTGGGTGTCACTATGGAGCTCTCACATGTGGAAGCTGCAAGGTCTTCTTCAAAAGAGCCGCT  360
ThrCysLeuIleCysGlyAspGluAlaSerGlyCysHisTyrGlyAlaLeuThrCysGlySerCysLysValPhePheLysArgAlaAla

                               130                                 140                                 150
GAAGGGAAACAGAAGTACCTGTGCGCCAGCAGAAATGATTGCACTATTGATAAATTCCGAAGGAAAAATTGTCCATCTTGTCGTCTTCGG  450
GluGlyLysGlnLysTyrLeuCysAlaSerArgAsnAspCysThrIleAspLysPheArgArgLysAsnCysProSerCysArgLeuArg

                               160                                 170                                 180
AAATGTTATGAAGCAGGGATGACTCTGGGAGCCCGGAAGCTGAAGAAACTTGGTAATCTGAAACTACAGGAGGAAGGAGAGGCTTCCAGC  540
LysCysTyrGluAlaGlyMetThrLeuGlyAlaArgLysLeuLysLysLeuGlyAsnLeuLysLeuGlnGluGluGlyGluAlaSerSer

                               190                                 200                                 210
ACCACCAGCCCCACTGAGGAGACAACCCAGAAGCTGACAGTGTCACACATTGAAGGCTATGAATGTCAGCCCATCTTTCTGAATGTCCTG  630
ThrThrSerProThrGluGluThrThrGlnLysLeuThrValSerHisIleGluGlyTyrGluCysGlnProIlePheLeuAsnValLeu

                               220                                 230                                 240
GAAGCCATTGAGCCAGGTGTAGTGTGTGCTGGACACGACAACAACCAGCCCGACTCCTTTGCAGCCTTGCTCTCTAGCCTCAATGAACTG  720
GluAlaIleGluProGlyValValCysAlaGlyHisAspAsnAsnGlnProAspSerPheAlaAlaLeuLeuSerSerLeuAsnGluLeu

                               250                                 260                                 270
GGAGAGAGACAGCTTGTACACGTGGTCAAGTGGGCCAAGGCCTTGCCTGGCTTCCGCAACTTACACGTGGACGACCAGATGGCTGTCATT  810
GlyGluArgGlnLeuValHisValValLysTrpAlaLysAlaLeuProGlyPheArgAsnLeuHisValAspAspGlnMetAlaValIle

                               280                                 290                                 300
CAGTACTCCTGGATGGGGCTCATGGTGTTTGCCATGGGCTGGCGATCCTTCACCAATGTCAACTCCAGGATGCTCTACTTCGCCCCTGAT  900
GlnTyrSerTrpMetGlyLeuMetValPheAlaMetGlyTrpArgSerPheThrAsnValAsnSerArgMetLeuTyrPheAlaProAsp

                               310                                 320                                 330
CTGGTTTTCAATGAGTACCGCATGCACAAGTCCCGGATGTACAGCCAGTGTGTCCGAATGAGGCACCTCTCTCAAGAGTTTGGATGGCTC  990
LeuValPheAsnGluTyrArgMetHisLysSerArgMetTyrSerGlnCysValArgMetArgHisLeuSerGlnGluPheGlyTrpLeu

                               340                                 350                                 360
CAAATCACCCCCCAGGAATTCCTGTGCATGAAAGCACTGCTACTCTTCAGCATTATTCCAGTGGATGGGCTGAAAAATCAAAAATTCTTT 1080
GlnIleThrProGlnGluPheLeuCysMetLysAlaLeuLeuLeuPheSerIleIleProValAspGlyLeuLysAsnGlnLysPhePhe

                               370                                 380                                 390
GATGAACTTCGAATGAACTACATCAAGGAACTCGATCGTATCATTGCATGCAAAAGAAAAAATCCCACATCCTGCTCAAGACGCTTCTAC 1170
AspGluLeuArgMetAsnTyrIleLysGluLeuAspArgIleIleAlaCysLysArgLysAsnProThrSerCysSerArgArgPheTyr

                               400                                 410                                 420
CAGCTCACCAAGCTCCTGGACTCCGTGCAGCCTATTGCGAGAGAGCTGCATCAGTTCACTTTTGACCTGCTAATCAAGTCACACATGGTG 1260
GlnLeuThrLysLeuLeuAspSerValGlnProIleAlaArgGluLeuHisGlnPheThrPheAspLeuLeuIleLysSerHisMetVal

                               430                                 440                                 450
AGCGTGGACTTTCCGGAAATGATGGCAGAGATCATCTCTGTGCAAGTGCCCAAGATCCTTTCTGGGAAAGTCAAGCCCATCTATTTCCAC 1350
SerValAspPheProGluMetMetAlaGluIleIleSerValGlnValProLysIleLeuSerGlyLysValLysProIleTyrPheHis

ACCCAGTGAAGCATTGGAAACCCTATTTCCCCACCCCAGCTCATGCCCCCTTTCAGATGTCTTCTGCCTGTTATTACTCTGCACTACTCC 1440
ThrGln

TCTGCAGTGCCTTGGGGAATTTCCTCTATTGATGTACAGTCTGTCATGAACATGTTCCTGAATTC                           1505
```

Fig. 2. Nucleotide sequence (1-1505) of clone 7A2M27 and deduced amino acid sequence (1-452) of the corresponding protein. The stop codon at the end of the open reading frame is underlined.

In order to identify further 7A2M27, particularly the ligand which binds the 7A2M27-protein product, the cDNA-clone was transiently expressed in COS cells. Relative binding affinities (RBA's) of several steroids were determined for binding proteins in the cytosol fraction (Table I). A relative high binding specificity was observed for the androgens: 5α-dihydrotestosterone, R1881 and testosterone. The nuclear localization of the binding protein was established by sucrose density gradient centrifugation of nuclear extracts obtained from cells incubated with 3H-labelled R1881 (a synthetic non-metabolizable androgen). A specific 3.6 S radioactive peak was detected, which could be completely abolished by a 100-fold molar excess of non-radioactive ligand. R1881-binding was not influenced by triamcinolone acetonide, indicating that

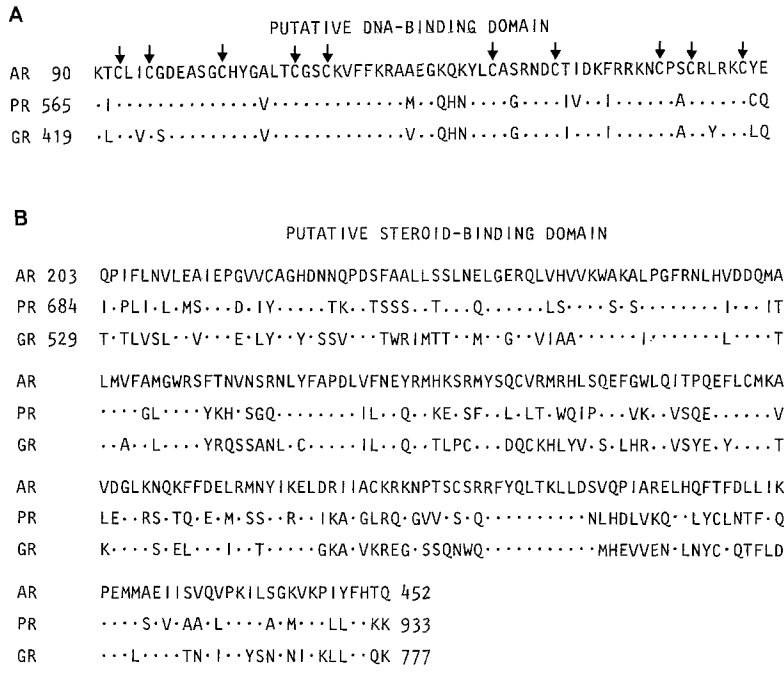

Fig. 3. Comparison of the amino acid sequences of the putative DNA-binding domains (3A) and the steroid binding domains (3B) of 7A2M27 (AR), progesterone receptor (PR) and glucocorticoid receptor (GR). Arrows indicate the conserved cysteines. Only amino acids which differ from AR are indicated.

progesterone receptors or glucocorticoid receptors were absent in the transfected COS cells [27]. Specific R1881-binding was absent in untransfected COS cells and in COS cells transfected with a control construct. Scatchard plot analysis of the binding of 3H-R1881 by transfected COS cells resulted in the demonstration of high affinity binding sites (Kd: 0.3 nM) with limited capacity.

We also investigated in various cell lines the expression of mRNA corresponding to 7A2M27 by Northern blotting, using the 250 bp StuI-EcoRI fragment and the

Table I

Relative Binding Affinities (RBAs) for the steroid binding protein present in the cytosol fraction of COS cells transfected with pSV-AR

DIHYDROTESTOSTERONE (DHT)	100
R1881 (METHYLTRIENOLONE)	85
TESTOSTERONE	15
PROGESTERONE	6
R5020 (PROMEGESTONE)	3
ESTRADIOL	4
TRIAMCINOLONE ACETONIDE	<1

The RBA for DHT was arbitrarily chosen at 100. The synthetic steroids R1881 and R5020 were used for a good discrimination between androgen and progesterone receptors [21].

500 bp EcoRI-EcoRI fragment of 7A2M27 (Figure 1) as probes. By far the strongest hybridization signal was obtained with mRNA from the LNCaP (prostate carcinoma) cell line which contains high levels of androgen receptor [28]. Both probes detected under stringent hybridization conditions three major mRNA species with a size of 11, 8.5 and 4.7 kb, respectively (Figure 4).

The chromosomal location of the 7A2M27 gene was determined, using a panel of human-hamster somatic hybrid cell lines. Table II summarizes the data obtained from chromosome and Southern blot analysis of the hybrids. In calculating the number of concordant chromosomes only those were taken into account which were completely present or absent in the various hybrids. In some hybrids only fragments of a particular human chromosome were present. This was especially the case for chromosome 11 (Table II). Therefore, data obtained from hybrids containing chromosome 11 fragments are shown in more detail in Table III. A3RS hybrids suggested concordance of 7A2M27 with 11q23-qter. However, hybrids A3Bi-1C and A3Bi-7C both contained this chromosome region and were negative for 7A2M27. Concordance 7A2M27 and X-chromosome was 100% in these hybrids. Summarizing, the results presented in Table II and III show that the 7A2M27 gene is situated on the X-chromosome.

The data presented provide several criteria that the isolated cDNA-product codes for a major part of the human androgen receptor. First the striking structural homology with the steroid binding and the DNA binding domain of

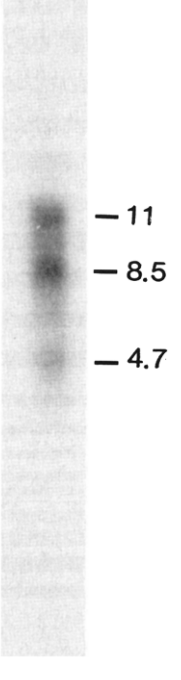

Fig. 4. Northern blot analysis of LNCaP RNA hybridized with 7A2M27 (StuI-EcoRI). Size of hybridizing bands is in kb. 28S and 18S rRNA were used as size markers.

Table II

Correlation between the presence/absence of 7A2M27 and human chromosomes in hamster-human hybrid cells

Hybrid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	7A2M27
A3RS-11B			+	*		+	+	+	+		*	+		+	+	+	+		+	+		+	+		+
A3RS-14A	+			*	+		+				*	+			+		+			+					-
A3RS-17A	+		+	+	+	+	+	+	+	+	*	+	+	+	+	+	+	+		+	+	+	+		+
A3RS-27A			+	*				±		+	*	+	±	±		+	+		+	+		+			-
A3Bi-5B	+		+	+	+	+	+	+	+	+	*	+	+	+	+	+	+	+	+	+	+	+	+		+
A3Bi-7C	+		+	+	+	+	±	±	±	+	*		+			+	+	+		+	+	+		+	-
A3Bi-1C				+	+	+	+	+	+		*	+	+	+		+	+	+	+		+	+			-
X/3-B	+		+	+	+	+	+	+		+	+	+		+	+	+	+	+	+	+	+	+	+		+
X/3-F2																	+		+	+	+				-
X/4-F4															+		+		+		+				-
A1W-BF2									+		+	*		+			+				+		+		+
A1W-CB						+	+		+		+	*		+			+				+	+	+		+
416 RO-6	+			+	+	*		+	+	±	+	+	±	±		+	+		+	+	±	+	+		+
17CB-3B	+	+		+	+	+	+		+			+	+	+	+	+	+	+	+	+	+	+			-
63-4-1-9	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
C9																									-
Concordancy (%)	60	47	60	60	53	70	64	78	71	64	87	70	46	84	60	60	53	53	46	53	57	67	100	40	

Concordancies are calculated, excluding hybrids containing chromosomes which are rarely present (±) and including hybrids containing chromosome fragments(*) (see also Table III). DNA's were hybridized with the 250 bp StuI-EcoRI fragment of 7A2M27 as a probe.

steroid receptors indicates that the cloned cDNA codes for a steroid ligand responsive regulatory protein. Second, the expressed nuclear protein product has a high specific affinity and a limited capacity for 5α-dihydrotestosterone and methyltrienolone. Third, the corresponding gene is localized on the X-chromosome the putative chromosome to which the androgen receptor gene has been assigned [29]. Fourth, high levels of hybridizing mRNA species were found in a human prostate cancer cell line, which contain high levels of androgen receptor protein [28]. At present experiments on the isolation of a full length AR cDNA are in progress. AR specific cDNA's are important tools in the investigation of regulation of receptor expression in normal androgen target cells, in prostate cancer as well as in androgen insensitivity syndromes.

Table III

Correlation between 7A2M27, human X chromosome, and chromosome 11 fragments in hamster-human hybrid cells

Hybrid	Chromosome 11 fragment	X-chromosome	7A2M27
A3RS-11B	11q23-qter	+	+
A3RS-17A	11q23-qter	+	+
A3RS-14A	11pter-q23	-	-
A3RS-27A	11pter-q23	-	-
A3Bi-5B	11pter-p12;p15-qter	+	+
A3Bi-7C	11pter-p12;p15-qter	-	-
A3Bi-1C	11pter-p12;p15-qter	-	-

ACKNOWLEDGEMENT

We are indebted to Dr. E. Milgrom and co-workers for the kind gift of the cDNA library; to Mrs. P. Delfos for photography and to Mrs. R. van der Ploeg for typing of the manuscript. This study was supported by grants of the Dutch Cancer Society KWF.

REFERENCES

1. Hollenberg,S.M., Weinberger,C., Ong,E.S., Cerelli,G., Oro,A., Lebo,R., Thompson,E.B., Rosenfeld,M.G. & Evans,R.M. (1985) Nature 318, 635-641.
2. Green,S., Walter,P., Kumar,V., Krust,A., Bornert,J-M., Argos,P. & Chambon,P. (1986) Nature 320, 134-139.
3. Greene,G.L., Gilna,P., Waterfield,M., Baker,A., Hort,Y. & Shine,J. (1986) Science 231, 1150-1154.
4. Misrahi,M., Atger,M., d'Auriol,L., Loosfelt,H., Meriel,C., Fridlansky,F., Guiochon-Mantel,A., Galibert,F. & Milgrom,E. (1987) Biochem. Biophys. Res. Commun. 143, 740-748.
5. Arriza,J.L., Weinberger,C., Cerrelli,G., Glaser,T.M., Handelin,B.L., Housman,D.E. & Evans,R.M. (1987) Science 237, 268-275.
6. Sap,J., Munoz,A., Damm,K., Goldberg,Y., Ghysdael,J., Leutz,A., Beug, H. & Vennström, B. (1986) Nature 324, 635-640.
7. Weinberger,C., Thompson,C.C., Ong,E.S., Lebo,R., Gruol,D.J. & Evans,R.M. (1986) Nature 324, 641-646.
8. Thompson,C.C., Weinberger,C., Lebo,R. & Evans,R.M. (1987) Science 237, 1610-1614.
9. Benbrook,D. & Pfahl,M. (1987) Science 238, 788-791.
10. Petkovich,M., Brand,N.J., Krust,A. & Chambon,P. (1987) Nature 330, 444-450.
11. Giguere,V., Ong,E.S., Segui,P. & Evans,R.M. (1987) Nature 330, 624-629.
12. Giguere,V., Hollenberg,S.M., Rosenfeld,M.G. & & Evans,R.M. (1986) Cell 46, 645-652.
13. Kumar,V., Green,S., Staub,A. & Chambon,P.: EMBO J. (1986) 5, 2231-2236.
14. Gronemeyer,H.,Turcotte,B., Quirin-Stricker,C., Bocquell,M.T., Meyer,M.E., Krozowski,Z., Jeltsch,J.M., Lerouge,T., Garnier,J.M. & Chambon,P.: EMBO J. (1987) 6, 3985-3994.
15. Maniatis,T., Fritsch,E.F. & Sambrook,J. (1982) Molecular Cloning: A laboratory manual (Cold Spring Harbor, NY).
16. Sanger,F., Nicklen,S. & Coulson,A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
17. Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. & Rutter,W. (1977) Biochemistry 18, 5294-5299.
18. Feinberg,A.P. & Vogelstein,P. (1983) Anal. Biochem. 132, 6-13.
19. Geurts van Kessel,A., Tetteroo,P.A.T., Borne,K.von dem, Hagemeijer,A. & Bootsma,D. (1983) Proc. Natl. Acad. Sci. USA 80, 3748-3752.
20. van Heuvel,M., Bosveld,I.J., Mooren,A.T.A., Trapman,J. & Zwarthoff,E.C. (1986) J. Gen. Virol. 67, 2215-2222.
21. Ojasoo,T. & Raynaud,J.P. (1978) Cancer Res. 38, 4186-4198.
22. Brinkmann,A.O., Bolt,J., van Steenbrugge,G.J., Kuiper,G.G.J.M., De Boer,W. & Mulder,E. (1987) The Prostate 10, 133-143.
23. McLaughlin,W.H., Milius,R.A., Gill,L.M., Adelstein,S.J. & Bloomer,W.D. (1984) J. Steroid Biochem. 20, 1129-1133.
24. Burmester,J.K., Maeda,N. & DeLuca,H.F. (1988) Proc. Natl. Acad. Sci. USA 85, 1005-1009.
25. McDonnel,D.P., Mangelsdorf,D.J., Pike,W.J., Haussler,M.R. & O'Malley,B.W. (1987) Science 235, 1214-1217.
26. Gehring,U. (1987) Trends in Biochem. 12, 399-402.
27. Zava,D.T., Landrum,B., Horwitz,K.B. & McGuire,W.L. (1979) Endocrinology 104, 1007-1012.
28. Brinkmann,A.O., Kuiper,G.G.J.M., Bolt-de Vries,J. & Mulder,E. (1988) J. Steroid Biochem. 29, 259-264.
29. Migeon,B.R., Brown,T.R., Axelman,J. & Migeon,C.J. (1981) Proc. Natl. Acad. Sci USA 78, 6339-6343.