Two Distinct Isoforms of cDNA Encoding Rainbow Trout Androgen Receptors

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Androgens play an important role in male sexual differentiation and development. The activity of androgens is mediated by an androgen receptor (AR), which binds to specific DNA recognition sites and regulates transcription. We describe here the isolation of two distinct rainbow trout cDNA clones, designated rtAR-α and rtAR-β, which contain the entire androgen receptor coding region. Comparison of the predicted amino acid sequence of rtAR-α to that of rtAR-β revealed 85% identity. Interestingly, despite this high homology, rtAR-α activated transcription of an androgen-responsive reporter gene in co-transfection assays, but rtAR-β did not. These results suggest that rainbow trout contains two distinct isoforms of androgen receptors whose functions differ. The region of rtAR-β responsible for its inactivity was mapped to its ligand binding domain by analyzing chimeras of the rtAR-α, rtAR-β, and rtGR-I (glucocorticoid) receptors. Alteration of any one of three out of four segments within this domain restored activity.

Extracts made from COS-1 cells transfected with an rtAR-α expression plasmid produced a high level of [3H]mibolerone binding, whereas no binding was observed by extracts of cells transfected with an rtAR-β expression plasmid. These data demonstrate that the lack of transactivation activity of rtAR-β is due to its inability to bind hormone.

In contrast to mammals, fish can undergo gonadal sex inversion in either direction by treatment with exogenous sex steroid if it is applied early enough during development (1, 2). These observations led to the postulate that androgens and estrogens are the substances responsible for sex differentiation of male and female fish, respectively (3), and has resulted in the development of protocols for the masculinization and feminization of large numbers of fish for experimental or economic purposes (1, 4, 5). The androgen receptor (AR) is a critical mediator of male sexual differentiation and development in both fish and mammals. Structurally and functionally, the AR belongs to the superfamily of ligand-responsive transcription modifiers, which encompasses the receptors for the steroid and thyroid hormones. Like other nuclear receptors, steroid receptors are composed of three major functional domains: an NH2-terminal hypervariable transcriptional activation domain (TAD), a central highly conserved DNA binding domain (DBD) consisting of two Cys-Cys zinc finger motifs, and a COOH-terminal ligand binding domain (LBD) (6–9). It has been reported that the AR regulates androgen target genes by binding to a specific DNA sequence, the androgen-responsive element (ARE; consensus = 5'-GGTACANNNTGTTCT-3'), which is similar to the glucocorticoid response element (12–14). The AR can either up- or down-regulate the expression of androgen target genes, the outcome probably depending on interactions with specific adapters or co-activators (10, 11).

At present, complete AR cDNAs have been cloned only from mammalian species (human, rat, and mouse) (6, 15). We have undertaken the isolation of the rainbow trout homologue of the androgen receptor in order to study its potential role in salmoid fish sexual differentiation, development, and general physiology and to further explore structure-function relationships through sequence comparisons. We report that there are two isoforms of AR mRNA in rainbow trout. These two isoforms, which were present in all tissues examined, encode 96.1- and 95.8-kDa proteins that are highly homologous to mammalian AR. Surprisingly, when the biological activities of the two isoforms were examined in a fish cell line using a transient expression assay, the 96.1-kDa protein was highly active, while the 95.8-kDa AR was inactive.

EXPERIMENTAL PROCEDURES

Isolation and Characterization of Rainbow Trout Androgen Receptor cDNAs—In order to isolate the rainbow trout androgen receptor cDNA, a probe was first generated by PCR. Oligonucleotide primers were designed based on the amino acid sequence of the highly central conserved region of the AR and then used to amplify rainbow trout testis cDNA as described previously (16). Products of the expected size were subcloned into the pSK vector (Stratagene) and sequenced. The primers, which yielded a 568-bp fragment highly homologous to human AR, were as follows: primer 1, TA(T/C)CT(T/C)TT/AA/AA/TA(T/C)CT/CG/AA/A/C/T/GG/AA/A/CA/T/A/GA/CT/CG/CT/TT/TC/TG; primer 2, CAT/AC/G/CT/GG/AA/A/CA/CT/AT/A/AA. A rainbow trout testis random hexanucleotide primed cDNA library in λZipII (Stratagene) was screened by hybridization with a probe made from the cloned PCR fragment. Positive clones were rescued as pBluescript plasmids by in vivo excision and sequenced. Two independent clones, pAR-α and pAR-β, were isolated.

Reverse Transcriptase PCR—Reverse transcriptase PCR was conducted as described previously (17) with some modifications. Briefly, poly(A)-selected RNA was prepared from various rainbow trout tissues. The cDNAs were synthesized from 100 ng of poly(A) selected RNA using a first strand synthesis kit (Stratagene). An aliquot of the first strand reaction was then subjected to PCR analysis using rtAR-α specific primers [GG(CAT/CAG)GC(GA/CAG)ATA] and [G(A/C)GCAG(GA/CAG)ATA] (which generated a 225-bp product) or rtAR-β-specific primers [β1 (ACAATATGGCAGGCGCA) and β2 (ATGGCCAGTCTCCTCCTC)] (which generated a 451-bp product).

Plasmid Construction—The pARE3TK-CAT reporter plasmid, which...
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Carries three androgen-responsive elements in front of a TK (thymidine kinase) promoter, was a kind gift of Dr. J. Trapani (18). The pMSG-CAT reporter plasmid, which carries the MMTV (mouse mammary tumor virus) promoter, was purchased from Amersham Pharmacia Biotech.

To construct the expression vectors, plasmids pAR-α and pAR-β were amplified by PCR using a sense primer homologous to the NH2-terminal end of the coding sequence and an antisense primer homologous to the COOH-terminal end. In addition, the coding sequences in each primer were flanked by EcoRI or HindIII sites for the rtAR-α and rtAR-β sequences, respectively. The resultant products were cleaved with EcoRI or HindIII to yield fragments of 2577 bp (rtAR-α) or 2574 bp (rtAR-β) and then subcloned into EcoRI- or HindIII-digested Blue-script SK+ (Stratagene) to yield pSK-rtAR-α and pSK-rtAR-β, respectively. Following verification of their sequences, the EcoRI and HindIII fragments were excised and subcloned into EcoRI or HindIII digested pDNA3 (Invitrogen) to yield pCMV-rtAR-α and pCMV-rtAR-β, respectively.

**Chimeric Plasmid Construction**—To facilitate construction of chimeric receptors, plasmids were first made in which the TAD, DBD, or LBD could be separately excised by flanking the DBD with ApaI and XbaI sites using site-directed mutagenesis as described previously (19, 20). Single-stranded DNA was prepared from pSK-rtAR-α, pSK-rtAR-β, and pSK-rtGR-I (21) for mutagenesis, and mutations were confirmed by DNA sequencing. The positions at which the indicated changes were made were as follows: at amino acids 495 and 562 for rtAR-α, yielding pSK-AX/rtAR-α; at amino acids 494 and 561 for rtAR-β, yielding pSK-AX/rtAR-β; and at amino acids 420 and 485 for rtGR-I (21), yielding pSK-AX/rtGR-I. Chimeras were then constructed by subcloning combinations of fragments from these three plasmids into appropriately digested pDNA3 as follows: pCMV-APABα contains the 1683-bp EcoRI/XbaI fragment from pSK-AX/rtAR-β and the 813-bp XbaI/EcoRI fragment from pSK-AX/rtGR-I; pCMV-APABβ contains the 1482-bp EcoRI/ApaI fragment from pSK-AX/rtAR-α and the 1008-bp ApaI/EcoRI fragment from pSK-AX/rtGR-I; pCMV-AGABαβ contains the 1077-bp ApaI/EcoRI fragment from pSK-AX/rtAR-β and and the 1260-bp EcoRI/ApaI fragment from pSK-AX/rtGR-I; pCMV-AGABαβ contains the 876-bp XbaI/EcoRI fragment from pSK-AX/rtAR-β and the 1008-bp EcoRI/XbaI fragment from pSK-AX/rtGR-I; and pCMV-APABαβα contains the 1683-bp EcoRI/XbaI fragment from pSK-AX/rtAR-β and the 813-bp XbaI/HindIII fragment from pSK-AX/rtAR-α.

Constructs chimeric for subregions of the LBD were created by using PCR methodology, and all chimeric constructs were verified by DNA sequencing. The LBD of rtAR-β was divided into four segments, corresponding to amino acid positions 561–630, 631–712, 713–784, and 785–853, carrying 7, 5, 4, and 5 amino acid substitutions, respectively, when compared with rtAR-α. Plasmids pCMV-AR-αββ, pCMV-AR-βββ, pCMV-AR-βββα, and pCMV-AR-ββββ were then constructed by replacing segment I, II, III, or IV of rtAR-β with the corresponding segment of rtAR-α, respectively (Fig. 6). Details of plasmid constructions are available on request.

**Tissue Distribution of Rainbow Trout AR-α and AR-β**—Comparison of the predicted amino acid sequence of the rtAR-β with that of rtAR-β reveals 85% identity with continuity. Comparison of the three different functional domains of the rtAR-α to those of rtAR-β shows a homology of 79% for the TAD (amino acids 1–495), 97% for the DBA (amino acids 496–562), and 93% for the LBD (amino acids 563–854) (Fig. 2). The observation that differences occur throughout the coding regions indicates that these two predicted proteins arise from different genes rather than by alternative splicing. However, since the testis cDNA library used to isolate the clones was constructed from pooled RNA from several fishes, it was also possible that they are encoded by two different alleles in the population. To test this, PCR fragments spanning the NH2-terminal portion of the two cDNAs were amplified and sequenced from one rainbow trout testis and ovary. The presence of the two isoforms was confirmed in each case (data not shown). Comparison of the amino acid sequence of rtAR-α and rtAR-β with that of the mouse and human ARs is shown in Fig. 2. The amino acid sequence of the DBD of rtAR-α and rtAR-β shows 92 and 95% identity with that of human AR (the DBD of human AR is identical to that of mouse AR). Similarly, the amino acid sequence of the LBD of rtAR-α and rtAR-β shows 65 and 68% identity with that of human AR (the LBD of human AR is 97% identical to that of mouse AR). Thus, both the DNA binding and ligand binding domains are highly conserved. On the other hand, the NH2-terminal TAD shows less sequence similarity than does the COOH-terminal portion (only 19% sequence identity between rtAR-α/rtAR-β and the human AR).

**Immunocytochemistry**—COS-1 cells were plated at 1 × 105 cells/well on two-well chamber slides and were transfected with 2 μg of rtAR-α expression plasmid or rtAR-β expression plasmid. Twenty-four hours after transfection, MT was added to 10−7 M, and after another 24 h the cells were washed in phosphate-buffered saline and fixed with 4% paraformaldehyde in phosphate-buffered saline. Immunocytochemical staining was performed as described previously (25), except that Cy3-conjugated sheep anti-rabbit IgG (Sigma) was used for visualization.

**RESULTS**

Isolation of Two Distinct cDNAs Encoding the Rainbow Trout Androgen Receptors—As a first step in isolating the rainbow trout AR cDNA clone, we designed a set of degenerate oligonucleotide primers homologous to an amino acid sequence that is highly conserved between the human and mouse AR. These primers were then used to generate PCR fragments from rainbow trout testis cDNA to isolate a fragment that would serve as a screening probe. A PCR product of the length predicted from the human AR gene was isolated, cloned, and sequenced. The sequence encoded 190 amino acids that had 70% identity with the corresponding region of the human AR. Using a probe generated from this fragment, approximately 1 × 106 plaques from a rainbow trout testis cDNA library were screened, and several strongly hybridizing clones were obtained and sequenced. The sequences obtained revealed two different AR homologues, designated rtAR-α, and rtAR-β (accession numbers for nucleotide sequences are AB012095 for rtAR-α and AB012096 for rtAR-β). Both sequences contain an ATG initiation codon followed by an extended open reading frame; the rtAR-α cDNA encodes an 854 amino acid protein of a molecular mass of 96.1 kDa, and the rtAR-β cDNA encodes an 853-amino acid protein of a molecular mass of 95.8 kDa (Fig. 1, A and B, respectively). Both of the encoded proteins contain all of the domains that characterize the steroid hormone receptor family, including a striking canonical leucine zipper structure in the COOH-terminal LBD.

**Structure of Rainbow Trout AR-α and AR-β**—Comparison of the predicted amino acid sequence of the rtAR-β with that of rtAR-β reveals 85% identity with continuity. Comparison of the three different functional domains of the rtAR-α to those of rtAR-β shows a homology of 79% for the TAD (amino acids 1–495), 97% for the DBA (amino acids 496–562), and 93% for the LBD (amino acids 563–854) (Fig. 2). The observation that differences occur throughout the coding regions indicates that these two predicted proteins arise from different genes rather than by alternative splicing. However, since the testis cDNA library used to isolate the clones was constructed from pooled RNA from several fishes, it was also possible that they are encoded by two different alleles in the population. To test this, PCR fragments spanning the NH2-terminal portion of the two cDNAs were amplified and sequenced from one rainbow trout testis and ovary. The presence of the two isoforms was confirmed in each case (data not shown).
homologous segments of the two sequences so that the presence of each transcript could be evaluated (Fig. 3, lanes 1 and 2). Both rtAR-α and rtAR-β mRNA expression could be detected in all tissues tested (Fig. 3). No amplification was seen when the AMV reverse transcriptase was omitted from the cDNA synthesis reaction (data not shown).

Biological Activities of Rainbow Trout AR-α and AR-β—To determine the biological activities of rtAR-α and rtAR-β, cells were co-transfected with an rtAR expression vector and an androgen-responsive reporter gene. The expression vectors were made by inserting the rtAR-α and rtAR-β cDNAs into a CMV expression vector to yield pCMV-rtAR-α and pCMV-rtAR-β. For the reporter gene we used pMSG-CAT, which carries the MMTV promoter driving expression of the bacterial CAT reporter gene (Fig. 4A). Although the expression of many genes is known to be androgen dependent, only a few have been shown to be directly regulated by androgen, and the MMTV promoter is the best studied among these (14). The MMTV promoter is regulated by glucocorticoids and progestins, but is also able to confer androgen responsiveness to a reporter gene, although less efficiently.

As it has been shown that the level of androgen inducibility in a transient transfection assay is dependent on the cell type used, we first screened several fish cell lines by co-transfection with the pMSG-CAT reporter plasmid and either the pCMV-rtAR-α or pCMV-rtAR-β expression plasmid. As a result of this screening, we selected the EPC cell line derived from carp (22). Transfection of the pMSG-CAT reporter plasmid into the EPC cell line produced very low basal expression of CAT, and it was not induced by MT (Fig. 4B). In contrast, upon co-transfection of the pMSG-CAT reporter plasmid and the rtAR-α expression plasmid, MT stimulated the level of CAT protein expression about 5-fold (Fig. 4B). Surprisingly, co-transfection of pMSG-CAT and pCMV-rtAR-β yielded no significant transactivation (Fig. 4B).

To further test the activities of rtAR-α and rtAR-β, we used another reporter plasmid, pARE3TK-CAT, which carries three androgen responsive elements derived from the prostate-specific antigen promoter (15) just upstream of the TK basal promoter (Fig. 4A). The results obtained were similar to those using the pMSG-CAT reporter plasmid; the CAT expression levels produced by pARE3TK-CAT were low in either the absence or presence of MT, but were significantly enhanced (about 7-fold) in the presence of MT upon co-transfection with pCMV-rtAR-α but not with pCMV-rtAR-β (Fig. 4C). These results indicate that the rtAR-α is capable of producing a fully functional receptor that mediates hormone-dependent transcriptional activation, whereas the highly homologous rtAR-β is inactive. When EPC cells were co-transfected with pCMV-rtAR-α and pARE3TK-CAT in the presence of the other steroids (dexamethasone, aldosterone, progesterone, and 17β-estradiol), CAT expression was not induced (data not shown). When we also used the co-transfection assay to examine the response of the rtAR-β to the same set of steroids including MT, no induction was observed with any of them (data not shown).

Functional Domain Mapping of the rtAR-β—To map the region(s) of rtAR-β that renders it inactive, a series of chimeric expression plasmids was constructed that carry various com-
binations of the TAD, DBD, and LBD regions from rtAR-β, rtAR-α, or the rainbow trout GR-I (21). To facilitate construction of the chimeric plasmids, we flanked the DNA binding domains of each receptor with ApaI and XbaI restriction sites. The chimeric constructs produced are illustrated in Fig. 5. These chimeric plasmids were transiently co-transfected with pARE3TK-CAT into EPC cells in the presence or absence of MT or dexamethasone. Co-transfection of pCMV-rtGR-I into EPC cells results in strong dexamethasone-dependent CAT expres-
sion from the pARE3TK-CAT reporter plasmid (Fig. 5), indi-
cating that the DBD of rtGR could functionally recognize the androgen-responsive element. The results from the various chimeric constructs suggest that the inactivity of the rtAR-β is due to its LBD; for example, a chimera composed of the rtGR TAD and DBD and the rtAR-β LBD is inactive, whereas one composed of the rtAR-β TAD and DBD and a LBD from either rtGR or rtAR-α is highly active (Fig. 5). The activity of the latter chimera, pCMV-AβAβ, is in fact about 2-fold greater than that of pCMV-rtAR-α.

To further localize the region of rtAR-β responsible for its inactivity, we subdivided its LBD into four segments and made constructs in which each was individually replaced with the corresponding segment from rtAR-α (Fig. 6). The four segments extended from amino acids 562–630, 631–712, 713–784, and 785–853 and contained 7, 5, 4, and 5 amino acid differences between the isoforms, respectively. The results of transfections

**FIG. 2.** Comparison of the deduced amino acid sequences for AR-α and AR-β of rainbow trout, human, and mouse. Sequence identities are shown by black boxes. The individual leucines of the putative leucine zipper structure in the COOH-terminal LBD are marked by asterisks. Gaps are indicated by dashes.
using the rtAR-αβ chimeric constructs, expressed in fold induction by MT, are shown in Fig. 6. Surprisingly, three of the four chimeric constructs supported a dramatic MT-regulated response, similar to that seen with pCMV-rtAR-α. Thus the inability of rtAR-β to activate transcription depends on the simultaneous presence of sequences distributed throughout the LBD.

**Ligand Binding Characteristics of the rtAR-α and rtAR-β**—Since the inability of rtAR-β to activate transcription resides in its LBD, we next examined the ligand binding abilities of the rtAR-α, rtAR-β, and chimeric rtARs. A high level of [3H]mibolerone binding was observed in extracts prepared from cells transfected with pCMV-rtAR-α or the active chimeric expression plasmids pCMV-AR-AβAβAα, -αβββ, -βαββ, and -βββα (Fig. 7). However, when cells were transfected with the pCMV vector as a negative control, or with the inactive chimeras pCMV-rtAR-β and pCMV-AR-βαβ, specific [3H]mibolerone binding was absent (Fig. 7). The complete correlation between mibolerone binding and transcriptional activation strongly suggests that the inability of rtAR-β to transactivate is due to its poor ligand binding affinity.

Next, we performed an analysis of [3H]mibolerone binding in cytosol fractions derived from COS-1 cells transiently transfected with pCMV-rtAR-α. Saturation ligand binding analysis and Scatchard analysis revealed a $K_d$ of 0.16 nM and $B_{max}$ of 178 fmol/mg of protein (data not shown). These values are similar to those of the human AR ($K_d$ = 0.5 nM and $B_{max}$ = 357 fmol/mg protein) (24).

**Nuclear Translocation Abilities of rtAR-α and rtAR-β**—If rtAR-β binds androgens poorly in vivo, it might remain in the cytoplasm in the presence of MT, whereas the rtAR-α would translocate to the nucleus. This possibility was tested by analyzing transfected COS-1 cells by fluorescent immunocytochemistry, using an antibody raised against bacterially expressed rtAR-α protein (NH$_2$-terminal 200 amino acids; see Fig. 8).
of any of the expression plasmids when preimmune antiserum was used (data not shown). In the presence of MT, the AR-α showed strong nuclear staining, whereas a striking cytoplasmic distribution was observed for AR-β (Fig. 8, B and C). The remaining chimeric expression plasmids (see Fig. 7) were also examined for their nuclear translocation ability. Only the AR-ββαβ protein showed cytoplasmic staining in the presence of MT (data not shown). Thus, the inability of rtAR-β to mediate transactivation correlated with failure to be translocated to the nucleus.

**DISCUSSION**

The function of steroid hormones in the development and sexual differentiation of fish has been well documented at the physiological and endocrinological levels. At the molecular level, both the estrogen receptor and glucocorticoid receptor have been cloned and characterized from various fish species, but sequence information for the AR has not been available. In order to elucidate the molecular mechanism of androgen action in the early stage of sexual differentiation and spermatogenesis, we isolated AR cDNA clones from rainbow trout testis.

By using PCR to generate a homologous probe, we isolated two distinct isoforms of rainbow trout AR cDNA clones that contain the entire coding sequence for the protein. Both encoded protein sequences are highly homologous to that of other species, especially in their COOH-terminal halves. Because this region includes the DNA binding domain, it is not unexpected that the DBD of both rtAR-α and rtAR-β could recognize the human AR response element (for rtAR-β based on activity of LBD chimeras; e.g. pCMV-AβAβG in Fig. 6). Homology between the human AR and rtAR-α is less strong at the NH2-terminal half (19% identical) than at the COOH-terminal half (DBD, 92% identical; LBD, 64% identical). The lower homology in the NH2-terminal half could be significant for regulation of gene activity, as this segment contains the region involved in transcriptional activation.

Precedence for isoforms of a sex steroid receptor was set recently by the discovery of a second estrogen receptor, ER-β (26, 27). ER-β shares many functional similarities with the classic estrogen receptor, ER-α, including substrate and antagonist binding affinities. However, there are differences in their tissue localization and in the mechanisms regulating their transcriptional activities. In the case of the AR, all data suggest that there is only a single gene in human (28). In contrast, our results demonstrate the existence of two distinct isoforms of ARs in rainbow trout. Interestingly, despite their highly similar amino acid sequences, one is active in our transient assay, and the other is not (Fig. 4).

Several lines of evidence demonstrate that the inactivity of rtAR-β in our assays is due to the sequence of its LBD. Interchanging the rtAR-α or GR-I LBD for that of rtAR-β in chimeric proteins produced an active receptor; and surprisingly, even replacement by the corresponding rtAR-α segment of any one of...
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three out of four subregions of the LBD was sufficient to produce activity. Consistent with this protein mapping data, ligand binding assays using cell extracts from transfected COS-1 cells revealed that rtAR-β bound [3H]mibolerone with an affinity comparable with that of the human AR, whereas no binding could be detected with rtAR-β (Fig. 7). An absence of ligand binding by rtAR-β in vivo was indicated by its failure to translocate to the nucleus when transfected COS-1 cells were treated with MT (Fig. 8). Furthermore, tests of the various chimeric constructs show a perfect correlation between transcriptional activation, ligand binding, and nuclear translocation. Thus, all our data indicate that the rtAR-β is inactive in the transient assay due to a lack of ligand binding.

The rtAR-α that we have isolated presumably mediates the androgen responses in the rainbow trout. The function of the rtAR-β, however, is unclear. We consider four possibilities. One is that the rtAR-β gene is a duplication of the rtAR-α that has devolved to nonfunctionality. This seems quite unlikely, however, as none of the 130 amino acid changes has resulted in an inactive protein. A second possibility is that rtAR-β is indeed inactive, but serves to modulate the activity of rtAR-α by forming a heterodimer. However, in preliminary experiments we have found no effect of co-transfection of the rtAR-β expression vector on the activity produced by rtAR-α. Generation of antibodies specific for either rtAR-α or rtAR-β, now under way, will permit assay for heterodimers in extracts of fish tissues. A third possibility is that the rtAR-β requires an accessory factor for hormone binding that is not present in either the EPC or COS-1 cells that we have used. The alteration in rtAR-β that prevents binding is apparently quite subtle, as replacements of small subsections of the LBD with the corresponding rtAR-α sequence restores activity. In fact, as the result of an inadvertent PCR-induced mutation we found that activity was restored by substitution of the glutamine at position 646 with an arginine (data not shown). The tenuousness of the binding deficit suggests it might be readily reversed by association of the rtAR-β with an accessory factor. On the other hand, whereas there is ample precedent for accessory proteins affecting the transcriptional potency of hormone receptors (10, 11), there is none for affecting ligand binding. Finally, it is possible that the ligand for rtAR-β differs from those that we have tested (methyltestosterone, dexamethasone, aldosterone, progesterone, and 17β-estradiol), perhaps being unique to fish. The antibody specific for rtAR-β that we are in the process of preparing should also be useful for examining this question, by determining if rtAR-β appears in the nucleus of fish cells.

In summary, the molecular cloning of the fish AR cDNAs described here identifies AR proteins that will be important for determining the mechanism of sex determination in fish. In addition, these proteins will be useful for comparative, structure-function analyses of mammalian ARs. Finally, the discovery of a highly homologous AR isoform, rtAR-β, which is inactive in the conventional assays we have used, raises the possibility of a novel function for a hormone receptor.

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