Transcriptional regulation by the androgen receptor (AR) requires its binding to hormone response element nucleotide sequences in DNA. A consensus glucocorticoid response element (GRE) can mediate transactivation by AR and other members of the AR/glucocorticoid (GR)/progesterone (PR)/mineralocorticoid (MR) receptor subfamily. We identified putative androgen response element (ARE) sequences by binding of a human AR DNA-binding domain fusion protein to DNA in a random sequence selection assay. A 17-base pair consensus nucleotide sequence, termed IDR17, containing three potential GRE-like core binding sites organized as both inverted and direct repeats, was determined from a pool of degenerate oligonucleotides. IDR17 was active in mediating androgen-dependent induction of reporter gene expression in transient transfection assays. Dissection of the IDR17 sequence revealed an 11-base pair sequence (DR-1), consisting of two potential core binding sites oriented as an overlapping direct repeat, as the most potent ARE. DR-1 demonstrated a strong preference for AR binding and transactivation when compared with GR. To our knowledge, this is the first observation that a direct repeat of GRE-like core motifs functions as a preferred hormone response element within the AR/GR/PR/MR subfamily of nuclear receptors.

Nuclear hormone receptors constitute a family of transcription factors that function by binding to specific DNA sequences in regulatory regions of target genes known as hormone response elements (HREs)1 (1–5). The general structure of HREs has been well characterized (6–8). They are generally composed of six base pair receptor binding sites, oriented as inverted or direct sequence repeats and separated by a variable number of spacing nucleotides. Specificity of an HRE is a property of the primary sequence of the individual binding sites as well as the spacing and orientation of the binding motifs (7–12). Specificity of hormone action may also be conferred by interaction between the receptor and other transcription factors (6, 7). In this case, the HRE is a more complex element that includes multiple simple HREs (partial palindromes or a single binding site) together with adjacent binding sites for other factors (13–16).

HREs comprise several subgroups corresponding to evolutionary conservation among subfamilies of the receptors. Androgen receptors (ARs) share a high degree of homology with glucocorticoid receptors (GRs), progesterone receptors (PRs), and mineralocorticoid receptors (MRs) in their DNA-binding domains (17–22). A well characterized HRE for this subfamily of receptors is the glucocorticoid response element (GRE), which is composed of inverted nonidentical hexamer binding sites separated by a spacing of 3 nucleotides, GTGACAnnT-GTTCT (6–8, 23). This sequence mediates androgen-induced, as well as glucocorticoid- and progesterone-induced, gene expression (23–26). Another subfamily of nuclear receptors consists of the thyroid hormone (TR), retinoic acid (RAR), and vitamin D (VDR) receptors (7–9). A striking feature of this latter subfamily of receptors is their recognition of a similar consensus nucleotide sequence binding site, with specificity of hormone response being determined by different spacing and orientation of the binding site motifs. Spacing of the consensus binding half-sites by 3, 4, or 5 nucleotides creates HREs for VDR, TR, and RAR, respectively (7, 10–12). In addition to direct repeats, both TR and RAR can recognize HREs consisting of inverted receptor binding sites (11, 12).

Naturally occurring androgen response elements (AREs) fall into two categories. Some AREs, such as those identified in the C3 subunit gene of rat prostate binding protein and mouse sex-limited protein (13, 27, 28), consist of GRE-like sequences with inverted binding sites separated by a 3-bp nucleotide spacer. In other AREs, like those in the probasin (29) and 20-kDa cystatin-like (16) genes, only single binding site sequences are apparent. However, a recent report showed that the 5'-subsequence, 5'-GGTTCT-3', within the partial palindrome of the probasin ARE-2 binding site, excludes GR binding but permits binding of AR (30). Another possibility for receptor-specific binding is that sequences adjacent to receptor binding sites create composite activation elements that also involve the binding of other factors (13–16). An additional hypothetical means of conferring hormone specificity is the spacing and orientation of binding sites within the DNA sequences that bind members of the AR, GR, PR, and MR subfamily. GR and AR were recently shown to bind to direct repeat DNA elements (30, 31) in addition to the more typical inverted repeat sequences. In this report, we provide evidence that a member of
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this subfamily, namely AR, can activate transcription of a reporter gene through an HRE consisting of a novel direct repeat nucleotide sequence that preferentially binds AR.

EXPERIMENTAL PROCEDURES

Plasmids—The following plasmid DNAs were generous gifts: OB7 (hGR) from Dr. R. Evans (Salk Institute for Biological Studies, La Jolla, CA); pCMV-hGR from Drs. F. French and J. Tan (University of North Carolina, Chapel Hill, NC); BPlclAT2 from Dr. C. Young (Mayo Clinic, Rochester, MN). MMTV-CAT and pCMV-hAR were used in our previous studies (32). Plasmid pGEX-2T was purchased from Pharmacia Biotech Inc., and pBluescript was obtained from Stratagene (La Jolla, CA).

Construction of hAR and hGR Prokaryotic Expression Vectors—Fragments of human AR and GR DNA-binding domains were prepared by PCR amplification under standard conditions from hAR (pCMV-hAR) and hGR (OB7) cDNAs. For hAR, the sense primer was 5'-CCCGGAATTCCTCGCTGTACGTGCGATGAA-3', which hybridizes to nucleotide positions 3035–2054, and the antisense primer was 5'-CCCCGGAATTCATTCTTCTCCTCTGATTGT-3', which hybridizes to nucleotides 2274–2297 (17). For hGR, the sense primer was 5'-CCCGGAATTCCCTCTGGTGTCGTCTGATGAA-3', which hybridizes to nucleotides 1392–1413, and the antisense primer was 5'-CCCCGGAATTCCTGCTGTTGCGCTGCTGAATTCC-3', which hybridizes to nucleotides 2274–2297 (17).

Expression and Purification of hAR and hGR Fusion Proteins—Freshly diluted cultures of pGEX-hAR or pGEX-hGR were grown for 1 h prior to induction of protein expression by growth in 1 mM isopropyl-β-D-thiogalactopyranoside for an additional 3.5 h. Cells were collected by centrifugation (5000 × g, 10 min, 4 °C) and lysed by sonication in 5 ml of ice-cold phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride. After the addition of 1% Triton X-100 and centrifugation (10,000 × g, 5 min, 4 °C), the supernatant was incubated (25 °C, 24 h) with 1 ml of 50% (w/v) glutathione-agarose beads with gentle mixing. The beads were washed three times with T4 DNA ligase buffer in the presence of dNTPs. The double-stranded Oligo 1 and Oligo 2 were eluted from the beads by incubation (25 °C, 4 min) with 0.5 ml of 50 mM Tris-HCl (pH 8.0) containing 15 mM reduced glutathione. The beads were recovered by centrifugation, and the elution step was repeated four or five times.

Oligonucleotide Probes—For random sequence selection assays, a pool of degenerate 55-bp oligonucleotides was synthesized. The 5' and 3' ends contained restriction endonuclease sites for BamHI and EcoRI, respectively, for subcloning purposes and were prepared for primer annealing to convert oligonucleotides from single- to double-stranded DNA and for subsequent PCR amplification. The sequence of Oligo 1 was 5'-GACGGAGATCCATGGAAATAN18ATCTGCTGATATCTGCGA-3'. The sequence of Oligo 2 was 5'-GACGGATCCATTGCAATAN18ATCCTGTAGGAATTCGGGA-3'. The pair of primers for both PCR products contained 18 nucleotides with a region of degenerate nucleotide sequence. The AR oligonucleotide was annealed to this subfamily, namely AR, can activate transcription of a reporter gene through an HRE consisting of a novel direct repeat nucleotide sequence that preferentially binds AR.

Reduction of nonspecific binding. The incubation mixtures were loaded on 5% non-denaturing polyacrylamide gels, and EMSAs were run at 10 V/cm for 4 h in 100 mM Tris-HCl (pH 8.0), 100 mM boric acid, 1 mM EDTA buffer at 4 °C. For analytical purposes, the gels were dried before autoradiography. For preparative purposes, gels were exposed directly for 1–2 h at 4 °C to X-Omat film for localization of the DNA and DNA-protein complex(es). Regions of the gel containing the shifted bands of DNA were excised and recovered by elution. Half of the recovered DNAs were amplified by PCR, radiolabeled, and used for successive rounds of selection. For EMSA in other experiments, oligonucleotides (GRE, IDR17, and DR-1) with defined sequences were used as probes. The DR-1 (23) probe represented a dimer of annealed and ligated DR-1 oligonucleotides derived by digesting the DR-1-pBlcAT plasmid (described below) with HindIII and BamHI. A 90-bp DNA fragment was isolated and radiolabeled by Klenow fragment in the presence of [α-32P]dCTP. For these experiments, 50–500 ng of either AR-DBD or GR-DBD fusion proteins were incubated with 25-bp-labeled DNAs in binding reactions. For competition assays, the proteins were first incubated with 10–1000-fold excess of unlabeled double-stranded oligonucleotide competitor at 25 °C for 10 min before the addition of the radiolabeled DNAs.

PCR Amplification of Selected DNA—Oligonucleotide DNAs recovered from EMSA were dissolved in 10 μl of 0.1 × TE buffer. For PCR amplification, 5 μl of the DNAs were used as templates in a standard 50-μl PCR reaction. DNA bands were detected with ethidium bromide and gelled on 5% polyacrylamide gels and recovered by elution as described above.

The DNAs amplified by PCR after the last round of selection were digested with BamHI and EcoRI to create cohesive ends and ligated into the plasmid, pBluescript KS1. Individual clones were sequenced by the dideoxy nucleotide termination method (Sequenase 2.0 kit, U.S. Biochemical Corp.)

Construction of Chimeric Reporter Genes—Single-stranded oligonucleotides IDR17, IR0, IR5, and DR-1 were phosphorylated, and equal amounts of the complementary strand of each oligonucleotide were annealed to generate cohesive ends with overhangs corresponding to XbaI recognition sites. The double-stranded oligonucleotides were converted to a single-stranded template, and the labeled DNA was separated on 5% polyacrylamide gels. Dimers of IDR17, IR0, IR5, and DR-1 were excised from the gel, eluted, recovered by ethanol precipitation, and ligated into the XbaI site of the pBlcAT2 plasmid. Positive clones were screened by restriction mapping and confirmed by sequencing.

Cell Culture and DNA Transfection—CV-1 cells were transfected with plasmid DNAs by the calcium phosphate/DNA co precipitation method, as described previously (32). The cells were subsequently cultured in Dulbecco’s modified Eagle’s medium containing 5% charcoal-stripped fetal bovine serum in the absence or presence of 0.01–10 nM R1881 (methyltrienolone) or 1–1000 nM dexamethasone for 16–24 h. The CAT reporter gene plasmids IDR17-R1, IR0-R5, and DR-1-pBlcAT2 or MMTV-CAT were cotransfected with the receptor expression vector or with the “empty” expression vector as negative controls for the specificity of steroid induction. The CAT and β-galactosidase enzyme activities were assayed in 50-μl aliquots of the cell extracts as described previously (32). CAT activity was quantitated by cutting and counting the radioactivity from the thin layer chromatography plates by scintillation spectrophotometry. CAT activities were normalized for transfection efficiency based upon their corresponding β-galactosidase activities.

RESULTS

Random Sequence Selection for an AR Binding Site—The method of random sequence selection and amplification originally described by Blackwell and Weintraub (34) was adopted to isolate AR binding sites. The DNA was a pool of oligonucleotides with a region of degenerate nucleotide sequence. The AR protein was a recombinant protein composed of amino acids 559–644 of the human AR-DBD fused to glutathione S-transferase (GST). This fusion protein was expressed in Escherichia coli and purified by affinity binding to glutathione agarose and elution in the presence of reduced glutathione. In preliminary
studies, the AR-DBD fusion protein was incubated with an oligonucleotide containing a consensus GRE/ARE nucleotide sequence and was shown to form a protein-DNA complex by EMSA. For the random sequence selection assay, the AR-DBD fusion protein was incubated with a 55-bp oligonucleotide containing an 18-bp central region of totally degenerate nucleotide sequence (N₁₈). The presence of an 18-bp degenerate region was based upon the assumption that a 15-bp consensus GRE sequence was sufficient for binding of an AR dimer and that a specific binding site for AR would resemble this model. On the initial round of selection, binding of the AR-DBD fusion protein to a small percentage of the degenerate oligonucleotides was detected by EMSA (data not shown). The DNA was isolated from the gel and amplified by PCR, to enrich the population of DNAs bound by the AR-DBD fusion protein, prior to the next round of selection. As expected, the proportion of selected and amplified DNA that bound to the AR-DBD fusion protein increased during successive rounds of selection by EMSA. Following six rounds of selection, DNA from the shifted protein-DNA complex was isolated and subcloned, and 39 individual clones were sequenced (Table I). Alignment revealed a single hexamer nucleotide consensus binding site, TGTTCT/TTC, identical to a GRE half-site, within the degenerate region as a preferred sequence for AR binding.

Based upon these preliminary studies and the similarity of the consensus hexamer binding site to that within known GREs that bind AR, we synthesized a second pool of 55-bp oligonucleotides containing a 13-bp region of degenerate nucleotide sequence (N₁₃) adjacent to a 6-bp (TGTTCT) consensus AR/GR/PR binding site, i.e. 5’T-GGTCT-3′. We predicted that the consensus binding site sequence would accommodate binding of one AR molecule and that the random sequence would allow identification of a second binding site with optimal binding of one AR molecule and that the random sequence selection assay, the AR-DBD fusion protein was incubated with an oligonucleotide sequence (N₁₃) adjacent to a 6-bp (TGTTCT) consensus oligonucleotides containing a 13-bp region of degenerate nucleotide sequence. Functional Activity of IDR17—To examine the IDR17 consensus AR binding site sequence as a functional ARE, two copies of IDR17 were cloned into the pBlCAT2 plasmid upstream of the thymidine kinase promoter. The IDR17-pBlCAT reporter gene construct was tested for androgen-dependent expression of CAT activity following cotransfection with the human AR cDNA expression vector, pCMVhAR, into CV-1 cells. The synthetic androgen, R1881, induced a dose-dependent increase of CAT activity, which was not observed in the absence of androgen or in cells transfected with the empty potential binding site is a 6-bp inverted repeat, -GGAACA-, which allows no intervening spacer between itself and the original binding site, -TGTTCT. In addition, there is another 5-bp sequence, GGAAC, which forms a direct repeat, overlapping by 1 bp with the adjoining 6-bp sequence, -GGAACA. We named this putative 17-bp AR binding site sequence IDR17 due to its content of both inverted repeats (IRs) and direct repeats (DRs).
lated by EMSA after the first (lane 2) IDR17 even at 100 nM dexamethasone. These results clearly lower stimulation of CAT activity by GR was evident with compared with those formed by GR with the identical DNA fragments, suggesting possible differences in their binding conformations. This experiment further validates the specificity of IDR17 as an androgen receptor binding site.

**Dissection of IDR17 Binding Sites**—Three potential dimeric binding sites can be proposed among the three putative hexamer sequences within IDR17. To determine which of these sequences acts as an ARE, we created the oligonucleotides shown in Fig. 4A. For oligonucleotide IR0, nucleotide bases n9 of IDR17 were substituted with nonspecific nucleotides, leaving only the hexamer IRs without a spacer (n8) of nonspecific nucleotides. For IR5, nucleotides n9,11 of IDR17 were substituted by five (n5) nonspecific bases, leaving the two 6-bp binding sites at the 5’ and 3’ ends, respectively, as IRs. For DR-1, the 3’ binding site specified by -TGTTCT was substituted by nonspecific nucleotides, leaving only an 11-bp sequence containing a 1-bp overlap (n9) of two hexameric DRs. Two copies of each IDR17 derivative were cloned into pBLCAT2 upstream of the thymidine kinase promoter and cotransfected with AR to test the activities of IDR17 and its derivatives in androgen-dependent induction of CAT reporter gene expression (Fig. 4B). When transfected CV-1 cells were incubated in the absence and presence of different concentrations of R1881, IR0-pBLCAT showed constitutive CAT activity that was independent of androgen induction. IR5-pBLCAT was not stimulated by androgen and showed only basal expression. DR-1-pBLCAT, however, was highly responsive to androgen induction, reaching a level 180-fold above base line, and CAT activity exceeded that achieved by IDR17-pBLCAT with equivalent concentrations of R1881. The transactivation function of DR-1 was strictly androgen-dependent and required cotransfection of the AR.

**Binding of AR to DR-1**—The finding that AR was able to activate transcription from the direct repeat sequence, DR-1, was unexpected in light of the structure of other ARE/GRE/PRE sequences identified to date. To determine whether DR-1 was a binding site for AR, the AR-DBD fusion protein was incubated with the DR-1 oligonucleotide and analyzed by EMSA. When a single DR-1 nucleotide sequence was present in the DNA, a low level of binding was observed for the shifted DNA-AR complex (Fig. 5A). However, when a tandem repeat of the DR-1 nucleotide sequence (DR-1, 2x) was present in the DNA fragment used as the probe, the binding of AR was greatly enhanced, and multiple shifted protein-DNA complexes were seen (Fig. 5B). We conclude that a single copy of the DR-1 sequence functions as a binding site for AR and that under conditions favoring cooperative binding of AR as in the head-to-tail tandem repeat (DR-1, 2x), this same nucleotide sequence functions as an even higher affinity AR binding site.

**DR-1 Is Specific for AR**—The binding of AR-DBD and GR-DBD fusion proteins bound quantitatively similarly to the consensus GRE at each of several protein concentrations. By contrast, the AR-DBD fusion protein bound with high affinity to IDR17 and displayed a distinctive pattern of protein-DNA complexes, whereas binding of the GR-DBD fusion protein to IDR17 was relatively weak. This pattern of protein-DNA complexes was also characterized by reproducible differences in the relative mobility of complexes formed by AR compared with those formed by GR with the identical DNA fragments, suggesting possible differences in their binding conformations. This experiment further validates the specificity of IDR17 as an androgen receptor binding site.

**Characterization of ARE Direct Repeat**

*Fig. 1.* Binding analysis of DNAs selected from partially random sequence oligonucleotides. The pool of 55-bp partially random sequence oligonucleotides in the initial mixture (lane 1) or those isolated by EMSA after the first (lane 2), third (lane 3), and fifth (lane 4) rounds of bindingselection were radiolabeled with $\gamma^32P$ATP. The radiolabeled DNA (10^6 cpm) was incubated with 200 ng of the AR-DBD protein in the binding reaction. The free and protein-bound DNA were separated by EMSA on 5% non-denaturing polyacrylamide gels and visualized by autoradiography. The two shifted DNA-protein complexes are indicated by the *arrows.*

pCMV5 expression vector (data not shown). These results indicate that the IDR17 sequence functions as an ARE in vivo.

To examine the specificity of hormone response, IDR17-pBLCAT was compared with MMTV-LTR-CAT, which contains four GRE/AREs in its 5’-flanking sequence. Expression plasmids containing either the human AR or GR cDNAs were co-transfected with a reporter gene plasmid, and cells were incubated with R1881 or the synthetic glucocorticoid, dexamethasone. In contrast to MMTV-LTR-CAT, where CAT activity was induced to a higher level by glucocorticoid than by androgen in the presence of their respective receptors, IDR17-pBLCAT was much more responsive in the presence of androgen and its receptor than in the presence of glucocorticoid and its receptor (Fig. 2). Nearly maximal induction of CAT activity by AR with IDR17 occurred at 1 nM R1881, whereas much lower stimulation of CAT activity by GR was evident with IDR17 even at 100 nM dexamethasone. These results clearly demonstrate that IDR17 not only functions as an efficient ARE but also that it is preferentially induced by androgens when compared with the generic GRE/ARE-like hormone response elements of MMTV-LTR that respond more favorably in the presence of GR rather than AR.

The binding site specificity of IDR17 for AR was also tested by EMSA. Binding of the AR-DBD fusion protein to IDR17 was compared with the binding of a similar human GR recombinant protein containing an analogous region of its DNA-binding domain amino acids (421–506) fused to GST. An oligonucleotide containing a perfect 15-bp palindromic GRE nucleotide sequence (-AGACAgcTGTTCT-) with the same flanking sequence as IDR17 was tested in parallel. As shown in Fig. 3, the AR-DBD and GR-DBD fusion proteins bound quantitatively similarly to the consensus GRE at each of several protein concentrations. By contrast, the AR-DBD fusion protein bound with high affinity to IDR17 and displayed a distinctive pattern of protein-DNA complexes, whereas binding of the GR-DBD fusion protein to IDR17 was relatively weak. This pattern of protein-DNA complexes was also characterized by reproducible differences in the relative mobility of complexes formed by AR compared with those formed by GR with the identical DNA fragments, suggesting possible differences in their binding conformations. This experiment further validates the specificity of IDR17 as an androgen receptor binding site.
of CAT reporter gene expression mediated through binding of AR or GR to the DR-1 nucleotide sequence in the presence of R1881 or dexamethasone, respectively. R1881 induced a 10–12-fold increase in CAT activity by AR compared with a minimal (0.8-fold) effect of dexamethasone on CAT activity by GR, when a single copy of the DR-1 element was present. Reversing the orientation of the DR-1 element had no effect on the induction of CAT activity by AR or GR. As shown previously in Fig. 4 B, tandem copies (2x) of DR-1 produced a synergistic response in CAT activity by AR in the presence of R1881, 87-fold above baseline, when compared with a single copy of DR-1 (11-fold above base line). In addition, stimulation of CAT activity by AR was maximal at 1 nM R1881 and 5-fold greater than the maximal CAT activity induced by GR at the highest concentration (100 nM dexamethasone) of glucocorticoid. In the same experiment, GR and AR induced CAT activity maximally by 92- and 35-fold, respectively, from the MMTV-LTR promoter, in the presence of 1 nM R1881 and 1 µM dexamethasone (data not shown). These results allow us to conclude that DR-1 binds AR selectively and with high affinity, leading to the preferential androgen stimulation of gene expression.

DISCUSSION

How specificity of steroid hormone action is achieved remains an important question. In principle, specificity can be generated at any step in a signal transduction pathway. One obvious level of cellular control is determined by the differential expression of specific steroid receptors in target cells. The presence of specific HREs as genetic codes for selective regulation of gene transcription also plays a key role in hormone action (1–8). Although a consensus HRE has been associated with transcriptional regulation by the AR/GR/PR/MR subfamily of nuclear receptors (23–27), it is not known how the specificity of these individual receptors is determined. For instance, prostate cells express AR, GR, and PR, yet the actions of these hormones and their specificity for activating or repressing gene expression differ considerably (35, 36). Within the subfamily of receptors composed of TR, RAR, and VDR, both spacing and orientation of similar core binding motifs within the HREs determine receptor specificity (7–12). In an effort to understand how cells might distinguish among AR, GR, and PR actions, we hypothesized that a specific binding site sequence might differentiate AR action from other receptors of its subfamily, and we searched for an ARE in the present studies.

To identify a specific ARE with a relatively unbiased, sensitive, and simple approach, we adopted the random sequence selection method (34) and utilized a two-stage approach. In the first stage, binding of the AR-DBD fusion protein to a pool of random oligonucleotides formed a single DNA-protein complex with the consensus receptor binding half-site, TGTTC(T/C), indicative of binding by a receptor monomer. We also observed a single shifted protein-DNA complex when the consensus GRE containing two half-sites as inverted repeats with a 3-bp nu-
cleotide spacer was bound to human AR-DBD and GR-DBD fusion proteins until greater concentrations of either fusion protein led to the formation of additional higher order complexes. Using β-galactosidase-human AR or protein A-rat AR fusion proteins, Young et al. (37) and De Vos et al. (38), respectively, also observed a single DNA-protein complex with the consensus GRE sequence on gel shift assays.

The absence or weak ability for dimerization or cooperative binding of the AR-DBD fusion proteins may be due to absence of the receptor N and C termini, which contain strong dimerization functions that are active in solution (39). Although a weak dimerization domain is present in the DNA-binding region of the steroid receptors (7, 8, 40–42), this interaction may occur predominantly following binding to DNA, which places the receptor monomers in the preferred orientation (12). The presence of prokaryotic protein domains in the fusion proteins may also alter the cooperative interaction of the receptor-DBD dimerization domains, since glutathione S-transferase is known to form homodimers. In normal target cells, the native receptor proteins may also interact with other factors involved in transcriptional regulation, and such interaction may increase the specificity, as well as the affinity, of receptors for the cognate response elements (6, 12, 43).

To enhance the probability of finding the optimal half-site spacing for an ARE, we synthesized a second pool of oligonucleotides containing the previously defined half-site sequence, TGTTCT, adjacent to a 13-bp random sequence. The appearance of a second lower mobility DNA-protein complex in the second and subsequent rounds of binding and selection suggests the involvement of more than one AR molecule in the binding to individual oligonucleotides. By contrast, a previous study by Roche et al. (44), using a random sequence selection method and a protein A-rat AR DNA-binding domain fusion protein, selected a 15-bp imperfect palindrome, 5'-GG(A/T)A-CAnnnTGTTCT-3', which is the same as the consensus GRE. Roche et al. (44) used oligonucleotides with a longer 26-bp random sequence, such that all potential sequences may not have been represented in the starting pool. Binding of AR protein to the selected oligonucleotides resulted in two shifted binding of the AR-DBD fusion proteins may be due to absence of the receptor N and C termini, which contain strong dimerization functions that are active in solution (39). Although a weak dimerization domain is present in the DNA-binding region of the steroid receptors (7, 8, 40–42), this interaction may occur predominantly following binding to DNA, which places the receptor monomers in the preferred orientation (12). The presence of prokaryotic protein domains in the fusion proteins may also alter the cooperative interaction of the receptor-DBD dimerization domains, since glutathione S-transferase is known to form homodimers. In normal target cells, the native receptor proteins may also interact with other factors involved in transcriptional regulation, and such interaction may increase the specificity, as well as the affinity, of receptors for the cognate response elements (6, 12, 43).

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AR activates transcription DBD was not created by the fusion partner, since full-length with binding to the DR-1 sequence. Binding of the GST-AR-GST did not restrict binding, but perhaps protein A interfered or restrict potential binding to DNA sequences. The inclusion of inverted and direct repeat configurations and functions as a random (n18) and partially random (n 13) oligonucleotide pools. GRE half-site sequence, was identified from both the totally consensus GRE. The primary half-site sequence, TGTTC(T/C) (or fundamental similarities and striking differences to the con-

dimer formation, the ability of homo- or heterodimers to bind properly spaced half-sites can be observed for DBDs alone (48–52). While these domains do not form dimers in solution in the absence of DNA, the target DNA serves as a scaffold that induces specific interactions between adjacent monomers, thus stabilizing the DNA-protein complex (53–55).

IDR17 contains three potential binding sites with both in-
verted and direct repeat configurations and functions as a preferred sequence for AR binding and androgen-dependent transactivation. When present in reporter gene constructs and cotransfected with the full-length AR into CV-1 cells, it is the truncated DR-1 sequence, however, instead of the full-length IDR17, that activates gene transcription most efficiently. Recent work has demonstrated that TR complexes can contact at least 20 nucleotides, including upstream and downstream flanking sequences, a spacer, and two half-sites (56, 57). In fact, random sequence selection assays determined that the optimum binding site for TR actually consisted of an octamer nucleotide sequence rather than a hexamer (57). Furthermore, direct repeats of the optimal binding site, separated by 1–5 bp, all functioned as equally strong TREs (12, 49). More recently, direct repeats of TGTTCT and RGGTCA motifs have been shown to function as response elements for OR and ER, respectively (31). In addition, binding of these receptors to direct repeats with different spacings between the half-sites suggested that binding to direct repeats was more flexible than binding to palindromic elements (31). However, binding of AR to a direct repeat, DR-1, with a one-base overlap remains unique. The asymmetrical head-to-tail arrangement of receptor dimers implied by the tandem of direct repeats suggests that the receptor subunits interact through a DNA-supported inter-

face involving the carboxyl-terminal extension of the DNA-binding domain (12) or the ligand-binding domain (31) rather than the dimerization domain in the D box of the DNA-binding domain, which appears to be nonfunctional under such conditions. Studies specific to homodimerization of the ligand-acti-

vated AR have suggested that monomers were oriented anti-
parallel to each other, allowing interaction between N- and C-terminal domains of the opposing monomers (58).
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A number of GRE-like sequences have been identified in the 5'-flanking or intron regions of some androgen-regulated genes, such as rat prostatic binding protein C3 subunit (27, 28), rat probasin (29), human prostate specific antigen (38), human genes, such as rat prostatic binding protein C3 subunit (27, 28), excluding the binding of GR (60). Interestingly, direct repeats that the left subsequence, 5'-TGTCCT-3' respectively interacts with the DNA-binding domain of the rat AR and not with that of the GR (60). Furthermore, it was concluded that the left subsequence, 5'-GGTTCT-3', was responsible for excluding the binding of GR (60). Interestingly, direct repeats of GGTTCT separated by a 3-bp spacer were able to specifically bind AR and not GR (60). The observation that direct repeats of binding sites can function as AREs provides the basis for a possible mechanism by which AR preferentially interacts with specific DNA sequences.

In summary, a 17-bp androgen receptor-specific binding site was identified. This sequence shares the same receptor binding site core motif as the consensus GRE but differs in the context in which these core motifs are arranged. The identified sequence showed preferential binding to AR in gel mobility shift assays and specific response to androgen induction in transfection assays, indicating that an ARE sequence distinct from the consensus GRE may confer androgen specificity.

Acknowledgments—We thank Dr. R. Evans (Salk Institute) for the gift of the human GR cDNA, Drs. F. French and J. Tan (University of North Carolina, Chapel Hill) for the pCMV-hGR expression plasmid, and Dr. C. Young (Mayo Clinic) for the pBL-CAT2 reporter gene construct.

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