Identification of a Functional Androgen Response Element in the Promoter of the Gene for the Androgen-regulated Aldose Reductase-like Protein Specific to the Mouse Vas Deferens*

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Mouse vas deferens protein (MVDP), a member of the aldo-keto reductase superfamily, is exclusively produced in the epithelial cells of the deferent duct under androgenic regulation. To better understand androgen-regulated MVDP gene expression, the location and sequences of androgen response elements (AREs) in the 5'-flanking DNA were determined. Sequence analysis revealed two putative AREs as follows: one between positions -1186 and -1171 (distal ARE) and the other between -111 and -97 (proximal ARE). To study hormonal regulation, fragments of the MVDP promoter region, extending from residue -1804 to +41, were linked to the chloramphenicol acetyltransferase (CAT) reporter gene and co-transfected with a human androgen receptor expression vector into T47D cells in a transient expression assay. A minimal region (-121 to +41) was identified as being sufficient for androgen-regulated gene expression. A mutation in proximal ARE almost completely abolished androgen induction of CAT. One copy of the sequence TGAAGT tcc TGTTCT, cloned in the opposite orientation in front of the thymidine kinase promoter, confers androgen responsiveness to the CAT reporter gene. Androgen transcriptional activity was not detected with the distal ARE.

The data provide strong evidence that transcriptional regulation of the MVDP gene occurs via the sequence TGAAGT tcc TGTTCT.

Transcription of eukaryotic genes by RNA polymerase II is mediated by two major cis-acting sequences as follows: promoter elements, which bind general transcription factors and define a basal transcriptional activity of the gene, and regulatable elements, which mediate either a positive or negative effect on the basal promoter activity (Maniatis et al., 1987). These regulatory sequences may act as hormone-dependent enhancers and confer hormone responsiveness to homologous and heterologous promoters irrespective of distance or orientation (Beato, 1989). In vivo, the expression of steroid-responsive genes is more complex, due to the action of tissue-specific factors, which also act via enhancer-like sequences (Adler et al., 1991). All of these cis-acting sequences serve as binding sites for sequence-specific DNA-binding proteins that are required in trans to mediate the transcriptional response. The classical steroid receptor proteins have been identified as the trans-acting factors required for function of the specific cis-acting steroid response elements (Beato, 1989).

The androgen receptor belongs to the superfamily of ligand-responsive transcription factors, which includes steroids, thyroid hormones, and retinoic acid receptors (Carson-Jurica et al., 1990). Specific cis-acting sequences with high affinity for glucocorticoids, progesterone, and estrogen receptors have been thoroughly characterized (for review, see Beato (1989)). The DNA-binding domains of the glucocorticoid, progesterone, mineralocorticoid, and androgen receptors (AR)1 are highly conserved sequences (homology of 80–90%), suggesting that they recognize similar cis-acting sequences (Evans, 1988; Lubahn et al., 1988). It has been found that in transfection experiments, glucocorticoids, progesterone, and androgens can all induce a reporter gene via a canonical glucocorticoid response element (GRE) (Ham et al., 1986). The DNA sequences to which steroid receptors bind are composed of two hexanucleotides arranged as an imperfect palindrome separated by three nonconserved base pairs (Beato, 1989; Nordeen et al., 1990). The consensus sequence of the responsive element for the glucocorticoid receptor and progesterone receptor is GGTACA nnn TGTT/CCT (Beato, 1989; Nordeen et al., 1990). It has been found that GREs from the mouse mammary tumor virus (MMTV) long terminal repeat and from the tyrosine aminotransferase gene also mediate transcriptional activation by androgens (Ham et al., 1998; Denison et al., 1989).

Although a large number of genes have been demonstrated to respond to androgens, there is little information regarding interactions between androgen receptors and specific sequences of androgen-responsive genes. Among the cellular genes that are transcriptionally regulated by androgens, the rat C3 (1) gene, which codes for one of the constituent peptides of prostatic binding protein, has been studied in more detail. Two restriction fragments located in the promoter region and in the first intron that are able to bind partially purified AR contain imperfect palindromic sequences resembling the GRE/PRE consensus sequence (Rushmere et al., 1987; Claessens et al., 1990). The intron fragment is shown to confer androgen responsiveness to the thymidine kinase promoter, and mutations within the GRE-like sequence suppress the response to androgens, suggesting that this sequence functions as an ARE (Claessens et al., 1989; Tan et al., 1992).

We have studied the mouse vas deferens protein (MVDP) gene, which is under hormonal, developmental, and tissue-specific control (Taragnat et al., 1988, 1990; Martinez et al., 1996.

The abbreviations used are: AR, androgen receptors; MVDP, mouse vas deferens protein; ARE, androgen response element; CAT, chloramphenicol acetyltransferase; kb, kilobase(s); GRE, glucocorticoid response element; MMTV, mouse mammary tumor virus; bp, base pair(s); DHT, dihydrotestosterone; PCR, polymerase chain reaction; PRE, progesterone response element.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U00149.

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1991). The amino acid sequence of MVDP, deduced from its cDNA nucleotide sequence, exhibits a high degree of homology with members of the aldo-keto reductase superfamily (Pailhoux et al., 1990), and the structure of the MVDP gene is very similar to that described for the human aldose reductase gene (Pailhoux et al., 1992). Although the physiological role of aldose reductase is still unclear, numerous studies suggest that this enzyme may play a key role in the etiology of some complications of diabetes mellitus (Kinoshta et al., 1981). Aldose reductase is a ubiquitous "housekeeping" enzyme, probably functional in all cells (Jeffery and Jornvall, 1983; Iwata et al., 1990).

It was shown in in vitro studies that in lens and corneal inner medulla, expression of the aldose reductase gene is highly regulated by hyperglycemia and hyperosmolarity (Bondy et al., 1989a, 1989b; Pettrash et al., 1992). However, to our knowledge, there is no example of an androgen-regulated aldose reductase.

MVDP is under androgen control, and testosterone is the signal that triggers MVDP gene expression at the protein and mRNA levels (Taragnat et al., 1988; Martinez et al., 1989). Hormonal induction of the MVDP gene is achieved mainly at the transcriptional level (Martinez et al., 1990). In addition, in vitro studies have shown that pure vas deferens epithelial cell cultures are able to produce MVDP in response to androgens in a medium devoid of other hormones, growth factors, and cAMP, suggesting that none of these factors exert a permissive influence on androgen action (Manin et al., 1992). The promoter of the MVDP gene contains a TATA-CATATA box, a CAAT box, a GC-rich motif, and a 5′-TGTTCT-3′ element that closely resembles the GRE/PER consensus sequence (Pailhoux et al., 1992).

Since only a limited amount of sequence information has been published for the 5′-upstream part of the MVDP gene (500 bp), we completed sequencing, including 1805 bp of the 5′-flanking region. To identify functional androgen response elements, transient expression experiments were performed using an androgen receptor vector and a reporter gene. As recipient cells, we have used the heterologous human breast cancer cell line T47D. By these means, we have identified a functional ARE that is essential for androgen-regulated expression of the MVDP gene.

**EXPERIMENTAL PROCEDURES**

**DNA Sequencing**—The mouse genomic clones employed in these studies were isolated from an amplified library (Clontech Laboratory) obtained by partial Sau3AI digestion of BALB/c liver DNA and insertion into pBluescript II KS(+) (Stratagene, La Jolla, CA). MVDP 5′-flanking regions were amplified by PCR with a pair of primers that was digested with restriction enzymes and the resulting fragments cloned into the pUC19 vector for DNA sequencing. DNA was sequenced by the dyeoxygenation termination method (Sanger et al., 1977) by using the T7 sequencing kit (Pharmacia LKB Biotechnology Inc.). Each subclone extremity was sequenced using universal and reverse pUC19 primers. Restriction maps of these clones were obtained by using the Mapma program (Promega).

**Construction of CAT Fusion Plasmids**—All plasmid constructs were prepared using standard methods (Sambrook et al., 1989). The promoterless basic plasmid pBLCAT2 (Luckow and Schütz, 1987) was used for cloning promoter restriction fragments from the MVDP gene upstream of the bacterial CAT gene. The genomic region investigated comprises 1846 bp of the 5′-flanking sequence. The vector pBLCAT2 contains a herpes simplex viral thymidine kinase promoter directing transcription of the CAT gene. The MMTV-CAT vector contains the upstream sequence of the long terminal repeat promoter of the mouse mammary tumor virus in front of a CAT reporter gene. The pSV-AR receptor vector (Brinkmann et al., 1989) contains an SV40 promoter directing the transcription of the full-length human androgen receptor cDNA. Recombinant plasmids were purified from clear lysates on cesium chloride gradients. Fragments from restriction enzyme-cleaved plasmids were analyzed by electrophoresis on 1% (w/v) agarose gels and identified in relation to molecular size markers by staining with ethidium bromide. Extensive restriction mapping was used to verify all constructs.

**Cell Culture and Transfection**—No androgenic induction was seen when fragments of the C3 (1) and prostate-specific antigen genes were cloned directly in front of the CAT gene or in front of a thymidine kinase-CAT construct and transfected in homologous prostate cell lines containing endogenous receptors (Parker et al., 1987; Riegman et al., 1991). We have chosen heterologous T47D cells in which testosterone-stimulated MMTV promoter activity (Parker et al., 1987). T47D cells were grown in Dulbecco’s modified Eagle’s medium supplemented with glutamine (2 mM), insulin (4 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% fetal calf serum. For each transfection experiment, cells from a subconfluent flask were seeded at a density of 1.6 × 10⁶ cells/dish and transfected 24 h later with plasmid DNAs using the DEAE-dextran method (Cato et al., 1986). Each transfection included 2.5 μg of pSV-AR vector (receptor expression) and 15 μg of MVDP DNA (regulation of the MMTV promoter) or 10 ng of MMTV-CAT (positive control of androgen induction) or 5 μg of pBLCAT2 vectors. Following a 1-h incubation with the DEAE-dextran/DNA precipitate and dimethyl sulfoxide shock, the cells were incubated in fresh Dulbecco’s modified Eagle’s medium supplemented with 10% steroid-free donor calf serum containing either 10⁻⁸ M dihydrotestosterone (DHT) or no additives. Cells were harvested after 48 h of hormone exposure for CAT assays.

**CAT Assays**—CAT activity of cell extracts was assayed according to the method of Neumann et al. (1987). Protein concentration was determined by the Bradford method (Bio-Rad). Samples containing 50–100 μg of total protein were incubated in 0.25 mL of 50 mM Tris pH 7.5, 0.1 mM acetyl coenzyme A, 0.2 μC of [³²P]chlorehemolhenol for 3 h at 37 °C. After thin-layer chromatography and autoradiography, acetylated and nonacetylated forms were cut out and quantitated by scintillation counting. CAT activity corresponds to the ratio of acetylated form radioactivity to total radioactivity (both forms). Average inductions and standard deviations were calculated from at least four independent transfections.

**PCR-Mediated Mutation of the ARE**—For the mutation of the ARE (-111–97) contained in the MVDP promoter, the fragment -125 to +75 was amplified by the PCR. The forward PCR primer (-125–86) carrying three mutations (underlined) for HindIII site production (position -119) and for ARE inactivation (positions -102 and -99) 5′-CACAAGCTT-GACATGAAGTTCC-CATGCCCCMCC-3′ and the reverse PCR primer (+51–+75) placed just downstream of a PstI site 5′-TGTGACT-GAGTTCACCGAAGTGGGC-3′ were used for p0.16 mCAT MVDP construct. A second forward PCR primer (+125–105) carrying one mutation (underlined) for HindIII site production (position -119) 5′-CACCAAGCTT-GACATGAAGTTCC-CATGCCCCMCC-3′ and the reverse PCR primer (shown previously) were used for p0.16 CAT MVDP construct.

The PCR products were cut with HindIII and PstI and isolated from a 2% agarose gel. The isolated fragment was ligated in HindIII-PstI digested plasmid pBLCAT3. The new plasmids p0.16 mCAT and p0.16 CAT were checked by sequencing.

**RESULTS**

**Sequence of the 5′-Flanking DNA Adjacent to the MVDP Gene**—The complete sequence of the MVDP gene (11 kb) and 0.5 kb of the 5′-flanking region have been previously determined (Pailhoux et al., 1992). We completed sequencing the gene and report here a sequence of 1.8 kb upstream from the transcription start site (Fig. 1). A CAT box, a GC box, and a TATA box sequence are respectively located 65, 51, and 31 bp upstream from the transcription start site. A 94-nucleotide long stretch at positions -746 and -472, respectively of purines. Numerous potential regulatory sites are present that bind proteins not known to affect MVDP gene expression (Table I). Other potential recognition elements for ATF, TCF, 1, 1, F-Act 1, USF, PCF, SIF, and NFκB (Faisst and Meyer, 1992) are also present in the 5′-flanking region of the MVDP gene. A poly(T) sequence of 14 nucleotides and a poly(A) sequence of 14 nucleotides are observed flanking the homopurine stretch at positions -746 and -472, respectively. A total of three potential cis-acting steroid response elements were identified in the 5′-flanking region of the MVDP gene by selecting for the right half-site of the consensus described. All are imperfect palindromes. A consensus sequence similar to that reported to function as an estrogen-responsive element is
Androgen Response Elements in MVDP Gene Promoter

A

-1804 GATCTCTCT TCAGATTCGAT TCAGAGGG GTCAATGCGA GAGACGTCAC
-1744 ACCTGGTAC GACCATGATA AGCTTGTGCT CATTACATGC ACAGCTGACAG
-1684 CCCTTTTTGT TTGGTCTCTTT CTGCTCTTTT CAGCTGCTGCT GAGCTCTCAC
-1624 TTCAATGAGG TCAGATTCGAT TCAGAGGG GTCAATGCGA GAGACGTCAC
-1564 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-1504 CTAGGTTCTA AACAGCTGCA AGCTTGTGCT CATTACATGC ACAGCTGACAG
-1444 ACCGTTCGAG CTCTAGTTTT GATCACTGAC AGCTTGTGCT CATTACATGC
-1384 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-1324 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-1264 TTCAATGAGG TCAGATTCGAT TCAGAGGG GTCAATGCGA GAGACGTCAC
-1204 GAAGGTTCG ACCATTTTTGT AACGCTCAGG GCCATGCGAC GCATGCGAC
-1144 ACCGTTCGAG CTCTAGTTTT GATCACTGAC AGCTTGTGCT CATTACATGC
-964 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-904 ACCTGGTAC GACCATGATA AGCTTGTGCT CATTACATGC ACAGCTGACAG
-844 CTAGGTTCTA AACAGCTGCA AGCTTGTGCT CATTACATGC ACAGCTGACAG
-784 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-724 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-664 ACCGTTCGAG CTCTAGTTTT GATCACTGAC AGCTTGTGCT CATTACATGC
-604 CACAAGGCT GGCTCTGGCT TGTCTGTGCT TGTCTGTGCT TGTCTGTGCT
-544 GAGATGCAAG GCACCAGGAC GCACCAGGAC GCACCAGGAC GCACCAGGAC
-484 TTGCAATGAGG TCAGATTCGAT TCAGAGGG GTCAATGCGA GAGACGTCAC
-424 CTCTAGTTTT GATCACTGAC AGCTTGTGCT CATTACATGC ACAGCTGACAG
-364 TTCTGCTCTGCT CATCACTGAC AGCTTGTGCT CATTACATGC ACAGCTGACAG
-304 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-244 TTCAATGAGG TCAGATTCGAT TCAGAGGG GTCAATGCGA GAGACGTCAC
-184 CTCTAGTTTT GATCACTGAC AGCTTGTGCT CATTACATGC ACAGCTGACAG
-124 CACAAGGCT GGCTCTGGCT TGTCTGTGCT TGTCTGTGCT TGTCTGTGCT
-64 ATGCTATGCT GAAGAGACGA AATGCTGTGC AATGCTGTGC AATGCTGTGC
-4 CACAGATGCAAG GCACCAGGAC GCACCAGGAC GCACCAGGAC GCACCAGGAC

FIG. 1. Sequence features of the MVDP gene promoter and 5'-flanking region. A, about 1800 residues of the sequence extending from nucleotides -1804 to +41 are shown. All negative numbering is relative to the start site of transcription (+1). The TATA box, GC box, CAAT box, and 94-bp homopurine stretch are underlined. GRE-like sequences are double underlined. B, restriction enzyme map of the MVDP gene promoter extending from nucleotides -1804 (Sau3AI) to +41 (PstI).

located at position -503. Two GRE-like sequences were identified at positions -1186 and -111 (Table I). The two AREs show a complete homology with the right half-site of the GRE/PRE consensus (5'-TGTTCT-3'). For the distal ARE, this homology is situated on the noncoding strand.

Analysis of MVDP Promoter Activity—As a control of the transfection efficiency and androgen induction, MMTV promoter activity was initially examined in T47D cells. Such constructs were described to be androgen-responsive in T47D cells that contain receptors for androgen, glucocorticoid, progesterin, and estrogen (Cato et al., 1987; Parker et al., 1987). In our experiments, MMTV promoter activity can be stimulated by progesterone but not by DHT (not shown). Cotransfection of T47D cells with MMTV-CAT and the androgen receptor expression vector (pSV-AR) gave positive results, suggesting that low levels of androgen receptor are responsible for the failure of DHT to induce MMTV promoter activity.

A series of MVDP putative promoter fragments were cloned in front of the CAT reporter gene. Since the transcription start site was found 40 nucleotides upstream of a PstI site, we ligated the Sau3AI-PstI (p1.8 CAT), HindII-PstI (p1.3 CAT), RsaI-PstI (p0.8 CAT), XbaI-PstI (p0.5 CAT), and HindIII-PstI (p0.16 CAT).
fragments containing this start point to the CAT reporter gene (Fig. 2). To delineate the responsive region, MVDP constructs were cotransfected with pSV-ARp in T47D cells in the absence or in the presence of 10⁻⁶ M DHT (T47D cells do not produce DHT, CAT acetylation). Cotransfection with pSV-ARp in the presence of DHT at various concentrations showed that DHT stimulated p0.5 CAT construct. Various concentrations of DHT showed stimulation of the thymidine kinase promoter activity (1.6-2.6-fold) in a dose-dependent fashion, irrespective of the orientation in front of the heterologous thymidine kinase promoter-CAT construct. Various concentrations of DHT showed stimulation of the thymidine kinase promoter activity (1.6-2.6-fold increase) in a dose-dependent manner (Fig. 3). This effect was not observed with the control pTk-CAT vector (pBLCAT2). Thus, within the -510 to +41 fragment, a DNA motif is able to function as an androgen-dependent enhancer.

Identification of Functional AREs—The GRE-like sequence at position -111 is the most likely candidate to be involved in the androgen responsiveness. To determine the role of this TGAAGT ccc TGTTCT sequence present in p0.16 CAT, it was mutated to TGAAGT ccc GTGGTAA in p0.16 mCAT (see "Experimental Procedures"). The mutated construct was cotransfected with the AR vector (pSV-ARp) into T47D cells. As shown in Fig. 3, deletion of the distal GRE-like sequence. Similarly, deletion of the homopurine stretch does not significantly affect the activity of the resulting fragments (p0.5 CAT compared with p0.8 CAT, Fig. 2).

Although a minimal region located -121 to +41 is sufficient for androgen-dependent transcriptional activity, the region -510 to +41 gave maximal androgen induction (about 12-fold). Cotransfection with pSV-ARp in the presence of DHT at various concentrations showed that DHT stimulated p0.5 CAT construct in a dose-dependent manner (Fig. 3). To determine whether the 0.5-kb fragment contains a functional ARE responsible for androgen responsiveness, it was cloned in opposite orientation in front of the heterologous thymidine kinase promoter-CAT construct. Various concentrations of DHT showed stimulation of the thymidine kinase promoter activity (1.6-2.6-fold increase) in a dose-dependent fashion, irrespective of the orientation of this fragment in the distal GRE-like sequence. Similarly, deletion of the homopurine stretch does not significantly affect the activity of the resulting fragments (p0.5 CAT compared with p0.8 CAT, Fig. 2).

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Androgen Response Elements in MVDP Gene Promoter

**Fig. 2.** Androgen-dependent transcriptional enhancer activities of the MVDP promoter and 5'-flanking region. A, autoradiogram of CAT gene expression assays measured on homogenates of cells cotransfected with different plasmid constructs and pSV-AR. B, induction of CAT activity by MVDP-CAT constructs. Restriction fragments of MVDP 5'-flanking region were cloned upstream of the CAT reporter gene in pBLCAT3. Nucleotide numbering is according to Fig. 1. (The arrow represents the transcription start site.) Constructs were cotransfected with pSV-AR into T47D cells. Cells were subsequently grown in the absence or presence of $10^{-6}$ M DHT. Asterisk, restriction site produced by PCR (experimental details are as described under "Experimental Procedures").

The mutation resulted in an almost total loss of the transcription activation by DHT compared with p0.16CAT. The results strongly suggest the importance of this motif in the androgen responsiveness of the MVDP gene.

As shown above, deletion of the distal GRE-like sequence does not decrease activity of the resulting fragments. However, to determine whether this sequence can act as a functional ARE, it was taken out of the MVDP promoter and linked to the thymidine kinase-CAT construct. We ligated the HincII-Sau3AI (about 180 nucleotides) and Sau3AI-XbaI (about 1300 nucleotides) to the pBLCAT2 plasmid, and these constructs were cotransfected with the AR vector in the presence of $10^{-6}$ M DHT. The results indicated that the distal GRE-like sequence, either alone (180 nucleotides) or associated with the homopurine stretch (1300 nucleotides), failed to enhance the activity of the thymidine kinase promoter (data not shown).

**DISCUSSION**

Sequence analysis of the upstream region from the androgen-regulated MVDP gene was carried out to locate conserved
sequence motifs that could possibly be involved in the androgen regulation or tissue specificity of expression. Sequences with proven GRE/PRE activity have been well characterized (Cato et al., 1986; Beato, 1989), and elements that mediate androgen-dependent regulation of gene transcription might be expected to be similar, since they share sequence similarity with the DNA-binding domain of glucocorticoid and progesterone receptors (Evans, 1988; Lubahn et al., 1988).

Two GRE-like sequences were identified within the 1.8 kb upstream of the MVDP gene, and we determined their activities in transcriptional enhancer assays. Gene fusion experiments with deleted fragments in the upstream region showed that a -121- to +41-bp fragment was sufficient for androgen-responsive CAT activity. We have identified a canonical ARE at that does not depend on tissue-specific factors from the vas deferens epithelial cells. The sequence 5'-TGAAGT tcc TTCT-3' of the functional ARE in the MVDP gene closely resembles one of the most effective synthetic oligonucleotides, 5'-GTTACA annn TTCTT-3', showing GRE and ARE activity (Ham et al., 1988; Roche et al., 1992). However, poor homology was observed with the left half-site of the GRE/PRE consensus.

Studies on the regulation of specific gene expression by androgen receptors have not been as successful as similar studies for other steroid hormones, owing to the fact that good model systems to study androgen-induced gene expression are in short supply. Most studies of androgen response elements have used the MMTV long terminal repeat, which responds to glucocorticoid, progesterone, and androgen via GRE/PRE consensus (Cato et al., 1987; Beato, 1989). Although the expression of a large number of genes is known to be androgen-dependent, relatively little is known about the interaction of the androgen receptor with the corresponding AREs. At present, among cellular genes, eight gene fragments have been shown to act as ARE. In the rat C3 (1) gene, a strong androgen binding region located in the first intron confers androgen responsiveness of the thymidine kinase promoter and contains a GRE half-site, which plays a critical role, as its mutation causes loss of induction (Rushmere et al., 1987; Classens et al., 1989, 1990; Tan et al., 1992). A 0.75-kb fragment, which was found to reside within the 5' long terminal repeat of an ancient endogenous provirus in the upstream region of the mouse sex-limited protein gene, was sufficient, in either orientation, for androgen-inducible expression (Loreni et al., 1988; Stavenhagen and Robins, 1988). The promoter of the prostate-specific antigen gene contains a functional ARE, the mutation of which almost completely abolishes androgen-inducible expression (Rieger et al., 1991). The mouse ornithine decarboxylase gene contains an ARE-like DNA motif starting at about -910 from the cap site, which was shown to be able to bind recombinant androgen receptor protein in vitro and to confer androgen responsiveness upon a reporter gene (Eisenberg and Jänne, 1989; Crozat et al., 1992). The plasma factor IX promoter contains a sequence resembling a consensus ARE that can bind androgen receptor in vitro but functions well only in CAT assays when multimerized (Crosley et al., 1992). The mouse β-glucuronidase gene contains an ARE-like sequence, located in the ninth intron, that mediates an-
In the C3 (1) gene, the substitution G to T in the core I1 hexamer mediated induction of the MVDP gene transcription. Similarly, the substitution ACA to GCC in the GREs of the MMTV long terminal repeat conferred nucleotides in this half-site is essential to maintain the ARE element (Luisi et al., 1989). This requirement for maximal androgen induction, the presence of the right half-site is not required for maximal androgen regulation (Rennie et al., 1991). Similarly, some androgen-regulated genes require the presence of additional DNA sequences (the nature of which remains to be determined) for full androgen induction (Riegman et al., 1991; Rennie et al., 1993). In addition to the potential cooperativity among the two AREs within the region upstream of the MVDP gene, androgen receptors may also interact cooperatively with other transcription factors to modulate MVDP gene expression. Several sequences, homologous with known recognition sites for gene regulatory proteins, were present in the 5'-flanking region of the MVDP gene (Table I). It has been previously shown that GREs can undergo synergistic interactions with a wide variety of heterologous binding sites, such as those for SP1, NF1, and CCAAT box binding factors (Schule et al., 1988; Strähle et al., 1988; Cato et al., 1988). Interestingly, consensus sequences for ubiquitous SP1, NF1, and CCAAT box binding factors were closely associated with the proximal ARE of the MVDP gene. Other consensus sequences for NFκB (Adler et al., 1991) and OCT-1 (Brüggemeier et al., 1991), present in the 5'-region, have been described as potential cooperating factors with AR and glucocorticoid receptor. The five potential recognition sequences for the PEA3/Ets1 factor, located in the homopurine stretch, are of particular interest, since it has been shown that the PEA3 protein is expressed in epididymis (Xin et al., 1992) and testis (Ito et al., 1993) of mouse. Numerous putative AP1- and AP2-binding sites are present in the 5'-flanking DNA of the MVDP gene. These transcription factors mediate induction by two different signal transduction pathways as follows: protein kinase C and cAMP (Imagawa et al., 1987). As the expression of the MVDP gene is confined to the vas deferens epithelial cells (Martinez et al., 1990), some transcription factors required for activation of the MVDP promoter should be cell-specific. Evidence was obtained that there are tissue-specific differences in the proteins that bind to the C3 (1) promoter region and that CAAAT box/enhancer binding protein itself may be one of the transcription factors involved (Zhang and Young, 1991). Whether these motifs and proteins are functional in the MVDP gene remains to be determined. The implications of some factors mentioned above in the full specific responsiveness of the transcription of the MVDP gene to androgens is suggested by cycloheximide experiments, showing that the induction of the MVDP gene by androgens depends on continuous protein synthesis (Dassouli et al., 1994).

Male accessory sexual organs of most mammals contain both aldose reductase and sorbitol dehydrogenase and provide the fructose and sorbitol of seminal plasma (Mann, 1964). Sperm contains sorbitol dehydrogenase, and it has been recently shown that a fructose transporter is expressed in human spermatozoa (Burant et al., 1992). Whether the expression of the human (Graham et al., 1991a) and rat (Graham et al., 1991b) aldose reductase and MVDP genes, which have a common evolutionary origin (Pailhoux et al., 1990), is subject to the same regulation remains to be determined.

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<td>Claessens et al., 1989</td>
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G to C mutation in the ARE sequence TGTTACT prevents any expression of the protein after puberty (Crossley et al., 1992). It appears that in vitro, as well as in vivo, a G nucleotide at position 2 of the right half-site of ARE is essential for AR/DNA interactions.

Based on sequence similarity, another potential ARE is present in the 5'-flanking region of the MVDP gene at position -1172. Although androgen transcriptional activity was not detected with this sequence and although androgen responsiveness was not affected by its deletion, the possibility exists that in homologous vas deferens epithelial cells, this distal ARE functions cooperatively with the proximal one. Cooperativity among AREs has been described in several genes regulated by androgens (Adler et al., 1991; Rennie et al., 1993).