Tissue-specific expression of two aldose reductase-like genes in mice: abundant expression of mouse vas deferens protein and fibroblast growth factor-regulated protein in the adrenal gland

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Aldose reductase (AR), the first enzyme in the polyol pathway, has been implicated in the pathogenesis of diabetic complications, although its physiological role is unclear. In mice, besides AR, two AR-like proteins, mouse vas deferens protein (MVDP) and fibroblast growth factor-regulated protein (FR-1), have been reported recently. Tissue-specific expression of these two genes was examined using the RNase protection assay method. Contrary to previous reports, MVDP was detected in a variety of tissues besides the vas deferens. High levels of MVDP mRNA were found in the adrenal glands, and low levels of expression were detected in eye, intestine, seminal vesicle, kidney, liver, testis and

lung. The major gene expression pattern for FR-1 was slightly different from that of MVDP, with the highest levels of mRNA detected in testis, heart, adrenal gland, and ovary; less was found in the lung and it was barely detectable in eye, intestine, liver and seminal vesicle tissue. Mouse embryos, as early as 10.5 days *post coitum*, expressed both genes, although the levels of expression were different. Human AR mRNA was found in human vas deferens, although not at the high level found in mice. The localization of both MVDP and FR-1 transcripts in the adrenal cortex by *in situ* hybridization led to the speculation that these two AR-like proteins could be related to hormone production.

INTRODUCTION

Aldose reductase (AR), a member of the monomeric aldoketoreductase superfamily, is the first enzyme in the polyol pathway. It has broad substrate specificity, and its reduction of glucose to sorbitol under hyperglycaemic conditions is thought to be responsible for diabetic complications such as cataractogenesis, retinopathy, neuropathy and nephropathy [1]. However, the normal physiological function of AR is not clear. AR cDNA has been isolated from different species including human, rat, cow and rabbit, and the deduced protein sequences of these ARs are highly homologous to each other, i.e. approx. 84–85% [2–5]. The mouse AR (MAR) cDNA was recently isolated and found to show 97% amino acid identity to rat AR [6]. Interestingly, two AR-like genes have also been reported in mice. Mouse vas deferens protein (MVDP), which shares 71%, 69% and 69% amino acid identity to human, rat and mouse AR respectively, is the major protein found in mouse vas deferens [6,7]. Its site of expression was found exclusively in the epithelial cells of the vas deferens by immunohistochemistry and *in situ* hybridization [8,9]. A functional androgen response element was mapped to the 5' end of the gene [10], conferring transcriptional regulation by the steroid hormone, androgen [11]. Another AR-like gene, fibroblast growth factor-regulated protein (FR-1), was found to have 82% and 69% amino acid identity to MVDP and MAR respectively [6,12]. FR-1 was identified as having properties similar to mitogen-inducible delayed-early genes, that is, it is inducible by fibroblast growth factors, phorbol myristate acetate, platelet-derived growth factor and epidermal growth factor in fibroblast culture. The transcript of FR-1 was reported in a number of mouse tissues and most abundantly in newborn liver, adult intestine, ovary and testis using Northern hybridization [12]. Since some regions of MVDP, FR-1 and MAR share more than 90% identity with each other, we were concerned that the

results of these studies, using full-length cDNAs as hybridization probes to determine the sites of expression of MVDP, FR-1 and MAR, may be distorted due to cross-hybridization with each other. We therefore investigated the gene expression pattern of MVDP and FR-1 using RNase protection assays with gene-specific probes. The results of these highly sensitive and specific assays showed that, contrary to the previous report, MVDP expression is not restricted to the vas deferens. MVDP and FR-1 are co-expressed in some tissues, but their major expression patterns are different. In addition, transcripts for both genes were found primarily in the zona fasciculata of the mouse adrenal cortex, suggesting that it may be involved in the production of hormones.

MATERIALS AND METHODS

Plasmid probes

A mouse genomic library (Stratagene, U.S.A.) was screened by standard hybridization methods [13] using three oligonucleotides derived from the published sequence of the MVDP gene [7]. Filters were washed finally in 1 × SSC/0.1% SDS at 50 °C (1 × SSC: 0.15 M NaCl, 15 mM sodium citrate), and a single clone containing the complete gene was obtained. From this clone, a 649 bp *Xba*I–*Stu*I fragment was inserted into pGEM7Zf(+) (Promega Biotec, U.S.A.) to give pEL359 which contains exons 5 and 6. In addition, a 1.5 kb *Eco*RI–*Sac*I genomic fragment consisting of intron 9, exon 10 (296 bp) and 3' downstream sequences was subcloned into pGEM7Zf(+) giving pEL365. Oligonucleotides 5'-GGCTGCAGGGACTGAAGAG-GACTATCC-3' and 5'-GGGAATTCCTATTATTGCTTTT-TATTGTTC-3' were used to prepare an MVDP exon 10 fragment and cloned into Bluescript KSII⁺ (Stratagene, U.S.A.) to give pDC101. pEL369 was subcloned from FR-1 cDNA (a gift of Dr. J. A. Winkles, American Red Cross, U.S.A.) representing the

Abbreviations used: AR, aldose reductase; DTT, dithiothreitol; FR-1, fibroblast growth factor-regulated protein; HAR, human placental AR; MAR, mouse AR; MVDP, mouse vas deferens protein; p.c., *post coitum*.

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269 bp 3'-most sequence. The plasmid pHAR contained the human placental AR (HAR) cDNA sequence [1] cloned into Bluescript KS⁺ (Stratagene, U.S.A.). This plasmid was linearized at an internal *Bam*HI site to give a 634-base riboprobe through *in vitro* transcription. Comparisons of sequences were done using the UWGCG computer programs of Devereux et al. [14].

RNA isolation

Male and female C57 adult mice (University of Hong Kong Laboratory Animal Unit, Hong Kong) were used for RNA isolation. Total RNAs were isolated from mouse kidney, brain, heart, liver, testis, seminal vesicle, vas deferens, small intestine, lung, adrenal gland, eye, leg muscle and ovary as well as 10.5-day- and 16.5-day-post coitum (p.c.) mouse embryos using a LiCl/urea differential centrifugation method [15]. Ovaries were dissected from mice at the non-oestrous stage of the cycle. Total RNAs from human vas deferens and human EA-hy926 [16] cells transfected with a full-length HAR cDNA were prepared by the same method.

RNase protection assay

In vitro transcription and RNase protection assays were performed as described by Lau et al. [17] with modifications. ³²P-labelled antisense RNA probes were generated from pEL365, pEL369 and pHAR by SP6, T7 and T3 RNA polymerases respectively. These were then hybridized to between 200 ng and 40 µg of total RNA in 80% formamide/40 mM Pipes (pH 6.7)/400 mM NaCl/1 mM EDTA at 45 °C for 18 h. Non-hybridized RNA was removed by digestion with RNase A (5 µg/ml) and RNase T1 (0.25 µg/ml) for 10 min at 30 °C. The solution was deproteinized with 50 µg/ml proteinase K/0.5% (w/v) SDS at 37 °C for 30 min, mixed with 10 µg of tRNA, and directly precipitated with 3 vol. of pure ethanol, then washed with 80% ethanol and dried. Electrophoresis was performed with a 6% polyacrylamide/8 M urea gel and the sizes of the protected fragments were determined with respect to DNA molecular-mass markers (pGEM4 digested with *Hpa*II). Quantification of MVDP mRNA was achieved by comparing the intensity ratio between the MVDP-specific protected fragment and a protected fragment from the internal control using the ImageQuANT software of the phospho-imager (Molecular Dynamics).

In situ hybridization

Adrenal gland and small intestine from adult mice and 13.5-day-p.c. mouse embryos were dissected, frozen with powdered solid CO₂ and kept in liquid nitrogen until required for use. Sections (10 µm) were cut using a cryostat, thaw mounted on to slides coated with 3-aminopropyltriethoxysilane (Sigma), and stored at -80 °C until *in situ* hybridization was performed. Mouse fetuses (15.5 days p.c.) were dissected, embedded in paraffin and deparaffinized as described by Wilkinson and Green [18]. Single-stranded ³⁵S-labelled sense and antisense RNA probes were generated from pDC101 (exon 10 of MVDP) using T7 and T3 RNA polymerases, and from pEL369 (exon 10 of FR-1) using SP6 and T7 RNA polymerases. Labelled riboprobes, 5 × 10⁴ c.p.m./µl, were hybridized to 10 µm cryostat sections or 6 µm paraffin mouse sections overnight at 50 °C. Sections were prehybridized at 25 °C for 2 h, hybridized at 50 °C overnight and washed as described by Chung et al. [19] with an additional step using 20 µg/ml RNase A in a buffer containing 0.5 M NaCl, 10 mM Tris/HCl, pH 7.5, and 5 mM EDTA. Dithiothreitol (DTT, 0.1 M) was added to all the washing solutions. Kodak

NTB-2 emulsion was used for autoradiography. Slides were exposed for 14 days (cryostat sections) or 12 days (paraffin sections), developed in D19 developer (Kodak), and stained for histology in eosin and/or haematoxylin. Sections were photographed using Kodak EPY-64T film under bright-field or dark-field illuminations.

RESULTS

MVDP and FR-1 gene-specific hybridization probes

Although some regions of MVDP, FR-1 and MAR cDNAs are highly homologous to each other, the 3' untranslated sequences of these three genes, corresponding to exon 10 of MVDP (intron/exon boundaries of MAR and FR-1 have not yet been determined), share little sequence similarity. Comparison between sequences of MVDP and FR-1 shows only 54% sequence identity with stretches of matching nucleotides amounting to no more than 10 bases (Figure 1). MAR sequence in the exon 10 region is 38% and 44% identical to MVDP and FR-1 respectively with the single longest stretch of 9 bp found to match between MAR and FR-1 (results not shown). Thus, probes created from the exon 10 regions of MVDP and FR-1 were specific to the relative genes. Plasmid pEL365 contained a genomic MVDP fragment which included exon 10 and the flanking sequences, giving a 296-base protected fragment specific to MVDP in the RNase protection assays. Plasmid pEL369 contained a *Bgl*I-*Eco*RI fragment subcloned from the exon 10 region of FR-1 cDNA and gave a 269-base protected fragment specific to FR-1. Plasmid pEL359 consisted of a genomic MVDP fragment including exon 5 and most of exon 6. Comparison between exons 5 and 6 of MVDP and FR-1 cDNA showed sequence similarity of 93% in their nucleotide sequences (results not shown). Only single base mismatches were observed within these sequences and these mismatches would not be cleaved by the concentration of RNase used in this RNase protection study [17]. Therefore, expected protected fragments observed by exon 5 (123 base) and part of exon 6 (104 base) using the pEL359 riboprobe in the protection assays represented hybridization to both MVDP and FR-1 mRNAs (results not shown). On the other hand, pEL359 riboprobe would protect approximately a 44-base fragment with MAR mRNA, since mismatches are found more frequently in the exon 5/6 region between MVDP/FR-1 and MAR.

Figure 1 DNA sequence comparison between exon 10 of MVDP and FR-1

Bold type represents sequence protected in the RNase protection assays. pEL365 contained the complete exon 10 of MVDP while pEL369 contained the 3' end of that of FR-1 from the *Bgl*I site to the end of sequence. The longest stretch of matching sequence is 10 bases.

Figure 2 Expression of MVDP mRNA in mouse tissues

RNase protection assays were performed using the pEL365 riboprobe and total RNA from mouse tissues. MVDP mRNA is represented by the 296-base protected fragment. Fragments smaller than 147 bases act as internal controls giving assessment of the amount of RNA used. The arrowhead points to a fragment used as internal control during quantification. Abbreviations: M, DNA marker, *Hpa*II digest of pGEM4; C, control where no RNA was added to the probe; K, kidney; B, brain; H, heart; L, liver; T, testis; SV, seminal vesicle; VD, vas deferens; I, intestine; Lu, lung; E, eye; AG, adrenal gland; Mu, skeletal muscle; ME (10.5, 16.5), mouse embryos 10.5 and 16.5 days p.c. Total RNA used are as indicated. Male tissues were used for K, B, L, T, SV, VD and Mu; tissues from female for H, I and G; and tissues from both male and female for E. Numbers on both sides represent sizes in nucleotide bases. Autoradiograph was exposed overnight at -70°C .

Figure 3 Expression of FR-1 mRNA in mouse tissues

RNase protection assays were performed using pEL369 riboprobe and total RNA from mouse tissues. FR-1 mRNA is represented by the 269-base protected fragment. Abbreviations and conditions are identical to Figure 2, except for the addition of O, ovary.

Expression of MVDP and FR-1 in mouse tissues

Tissue distributions of MVDP mRNA and FR-1 mRNA were examined by RNase protection assays using pEL365 and pEL369 riboprobes respectively. Thirteen adult mouse tissues and embryonic tissues from two different stages were examined. Experiments were repeated 3–10 times depending on the tissues used and with RNA collected on at least three separate occasions. Expression patterns were found to be slightly different between the two genes. High levels of expression of MVDP mRNA were found in vas deferens and adrenal gland. In Figure 2, the 296-base protected fragment from vas deferens and adrenal gland represents hybridization to only 200 ng and 500 ng of total RNA respectively. When 20 μg of total RNA from each of kidney, brain, heart, liver, testis, seminal vesicle, ovary, intestine, lung and eye were hybridized to the MVDP-specific probe, relatively high levels of mRNA expression were detected in intestine, seminal vesicle and eye tissues. Transcript of MVDP was

Figure 4 No major difference in MVDP mRNA expression between male and female non-reproductive tissues

RNase protection assays were performed using pEL365 riboprobe and total RNA from male (m) and female (f) mouse tissues. MVDP mRNA is represented by the 296-base protected fragment. Abbreviations and conditions are identical to Figure 2 plus O, ovary.

expressed at a low level in kidney, liver, testis and lung, and was undetectable in ovary (see Figure 4), brain, heart and 40 μg of muscle. Low levels of MVDP mRNA were detected in 10.5-day-p.c. mouse embryo (40 μg), with expression levels increased by 16.5 day p.c. (40 μg). Intense hybridization to several fragments (147 bases and smaller) was also observed with pEL365 riboprobe, but not with pDC101 riboprobe which contained the PCR product of MVDP exon 10 alone. Thus, these lower-molecular-mass protected fragments, representing hybridization probably to another gene 3' of MVDP, had provided an internal control giving an assessment of the amount of RNA used (see Figures 2 and 4). Quantification of MVDP mRNA using the phospho-imager showed an estimated 400-fold increase in expression in vas deferens compared with kidney; a 45-fold increase in adrenal gland, and an approximately 5-fold increase in eye, seminal vesicle and intestine compared also to kidney expression.

For FR-1, a major 269-base protected fragment was detected with its highest level of expression found in testis and adrenal gland tissues. The expression in the adrenal gland was much lower compared with MVDP since the protected fragment was barely observable when 500 ng of total RNA was used. A relatively high level of FR-1 transcript was detected in the heart, while lower levels were observed in the ovary, lung, intestine, eye and male skeletal muscle (Figure 3). FR-1 mRNA was not detectable in brain, liver, seminal vesicle, 200 ng of vas deferens (Figure 3), nor in 20 μg of vas deferens (results not shown). Contrasting to MVDP, levels of FR-1 gene expression were higher in the 10.5-day-p.c. mouse embryo when compared with 16.5-day-p.c. mouse fetus.

Expression pattern between male and female mice in non-reproductive tissues

Since a functional androgen response element was found in the promoter region of the MVDP gene and a high level of gene expression was detected in the vas deferens, we investigated the expression of MVDP mRNA in other tissues to see if it was also sex-dependent. Results indicate that no major difference was detected in MVDP mRNA expression between male and female mice in non-sexual tissues. Similar levels of MVDP transcript were detected in kidney, intestine, eye and adrenal gland tissues of male and female adult mice (Figure 4). In the reproductive

Figure 5 Localization of MVDP/FR-1 mRNA in adult mouse tissues

In situ hybridization was performed on neighbouring sections of adult adrenal gland (a–d) and small intestine (e and f). Abundant amount of transcripts (silver grains) are found in the adrenal cortex (ct) but not in the medulla (md) using antisense probes of MVDP exon 10, pDC101 (b), and FR-1 exon 10, pEL359 (d). Significantly lower MVDP gene expression was found in small intestine in the epithelial cells of the villi (v) using the antisense probe for MVDP exon 10, pDC101 (f). Sections were exposed for 14 days before being developed. (a), (c) and (e) represent photomicrographs taken under bright-field illumination, and (b), (d) and (f) represent corresponding sections taken under dark-field illumination. Abbreviation: mu, muscle. Scale bar, 100 μ m.

organs, MVDP mRNA was undetectable in the ovary, expressed at a low level in the testis, but found in abundance in the vas deferens. Thus, other than in the sex organs, the expression of MVDP is not sex-dependent. For FR-1, no major difference in mRNA expression was detected between male and female adrenal gland, heart, lung, intestine and eye (results not shown). However, unlike male skeletal muscle, a protected fragment was not detected in the female skeletal muscle (results not shown).

Expression in the adrenal cortex

In situ hybridization was performed using sense and antisense RNA probes transcribed from pDC101 (MVDP exon 10) and pEL369 (FR-1 exon 10), on neighbouring sections of adult adrenal gland and adult small intestine. Very high levels of hybridization (silver grains) were observed in the adult and foetal adrenal glands using an antisense probe from pDC101 (Figures

5b and 6b) which shows expression specific to MVDP. A considerably high level of mRNA expression, specific to FR-1 (pEL369), was also detected in the adult mouse adrenal gland (Figure 5d) as well as in the 13.5-day-p.c. embryo (Figure 6d). Hybridization signals in the adrenal gland were localized in the cortex in both adult and 15.5-day-p.c. foetal mice, but no hybridization was observed in the medulla. In the small intestine, a significantly lower level of MVDP transcript was detected in the intestine, and signals were localized in the epithelial cells of the villi (Figure 5f). Negative hybridization resulted from using both sense probes specific to MVDP and FR-1 (results not shown).

Expression of human AR in vas deferens

The high level of MVDP mRNA in mouse vas deferens prompted us to see if human AR is also abundant in the human vas

Figure 6 Localization of MVDP/FR-1 mRNA in foetal mouse adrenal cortex

In situ hybridization was performed on developing mouse foetuses. Abundant amounts of MVDP transcript (silver grains) were found in the adrenal cortex but not in the medulla of 15.5-day-p.c. mouse foetus using antisense probes of MVDP exon 10, pDC101 (b). Expression of FR-1 mRNA is detected in the adrenal gland of 13.5-day-p.c. mouse foetus using antisense probe for FR-1 exon 10, pEL369 (d). Sections were exposed for 12 days (a and b) and 14 days (c and d) before being developed. (a) and (c) represent photomicrographs taken under bright-field illumination, and (b) and (d) represent corresponding sections taken under dark-field illumination. Abbreviations: ag, adrenal gland; k, kidney. Scale bar, 100 μ m.

Figure 7 Expression of HAR in human vas deferens

RNase protection assay was performed using the pHAR riboprobe and 10 μ g of total RNA from human vas deferens (lane 2), 3 μ g of total RNA from EA-hy926 cells transfected with HAR full-length cDNA (lane 3). A control was also performed where no RNA was added (lane 1). HAR mRNA is represented by the 634-base protected fragment. The autoradiograph was exposed overnight at -70°C . Shorter exposure showed lane 3 to be identical to lane 2.

deferens. An RNase protection assay was performed on total RNA isolated from human vas deferens using the pHAR riboprobe. In Figure 7, a 634-base protected fragment specific to human AR was detected using 10 μ g of human vas deferens total

RNA (lane 2). The presence of the higher-molecular-mass fragment represents self-hybridization, since the same fragment was observed when no RNA was added to the probe (Figure 7, lane 1). Identical size fragments were also observed (with shorter exposure not shown here) in 3 μ g of the control RNA from EA-hy926 cells transfected with HAR cDNA (Figure 7, lane 3). Thus, AR mRNA was found to be expressed in human vas deferens but in much lower amounts than MVDP in mouse vas deferens. If a highly expressed MVDP-like protein were present in human vas deferens tissue, the nucleotide sequence of its gene would most likely be significantly different from AR such that no large fragments of the AR probe were protected.

DISCUSSION

Expression of MVDP has been well studied [8,9,20,21]. The site of MVDP mRNA synthesis was reported, by *in situ* hybridization using its cDNA probe, to be restricted to the epithelial cells of the vas deferens. It was also reported that the MVDP transcript was not detected in other mouse tissues [9]. In this study, a highly sensitive assay was used in combination with a specific probe for the MVDP gene, showing that its transcript is more widely expressed than expected. Apart from an abundant MVDP mRNA found in vas deferens, a very significant level of MVDP transcript was detected in the mouse adrenal gland. A recent study has also shown the presence of an AR in rat adrenal gland among the six aldo-ketoreductases purified [22]. In addition, expression of MVDP mRNA was detected in intestine, seminal vesicle, eye, kidney, liver, testis and mouse embryos. Expression of MVDP mRNA in vas deferens was found to be approx. 400-fold that of the kidney, approx. 80-fold that of intestine, seminal vesicle and eye; and approx. 9-fold that of adrenal gland. Thus,

the overexpression of MVDP in vas deferens may have masked the detection in other tissues during previously published studies. Messenger RNA distribution for the other AR-like gene, FR-1, was found to be slightly different. Higher levels of FR-1 gene expression were detected in testis, heart, adrenal gland and ovary; less in lung and mouse embryos, and it was barely detectable in eye, intestine, liver and seminal vesicle. Thus, the major tissue-specific expression for MVDP mRNA was found to be different from that of FR-1 mRNA, although they co-expressed at a low level in most tissues. A high level of expression in the intestine was not detected for FR-1 in this study as was reported by Donohue et al. [12] using Northern blot hybridization with FR-1 cDNA. Their signal, however, could represent hybridization to other RNAs homologous to FR-1. Interestingly, expression of FR-1 was found in male skeletal muscle and was undetectable in female skeletal muscle of the mouse, similar to expression observed by Cromlish and Flynn [23] where an AR purified from rabbit skeletal muscle was found to be sex-dependent (being expressed in the male but only in trace amounts in the female muscle). The reason for such expression is as yet unknown. MVDP, however, does not appear to be sex-dependent in non-reproductive tissues including adrenal gland.

The structural organization of the MVDP gene [24] resembles that of the AR gene from different species including human [25], rat [26], mouse [6] and rabbit [27] in having 10 exons and nine introns, and the amino acid sequences of MVDP and FR-1 are highly homologous to human, rat and mouse AR. A functional androgen response element was located in the upstream sequence of the MVDP gene [10], while a consensus sequence for the androgen response element was also found in human AR [28]. In addition, the catalytic residues (Asp-44, Tyr-48, Lys-77 and His-110 [29]) which mediate the binding of NADPH were found to be conserved in all AR genes, as well as MVDP and FR-1. The fact that most tissues known to express AR in different species express either one of MVDP or FR-1 indicates that these two genes belong to the same family as AR.

RNase protection assays using riboprobe transcribed from pEL359 (exons 5 and 6) showed a higher level of expression in kidney and brain when compared with the combined expression of MVDP and FR-1 (results not shown). Thus, more members from the AR family with very similar sequences (especially in exon 5) may exist. Recently, the cDNA sequence for MAR was reported to be isolated from kidney with 97% identity to rat lens AR [6]. Nucleotide sequence comparison between MVDP and MAR, however, could not explain the extra expression in kidney and brain, thus more AR-like proteins may yet be found. It is possible that multiple AR genes may be common in other species with specificities to their corresponding tissues. It would be of interest to find out if the isoenzymes reported in kidney, muscle, lens and heart from a variety of species [24,30–33] are in reality coded by different AR genes.

Although the role of AR in the pathogenesis of diabetic complications is well documented [34], its physiological functions remain unclear. It has been suggested that under normal physiological conditions, AR may participate in osmoregulation in kidney medulla [35], and a putative osmotic response element was located in the 5' sequence of the rabbit AR gene [29]. In plants, AR was found to be expressed constitutively during embryo maturation and may play a role in the osmoprotection involving desiccation tolerance [36]. AR is also implicated in differentiation of lens fibre cells [37], proliferation of Schwann cells in peripheral nerves [38], and is found as a tumour-associated protein in hepatocarcinogenesis [39]. In this study, both MVDP and FR-1 were found in adult and embryonic adrenal glands. The expression of these mRNAs in adrenal glands in zones where

steroid hormones (glucocorticoids and androgen) are produced led to the speculation that a function of the AR-like proteins may be associated with hormone production. Complementary DNA encoding 20 α -hydroxysteroid dehydrogenase has been isolated recently and found to be identical to the cDNA sequence of bovine lens AR [40]. The enzyme 20 α -hydroxysteroid dehydrogenase catalyses the reduction of 17-hydroxyprogesterone [41], a major precursor of biologically active androgens, oestrogens and glucocorticoids. However, the AR in mouse was shown to be incapable of catalysing 17-hydroxyprogesterone reduction [6]. Perhaps in mouse, the hydroxysteroid dehydrogenase activity is being taken over by MVDP or FR-1.

It is also of interest that strong *in situ* hybridization signals were expressed by MVDP in the fetal adrenal gland at 15.5 days p.c., yet signals for FR-1 mRNA was much lower at this stage (results not shown). However, positive signal specific to FR-1 was localized in the adrenal gland in 13.5-day-p.c. embryos. This is consistent with the RNase protection assay results where much lower levels of FR-1 transcript were detected during more advanced embryonic development. Transcripts from both MVDP and FR-1 were found in mouse embryos as early as 10.5 days p.c. It is not yet known whether the signals in early embryos were associated with developing adrenal glands, which are seen around 11 days p.c. [42]. The importance of AR in developing embryos is unknown; however, uncoordinated levels of expression between MVDP and FR-1 mRNAs at 10.5 days p.c. and 16.5 days p.c. may indicate stage specificity of these AR-like proteins during development. Functional roles of these genes during development may be elucidated by further studies localizing the temporal and spatial expression of these transcripts in developing embryos.

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