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Novel Androgen-Dependent Promoters Direct Expression of the C4b-Binding Protein α-Chain Gene in Epididymis

Mayumi I. Nonaka,*† Guixian Wang, † Takao Mori,* Hidechika Okada, † and Masaru Nonaka 1 *

C4b-binding protein (C4BP) is a large plasma protein composed of seven α-chains and one β-chain and is involved in the fluid phase regulation of the classical pathway of the complement system. Complement inhibitory activity is located in the α-chain, and its mRNA has been detected only in liver to date. Here, we have isolated cDNA clones encoding the α-chain of guinea pig C4BP (C4BPα) and have demonstrated significant C4BPα mRNA expression in epididymis as well as liver. The level of C4BPα transcripts increased in the epididymis after birth, while it remained constant in the liver. C4BPα mRNA was also detected in the normal murine epididymis at a significant level, but it decreased drastically after castration, suggesting that epididymal expression of the C4BPα gene is regulated by androgen. Gene analysis of guinea pig C4BPα indicated that liver and epididymis C4BPα mRNA share the coding region and 3'-untranslated region, but are transcribed from independent promoters on a single-copy gene. Two novel epididymis-specific promoters were identified in the region corresponding to the first intron of liver transcripts. The binding motif for hepatocyte NF-1 occurs in the promoter used for transcription of liver C4BPα, whereas androgen-responsive elements occur in both promoters used in the epididymis. These findings present a novel link between complement regulators and reproduction. Furthermore, variation in the 5'-untranslated regions, arising from alternative splicing of the newly identified exons, is demonstrable in the guinea pig C4BPα transcripts. The Journal of Immunology, 2001, 166: 4570 – 4577.

The regulation of complement is essential to avoid its excessive activation and to protect self cells against complement attack. Many secretory and membrane-associated regulators mediating such activity have been identified in plasma and on the surfaces of blood cells and various epithelial cells. In the reproductive organs the distribution of membrane-associated complement regulators, such as decay-accelerating factor (DAF), membrane cofactor protein (MCP), and C59, has been well characterized in humans (1–6) and in guinea pigs (7, 8). These proteins have been detected mainly in the female reproductive organs, such as the uterus, oviduct, ovary, and placenta, although the expression patterns of these proteins are distinct. All these proteins have also been localized on spermatozoa. It has been suggested that these regulators play a pivotal role in the protection of tissues and spermatozoa from complement attack caused by sperm Ag or invasive micro-organisms in the female reproductive tract, where complement-hemolytic activity has been detected (1). Furthermore, a recent gene-targeting study indicated that Crry, another murine membrane-associated complement inhibitor, plays a critical role in protecting embryos from complement attack during pregnancy (9).

Recently, a sperm protein involved in sperm-egg interaction was isolated from mice and guinea pigs, referred to as sp56 and AM67, respectively (10, 11). This protein shows significant structural similarity to the α-chain of C4b binding protein (C4BPα), one of the fluid phase complement regulators. This finding instigated our investigation of C4BPα in the reproductive organs.

Human C4b-binding protein (C4BP) is a large, hetero-oligomeric plasma glycoprotein (~550 kDa) and is recognized as an acute phase protein. The major form is composed of seven α-chains (70 kDa) and one β-chain (45 kDa), which are disulfide-linked at their C-terminal regions. Each α-chain is composed of eight short consensus repeat (SCR) domains, followed by the C-terminal region. The β-chain is composed of three SCR domains, followed by the C-terminal region. C4BP binds to complement component C4b through each α-chain and regulates complement activation by decay acceleration of the C3 convertase of the classical pathway (C4b2a) and/or by acting as a cofactor for the cleavage of C4b by factor I (12–14). The β-chain contains a binding site for the anticoagulant vitamin K-dependent protein S, which suggests that C4BP also acts as a regulator in the protein C coagulant pathway (15, 16). Furthermore, it has been reported that the α-chain of C4BP interacts with serum amyloid protein (SAP) in plasma (17), and that the binding of SAP to C4BP decreases the complement regulatory activity of C4BP (18).

In this paper we describe the significant expression of C4BPα in both guinea pig and murine epididymis and demonstrate the tissue-specific transcription of the guinea pig C4BPα gene from alternate promoters. We describe here a novel interaction between complement regulators and the reproductive organs.

Materials and Methods

Molecular cloning of guinea pig C4BPα

A cDNA library, constructed from poly(A) mRNA from the testis of an approximately 7-wk-old guinea pig (19), was screened with a 0.2-kb C4BPα-like cDNA fragment homologous to the SCR2-SCR3 region of human C4BPα, which was unexpectedly isolated by RT-PCR during amplification of guinea pig DAF cDNA. The probe was labeled with [α-32P]dCTP using the Rediprime DNA labeling system (Amersham Pharmacia Japan, Tokyo, Japan), and hybridization was performed for 20 h in 1 M NaCl, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 10% Denhardt’s solution, 1% salmon sperm DNA, and 0.1% SDS at 55°C. Thirteen positive

*Department of Biological Sciences, University of Tokyo, Tokyo, Japan; and †Department of Molecular Biology, Nagoya City University School of Medicine, Nagoya, Japan

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1 Address correspondence and reprint requests to Dr. Masaru Nonaka, Department of Biological Sciences, University of Tokyo, Hongo 7-3-1, Tokyo 113-0033, Japan. E-mail address: mnonaka@biol.s.u-tokyo.ac.jp

2 Abbreviations used in this paper: DAF, decay-accelerating factor; MCP, membrane cofactor protein; C4BP, C4b-binding protein; C4BPα, α-chain of C4BP; SCR, short consensus repeat; SAP, serum amyloid protein; RACE, rapid amplification of cDNA ends; UT, untranslated; ARE, androgen response element; HNF-1, hepatocyte NF-1.
plagues were isolated in the screening of $5 \times 10^5$ recombinant plaques. These were plaque-purified and converted into plasmid clones by the inherent excision process according to the manufacturer’s directions. The nucleotide sequences were determined using the chain termination method, with an ALF DNA sequencer (Pharmacia LKB Biotechnology, Uppsala, Sweden) or an ABI 370 DNA sequencer (Applied Biosystems, Foster City, CA).

Guinea pig liver and epididymal C4BP cDNA were isolated by 5'- and 3'-rapid amplification of cDNA ends (RACE), using the adapter primer 5'-GACGTCGAGTCGACATCG-3', a dT17-adaptor primer, and terminal transferase (Roche Japan, Tokyo, Japan). Other oligonucleotides used were as follows: GBP-2 (5'-TGCTGTACCACTGCA-3') and GBP-4 (5'-CTCTGGATTTCCTGCA-3') for 5'-RACE, and GBP-1 (5'-AGGTACTGTCGCGCAAG-3') and GBP-3 (5'-CAACAGTTGTGGA-3') for 3'-RACE. GBP-2 and GBP-1 were used for the RT of RNA. Products amplified by 5'-RACE were gel-purified and subcloned into the pCR2-TOPO vector (Invitrogen, San Diego, CA). Products amplified by 3'-RACE, in both the liver and the epididymis, were detected as a single band by agarose gel electrophoresis, and were directly sequenced after pretreatment with exonuclease I and shrimp alkaline phosphatase (Amersham Pharmacia Japan).

**Northern blotting analysis**

Total RNA was isolated from various tissues of Hartley guinea pigs of various ages (Japan SLC, Shizuoka, Japan) and from 10-wk-old BALB/c mice (Japan Clea, Tokyo, Japan), using the guanidine thiocyanate-CsCl method. Four 7-wk-old mice were castrated, and RNA was isolated on the eighth day after castration. RNA was also isolated from three noncastrated mice of the same age as controls. Approximately 5 µg of total RNA was denatured with glyoxal and DMSO as described previously (20), separated electrophoretically on 1% agarose gels, and transferred to Hybond-N nylon membrane (Amersham Pharmacia Japan). These were plaque-purified and converted into plasmid clones by the in-vitro Transcription System (Promega, Madison, WI). PCR-amplified 382-, 417-, and 455-bp fragments in the U3, U5, and U1 regions, respectively, were cloned into pGEM3Zf+ vectors, and sequenced as described above.

**RT-PCR amplification**

The following oligonucleotides were synthesized: A1–1, 5'-CAAGGCCCTTAGCCACAC-3'; A3–5, 5'-AAAAAGCGAGAGGTAT-3'; and A5–1, 5'-GCTTAGTCCACGTCACA-3', which bind to a site in the 5'-untranslated (UT) region of the U1, U3, and U5 types, respectively (sense primer). GBP1A2 (5'-CTACAGAGAGCTACATA-3') was designed to bind to a site in the common SCR1 region (antisense primer; shown by dotted line in Fig. 2 and 4b). Total RNA from the liver, epididymis, and testis was reverse transcribed, and cDNA fragments were amplified by PCR, with denaturation at 95°C for 3 min, followed by 30 cycles of 95°C for 0.5 min, 42°C for 0.5 min, and 72°C for 1 min and a final extension at 72°C for 5 min.

**Isolation of genomic clones encoding the 5'-UT region of guinea pig C4BPα**

A guinea pig genomic library constructed in the EMBL3 vector was purchased from Clontech (Palo Alto, CA). Fragments including the 5'-UT regions of the isolated clones of guinea pig C4BPα were used as probes to screen 10 5 recombinant plaques. Four clones were plaque-purified, and the phage DNA was isolated using standard methods. The inserts were digested with the appropriate restriction enzymes, subcloned into pGEM3Zf+ or pBlue script SKII vectors, and sequenced as described above.

**RNase protection assay**

Five or 10 µg of total RNA were hybridized with [α-32P]CTP-labeled RNA probes using the RNase protection assay kit RPAII (Ambion, Austin, TX). Probes were labeled using RiboProbe In Vitro Transcription Systems (Promega, Madison, WI). PCR-amplified 382-, 417-, and 455-bp fragments in the U3, U5, and U1 regions, respectively, were cloned into pGEM3Zf+, linearized, and used to synthesize probes. However, the U3 probe did not work well, detecting many nonspecific bands.

**Results**

**Molecular cloning of guinea pig C4BPα**

Thirteen positive clones were isolated when a guinea pig testis cDNA library was screened with a 0.2-kb fragment of guinea pig C4BPα, previously isolated by RT-PCR. These clones were divided into two groups according to the nucleotide sequences of their coding regions. Four of these clones encoded seven SCRs followed by the C-terminal region and were identified as the previously isolated sperm protein, AM67 (11). The other nine clones encoded a new protein, composed of eight SCRs followed by a C-terminal region, similar to C4BPα of other animals (except for mice) (21–25), and the protein-coding region showed 57 and 73% identity to that of human C4BPα at the amino acid and nucleotide levels, respectively, suggesting that these clones encode a guinea pig counterpart of human C4BPα. However, guinea pig C4BPα cDNA included three transcript types with different 5'-UT regions, termed U1, U3/U2, and U3 types (Fig. 1). The U3/U2 type 5'-UT region occurs when the U2 sequence is inserted into the U3 type, suggesting that guinea pig C4BPα mRNAs are transcribed from two promoters. The schematic models of the isolated clones and the percent identity between guinea pig C4BPα and AM67 in each domain are shown in Fig. 1. Guinea pig C4BPα shows 55% overall identity with AM67. It is noteworthy that the SCR2 and SCR3 regions of both show strong identity, since SCR2 and SCR3 are important domains for binding to C4b (26). However, it is unclear whether AM67 interacts with C4b.

The nucleotide and deduced amino acid sequences of the guinea pig C4BPα cDNA are shown in Fig. 2. The amino acid identity scores of each SCR between guinea pig C4BPα and that of other animals, and between AM67 and sp56, the mouse homologue of AM67, are summarized in Table I. In C4BPα, overall identity scores are relatively low, and the identity scores of each SCR vary widely. On the other hand, sperm protein AM67/sp56 is generally more conserved than C4BPα, and the identity scores of each SCR vary widely.

**FIGURE 1.** Schematic model of the cDNA clones encoding guinea pig C4BPα and AM67 isolated from a testis cDNA library. All cDNA clones encoding C4BPα share an identical coding region, including signal peptides (SP), eight SCRs followed by the C-terminal (CT) region, and the 3'-UT region (□). However, they contained three 5'-UT regions, termed U1, U3/U2, and U3. The U3 type lacks the U2 region in contrast to the U3/U2 type. The numbers between C4BPα and AM67 represent the percentage of identity between each SCR at the amino acid (a.a., top) and nucleotide (nuc, bottom, in parentheses) levels. The horizontal arrows indicate the probe sites used in Northern blotting analysis (see Fig. 3).
are relatively constant, although SCR2 is its most highly conserved domain. These results suggest a functional difference between AM67/sp56 and C4BPa.

Northern blotting analysis

To examine the tissue distribution of C4BPa and AM67, Northern blotting analysis was performed using RNA from various tissues of 14-wk-old guinea pigs and the 0.6- and 0.4-kb fragments of C4BPa and AM67, respectively, as probes (shown as Probe A and Probe B in Fig. 1). These probes did not cross-hybridize under the experimental conditions described. As shown in Fig. 3A, significant expression of C4BPa mRNA was observed in the epididymis as the liver, but not in the testis, whereas AM67 mRNA was detected only in the testis.

Further analysis, using RNA taken from tissues at various developmental stages, indicated that epididymal C4BPa transcripts increase significantly after birth, in concert with the testicular androgen levels (27), whereas liver C4BPa transcripts are expressed at constant levels at all ages tested. This implies that the expression of epididymal C4BPa is affected by androgen.

Table 1. Amino acid identities (%) in each SCR between guinea pig C4BPa and its counterparts in other animals (21–25) and between AM67 and sp56

<table>
<thead>
<tr>
<th>Domain</th>
<th>Guinea Pig C4BPa</th>
<th>AM67</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCR1</td>
<td>59</td>
<td>45</td>
</tr>
<tr>
<td>SCR2</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td>SCR3</td>
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<td>55</td>
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<tr>
<td>SCR6</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>SCR7</td>
<td>47</td>
<td>44</td>
</tr>
<tr>
<td>SCR8</td>
<td>66</td>
<td>64</td>
</tr>
<tr>
<td>CT</td>
<td>60</td>
<td>56</td>
</tr>
</tbody>
</table>

Mouse C4BPa lacks the domains corresponding to SCR3 and SCR6.

FIGURE 2. a, Nucleotide and deduced amino acid sequences of the U3/U2 type of guinea pig C4BPa cDNA. N-terminal residue of guinea pig C4BPa shown as +1 was estimated by alignment with other species C4BPa. The potential signal peptide sequence is written in italics. Although Met(-55) is the first ATG codon from the 5' end, Met(-12) is more likely to be the translation start site, since the sequence written in italics contains many hydrophilic amino acids. Five potential N-glycosylation sites were found in SCR1, SCR3, SCR4, and SCR8, as shown by arrowheads.

b, Nucleotide sequence of the 5'UT region of the U1 type C4BPa cDNA. The sequences shown have been entered in the EMBL/GenBank/DDBJ database under accession numbers AB049465 and AB049466.
of liver C4BPα is constitutive. In testis, the expression of C4BPα was very low at all ages (Fig. 3B), although extended autoradiographic exposure detected a faint band at 12 days of age (data not shown). On the other hand, AM67 transcripts were detected from 9 wk of age, with no increase thereafter (Fig. 3B), suggesting that AM67 mRNA is expressed in round spermatids, as has been reported for sp56 (10). The distinct tissue distributions of C4BP and AM67 suggest a functional difference between them.

Isolation of epididymal and liver C4BPα cDNA by 5'- and 3'-RACE

Because the transcript size of epididymal C4BPα is smaller than that of liver C4BPα, as shown in Fig. 3, epididymal and liver C4BPα cDNAs were isolated by 5'- and 3'-RACE. Sequence analysis of the isolated cDNAs indicated that epididymal C4BPα cDNA is identical with testis C4BPα cDNA, including the 5'-UT sequences. However, although liver C4BPα cDNA was identical with the epididymal and testis C4BPα cDNAs in the protein coding and 3'-UT regions, they differed in the 5'-UT region. The 5'-UT region of liver C4BPα transcripts included one of two sequences, termed U5 and U5/U4. The sequence U5/U4 occurred when the U4 sequence was inserted at the 3'-end of the U5 sequence, suggesting that these two mRNA species are transcribed from a common promoter. The schematic model of the epididymal and liver C4BPα and the sequence of the 5'-UT region of the U5/U4 type are shown in Fig. 4. The sequence U5 is highly homologous (>65%) to the 5'-UT region of C4BPα of other animals, except for bovine C4BPα (21–25), indicating that the U5 type transcript corresponds to C4BPα mRNA of these animals. The 5'-UT sequence of bovine C4BPα showed no significant homology with any of the 5'-UT sequences of guinea pig C4BPα mRNA.

To confirm the tissue specificity of these mRNA species and to examine which mRNA species are dominant in each tissue, RT-PCR was performed using oligonucleotides designed to bind sites in the U1, U3, and U5 regions as sense primers and to a site in the SCR1 region as an antisense primer (underlined in Figs. 2 and 4b). As shown in Fig. 5, the U1 type transcript was detected in the epididymis and the testis, but not in the liver. A band corresponding to the U1/U2 type transcript, which was not isolated during cDNA cloning, was also detected, although the level of expression seemed very low. Similarly, the U3/U2 and U3 type transcripts were detected at significant levels in the epididymis and only when the U4 sequence was inserted at the 3'-end of the U5 sequence, suggesting that these two mRNA species are transcribed from a common promoter. The schematic model of the epididymal and liver C4BPα and the sequence of the 5'-UT region of the U5/U4 type are shown in Fig. 4. The sequence U5 is highly homologous (>65%) to the 5'-UT region of C4BPα of other animals, except for bovine C4BPα (21–25), indicating that the U5 type transcript corresponds to C4BPα mRNA of these animals. The 5'-UT sequence of bovine C4BPα showed no significant homology with any of the 5'-UT sequences of guinea pig C4BPα mRNA.

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faintly in the testis, but not in the liver. The levels of the U3/U2 and U3 type transcripts were almost equal. Conversely, the U5 and U5/U4 type transcripts were detected preferentially in the liver, although faint bands were also observed in the epididymis. Interestingly, the U5/U4 type transcript, but not the U5 type, was dominant in the guinea pig liver, while the U5 type transcript corresponds to C4BPα mRNA of other animals.

Analysis of the guinea pig C4BPα gene

To clarify the organization of the multiple 5′-UT regions of guinea pig C4BPα mRNA, four genomic clones encoding these areas were isolated and analyzed. As shown in Fig. 6, the restriction enzyme maps of these clones were consistent with each other, indicating that the guinea pig C4BPα gene is a single copy gene. The exon/intron boundaries for the exons encoding a part of the 5′-UT and the signal peptide region (5′-UT/SP), SCR1, and SCR2a, were found in the same positions as the second, third, and fourth exons, respectively, of the human and mouse C4BPα genes. The exon encoding U5 corresponds to the first exons of the human and mouse C4BPα genes and shows >60% homology with them over the 500 or more base pairs analyzed to date (28, 29). The exons encoding U3 and U1 were found separately between the exons encoding U5 and 5′-UT/SP, suggesting that three independent promoters exist in the guinea pig C4BPα gene, and that tissue-specific expression of C4BPα is regulated by differential use of these promoters. The U4 sequence was encoded by one exon, located close to the 5′-end of the U3 region, and alternate splicing of this exon generates two C4BPα mRNA species in the liver. The U2 sequence was found spliced to the 5′-end of the 5′-UT/SP exon, indicating that use of alternative splice acceptor sites produces two species in both epididymal C4BPα mRNAs transcribed from two independent promoters.

Characterization of the promoter regions

To determine the transcription-initiation sites and to confirm the tissue specificity of these promoters, an RNase protection assay was performed using probes containing the U5 and U1 regions together with their 5′-flanking regions. As shown in Fig. 7a, the U5 probe was protected by transcripts expressed in the liver, and the U1 probe was protected by transcripts expressed in the epididymis. In addition, both promoters contained multiple transcription-initiation sites.

Fig. 7b shows the nucleotide sequences of three promoter regions. The approximate positions of the transcription-initiation sites described above are shown by arrows. In the U5 promoter region, a hepatocyte NF-1 (HNF-1) response element, GGTAAT NATTAAC, was found in the same position as those in the human, mouse, and rat C4BPα genes (28–30), explaining the preferential transcription from this promoter in the liver (31). A TATA box was found at the same position as that in the mouse C4BPα gene (29). On the other hand, an androgen response element (ARE) consensus sequence (32), GG(AT)ACANNNTGTTCT, was found with three mismatches in each of the 5′-flanking regions of U1 and U3, explaining the androgen-dependent transcription from these promoters, in the epididymis. A TATA box was also found in both these regions.
Androgen-dependent expression of murine C4BPα in the epididymis

To investigate whether the significant expression of C4BPα in the epididymis is specific to guinea pigs, we examined the tissue distribution of murine C4BPα mRNA. As shown in Fig. 8A, normal adult mice express a significant level of C4BPα mRNA in the epididymis as well as in the liver in a pattern similar to that in guinea pigs. To confirm that the expression of epididymal C4BPα mRNA depends on androgen, we analyzed the C4BPα transcript levels in castrated mice. As shown in Fig. 8B, the level of epididymal C4BPα mRNA was significantly reduced in castrated mice compared with that in noncastrated mice, while transcript levels in the liver showed no significant difference, suggesting that expression of epididymal C4BPα mRNA is androgen dependent.

Discussion

C4BPα is the major component of C4BP participating in the fluid phase regulation of the complement system, and its mRNA had been detected only in liver. In this report we describe significant androgen-dependent expression of guinea pig and murine C4BPα mRNA in the epididymis, one of the male reproductive organs. Analysis of the cDNA sequences and the gene structure of guinea pig C4BPα indicates that epididymal and liver C4BPα mRNA are identical in their coding regions, 3’-UT regions, and parts of their 5’-UT regions. However, they are transcribed in a tissue-specific manner from independent promoters on a single-copy gene.
Guinea pig C4BP has been isolated previously from acrosome-intact spermatozoa (33), as well as from plasma (34). When guinea pig fertilin (as called PH-30), which is a potential sperm-egg membrane fusion protein, was isolated from cauda epididymal spermatozoa using an mAb column against fertilin, two additional proteins coeluted. One was identified as a new member of the pentaxin protein family, termed apexin. The other was shown to be homologous to human C4BP using the SDS-PAGE, Western blotting, and amino acid sequence analyses. However, since its mobility was slightly different from that of serum C4BP, it was termed sp-C4BP. The two partial amino acid sequences of sp-C4BP described in that report, EGGYLSALSYYVECDGDGVQGN and NPGDLPHGTIEVK, are completely identical with regions of the amino acid sequence of guinea pig C4BP deduced from the cDNA clones isolated here (Fig. 2), indicating that sp-C4BP is definitely guinea pig C4BP. The slight difference observed in their mobilities may be the result of post-translational modification, such as glycosylation. Furthermore, since C4BP mRNA was detected at negligible levels in adult testes, sp-C4BP is likely to originate in the epididymis and attached to spermatozoa via apexin or fertilin, or directly.

Although it is unclear whether the association of C4BP, apexin, and fertilin is physiologically relevant (33), it is tempting to speculate that epididymal C4BP may interact with apexin in a way comparable to the complex formed by serum C4BP with SAP, another member of the pentaxin family (17, 18). However, because apexin is an intracellular protein, located in the acrosomal region (33), it is unlikely that epididymal C4BP binds to apexin during the passage of spermatozoa through the epididymis. The binding of epididymal C4BP to apexin may be an artifact arising during protein isolation. Otherwise, C4BP may interact with fertilin, which is expressed on the surface of the spermatozoa and proteolytically processed during sperm maturation in testis and epididymis (35).

Analysis of the guinea pig C4BPα gene identified three promoters. One is used in liver and shows significant sequence homology to the promoters already reported for the human, mouse, and rat C4BPα genes. All these promoters contain an HNF-1 consensus sequence (28–30) that is essential for the hepatic activity of the promoter of the C4BPα gene (31). The other two promoters are newly identified in the C4BPα gene and are transcriptionally active in the epididymis. Neither of the promoters active in the epididymis contains consensus sequence for HNF-1, but they do contain the consensus sequence for an ARE. The promoter used in liver contains no consensus sequence for ARE. These findings support the tissue-specific expression of C4BPα mRNA and explain why epididymal C4BPα mRNA is transcribed in an androgen-dependent manner.

The guinea pig C4BPα gene is unusual in that it generates several mRNA species by alternate splicing of the newly identified 5’-UT exons, which occur between the exons corresponding to the first and second exons of the human and mouse C4BPα genes. As a result of this alternate splicing of the additional 5’-UT exon, liver C4BPα transcripts possess at least two possible 5’-UT sequences. On the other hand, utilization of alternate splice acceptor sites in the exon encoding the U2 and 5’-UT/SP regions in combination with the use of alternate promoters produces at least four epididymal C4BPα transcripts with different 5’-UT regions, as detected by RT-PCR (Fig. 5). Such heterogeneity in the 5’-UT region has been found in many proteins. In the expression of acetyl-coenzyme A carboxylase, it is considered to be involved in the regulation of translation (36). However, the reason why the 5’-UT region of C4BPα mRNA is heterogeneous in guinea pigs, but not in other animals, remains unclear.

Significant androgen-dependent expression of C4BPα in the epididymis is observed not only in guinea pigs but also in mice, suggesting a novel role for C4BP in reproduction. No other reproductive organs, such as seminal vesicle, uterus, or ovary, express C4BPα mRNA at detectable levels in either guinea pigs (Fig. 3A) or mice (Fig. 8A and our unpublished observations). Guinea pig epididymal C4BP exists as a high molecular mass oligomeric protein (540–590 kDa), similar to serum C4BP (33, 34), suggesting that epididymal C4BP may possess a complement inhibitory activity. Clusterin (also known as SP-40), which is one of the major secreted proteins in the epididymis, has been thought to function as an inhibitor of the membrane attack complex in the complement system (37). However, clusterin is ubiquitously expressed (38), and recent findings indicate that under physiological conditions clusterin is unlikely to be an important complement regulator (39). Since clusterin possesses multiple functions besides complement inhibition, such as its roles in the regulation of apoptosis and in lipid transport (38, 39), it may play a noncomplement role in epididymis. Therefore, C4BP seems to be the first complement regulator to be identified as an epididymal protein, and the first fluid phase complement inhibitor synthesized in the reproductive organs to be identified.

On the other hand, the epididymis is protected from the immune system by the blood-epididymis barrier, similar to the blood-testis barrier under normal conditions, and no complement has been detected in this organ (1, 37). Therefore, if epididymal C4BP functions as a complement regulator, it may bind to spermatozoa and play a role in the protection of spermatozoa in the female reproductive tract, where complement activity has been detected (1). However, since spermatozoa express many membrane-associated complement inhibitors, such as DAF, MCP, and CD59, on the entire surface and/or on the inner membrane (4–6, 8), further association of C4BP is unlikely to be essential for protection of the spermatozoa. C4BP may be involved in sperm maturation.

In this context other roles in reproduction have been speculated for some of the complement regulatory proteins, over and above their roles in complement regulation. For example, it has been suggested that human MCP, expressed on spermatozoa, is involved in sperm-egg recognition (40, 41). Negligible expression of
rodent MCP in any cells except spermatozoa also suggests a specific role for this protein in reproduction (19, 42, 43). In addition, guinea pig DAF is significantly expressed on the epithelial cells of seminal vesicle, where no complement has been detected (7, 8).

This distribution is suggestive of a novel function for DAF. Furthermore, human C4BPβ, but not C4BPα, was recently detected in the regressing corpus luteum and corpus albicans of the adult human ovary (44). As C4BPβ contains no complement inhibitory activity, ovarian C4BPβ is obviously involved in a system distinct from the complement system. These proteins, C4BPα, C4BPβ, MCP, and DAF, as well as sperm protein AM67/sp56 are all composed of SCR domains. The SCR proteins might have developed in close contact with the reproductive system. Further investigation of the roles of these proteins in reproduction would define the diversified functions of the SCR proteins recognized as complement regulators.

References