NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH Public Access
Author Manuscript
Mol Reprod Dev. Author manuscript; available in PMC 2007 April 25.

Published in final edited form as:
Mol Reprod Dev. 2006 June ; 73(6): 767–773.

Calpain 11 Is Unique to Mouse Spermatogenic Cells

Irit Ben-Aharon[1], **Paula R. Brown**[2], **Ruth Shalgi**[1], and **Edward M. Eddy**[2]

1 *Department of Cell and Developmental Biology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel*

2 *Gamete Biology Group, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina USA*

Abstract

The calpains are a family of calcium-dependent thiol proteases involved in intracellular processing of proteins. They occur as heterodimers containing one of various large subunits and a common small subunit. Some of the large subunits are expressed ubiquitously and others are expressed in a restricted set of tissues. We have cloned the cDNA for mouse calpain 11 and demonstrated that it is expressed specifically in the mouse testis. The mRNA begins to accumulate in the testis between days 14 and 16 days after birth, corresponding to the period of pachytene spermatocyte development. The protein is detected by day 18 after birth, during mid to late pachytene spermatocyte development, and is present in the acrosomal region of spermatozoa from the cauda epididymis. The expression of calpain 11 during spermatogenesis and its localization is spermatozoa suggest that it is involved in regulating calcium-dependent signal transduction events during meiosis and sperm functional processes.

Keywords

calcium-dependent proteases; signaling; gene expression; gene families

INTRODUCTION

Calpain family members are calcium-dependent thiol proteases that carry out intracellular processing of proteins. They have been implicated in calcium-mediated cell-signaling pathways, including those involved in cytoskeleton remodeling, cell cycle regulation, cell differentiation, and membrane fusion (Croall and DeMartino, 1991; Carafoli and Molinari, 1998; Barnoy et al., 1999). They exist as heterodimers of one of various large (~80 kDa) subunits and a common small (~30kDa) subunit (Murachi et al., 1981; Carafoli and Molinari, 1998). There are 14 known genes in the human for large subunit calpains, some of which are probably calcium-independent or lack protease activity (Dear and Boehm, 2001), and 11 calpain genes have been characterized in the mouse (http://www.informatics.jax.org/). Some calpains are expressed ubiquitously, while others have restricted tissue expression patterns. Large subunits calpain 1 (CAPN1) and calpain 2 (CAPN2), as well as small subunit calpain 4 (CAPN4), are expressed ubiquitously and are the best characterized calpain family members. CAPN1 (μ-calpain) is activated in vitro by micromolar amounts of calcium, while CAPN2 (m-calpain) requires millimolar amounts for activation. Additional mechanisms of calpain activation in vivo include phospholipid binding, autolysis, escape from endogenous inhibitors, protein coactivators, phosphorylation, and cell adhesion (Glading et al., 2002).

[1]Correspondence to: Dr. E. M. Eddy, LRDT, C4-01, NIEHS, NIH, 111 T.W. Alexander Drive, Research Triangle Park, NC 27709 USA, Tel. (919) 541-3015, Fax (919) 541-3800, E-mail eddy@niehs.nih.gov.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

There are dramatic shifts in the patterns of gene expression and changes in cell morphology and function during spermatogenesis. Many male germ cell-specific transcripts and proteins are produced during this process and have developmentally-regulated patterns of expression. Some are products of genes expressed only in spermatogenic cells that are homologues of genes expressed in somatic cells, while others are products of novel genes. Many encode proteins essential for novel functional processes of spermatogenesis or for the formation of novel sperm-specific components of spermatozoa (Hecht, 1993; Eddy and O'Brien, 1994). A human testis-specific calpain 11 (*CAPN11*) gene was identified recently that contains motifs for calcium binding and protease activity (Dear et al., 1999). It was reported also that an orthologue of human *CAPN11* is expressed in the mouse testis (Dear and Boehm, 1999). Because the CAPN11 protein is a candidate for serving an important role in the development and function of spermatogenic cells, we have isolated and sequenced the mouse *Capn11* cDNA, defined when the RNA and protein are expressed during spermatogenesis, and determined where the protein is localized in spermatozoa.

MATERIALS AND METHODS

Isolation of mouse Capn11 cDNA

Primers based on sequences reported previously (Dear and Boehm, 1999) were designed to include terminal SalI and NotI restriction enzyme sites (forward 5'-ccggtcgacagcggccaaaggatat, reverse 5'-ccggcggccgcatcatccttcttgtg) and used to amplify in a polymerase chain reaction (PCR) a region of the *Capn11* sequence using PFU polymerase (Stratagene, La Jolla, CA) and mouse testis cDNA (BD Biosciences Clontech, Palo Alto, CA). The 735 bp PCR product was digested with SalI and NotI, ligated into pBluescript (Stratagene) using the TAKARA DNA Ligation kit version 2 (Panvera, Madison, WI), and transformed into *E. coli* DH5α competent cells (Life Technologies, Inc., Grand Island, NY). Resulting clones were sequenced with the BigDye™ Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA) as recommended by the supplier. Additional 5' and 3' sequences were determined using mouse testis Marathon-Ready™ cDNA and 5' and 3' RACE procedures as recommended by the supplier (BD Biosciences Clontech).

Expression of Capn11 gene

Primers specific for *Capn11* were used for semi-quantitative reverse transcriptase (RT) PCR assays (Table 1). Total RNA was extracted from testes of mice from 6 to 30 days of age and from 11 other tissues with TRIzol Reagent (Invitrogen, Carlsbad, CA) and used as templates to synthesize first-strand cDNA. The RNA samples (2 μg each) were treated with amplification grade DNase I (Invitrogen) prior to addition of 200U of SuperScript™ II RNase H reverse transcriptase (Invitrogen), according to manufacturer's recommendations. The RT products were then used as PCR templates (1 μl each) to amplify double-stranded cDNAs of *Capn11* and βactin. These reactions were repeated three times. The Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) system was used to analyze the double-stranded cDNA products (Muller et al., 1995) following the manufacturer's recommended protocol.

Real-time PCR

Gene specific primers for *Capn11* and ribosomal protein L7 (*Rpl7*) mRNA (Table 1) were used in real-time PCR experiments to determine expression during post-natal testis development. Using the RT products as template at a concentration of 5 ng/μl in SYBR Green Master Mix (Applied Biosystems), the specific primers amplified 188 bp and 77 bp of *CAPN11* and *Rpl7*, respectively. A 7900 HT Sequence Detection System (Applied Biosystems) produced a raw data file using a standard profile of denaturing 10 m. at 95° C and amplifying 15 s at 95° C, 1 m at 60° C for 40 cycles. Standard curves for both *Capn11* and *Rpl7* were used to produce a relative quantification of the raw Ct scores. The average of three intra-assay Ct scores was

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

then taken for both genes at each age and the *Rpl7* age matched data was used to normalize the expression level of *Capn11*. All real-time PCR products were examined by electrophoresis on 3% NuSieve agarose gels to verify that they contained a single amplicon.

Production of antisera

Peptides identified from the deduced sequence of mouse CAPN11 were synthesized, purified by HPLC, conjugated to keyhole limpet hemocyanin (Sigma-Genosys, The Woodlands, TX) and used to immunize adult female New Zealand white rabbits (Covance, Denver, PA). The antisera were screened by immunoblotting on lysates of adult mouse testis and the antiserum against the peptide containing amino acids 308-324 (SDKATEWEEVSPDVR) was used for the studies reported here. This antiserum was affinity-purified over a column containing the peptide coupled to Sepharose 4B. Non-specifically bound proteins were removed by repeated washes with DPBS pH 7.0, and the antibodies were eluted using a pH 2 buffer and immediately neutralized. All procedures involving laboratory animals were approved by the NIEHS Animal Care and Use Committee.

Immunoblotting procedures

Spermatozoa were collected from the cauda epididymis of adult C57BL/6 mice and diluted in ice-cold buffer containing 20 mM Tris, 130 mM NaCl, 2 mM EGTA, pH 7.15 (TN/EGTA buffer) as described (Kalab et al., 1994). Following centrifugation at 1000 *RCF* for 10 min at 4°C, spermatozoa were suspended in TN/EGTA buffer and counted. Aliquots of $1x10^6$ spermatozoa were stored at −70°C until use. Samples were mixed with Laemmli buffer (New England BioLabs, Beverly, MA), boiled for 5 minutes, and proteins separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) at 30mA on 10% gels (Bio-Rad Laboratories, Hercules, CA) in 25mM Tris, 192mM glycine and 0.1% SDS (Leammli, 1970). Proteins were transferred electrophoretically from gels onto nitrocellulose membranes (Amersham, Buckinghamshire, UK) overnight at 40mA in transfer buffer containing 25mM Tris, 192mM glycine and 20% methanol (Towbin et al., 1979).

Testes were collected at two-day intervals from 6 to 30 day old CD-1 mice and homogenized at a concentration of 50 mg (wet weight)/ml in lysis buffer containing 20mM HEPES (pH 7.4), 140mM NaCl, 0.1% Triton X100, and Complete™ proteinase inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Testis lysates (0.5 mg wet weight) were separated by SDS-PAGE on 10% (w/v) acrylamide gels and transferred onto Imobilon™ nylon membrane (Milllipore Corp., Bedford, MA). Molecular mass was estimated by comparison with the migration of prestained protein standards (Amersham, Buckinghamshire, UK).

Membranes were soaked for two h at room temperature in blocking solution containing 150 mM NaCl, 10 mM Tris, 0.5% Tween 20 (TBS/Tween), and 5% nonfat dry milk. They were then incubated for 18h at 4°C with antiserum to CAPN11 diluted 1:100 in Tris-buffered saline (TBS)/Tween and 1% BSA. Following 3 washes in TBS/Tween, membranes were incubated for 1 hour in donkey anti-rabbit antibody conjugated to horseradish peroxidase (Jackson Laboratories, West Grove, PA) diluted 1:5000 in blocking solution. Membranes were washed 5 times in TBS/Tween solution, processed using the Supersignal chemiluminescence detection system (Pierce, Rockford, IL) and exposed to X-ray film.

Immunofluorescence procedures

Spermatozoa isolated from the cauda epididymis of adult C57BL/6 mice were suspended in Dulbecco’s phosphate-buffered saline (DPBS) supplemented with 3% fetal calf serum. Cells were permeabilized by slowly dripping them along the side of a test-tube immersed in ice (Jones et al., 1983). Samples were warmed to room temperature and the procedure was then repeated. Permeabilized spermatozoa were incubated for 2 h in antiserum to CAPN11 (1:75)

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

and washed three times in blocking solution. Spermatozoa were then transferred into blocking solution containing Cy2-labeled donkey anti-rabbit IgG (1:500) and 1 lg/ml of Hoechst 33342 (Sigma, St. Louis, MO) for 30 minutes in the dark, washed 3 times in blocking solution, and mounted between a slide and a cover slip.

CAPN11 and DNA staining were visualized and photographed using a Zeiss LSM410 confocal laser scanning microscope (Oberkochen, Germany) equipped with a 25-mW krypton-Argon laser, a 10-mW helium-neon laser (488, 543 and 633 maximum lines), and an UV laser (Coherent Inc. Laser Group, Santa Clara, CA). A 40X NA/1.2 planapochromat water-immersion lens (Axiovert 135 M; Zeiss) was used for all imaging. Spermatozoa were scanned through the Z-axis to capture an optical section at the equatorial plane.

RESULTS

Characterization of mouse *Capn11* cDNA

A 2613 bp mouse *Capn11* cDNA generated by PCR contained an in-frame stop codon 50 bp upstream of the putative transcription start site, a poly-adenylation signal at bp 2557–2562, and a poly A tract beginning at bp 2575 (GenBank accession number AY578330). It encoded a deduced protein of 714 amino acids with a calculated pI of 6.03 and molecular mass of 82970 daltons. By querying the NCBI mouse genome database with the *Capn11* cDNA sequence, it was determined that the *Capn11* gene is approximate 28.4 kb in length, contains 20 exons, and is located on chromosome 17 in region 17B3 (contig Mm17_39695_30). The mouse *Capn11* cDNA sequence is 80% identical to human *CAPN11*, 65% identical to chicken l/m calpain, 63% identical to mouse *Capn1,* and 62% identical to mouse *Capn2* (data not shown). The mouse CAPN11 protein is 75% identical to human CAPN11, 55% identical to chicken μ/m calpain, 52% identical to mouse CAPN1, and 51% identical to mouse CAPN2 (Fig. 1). Like other calpains, the mouse CAPN11 protein also contains a cluster of five repetitive EF-hand motifs near the C terminus, and another EF-hand motif closer to the N terminus (Fig. 1).

Expression of Capn11 mRNA

When RNA levels from 12 different mouse tissues were compared by semi-quantitative RT-PCR, *Capn11* was detected only in testis and mixed germ cells (Fig. 2A). In addition, the expression pattern of *Capn11* during spermatogenesis was assessed. Because the first wave of spermatogenesis is relatively synchronous in juvenile mice (Bellvé et al., 1977), Real-time PCR was carried out using RNA extracted from testes of mice collected at 2-day intervals from 8 to 28 days of age to determine when the gene is expressed during spermatogenesis. The steady-state level of *Capn11* mRNA is shown (Fig. 2B) as fold increases relative to day 8 testes. These results indicate that mRNA begins to accumulate between days 14 and 16 after birth, and peaks on day 20, corresponding to the period when pachytene spermatocytes are first seen and then accumulate. *Capn11* expression declined on days 22-24, but increased on day 26 and remained elevated thereafter (Fig. 2B).

Expression of *CAPN11* protein

Immunoblotting studies were carried out to determine when CAPN11 protein first appears during spermatogenesis, using protein extracts of testes collected at 2 day intervals from mice 6 to 30 days of age. CAPN11 first was observed clearly on day 18 after birth (Fig. 3), corresponding to the appearance of late pachytene spermatocytes. The steady-state level of CAPN11 appears to plateau on day 24 (Fig. 3A). Staining of the lower (83 kDa), but not of the non-specific upper band (Fig. 3B), was greatly reduced when CAPN11 antiserum was incubated with an excess amount of the CAPN11 peptide prior to immunostaining the blot. The cross-reacting protein in the upper band remains to be identified.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

CAPN11 in mouse spermatozoa

Immunoblotting with antiserum to CAPN11 demonstrated that the 83 kDa protein is present in spermatozoa from the cauda epididymis of adult mice (Fig. 4A). An extract of whole testis served as a positive control and an extract of whole kidney served as a negative control because *Capn11* mRNA was not detected in kidney (Fig. 2A). An 83kDa band was detected in sperm and testis (Fig. 4A). The lower band seen in the kidney extract was not eliminated when the antiserum was blocked with the immunizing peptide, strongly suggesting that this staining is due to non-specific cross-reaction (Fig 4B).

Immunofluorescence combined with confocal microscopy suggests that CAPN11 localizes to the acrosome in mouse spermatozoa (Fig. 5). Using second antibody along (Fig. 5C) or incubating the antiserum with the immunizing peptide significantly reduced the immunostaining of spermatozoa (data not shown).

DISCUSSION

Previous northern analysis and *in situ* hybridization studies suggested that mouse *Capn11* transcription is restricted to pachytene spermatocytes in the testis (Dear and Boehm, 1999). We cloned a 2613 bp mouse *Capn11* cDNA and confirmed by northern analysis that expression of *Capn11* is testis-specific and is initiated during early pachytene spermatocyte development. Western blotting and immunofluorescence studies demonstrated that synthesis of CAPN11 protein begins during late pachytene spermatocyte development, indicating that expression of CAPN11 is under translational regulation. By using the cDNA sequence to query the NCBI mouse genome database, we determined that the *Capn11* gene contains 20 exons that span 28.4 kb of mouse chromosome 17B3.

Many genes and transcripts are expressed only during spermatogenesis and have developmentally regulated patterns of expression (Eddy and O'Brien, 1994). Some of these genes encode entirely novel proteins associated with spermatogenic cell-specific structural components or functional activities. Others encode proteins that are homologous to proteins present in somatic cells, but have specialized roles in the development and function of male gametes, and are expressed instead of or in addition to other family members (Eddy, 1995). Our findings indicate that CAPN11 fits into the category of spermatogenic cell-specific proteins whose expression occurs concurrently with other family members and is developmentally regulated.

Human CAPN11 has higher identity to chicken large subunit l/m calpain than to other mammalian large subunit calpains (Dear et al., 1999a). We found that the deduced sequence of mouse CAPN11 is 80% identical to its human counterpart, 65% identical to chicken l/m calpain, 63% identical to mouse CAPN1, and 62% identical to mouse CAPN2. The greatest difference between mouse and human CAPN11 is a 13 amino-acid sequence present only on the N-terminus of mouse CAPN11. Calpains characteristically contain EF-hand motifs, helix-loop-helix structures that coordinate the binding of a calcium molecule (Persechini et al., 1989; Maki et al., 2002). The deduced amino acid sequence of mouse CAPN11 contains a cluster of five putative EF-hand motifs in the C terminus and another nearer the N terminus, comparable to human CAPN11 and chicken l/m calpain.

Several calpains are expressed preferentially in specific human tissues. These include CAPN3 (skeletal muscle), CAPN6 (placenta), CAPN8 (smooth muscle), and CAPN9 (stomach, small intestine) (Dear et al., 1999). Some calpains also have particularly important roles in specific tissues. Mutations in the *CAPN3* gene in humans are associated with limb girdle muscular dystrophy type 2A (Richard et al., 1995) and *CAPN3* knockout mice have a similar phenotype (Richard et al., 2000). These mice also display alterations in apoptosis and disruption of the

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

IκBα/NFκB pathway. In addition, genetic variations in intron 3 of the *CAPN10* gene are associated with type-2 diabetes mellitus in humans (Horikawa et al., 2000). However, it is clear that *Capn11* has the most restricted pattern of expression reported thus far for calpain gene-family members.

CAPN1 and CAPN2 protein (Ben-Aharon et al., 2005) and *Capn5*, *Capn6*, and *Capn7* transcripts are present in mouse testis (Dear and Boehm, 1999), and *CAPN13* transcripts are present in human testis (Dear and Boehm, 2001). In addition, ESTs for *CAPN1, CAPN2, CAPN5,* and *CAPN10* have been identified in human testis libraries (UniGene build #186). However, it is difficult to determine the significance of these findings without knowing the levels of expression and cellular distribution of these calpains in the testis.

Several mechanisms have been suggested to be responsible for the activation of calpains that leads to the precise and limited cleavage of specific intracellular proteins. While calcium binding is sufficient for activation in vitro, the levels required are seldom achieved in vivo and other mechanisms are suggested to either lower or replace the calcium requirement (Glading et al., 2002). Among these is the release from binding by calpastatin, a ubiquitously expressed endogenous calpain inhibitor. A variety of tissue-specific calpastatin isoforms are produced by alternative splicing, one of which is found only in testis. Both the human (Li et al., 2000) and mouse (Li and Goldberg, 2000) testis isoforms (tCAST) contain a novel 40 amino acid N-terminal peptide sequence transcribed from an intronic promoter. This was shown in the mouse to remove a nuclear localization signal and to add a site for myristylation that confers membrane targeting (Li and Goldberg, 2000). Calpastatin and calpain activity were present in human spermatozoa (Rojas et al., 1999) and calpastatin and calpains were detected by immunostaining between the plasma membrane and outer acrosomal membrane of macaque monkey spermatozoa (Yudin et al., 2000). It was determined that tCAST is expressed in round spermatids (Li and Goldberg, 2000), suggesting that it might be involved in the regulation of CAPN11 activity in spermatids and spermatozoa. A synthetic inhibitor of calpain protected against injury and reduced the level of apoptosis following ischemia/reperfusion of the testis, suggesting that calpain activity contributes to the high level of apoptosis that occurs during spermatogenesis (Shiraishi et al., 2000).

A variety of substrates for calpains have been identified and calpain activation produces diverse effects (28). Many of the known substrates are kinases and phosphatases involved in signal transduction processes or are cytoskeletal proteins associated with cell motility (Glading et al., 2000; Goll et al., 2002; Sato and Kawashima, 2001). Most of these studies have been in cell-free systems or in cultured cells and have focused on CAPN1 and CAPN2. However, a number of known calpain substrates are present in spermatogenic cells that are potential CAPN11 targets. CAPN11 is present in spermatogenic cells during the meiotic divisions and spermiogenesis, during which time these cells undergo dramatic structural and biochemical modifications. In addition, CAPN11 localizes to the acrosomal cap of spermatozoa and calpains are involved in modulating the organization of cytoskeletal elements during membrane fusion events (Barnoy et al., 1998; Potter et al., 1998; Spira et al, 2003). The transient decrease in *Capn11* mRNA levels observed around day 22 correlate with a temporary increase in CAPN1 immunostaining observed during the same period (Ben-Aharon et al., 2005). CAPN1, CAPN2 and CAPN 11 are co-expressed during this period, suggesting that a homeostatic relationship might be present between CAPN1 and CAPN11, although further studies will be needed to test this possibility. In conclusion, these studies suggest that CAPN11 has the appropriate temporal and spatial distribution to be involved in regulating key signal transduction events and processes of cytoskeletal remodeling during meiosis, spermiogenesis and sperm function. However, additional studies will be needed to identify the substrates and to determine which of these roles CAPN11 serves in spermatogenic cells.

NIH-PA Author Manuscript

Acknowledgements

We thank Clyde Rogers for his excellent care of the animals used in these studies, April McIver for her important contributions to the research, and the other members of our laboratories for valuable discussions and helpful suggestions. These studies were supported in part by the National Institutes of Health Scholars in Tel Aviv University, Sackler Faculty of Medicine Women Health Studies: the Biomedical Science Program (I. B-H.), and in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

References

Barnoy S, Glaser T, Kosower NS. The calpain-calpastatin system and protein degradation in fusing myoblasts. Biochim Biophys Acta 1998;1402:52–60. [PubMed: 9551085]

Barnoy S, Zipser Y, Glaser T, Grimberg Y, Kosower NS. Association of calpain (Ca2+-dependent thiol protease) with its endogenous inhibitor calpastatin in myoblasts. J Cell Biochem 1999;74:522–31. [PubMed: 10440922]

Bellvé AR, Cavicchia JC, Millette CF, O'Brien DA, Bhatnagar YM, Dym M. Spermatogenic cells of the prepubertal mouse: isolation and morphological characterization. J Cell Biol 1977;74:68–85. [PubMed: 874003]

Ben-Aharon I, Brown PR, Etkovitz N, Eddy EM, Shalgi R. The expression of calpain 1 and calpain 2 in spermatogenic cells and spermatozoa of the mouse. Reproduction 2005;129:435–442. [PubMed: 15798018]

Carafoli E, Molinari M. Calpain: a protease in search of a function? Biochem Biophys Res Commun 1998;247:193–203. [PubMed: 9642102]

Croall DE, DeMartino GN. Calcium- activated neutral protease (calpain) systems structure, function, and regulation. Physiol Rev 1991;71:813–847. [PubMed: 2057527]

Dear TN, Boehm T. Diverse mRNA expression patterns of the mouse calpain genes Capn5, Capn6 and Capn11 during development. Mech Develop 1999;89:201–209.

Dear TN, Boehm T. Identification and characterization of two novel calpain large subunit genes. Gene 2001;274:245–252. [PubMed: 11675017]

Dear TN, Möller A, Boehm T. CAPN11: a calpain with high mRNA levels in testis and located on chromosome 6. Genomics 1999;59:243–247. [PubMed: 10409436]

Eddy EM. Chauvinist genes of male germ cells: gene expression during mouse spermatogenesis. Reprod Fertil Develop 1995;7:695–704.

Eddy, EM.; O'Brien, DA. The Spermatozoon. In: Knobil, E.; Neill, JD., editors. The Physiology of Reproduction. 1. New York: Raven Press; 1994. p. 29-77.

Glading A, Lauffenburger DA, Wells A. Cutting to the chase: calpain proteases in cell motility. TRENDS Cell Biol 2002;12:46–54. [PubMed: 11854009]

Goll DE, Thompson VF, Li H, Wei W, Cong J. The calpain system. Physiol Rev 2002;63:731–801.

Hecht, NB. Gene expression during male germ cell development. In: Desjardins, C.; Ewing, LL., editors. Cell and Molecular Biology of the Testis. Oxford University Press; Oxford: 1993. p. 464-503.

Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinori Y, Linder TH, Mashima H, Schwarz PEH, del Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nature Gen 2000;26:163–175.

Jones R, Brown CR, Cran DG, Gaunt SJ. Surface and internal antigens of rat spermatozoa distinguished using monoclonal antibodies. Gamete Res 1983;8:255–65.

Kalab P, Visconti P, Leclerc P, Kopf GS. p95, the major phosphotyrosine-containing protein in mouse spermatozoa, is a hexokinase with unique properties. J Biol Chem 1994;269:3810–17. [PubMed: 7508920]

Leammli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 1970;227:680–685. [PubMed: 5432063]

Li S, Goldberg E. A novel N-terminal domain directs membrane localizatin of mouse testis-specific calpastatin. Biol Reprod 2000;63:1594–1600. [PubMed: 11090425]

Li S, Liang Z-G, Want G-Y, Yavetz B, Kim ED, Goldberg E. Molecular cloning and characterizatin of functional domains of a human testis-specific isoform of calpastatin. Biol Reprod 2000;63:172–178. [PubMed: 10859257]

Maki M, Kitaura Y, Satoh H, Ohkouchi S, Shibata H. Structures, functions and molecular evolution of the penta-EF-hand Ca2+-binding proteins. Biochem Biophys Acta 2002:1600-51–60.

Muller D, Molinari I, Soldati L, Bianchi GA. Genetic deficiency in calpastatin and isovalerylcarnitine treatment is associated with enhanced hippocampal long-term potentiation. Synapse 1995;19:37–45. [PubMed: 7709342]

Murachi T, Tanaka K, Hatanaka M, Murakami T. Intracellular Ca2+-dependent protease (calpain) and its high-molecular weight endogenous inhibitor (calpastatin). Adv Enzyme Reg 1981;19:407–424.

Persechini A, Moncrief ND, Kretsinger RH. The EF-hand family of calcium-modulated proteins. Trends Neurosci 1989;12:462–267. [PubMed: 2479149]

Potter DA, Tirnauer JS, Janssen R, Croall DE, Hughes CN, Fiacco KA, Mier JW, Maki M, Herman IM. Calpain regulates actin remodeling during cell spreading. J Cell Biol 1998;141:647–662. [PubMed: 9566966]

Richard I, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, Bourg N, Brenguier L, Devaud C, Pasturaud P, Roudaut C, Hillaire D, Passos-Bueno M, Zatz M, Tischfield JA, Ferdeau M, Jackson CE, Cohen D, Beckmann JS. Mutations n the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. Cell 1995;81:27–40. [PubMed: 7720071]

Richard I, Roudaut C, Marchand S, Baghdiguian S, Herasse M, Stockholm D, Ono Y, Suel L, Bourg N, Sorimachi H, LeFranc G, Fardeau M, Sébille A, Beckmann JS. Loss of calpain 2 proteolytic activity leads to muscular dystrophy and to apoptosis-associated I Ba/nuclear factor B pathway perturbation in mice. J Cell Biol 2000;151:1583–1590. [PubMed: 11134085]

Rojas FJ, Brush M, Moretti-Rojas I. Calpain-calpastatin: a novel complete calcium-dependent protease system in human spermatozoa. Mol Hum Reprod 1999;5:520–526. [PubMed: 10340998]

Sato I, Kawashima S. Calpain function in the modulation of signal transduction molecules. Biol Chem 2001;3882:743–751. [PubMed: 11517927]

Shiraishi K, Naito K, Yoshida K. Inhibition of calpain but not caspase protects the testis agains injury after experimental testicular torsion of rat. Biol Reprod 2000;63:1538–1548. [PubMed: 11058563]

Spira ME, Oren R, Dormann A, Gitler D. Critical calpain-dependent ultrastructural alterations underlie the transformation of an axonal segment into a growth cone after axotomy of cultured Aplysia neurons. J Comp Neurol 2003;457:293–312. [PubMed: 12541311]

Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4. [PubMed: 388439]

Yudin AI, Goldberg E, Robertson KR, Overstreet JW. Calpain and calpastatin are located between the plasma membrane and outer acrosomal membrane of cynomolgus macaque spermatozoa. J Androl 2000;21:721–9. [PubMed: 10975419]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

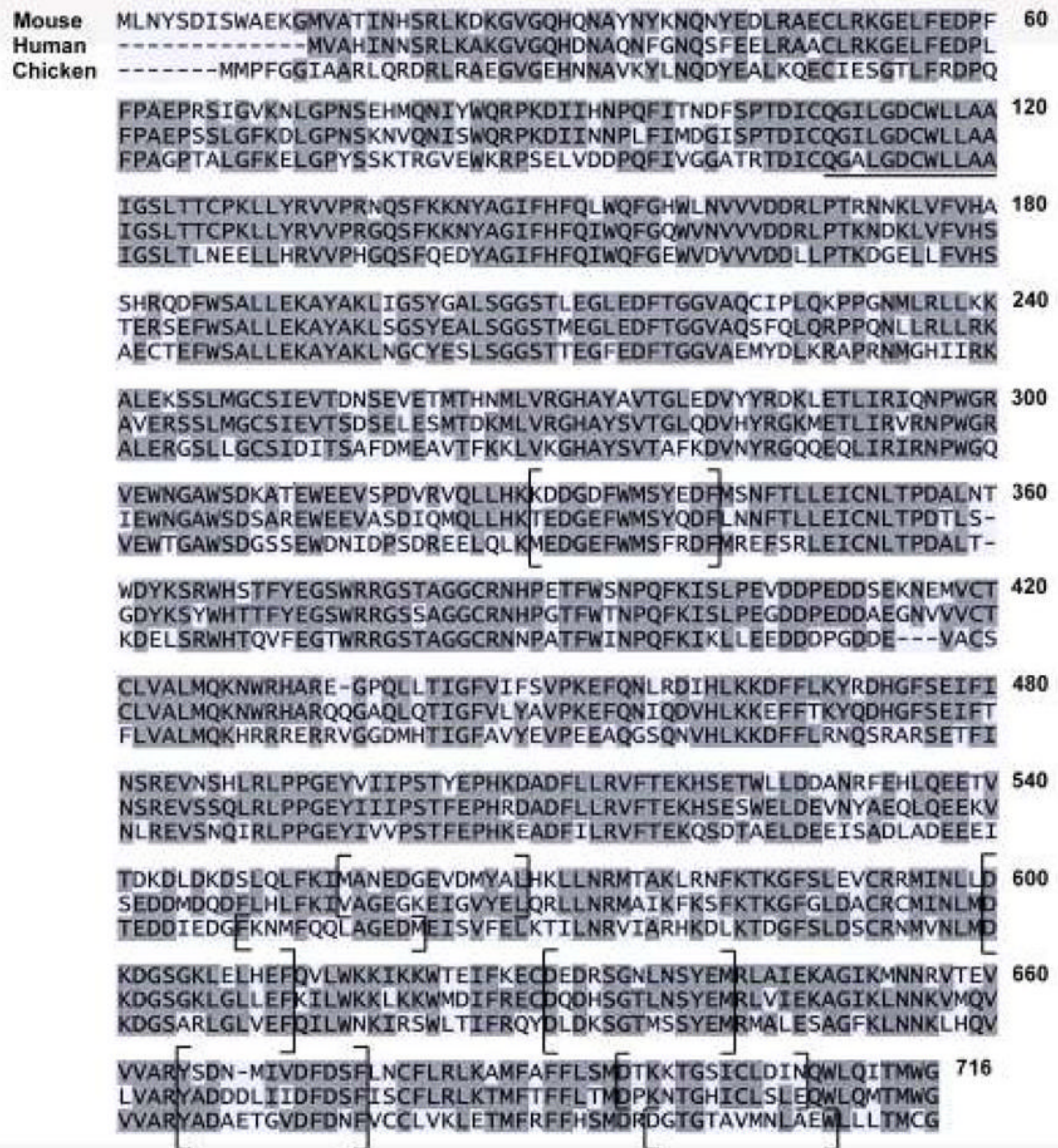

Figure 1.

Comparison of the deduced amino acid sequences of mouse CAPN11 with those of human CAPN11 and chicken m/μ calpain. Shaded areas indicate amino acid homology. Brackets define EF-hand motifs.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

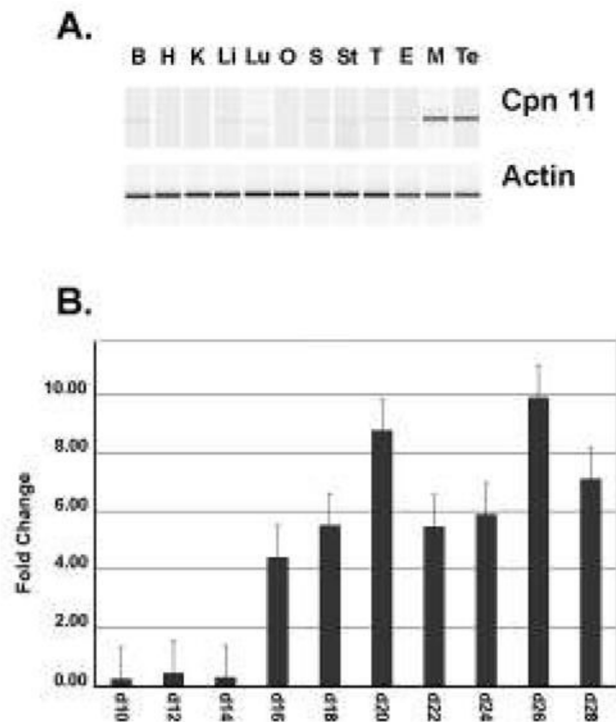

Figure 2.

Capn11 mRNA expression. A. Expression of *Capn11* mRNA as determined by RT-PCR in mouse brain (B), heart (H), kidney (K) liver (Li), lung (Lu), ovary (O), spleen (S), stomach (St), thymus (T), epididymis (E), mixed germ cells (M), and testis (T). Actin was detected in all tissue samples at approximately equal levels. Images are from an Agilent 2100 Bioanalyzer readout. B. Expression of *Capn11* mRNA as determined by real-time RT-PCR in testes from mice at days 10 to 28 after birth. Levels are expressed in terms of fold change compared to day 8.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Figure 3.

Developmental expression of CAPN11. A. Testes were collected from 6 to 30 day old mice and crude protein extracts were isolated by homogenizing in lysis buffer. Proteins were resolved by SDS-PAGE analysis and transferred onto ImobilonTM nylon membrane. The blots were incubated with the antiserum (1:100) to the CAPN11 peptide, followed by ECL detection. The 83 kDa CAPN11 band (arrow) is distinct by day 18. B. Preincubating the antiserum with the CAPN11 peptide (1:1) considerably reduced the immunostaining of the 83 kDa CAPN11 band, but not the cross-reaction with the higher molecular weight band.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

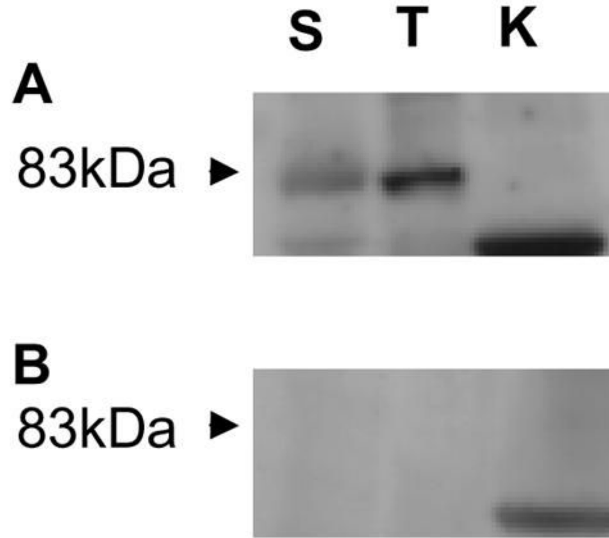

Figure 4.

Presence of CAPNP11 in spermatozoa. A. Sperm proteins (lane S) were resolved by SDS-PAGE and transferred onto PVDF membrane. The blots were incubated with antiserum to CAPN11 peptide (1:100), followed by an ECL detection. Mouse testis protein extract served as a positive control (lane T); mouse kidney protein extract served as negative control (lane K). The arrow indicates the 83 kDa CAPN11 band. B. CAPN11 antiserum was incubated with the peptide used for the immunization (1:1) for 1h prior to immunoblotting. Following this treatment, this antiserum cross-reacted with the unknown higher molecular weight protein, but did not detect CAPN11.

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

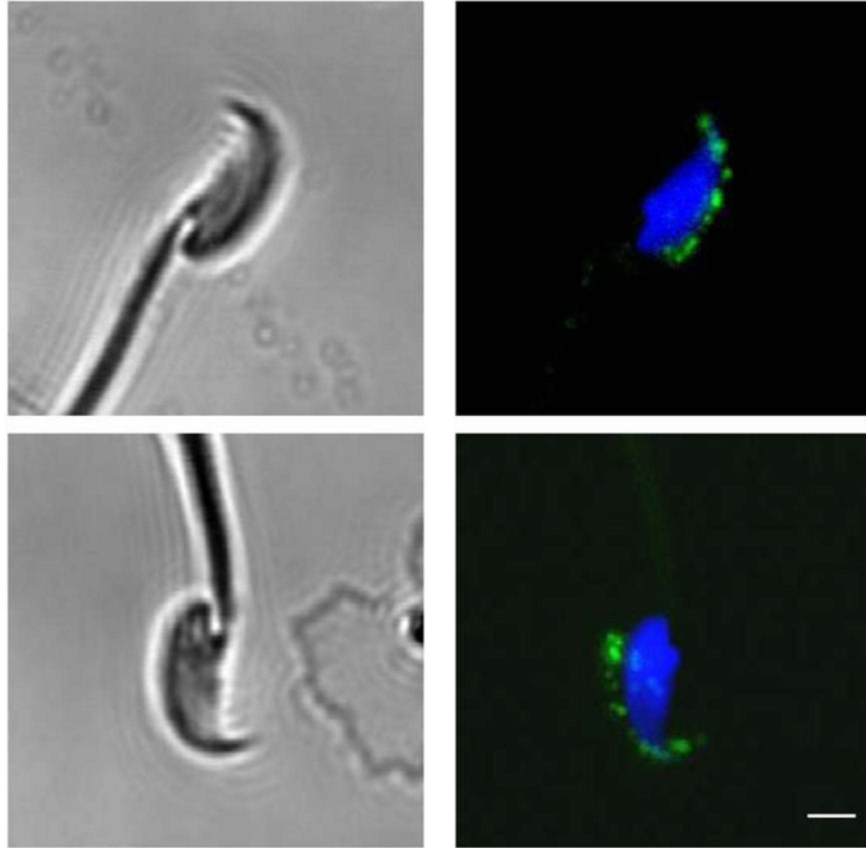

Figure 5.

Localization of CAPN11 in spermatozoa. Phase contrast (A, C) and immunofluorescence pictures (B, D) of spermatozoa are shown. B. DNA is stained blue by Hoechst dye and CAPN11 is immunostained green. D. Second antibody only negative control. Bar= 10lM

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 1

		cDNA (bp)	Genomic (bp)
Calpain 11	5’ attacaagagccgctggcac 3’ aaaccatttcgtttttctcag	171	~370
Ribosomal L7	5’ agctggcctttgtcatcagaa 3’ gacgaaggagctgcagaacct	77	
Mouse β-actin	5’ ttccgatgccctgaggctcttttc 3’ cttgctgatccacatctgctggaa	314	~525

Sequences of primers used for real-time RT-PCR.