Selenium is a micronutrient that is essential for the production of normal spermatozoa. The selenium-rich plasma protein selenoprotein P (Sepp1) is required for maintenance of testis selenium and for fertility of the male mouse. Sepp1 trafficking in the seminiferous epithelium was studied using conventional methods and mice with gene deletions. Immunocytochemistry demonstrated that Sepp1 is present in vesicle-like structures in the basal region of Sertoli cells, suggesting that the protein is taken up intact. Sepp1 affinity chromatography structures in the basal region of Sertoli cells, suggesting that its uptake have not been identified.

Selenium deficiency results in the production of abnormal spermatozoa. Feeding a selenium-deficient diet reverses this effect. We recently demonstrated that Sepp1−/− male mice have dramatically reduced testis selenium levels, produce abnormal spermatozoa that appear identical to those of selenium-deficient wild type mice, and are infertile (8, 9). Unlike selenium-deficient wild type animals, the sperm defects, testis selenium levels, and infertility of Sepp1−/− males are not reversed by dietary selenium supplementation. These findings demonstrate a requirement for Sepp1 in male fertility.

The present study was undertaken to define the uptake mechanism and trafficking pathway by which Sepp1 delivers its selenium to developing germ cells. We demonstrate that a member of the lipoprotein receptor family (10), apolipoprotein E receptor 2 (ApoER2), is required for Sepp1 uptake by the seminiferous epithelium.

**EXPERIMENTAL PROCEDURES**

**Animals**—Care and use of animals conformed to National Institutes of Health guidelines for humane animal care and use in research. The Vanderbilt Institutional Animal Care and Use Committee approved all animal protocols. Rats and mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved facility on a 14 h:10 h light:dark cycle with food and water provided *ad libitum*; diets were either standard laboratory chow or a *Torula* yeast diet supplemented with 0.25 parts/million selenium as sodium selenite (11). Sepp1−/− mice were produced and genotyped by PCR as described previously (9). The primers for identifying the *Sepp1* genotypes included primer 1 (5′-GCCATCAGGGCTCAGTGCGAG-3′), primer 2 (5′-GTTAGTTGGTCGTGTTGTTAGCGA-3′), and primer 3 (5′-GGATGCCTGACGACACACAAAT-3′). Homozygous wild type (*Sepp1*+/+) mice yielded a 600-bp PCR product with primers 1 and 2, whereas *Sepp1*−/− mice yielded a 500-bp PCR product using primers 1 and 3. ApoER2 null mice (strain name B6:129S6-Lrp8tm1Her/J, The Jackson Laboratory) were provided by Dr. Edwin Weeber, Department of Molecular Physiology.
and and Biophysics, Vanderbilt University, and were genotyped by PCR. The PCR primer pair to identify homozygous (apoER2<sup>−/−</sup>) mice was 5′-GATTGGAGAACATAAGCA- GGCATG-3′ and 5′-GCTGTGGAATTCAGCCAT- ACC-3′ and yielded a 420-bp PCR product; the primers to identify wild type mice were 5′-CCACAGTGTCACACAG- GTAATGTG-3′ and 5′-ACGATGACCCATATGACGC- AGGC-3′, which yielded a 520-bp product. Mice were sacrificed using CO<sub>2</sub>, and their organs were removed and immediately utilized in the following protocols.

**Materials**—Mouse monoclonal and rabbit polyclonal antibodies against rat Sepp1 have been previously described (12, 13). Rat hybridomas expressing antibodies to mouse Sepp1 were a gift of Dr. T. Naruse, Kaketsuken, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan. IgG was purified from hybridoma culture medium using protein G-Sepharose (GE Healthcare). Affinity-purified rabbit antibody against mouse ApoER2 were produced commercially (Rockland Immunochemicals Inc.). Two peptides, CEVRRDLVKRDSR and CPDGSDESKAT, representing motifs in the extracellular domain of mouse ApoER2 were conjugated to hemocyanin for immunization. Immune serum was affinity-purified on peptide-agarose affinity columns.

**Affinity Purification and Proteomic Identification of Testicular Sepp1-binding Proteins**—Sepp1 was purified from rat plasma using a monoclonal antibody affinity column (14) and then coupled to AminoLink Plus resin at pH 10.0 (Pierce). The Sepp1-AminoLink resin was stored in a column buffer of TNI (150 mM NaCl, 25 mM Tris-HCl, pH 7.5, 2 mM magnesium chloride, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM sodium fluoride, 1 mM sodium vanadate, and 0.05% sodium azide) containing 1 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, and 0.2% Triton X-100. A membrane preparation was prepared from the testes of adult Sprague-Dawley rats. The testes were suspended in ice-cold TNI and homogenized with a Brinkman polytron set at medium power for five 5-s bursts. The suspension was centrifuged at 40,000 revolutions/min for 30 min in a Beckman TLA100.3 rotor, and the cytosol was discarded, and the pellet was washed by resuspension in TNI followed by centrifugation at 40,000 revolutions/min for 30 min in a Beckman TLA100.3 rotor. Membrane proteins were solubilized at 4 °C in TNI containing 0.2% Triton X-100, and insoluble material was pelletted by centrifugation at 40,000 revolutions/min for 30 min in a Beckman TLA100.3 rotor. The Triton-soluble fraction was adjusted to 1 mM each CaCl<sub>2</sub> and MgCl<sub>2</sub> and then incubated with the Sepp1-AminoLink resin. The column was washed with 10 volumes of column buffer, and bound proteins were sequentially eluted with 1 M NaCl, 1 mM Tris-HCl, pH 7.5, followed by 0.1 M glycine-HCl, pH 2.5. The acid-eluted fraction was neutralized with 1 M Tris base, dialyzed against water, and lyophilized to powder. Polypeptides in the acid-eluted fraction were trypsin-digested and identified by liquid chromatography-tandem mass spectrometry by the Vanderbilt Proteomics Laboratory in the Mass Spectrometry Research Center using the Sequest algorithm and the National Center for Biotechnology Information (NCBI) Blast programs.

**SDSL-ApoER2 and Western Blotting**—Protein samples were diluted in SDS sample buffer containing 50 mM dithiothreitol and heated at 95 °C for 5 min. SDS-PAGE was performed on 10% BisTris NuPAGE Novex acrylamide gels (Invitrogen). Polypeptides were visualized with Coomassie Blue or transferred to nitrocellulose membranes for Western blot analyses. Blots were rinsed in phosphate-buffered saline (150 mM NaCl, 10 mM sodium phosphate, pH 7.4) and blocked in Odyssey blocking buffer (Li-Cor Biosciences, Lincoln, NE). Blots were then incubated in primary antibody diluted 1:2,500–1:5,000 in Odyssey blocking buffer containing 0.1% Tween 20; parallel control blots substituted equivalent levels of the appropriate non-immune IgG. Blots were washed four times with phosphate-buffered saline (150 mM NaCl, 10 mM sodium phosphate buffer pH 7.4, 0.025% sodium azide) containing 0.1% Tween (PBST) and then incubated in secondary antibody of Alexa 680-conjugated goat anti-rat or goat anti-mouse IgG (Invitrogen) diluted 1:5,000 in PBST. Blots were washed in PBST and imaged using an Odyssey infrared imager.

**Cloning of Mouse Testis ApoER2**—Total RNA was isolated from mouse testes using TRIzol reagent (Invitrogen), and a full-length ApoER2 cDNA was amplified with a one-step reverse transcription (RT)-PCR System (Invitrogen) using primers corresponding to the N terminus (P1 = 5′-ATGGGCCGCCCAG- AACTGGGGCGCG) and C terminus (P2 = 5′-GGCCAGTCC- ATCATTTCAAGGACTAATGCCAC-3′) of mouse ApoER2 (15). PCR products were separated on 1.2% agarose gels (E-gels, Invitrogen), and DNA was extracted from excised bands using a Qiaex II gel extraction kit (Qiagen Inc., Valencia, CA). Nested PCR analysis of the RT-PCR product was performed using primer pairs representing the predicted N terminus of mature ApoER2 (P3 = 5′-GGCCAGTCAAGGAGTTGGAAGAG-3′) and a sequence in the epidermal growth factor homology domain (P4 = 5′-GTAAGCCCGTCTCACCGGTAT-3′). To sequence testis ApoER2, the full-length RT-PCR product was cloned into pCR4-TOPO plasmid (Invitrogen), and the cDNA of three clones was sequenced in both directions (Gene Pass, Nashville, TN).

**Immunocytochemistry**—Mouse and rat testes were fixed 1 h at 4 °C with 4% formaldehyde in 0.1 M sodium phosphate, pH 7.4. The tissue was then rinsed in phosphate buffer and infiltrated overnight at 4 °C in phosphate buffer containing 20% sucrose. Tissues were then placed in OCT (optimal temperature cutting compound, Fisher Scientific, Atlanta GA), frozen in liquid nitrogen, and stored at −70 °C. Cryosections were rinsed with TBS (20 mM Tris-HCl, pH 8.0, 150 mM sodium chloride, 0.025% sodium azide) containing 0.05% Tween 20 (TBST) and blocked with TBST containing 1% bovine serum albumin and 0.1% glycine. Sections were then incubated in monoclonal rat anti-mouse Sepp1 or mouse anti-rat Sepp1
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diluted in blocking solution for 1 h at room temperature. Control slides were incubated with equivalent levels of non-immune rat or mouse IgG. Sections were washed three times for 5 min in TBST and then incubated for 1 h in affinity-purified Cy3- or Cy5-conjugated secondary antibodies to rat or mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Slides were washed three times for 5 min in TBST and mounted in 50% glycerol in TBS. Specimens were examined using phase contrast, differential interference contrast, and fluorescence microscopy; fluorescence images comparing experimental and control specimens used identical exposures. At least three animals were used for each experiment and representative figures are shown.

Immunostaining was also performed on enzyme-dissociated testicular cells. Testes were minced in Hanks’ balanced salt solution (Sigma) and then gently agitated at 32 °C for 30 min in Hanks’ balanced salt solution containing 1.0 mg/ml collagenase, 0.5 mg/ml soybean trypsin inhibitor, and 25 μg/ml DNase (Sigma). The cells were rinsed three times in Hanks’ balanced salt solution and immunostained as described above.

Co-immunoprecipitation Analyses—A Triton X-100 lysate of mouse testis membranes prepared as described above was incubated with rat anti-mouse Sepp1 conjugated to AminoLink beads for 1 h. The beads were pelleted by centrifugation at 1500 × g for 1 min, and the pellet was washed three times in TBST. The final pellet was incubated in SDS-PAGE sample buffer, heated to 75 °C for 5 min, and centrifuged. The supernatant fraction was used for SDS-PAGE and Western blot analysis as described above.

Co-localization Protein Probes—Non-isotopic in situ hybridization was performed (16) using digoxigenin-labeled sense and antisense ApoER2 riboprobes and formaldehyde-fixed cryosections of mouse testis. Digoxigenin (Dig)3-labeled nucleotides and alkaline phosphatase-conjugated anti-Dig antibody were obtained from Roche Diagnostics. Hybridizations were performed overnight at 58 °C using identical concentrations of sense or antisense probes.

Analysis of Sperm Phenotypes—The caput and cauda regions of the epididymis were minced in Dulbecco’s phosphate-buffered saline. Released spermatozoa were examined by phase contrast microscopy either prior to or following fixation with 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4.

Measurement of Tissue Selenium Levels—Selenium was measured using a modification of the fluorometric assay of Koh and Benson (17, 18). Tissue and blood plasma were digested in a mixture of perchloric and nitric acids and then reduced with hydrochloric acid followed by derivatization with 2,3-diaminonaphthalene. Fluorescence was measured using an excitation wavelength of 375 nm and an emission wavelength of 525 nm and compared with a sodium selenite standard curve. The detection limit of this assay is 1 ng of selenium.

RESULTS

Localization of Sepp1 in Rat and Mouse Testes—We previously demonstrated that Sepp1, the major form of selenium in plasma, is required to maintain testis selenium, normal sperm development, and male fertility (8, 9). However, it is not known how the selenium content of Sepp1 is delivered to spermatogenic cells. To identify cell types of the seminiferous epithelium involved in Sepp1 uptake and trafficking, cryosections of rat and mouse testes were immunostained with monoclonal antibodies to rat or to mouse Sepp1. In both species, Sepp1 localized to punctate foci at the basal aspect of the seminiferous epithelial within Sertoli cells (Fig. 1, A and C). Neither peritubular cells nor spermatogenic cells displayed detectable immunostaining with anti-Sepp1. Control specimens, prepared by substituting equivalent levels of non-immune IgG for anti-Sepp1, displayed no detectable immunostaining (Fig. 1B). As an additional control, testes of Sepp1−/− mice were immunostained with anti-Sepp1, and no fluorescence was detected (Fig. 1D). Because these animals lack Sepp1, this result demonstrates a lack of antibody cross-reactivity with other tissue proteins. Suspensions of collagenase-dissociated testis cells were also immunostained with anti-Sepp1. Sepp1 localized both to the basal surface of Sertoli cells and to vesicle-like elements within

3 The abbreviations used are: Dig, digoxigenin; RT, reverse transcription.
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Identification of Testicular Sepp1-binding Proteins—To identify candidate membrane receptors for Sepp1, a Triton X-100 lysate of rat testis membranes was prepared and fractionated on a rat Sepp1 affinity column. Liquid chromatography/tandem mass spectrometry analysis of the Sepp1-binding fraction yielded 17 peptides matching apolipoprotein E receptor 2 (ApoER2 or LRP8; GI:6249384) and 20 peptides that matched the rat low density lipoprotein termed RAP (receptor-associated protein (RAP)).

Identification of Testicular ApoER2 Splice Variants—Alternative splicing of the ligand-binding domain of mouse ApoER2 mRNA produces multiple receptor variants of distinct ligand-binding specificity (15, 23). However, the isoform(s) expressed in the testis have not been identified. Mouse testis mRNA was amplified by RT-PCR using a primer pair representing the N terminus and C termini of the mouse ApoER2 mRNA coding sequence (15). A ~2.3-kb cDNA product was observed (Fig. 3B, lane 1), whereas the brain cDNA yielded a larger single band of the predicted size of 941 bp (Fig. 3B, lane 2). Electrophoretic comparison of the full-length tests and brain cDNAs revealed that the testis cDNA was ~300-bp shorter than the brain cDNA (Fig. 3C). These data suggested that the testis and brain cDNAs differed in the structure of both their extracellular and cytoplasmic domains and represented distinct ApoER2 splice variants. To identify the testis ApoER2 isoform, the 2.3-kb RT-PCR product was subcloned into a pCR plasmid, and three clones were fully sequenced. All testis ApoER2 clones lacked ligand-binding repeat 8 in the extracellular domain as well as a 59-amino-acid insert in the cytoplasmic domain that is encoded by exon 19 (Fig. 3D). In addition, two of three testis clones possessed a 13-amino-acid insertion in the extracellular domain immediately following ligand-binding repeat 7, which contains a furin cleavage site (Fig. 3D). The two testis ApoER2 variants, with and without the furin cleavage site, encoded full-length polypeptides of 782 amino acids (86,998 Da) and 769 amino acids (84,998 Da).

Table 1

| ApoER2 peptides | RAP peptides |
|-----------------|--------------|
| ATCSESEBCAEK    | TSGKFSSEELD |
| TVSVATDDGR      | FSSEELDK    |
| SPSLIFTFRN      | VBGLODDGIEK |
| SPSLIFTFRNEVR   | LVKSGLODDGIEK|
| AVAGRPALSIFTNR  | DELMKWK    |
| WKCQDDECPCPODSK | DELMKW     |
| IERAGINNGADR    | HVESIGDPBHSR |
| LLYWIDSR        | LKVHEISGDPBHSR|
| ECEEDQFR        | LABHSDLK   |
| RCTLPFRN        | EKVVLLEEK  |
| CTLFRN          | YVVLLEEK   |
| CECHPGYOMTTLT    | VKEHLQDLSSR|
| GYFKCCECPGYMDTTLTKCK | HLDQDLSSR |
| GYFK             | KHLQDLSSR  |
| AIAVDPRL        | EEKHFEEAK  |
| DLSEPRAIAVDPRL  | LRSTIQGLDR |
| RCNQKR          | SINGGLDR   |
| NQDKESERF       | RSEGEFRR   |
| NQDKESERF       | YQLDGRK    |

Cell-specific ApoER2 mRNA Expression in the Testis—In situ hybridization was used to localize the ApoER2 mRNA expression in the mouse testis. Sections hybridized with antisense ApoER2 Dig riboprobes displayed specific labeling of the Sertoli cells, and no signal was detected in other cell types of the seminiferous epithelium (Fig. 4A). Control specimens hybridized with sense ApoER2 Dig riboprobes displayed no detectable signal (Fig. 4B). This result supports the idea that Sertoli cell ApoER2 expression is linked to Sepp1 uptake.

Demonstration of Sepp1 and ApoER2 Binding by Co-immunoprecipitation—An anti-ApoER2 peptide antibody was prepared for immunoprecipitation analysis, and it displayed strong immunoreactivity to a bacterially expressed 50-kDa ApoER2 extracellular domain fusion protein (Fig. 5A). To test for in vivo binding between Sepp1 and ApoER2, monoclonal domain to compare the testis ApoER2 cDNA to a mouse brain ApoER2 cDNA encoding five ligand-binding repeats, including numbers 1, 2, 3, 7, and 8 (Fig. 3B). PCR of the testis ApoER2 cDNA yielded a single amplicon of ~830 bp (Fig. 3B, lane 1), whereas the brain cDNA yielded a larger single band of the predicted size of 941 bp (Fig. 3B, lane 2). Electrophoretic comparison of the full-length tests and brain cDNAs revealed that the testis cDNA was ~300-bp shorter than the brain cDNA (Fig. 3C). These data suggested that the testis and brain cDNAs differed in the structure of both their extracellular and cytoplasmic domains and represented distinct ApoER2 splice variants. To identify the testis ApoER2 isoform, the 2.3-kb RT-PCR product was subcloned into a pCR plasmid, and three clones were fully sequenced. All testis ApoER2 clones lacked ligand-binding repeat 8 in the extracellular domain as well as a 59-amino-acid insert in the cytoplasmic domain that is encoded by exon 19 (Fig. 3D). In addition, two of three testis clones possessed a 13-amino-acid insertion in the extracellular domain immediately following ligand-binding repeat 7, which contains a furin cleavage site (Fig. 3D). The two testis ApoER2 variants, with and without the furin cleavage site, encoded full-length polypeptides of 782 amino acids (86,383 Da) and 769 amino acids (84,998 Da).

Table 1

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|-------------------------------|
| Liquid chromatography/tandem mass spectrometry identification of rat testis proteins eluted from a rat Sepp1 affinity column yielded 17 peptides for apolipoprotein receptor 2 (ApoER2) and 20 peptides for receptor-associated protein (RAP). |
| ApoER2 peptides | RAP peptides |
|-----------------|--------------|
| ATCSESEBCAEK    | TSGKFSSEELD |
| TVSVATDDGR      | FSSEELDK    |
| SPSLIFTFRN      | VBGLODDGIEK |
| SPSLIFTFRNEVR   | LVKSGLODDGIEK|
| AVAGRPALSIFTNR  | DELMKWK    |
| WKCQDDECPCPODSK | DELMKW     |
| IERAGINNGADR    | HVESIGDPBHSR |
| LLYWIDSR        | LKVHEISGDPBHSR|
| ECEEDQFR        | LABHSDLK   |
| RCTLPFRN        | EKVVLLEEK  |
| CTLFRN          | YVVLLEEK   |
| CECHPGYOMTTLT    | VKEHLQDLSSR|
| GYFKCCECPGYMDTTLTKCK | HLDQDLSSR |
| GYFK             | KHLQDLSSR  |
| AIAVDPRL        | EEKHFEEAK  |
| DLSEPRAIAVDPRL  | LRSTIQGLDR |
| RCNQKR          | SINGGLDR   |
| NQDKESERF       | RSEGEFRR   |
| NQDKESERF       | YQLDGRK    |

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FIGURE 3. A, RT-PCR amplification of mouse testis RNA with primers (P1, P2), representing the N and C termini of mouse brain ApoER2 (see panel D), yielded an amplicon of ~2.3 kb. B, nested PCR reactions of the 2.3-kb testis cDNA product (lane 1) and of brain ApoER2 cDNA (lane 2) with internal primers P3 and P4 (see panel D) indicating that the tests ApoER2 represented a different splice variant(s). C, PCR of full-length testis (lane 1) and brain (lane 2) ApoER2 revealed a size difference of ~0.3 kb between the two isoforms. D, diagrams comparing the domain structure of brain and testis ApoER2. All sequenced testis clones lacked ligand-binding domain 8 as well as the 59-amino-acid cytoplasmic domain encoded by exon 19 (Ex 19). Two testis variants were identified that differed in the presence or absence of the furin cleavage sequence. EGF, epidermal growth factor. SS, signal sequence; 1, 2, 3, 7, 8, ligand binding repeats; A, B, C, cysteine-rich repeats in epidermal growth factor precursor homology domain; TM, transmembrane domain.

anti-mouse Sepp1 conjugated to Affi-gel plus beads was utilized for immunoprecipitation of mouse testis membrane lysates. Immunoprecipitation pellets were fractionated by SDS-PAGE and analyzed by Western blotting (Fig. 5B). Staining with anti-Sepp1 revealed a single band of ~50,000 in the IP pellets that co-migrated with purified mouse Sepp1 (Fig. 5B, lane 1). Companion blot lanes stained with a rabbit anti-peptide antibody to mouse ApoER2 displayed a major immunoreactive band of ~85,000, the predicted size of the processed testicular ApoER2 isoform (Fig. 5B, lane 2). In addition, two faint but specifically stained bands of higher molecular mass were also detected. They may represent incompletely processed ApoER2 precursor(s) or other ApoER2 isoforms expressed at low levels in the testis. Companion control lanes stained using non-immune IgG displayed no positive bands (Fig. 5B, lane 3). These data demonstrate that Sepp1 and ApoER2 form a complex in vivo.

ApoER2−/− Mice Have Low Testis Selenium Levels—The above data suggested that ApoER2 is a Sepp1-binding protein, localized to Sertoli cells with a potential function in testis Sepp1 uptake. To test whether ApoER2 functions in testis selenium homeostasis, we measured total selenium levels in the liver and testes of apoER2−/−, apoER2+/−, and apoER2+/+ mice. No differences in liver selenium levels were observed between genotypes (Fig. 6). In striking contrast, however, the selenium level of testes from apoER2−/− mice was sharply reduced from those in both apoER2+/− and apoER2+/+ mice (Fig. 6). These data show that ApoER2 is essential to maintain normal testis selenium levels.

Sepp1 Does Not Bind Seminiferous Tubules of apoER2−/− Mice—The above data suggested that ApoER2 participates in selenium delivery to germ cells via a Sepp1-dependent pathway. To test this hypothesis, testes of apoER2+/− and apoER2−/− mice were compared for Sepp1 localization by immunocytochemistry. Although the seminiferous epithelium of apoER2+/− mice displays basal Sepp1 staining (Fig. 7A), no Sepp1 was detected in the seminiferous tubules of apoER2−/− mice (Fig. 7B). These data indicate that ApoER2 is necessary for Sepp1 binding and/or uptake by the seminiferous epithelium.

Analysis of Sperm Phenotypes in apoER2−/− Mice—We previously demonstrated that both selenium-deficient Sepp1+/− mice and selenium-replete Sepp1+/+ mice have low testis selenium levels and a similar set of sperm defects (8, 9). However, feeding a high selenium diet to Sepp1−/− males did not restore testis selenium levels or a normal sperm phenotype, although it does restore normal sperm morphology in selenium-deficient Sepp1+/− mice. Phase contrast microscopy of caput spermatozoa of apoER2+/− or apoER2−/− males reveals a normal flagellar architecture (Fig. 8A). In contrast, caput spermatozoa of apoER2−/− mice revealed premature truncation of the mitochondrial sheath that appeared as a narrowing of the flagellum near the midpiece principal piece junction (Fig. 8B). Cauda spermatozoa of both apoER2+/− and apoER2−/− males possessed normal flagella (Fig. 8C). Most cauda epididymal spermatozoa from apoER2−/− males displayed a
hairpin bending of the flagellum at the midpiece principal piece junction (Fig. 8D). These data demonstrate that spermatzoa of apoER2−/− males display the same defective sperm phenotype that was previously described in both Sepp1−/− mice and in selenium-deficient Sepp1+/+ mice (8, 22).

DISCUSSION

The molecular form of selenium taken up by the seminiferous epithelium to support spermatogenesis has been an unre-
solved question. However, the recent finding that Sepp1−/− males fed a high selenium diet exhibit a selenium-deficient sperm phenotype, low testis selenium levels, and infertility indicated that Sepp1, not low molecular weight selenium compound(s), was essential for testis selenium homeostasis (8, 9). This study demonstrates that Sertoli cells of the seminiferous epithelium function in Sepp1 uptake from the interstitial fluid and that both Sepp1 binding and internalization require ApoER2, a member of the lipoprotein receptor family.

A link between ApoER2 and male infertility has been demonstrated previously, and it was shown that the cauda epididymal spermatozoa of ApoER2-null mice exhibit abnormal flagellar bending at the midpiece principal piece junction (21, 22). However, because spermatozoa from the caput epididymis of apoER2−/− males appeared normal and immunocytochemical data suggested that ApoER2 was highly expressed in the epididymal initial segment, it was proposed that epididymal dysfunction played a primary role in producing abnormal spermatozoa in apoER2−/− males (22). However, our results demonstrate that ApoER2 is expressed in testicular Sertoli cells, a finding consistent with published Northern blot and RT-PCR data showing high ApoER2 mRNA expression in human, rabbit, and mouse testes (24–26). Moreover, in apoER2−/− mice, we found that testis selenium levels were dramatically reduced to levels comparable with those previously reported for Sepp1−/− males (9) and that feeding sodium selenite-supplemented diets does not reverse the selenium-deficient status of the apoER2−/− testis. Thus, similar to Sepp1, ApoER2 also is essential for maintaining normal testis selenium levels. In its absence, spermatozoa display a selenium-deficient phenotype.

The lipoprotein receptor family encompasses a diverse family of transmembrane proteins that function both in the endocytic uptake of a variety of extracellular ligands and in intracellular signaling pathways. In the brain, ApoER2 participates in signaling pathways crucial for brain development as well as for normal synaptic function in adults, and several extracellular ligands for ApoER2 have been identified (27, 28). The present study is the first to show that ApoER2 binds Sepp1. Co-immunoprecipitation analyses demonstrate that ApoER2 and Sepp1 are associated in vivo in the testis. However, it remains to be established whether they directly bind to each other or are constituents of a multiprotein complex. Alternative splicing of ApoER2 mRNA results in the expression of ApoER2 isoforms with different ligand-binding specificities (15, 23). Whereas the major mouse brain ApoER2 isoform contains five ligand-binding repeats in its extracellular domain (15), the two mouse testis ApoER2 isoforms identified in this study each possess four ligand-binding repeats. It is possible that brain and testis ApoER2 isoforms bind different sets of extracellular ligands.

ApoER2-dependent Sepp1 uptake by Sertoli cells represents an essential step in the trafficking pathway that provides selenium to support spermatid selenoprotein synthesis. Spermatids reside above the blood-testis barrier formed by Sertoli cell tight junctions, therefore they are not exposed to Sepp1 or low molecular weight selenium compounds present in peritubular fluid. Nonetheless, spermatids require selenium to synthesize selenoproteins such as glutathione peroxidase 4, the major selenoprotein of mature spermatozoa, which is a structural com-

FIGURE 8. Phase contrast photomicrographs of caput (A and B) and cauda epididymal spermatozoa (C and D) from wild type (A and C) and apoER2−/− (B and D) mice. A, note that caput epididymal spermatozoa of wild type mice display a typical flagellar structure and no discernable abnormality at the midpiece principal piece junction. B, in contrast, caput spermatozoa of apoER2−/− mice show an abrupt narrowing of the posterior midpiece (arrowhead). C, cauda epididymal spermatozoa of wild type mice with normal flagella. D, cauda epididymal spermatozoa from apoER2−/− mice display a hairpin-like bend at the junction of the midpiece and principal piece segments (arrowheads). CD, cytoplasmic droplet; Mp, midpiece; PP, principal piece.
ponent of the mitochondrial capsule in the midpiece (29). The molecular form of selenium that Sertoli cells provide to spermatids remains unresolved. Immunoreactive Sepp1 is not detected within spermatids, suggesting that Sertoli cells do not deliver intact Sepp1 by transcytosis. Sepp1 may be hydrolyzed within Sertoli cell lysosomes, resulting in release of selenocysteine. However, free selenocysteine cannot be incorporated into selenoproteins, and its selenium content must be released as selenide and be converted to selenophosphate by two enzymes, selenocysteine β-lyase and selenophosphate synthetase (30–32). Selenophosphate is the selenium-containing molecule utilized for the co-translational synthesis of the selenocysteine residues that are inserted co-translationally into selenoproteins (33, 34). Both selenocysteine β-lyase and selenophosphate synthetase are expressed in the testis (30, 32), and the identification of their cell-specific distribution patterns should provide insights into whether Sertoli cells provide selenocysteine or another form of selenium to spermatids.

Sepp1 and ApoER2 are functionally linked in the trafficking pathway providing selenium to spermatogenic cells. Previously it was shown in Sepp1<sup>−/−</sup> males that testis selenium levels are reduced to 19% of those in Sepp1<sup>+/+</sup> mice, whereas brain and kidney selenium levels were reduced to 43 and 76% of controls (9). Our data show that testis selenium levels are also depleted in apoER2<sup>−/−</sup> males, even though they have a normal level of liver selenium. Interestingly, unlike the brain or kidney, testis selenium levels in Sepp1<sup>−/−</sup> males could not be elevated by feeding increased selenium, indicating that the brain and kidney can take up other plasma forms of selenium, but that the testis requires Sepp1 (8).

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