A hallmark of fertilization is a high degree of species specificity, implying gamete-specific recognition signals. To identify sperm-specific plasma membrane proteins, an antiserum to sperm plasma membranes was produced in female guinea pigs. The screening of a testis cDNA expression library with this antiserum resulted in the isolation of two clones encoding a predicted protein containing two extracellular immunoglobulin-like domains, a transmembrane segment, and an intracellular proline-rich domain. The predicted protein (named sperad) is closely related to a large family (biliary glycoproteins) of putative cell adhesion molecules. Sperad is first expressed by the haploid spermatid and is localized to the plasma membrane overlying the acrosome, supportive of a role in cell adhesion/signaling. However, sperad expression in Sf9 cells does not result in Sf9 cell aggregation or in sperm adhesion to the infected insect cells, suggesting that sperad is involved in heterotypic interactions. The open reading frame of the two cDNA clones predicts proteins of either 32.2 or 33.3 kDa. Antibody produced to sperad recognizes three sperm plasma membrane proteins on immunoblots (Mr 55,000, 36,000, and 28,000), but the lower molecular weight proteins are degradation products; deglycosylation confirmed that Mr 55,000 sperm plasma membrane represents the full-length protein encoded by the clone. Induction of the acrosome reaction does not appear to alter the molecular weight of sperad but does result in its loss from the sperm cells. Thus, sperad is likely involved in heterotypic interactions prior to interaction of spermatozoa with the egg plasma membrane.

Mammalian spermatozoa undergo many changes as they move through the male and female reproductive tracts, ultimately delivering their haploid genome to the egg. As spermatozoa traverse the epididymis, they become progressively motile and capable of binding to the egg zona pellucida (1). Coincidentally, various secreted epididymal proteins bind to spermatozoa followed by a complement of accessory gland-derived proteins (2). In the female reproductive tract, several interactions between the spermatoozon and its environment are also evident, including: 1) oviductal epithelial cell binding, 2) development of the competence to fertilize an egg, known as capacitation, 3) induction of an exocytotic event known as the acrosome reaction, 4) hyperactivation of motility, 5) cumulus mass and zona pellucida binding and penetration, and 6) egg plasma membrane binding and fusion. Many of these interactions appear to show relative or absolute species specificity, suggesting sperm-specific adhesion and signaling molecules.

Antibodies can serve as powerful reagents for the identification of sperm-specific proteins. Auto- and allo-antisera, as well as monoclonal antibodies, have been raised against sperm cells from several species, and these antisera often recognize proteins that appear to participate in sperm and egg interactions (3–5). Some of these antigens, such as rabbit sperm autoantigen, hyaluronidase, and fertilin, have been characterized at the molecular level (6–8).

To identify novel sperm-specific membrane proteins, we produced antiserum to guinea pig sperm membranes in female guinea pigs based on the hypothesis that only male-specific proteins would stimulate an immune response. The antiserum was then used to identify cDNA clones from an enriched spermatogenic cell cDNA expression library.

Using this approach, we isolated a cDNA clone that predicted a novel glycoprotein related to a family of cell adhesion molecules originally found in the liver (9). The protein (named sperad), first expressed by the haploid spermatid, was localized to the periacrosomal plasma membrane of mature spermatoozon, suggesting a role in cell-cell interaction. Although many members of this large family display homotypic adhesion properties, the expression of sperad in Sf9 cells did not result in cell aggregation. Therefore, sperad is likely to function in heterotypic cell interactions. Since the glycoprotein also contains a proline-rich intracellular domain, a feature different from other members of this family, unique intracellular signaling may also represent a function of this plasma membrane protein.

**MATERIALS AND METHODS**

**Sperm Membrane Preparation**—Guinea pig caudal epididymal spermatozoa were washed in a solution containing 4 mM HEPES, 140 mM NaCl, 4 mM KCl, 10 mM glucose, 2 mM MgCl₂, 100 µM EDTA, pH 7.4, as described by Hardy et al. (10). Spermatozoa were allowed to acrosome react in 4 mM HEPES, 140 mM NaCl, 4 mM KCl, 10 mM glucose, 10 mM CaCl₂, pH 7.4, containing 20 µg/ml A23187 for 10 min at 37 °C; a protease inhibitor mixture (1 mM diisopropyl fluorophosphate, 10 mM EDTA, 0.1 mM leupeptin, and 10 µM E-64) was then added. The cells were cooled on ice (5 min) and centrifuged at 400 x g, 5 min, 4 °C. The supernatant solution was recovered, and the sperm pellet was washed in 10 volumes of the buffer containing protease inhibitors, centrifuged at 400 x g, 5 min, and the solution pooled with the first supernatant solution. The combined solution was centrifuged at 12,000 x g, 15 min, 4 °C to remove cell debris, and the resulting supernatant fraction further centrifuged at 100,000 x g, 1.5 h, 4 °C to recover membrane vesicles. This membrane pellet was washed twice with 10 mM HEPES, pH 7.4, 500 mM NaCl, 10 mM KCl, 10 mM EDTA, and stored in 20 mM HEPES, 150 mM NaCl, pH 7.4, at −20 °C.

A second membrane fraction was prepared by resuspending the 400 x g pellet of acrosome reacted spermatoozon in 5 volumes of buffer
containing protease inhibitors, followed by cavitation of the suspension at 70°C for 5 min, 20 min, 2–4°C. The cavity was centrifuged at 30,000 × g, 30 min, 4°C to remove cell debris. This pellet was washed, centrifuged again, and the two supernatant fractions combined. The resultant solution was centrifuged at 260,000 × g, 45 min, 4°C to recover the membrane material. The pellet was resuspended in 10 mM HEPES, pH 7.4, 150 mM NaCl, 10 mM EDTA, 10 mM benzamidine, 10% sucrose, overlaid on a 30/40% sucrose step gradient in the same buffer, and centrifuged at 200,000 × g, 30 min, 4°C. The membranes at the 10/50% interface were collected, washed with the high salt buffer, and stored as described above.

Production of Antisera—Antisera were produced by intradermal immunization of female guinea pigs with 700 μg of sperm membrane protein in Freund's complete adjuvant. The animals were boosted once (intramuscular) with 550 μg of sperm membrane protein in Freund's incomplete adjuvant. In each case, 90% of the immunogen was derived from membranes isolated from the acrosome reaction supernatant solution.

Antisera specific for sperm were produced against protein expressed in E. coli BL-21(DE3) using the pRSET vector (Invitrogen). Two constructs were expressed, representing the mature protein (Ala37–Val330) and the immunoglobulin domains (Ala37–Asn255). The two constructs were expressed, representing the mature protein (Ala37–Val330) and the immunoglobulin domains (Ala37–Asn255). The expressed proteins were isolated from bacterial lysates by Ni2+-iminodiacetic acid affinity chromatography and preparative electrophoresis. Rabbits were immunized and boosted once with 100 μg of protein/animal.

RNA Isolation—Total RNA was prepared using the guanidinium thiocyanate method (11). Poly(A)+ RNA was isolated with oligo(dt)-cellulose (12).

cDNA Cloning—An enriched spermatogenic cell population was prepared according to Arboleda and Gerton (13). Poly(A)+ RNA from this material was used to synthesize a cDNA library in ZAPII according to the manufacturer's directions (Stratagene). Fusion protein expression was induced with 10 mM isopropyl-β-D-thiogalactopyranoside saturated nitrocellulose filters. The filters were rinsed with TBST (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20) containing 0.05% NaNO3 twice, and nonspecific protein binding sites blocked by overnight incubation in TBST containing 0.05% NaNO3 + 5% nonfat milk. The filters were rinsed with TBST (two 1-liter washes), and probed with 1:5000 sperm antisperm immunodot membrane dilution in TBST (2 h). Following washing with TBST (four 1-liter washes), the filters were incubated with 1:5000 horse-rasderasiliconconjugated rabbit anti-guinea pig IgG (Zymed) in TBST (1 h). The filters were then washed with TBST (two 1-liter washes) and peroxidase activity detected with enhanced chemiluminescence (Amersham). Phage clones were rescued as Bluescript plasmids and analyzed by Southern blot. Identified partial clones were randomly primed with [α-32P]dCTP and used to reprobe the library to identify complete clones. Plaque hybridization was at 42°C overnight, followed by washing in 0.1 × SSC, 0.2% SDS, 0.1% Na2PO4 (1 h at 23°C, three 1-liter washes at 65°C) (1 × SSC is 150 mM NaCl, 15 mM sodium citrate). Full-length clones were sequenced on both strands, and sequences were analyzed using DNASTAR software (Madison, WI).

Northern Blot—Total RNA (25 μg) from various guinea pig tissues and an enriched spermatogenic cell fraction was electrophoresed through 1% formaldehyde agarose gels and blotted overnight to nylon membranes (14). Blots were hybridized with an [α-32P]dCTP-labeled polymerase chain reaction product (950 base pairs corresponding to nucleotides C259–C1210) as described above for library screening. Blots were washed in 2 × SSC, 0.1% SDS (10 min) and 0.5 × SSC, 0.1% SDS (two 20-min washes) at 65°C.

Indirect Immunofluorescence—Spermatozoa, washed as described above, were further washed with 0.1 M Na3PO4, pH 7.2. The sperm pellet was fixed in 10 volumes of 0.1 M Na3PO4, pH 7.2 containing 4% paraformaldehyde, 1% glutaraldehyde (30 min, 23°C). The fixation was quenched with 0.05 volumes of 1.0 mM Tris-HCl, pH 6.8 (1 h). The fixed spermatozoa were washed twice with Dulbecco's PBS, spotted onto slides, air dried, and rinsed briefly in water. Spermatozoa were permeabilized in Dulbecco's PBS, 10% normal goat serum, 1% Triton X-100, 0.2% 2-mercaptoethanol, 100°C for 5 min) were diluted 10-fold into 50 mM NaPO4, 0.2 mM EDTA, 1% Triton X-100, pH 8.0, and deglycosylated using N-glycosidase F (Boehringer Mannheim) at 20 units/mg protein. SDS-polyacrylamide gel electrophoresis was done according to the method of Laemmli (17). Electrophoretically separated samples were transferred to nitrocellulose membranes by Western blotting (50 V, 1 h) (18). Blots were probed with primary antibody in TBST (1 h). After washing with TBST (four changes over 30 min), the blots were probed with peroxidase-conjugated secondary antibody, washed with TBST (two changes over 30 min), and bound secondary antibody detected with enhanced chemiluminescence.

RESULTS AND DISCUSSION

Several proteins of isolated guinea pig sperm membranes were detected with the antisera produced in female guinea pigs (Fig. 1). The membrane proteins ranged in size from M, 28,000 to M, 120,000. No guinea pig sperm membrane proteins were detected with the preimmune serum. That the identified proteins were components of the membrane fraction was also indicated by the absence of detectable immunoreactive components in the soluble fraction released during the acrosome reaction. Some of these proteins were glycoproteins as determined by enzymatic deglycosylation with N-glycosidase F. Treatment with N-glycosidase F did not change the number of immunoreactive proteins, indicating that N-linked oligosaccharides were not solely responsible for the antigenicity.

An enriched spermatogenic cell cDNA expression library prepared from four animals was screened with the preimmune serum to identify clones encoding the sperm membrane proteins. Five clones were detected, four of which represented the same novel transcript. Subsequent screening with this cDNA insert iden-

1 The abbreviations used are: TBST, Tris-buffered saline with Tween 20; PBS, phosphate-buffered saline; kb, kilobase(s).
tified two closely related full-length cDNAs of 1.51 and 1.56 kb (Fig. 2). The clones were full-length as suggested by three observations. First, the predicted initiating methionine represented a strong Kozak consensus sequence (19). Second, an apparent signal peptide followed the predicted start methionine (20). Finally, all three reading frames to the predicted initiating methionine contained a stop codon, demonstrating that no longer open reading frame was present. Each clone encoded a protein consisting of a putative signal peptide, two immunoglobulin-like domains (one variable and one constant based on conserved sequence characteristics), a transmembrane segment, and a repetitive, proline-rich domain (Fig. 4B) (21). The predicted mature proteins had a calculated molecular mass of 32.2 or 33.3 kDa in the absence of glycosylation. Both clones contained three conserved potential N-linked glycosylation sites in the immunoglobulin domains with an additional site in the 1.51-kb clone. It is likely, therefore, that the repetitive proline-rich domain is within the cytoplasmic compartment of the cell.

Several nucleotide differences were spread throughout the sequences. Thus, the two sequences may represent either alleles of the same gene or two closely related genes with the same expression pattern. It is unlikely that the two sequences are splice variants of one gene based on the conserved gene structure of homologous proteins (see below).

That the cDNAs were expressed only in the testis was determined by Northern blot analysis of various guinea pig tissues and an enriched spermatogenic cell sample (Fig. 3). The samples were probed with a polymerase chain reaction product (950 base pairs) corresponding to the coding sequence of the mature protein. A signal of approximately 1.8 kb was detected in the testis and spermatogenic cell RNA samples. No signal was detected in brain, heart, kidney, liver, ovary, or spleen. The broad hybridizing band suggested the presence of each of the two similar mRNA species within a single testis. Using oligonucleotide primers, which flanked the proline-rich repeat region of the mature protein, the putative transmembrane segment of the protein was identified.

**Fig. 2.** Nucleotide and deduced amino acid sequences of the two isoforms of sperad (L, long; S, short). Amino acids are numbered from the predicted initiating methionine. The putative signal peptide sequences (amino acids 1–34) are shown in lowercase. Identical residues and gaps used to align the sequences are indicated by periods and dashes, respectively. Potential N-linked glycosylation sites are double underlined, and the putative transmembrane segment is underlined.
gion, each cDNA was detected in a single testis by reverse transcriptase-polymerase chain reaction (data not shown). Comparison of the clones to the GenBank™ data base indicated that they were novel transcripts related to the biliary glycoprotein family of cell adhesion molecules. The sequence similarity to the biliary glycoproteins was highest in the signal peptide, and gradually decreased through the immunoglobulin domains until the sequences completely diverged carboxyl-terminal to the transmembrane segment (Fig. 4A). This site of sequence divergence corresponded to the end of an exon in the conserved biliary glycoprotein gene structure of rat, mouse, and human (22–24). At the nucleotide level, additional sequence similarity between the 3′-untranslated region of the guinea pig clones (nucleotides 1182–1467 of the 1.56-kb clone) and the biliary glycoprotein intracellular domain encoding exons 7–9 was detected. This sequence was not translated in the encoded guinea pig proteins, since the sperm membrane antisemum recognized the proline-rich region expressed as a glutathione S-transferase fusion protein in bacteria (data not shown). In addition, it is unlikely that a protein containing these biliary glycoprotein intracellular domain exon sequences is produced in guinea pig based on two observations. First, the guinea pig sequence contained a single nucleotide insertion (nucleotide 1228 of the 1.56-kb clone), which would produce a frameshift in the potential biliary glycoprotein reading frame. Second, a nucleotide substitution (nucleotide 1280 of the 1.56-kb clone) produces an in-frame stop codon in the potential biliary glycoprotein reading frame. These changes are probably the result of the elimination of this nucleotide sequence from the protein coding sequence in the guinea pig.

The biliary glycoproteins are members of the carcinoembryonic antigen family, a subdivision of the immunoglobulin superfamily (9). These glycoproteins are widely distributed in epithelial, endothelial, and myeloid lineage cells with high levels of expression found in the liver, intestine, and granulocytes (25). The predicted domain organization of the biliary glycoproteins has been determined in mouse, rat, and human from cloning of the corresponding mRNAs (26–28). In each case, these glycoproteins possess a signal peptide, an immunoglobulin V-like N-domain, a variable number of immunoglobulin C2-like domains (0–3), a transmembrane segment, and an intracellular domain. Each of the immunoglobulin type domains corresponds to a single exon while the intracellular domain of the biliary glycoproteins is encoded by three exons in the conserved gene structure. Several forms of biliary glycoproteins are produced in all three species. This variation arises as the result of alternative splicing of the exons encoding the C2-like immunoglobulin domains and the intracellular domain producing a variable number of C2-like domains, and either a short (approximately 10 amino acids) or long (approximately 73 amino acids) intracellular domain.

The function of the biliary glycoproteins has been addressed in expression studies, where it has been concluded that they act as cell adhesion proteins, at least in vitro (26, 29). Although these glycoproteins have been suggested to principally mediate homotypic adhesion, heterotypic adhesion to other members of the carcinoembryonic antigen family has been observed (30, 31). Characterization of the adhesive activity of biliary glycoproteins has shown dependence on both calcium and physiological temperatures; the immunoglobulin variable type domain at
Molecular Cloning of Sperad

33513

Fig. 5. Detection of sperad in guinea pig sperm membranes. Immunoblot of guinea pig sperm membrane protein (10 μg lane) probed with anti-sperad, 1:40,000. Lane –, without N-glycosidase F digestion; lane +, with N-glycosidase F (20 units/mg protein) digestion.

Fig. 6. Indirect immunofluorescent localization of sperad on spermatozoa. Paired phase contrast and epifluorescence images of fixed and permeabilized caudal epididymal guinea pig spermatozoa probed with sperad immune serum (A and B), or preimmune serum (C and D) (1:500). Epifluorescence exposure time was 1 s for each image.

The N terminus appears to be the functional binding domain (29, 32). Due to the homology of the guinea pig protein to these cell adhesion molecules and its specific cellular location (see below), we have designated it sperad.

The intracellular domain of sperad is proline-rich containing an extended region where every third amino acid is a proline residue. This proline-rich motif is predicted to form a polyproline II helix consisting of three residues per turn (33). The majority of this region comprises the short repetitive sequence PPQPEQ. This would result in one side of the three-sided polyproline II helix possessing a negative charge approximately every 19 Å along the helix axis at physiological pH. Polyproline II helices are known to mediate low affinity (micro)molar binding interactions between proteins (33). In many cases, the binding of polyproline II helices involves the SH3 or WWP domains that are often found in signal-transducing proteins (34, 35). In the case of sperad, its facile extraction with detergent suggests that it does not interact with cytoskeletal components, and no binding protein has yet been identified.

To identify which sperm membrane protein was represented by sperad, we generated a specific polyclonal antibody to a protein representing the N terminus appears to be the functional binding domain (29, 32). Due to the homology of the guinea pig protein to these cell adhesion molecules and its specific cellular location (see below), we have designated it sperad.

The intracellular domain of sperad is proline-rich containing an extended region where every third amino acid is a proline residue. This proline-rich motif is predicted to form a polyproline II helix consisting of three residues per turn (33). The majority of this region comprises the short repetitive sequence PPQPEQ. This would result in one side of the three-sided polyproline II helix possessing a negative charge approximately every 19 Å along the helix axis at physiological pH. Polyproline II helices are known to mediate low affinity (micro)molar binding interactions between proteins (33). In many cases, the binding of polyproline II helices involves the SH3 or WWP domains that are often found in signal-transducing proteins (34, 35). In the case of sperad, its facile extraction with detergent suggests that it does not interact with cytoskeletal components, and no binding protein has yet been identified.

To identify which sperm membrane protein was represented by sperad, we generated a specific polyclonal antibody to a protein representing the N terminus appears to be the functional binding domain (29, 32). Due to the homology of the guinea pig protein to these cell adhesion molecules and its specific cellular location (see below), we have designated it sperad.

The intracellular domain of sperad is proline-rich containing an extended region where every third amino acid is a proline residue. This proline-rich motif is predicted to form a polyproline II helix consisting of three residues per turn (33). The majority of this region comprises the short repetitive sequence PPQPEQ. This would result in one side of the three-sided polyproline II helix possessing a negative charge approximately every 19 Å along the helix axis at physiological pH. Polyproline II helices are known to mediate low affinity (micro)molar binding interactions between proteins (33). In many cases, the binding of polyproline II helices involves the SH3 or WWP domains that are often found in signal-transducing proteins (34, 35). In the case of sperad, its facile extraction with detergent suggests that it does not interact with cytoskeletal components, and no binding protein has yet been identified.

The major intracellular domain of sperad is proline-rich containing an extended region where every third amino acid is a proline residue. This proline-rich motif is predicted to form a polyproline II helix consisting of three residues per turn (33). The majority of this region comprises the short repetitive sequence PPQPEQ. This would result in one side of the three-sided polyproline II helix possessing a negative charge approximately every 19 Å along the helix axis at physiological pH. Polyproline II helices are known to mediate low affinity (micro)molar binding interactions between proteins (33). In many cases, the binding of polyproline II helices involves the SH3 or WWP domains that are often found in signal-transducing proteins (34, 35). In the case of sperad, its facile extraction with detergent suggests that it does not interact with cytoskeletal components, and no binding protein has yet been identified.

The inability to detect sperad without permeabilization suggests that the proline-rich region is intracellular. Following the ionophore-induced acrosome reaction, little or no immunofluorescence remained associated with the sperm cells. The acrosomal location of sperad suggested that it was associated either with the plasma membrane or the outer acrosomal membrane. Immunoelectron microscopy of caudal epididymal spermatozoa with the anti-sperad antibody demonstrated labeling of the periacrosomal plasma membrane (Fig. 7). There was no difference in labeling pattern between the regions of the the two immunoglobulin domains of sperad expressed in bacteria (anti-sperad ECD: Ala37–Asn255). This antiserum also recognized the same set of proteins. In addition, antibody microaffinity purified from the guinea pig sperm membrane antiserum with the M, 55,000 sperm membrane protein also recognized all three proteins. The sperm membrane antiserum recognized only the full-length protein and not the truncated immunoglobulin domain protein expressed by bacteria. Thus, based on these observations, the M, 36,000 and 28,000 proteins probably represent degradation products of the M, 55,000 protein.

Deglycosylation of the M, 55,000 protein with N-glycosidase F resulted in a shift to M, 44,000 (Fig. 5). The mobilities of the M, 36,000 and 28,000 components were not affected by N-glycosidase F, suggesting that they do not contain N-linked oligosaccharides. When the full-length clone (Ala37–Val330) was expressed in bacteria, the isolated fusion protein had a M, 44,000 rather than the calculated 32,000. The agreement between the size of the protein expressed in bacteria and the size of the deglycosylated M, 55,000 membrane protein confirmed that the clones were complete.

Indirect immunofluorescence of fixed, permeabilized guinea pig spermatozoa with anti-sperad localized the protein specifically over the acrosome (Fig. 6). No signal was obtained with acrosome intact spermatozoa using preimmune serum, or using the specific antiserum without a permeabilization step. The predominant epitope recognized by the anti-sperad antibody is the proline-rich repeat as determined by immunoblotting of sperad protein fragments expressed in bacteria (data not shown). Therefore, the inability to detect sperad without permeabilization was further evidence that the proline-rich region was intracellular. Following the ionophore-induced acrosome reaction, little or no immunofluorescence remained associated with the sperm cells.

The acrosomal location of sperad suggested that it was associated either with the plasma membrane or the outer acrosomal membrane. Immunoelectron microscopy of caudal epididymal spermatozoa with the anti-sperad antibody demonstrated labeling of the periacrosomal plasma membrane (Fig. 7). There was no difference in labeling pattern between the regions of the

The majority of this region comprises the short repetitive sequence PPQPEQ. This would result in one side of the three-sided polyproline II helix possessing a negative charge approximately every 19 Å along the helix axis at physiological pH. Polyproline II helices are known to mediate low affinity (micro)molar binding interactions between proteins (33). In many cases, the binding of polyproline II helices involves the SH3 or WWP domains that are often found in signal-transducing proteins (34, 35). In the case of sperad, its facile extraction with detergent suggests that it does not interact with cytoskeletal components, and no binding protein has yet been identified.

To identify which sperm membrane protein was represented by sperad, we generated a specific polyclonal antibody to a hexahistidine-tagged protein representing the mature sperad protein expressed in bacteria (anti-sperad: Ala37–Val370). Immunoblots of guinea pig sperm membranes using this antiserum identified three proteins of molecular weight 55,000, 36,000, and 28,000 (Fig. 5). This electrophoretic profile was constant throughout the epididymis as well as through in vitro capacitation and the acrosome reaction. All three of these proteins corresponded to proteins detected with the sperm membrane antiserum as determined by immunoprecipitation with anti-sperad, and subsequent detection of these proteins on immunoblots with the sperm membrane antiserum. We also generated a separate polyclonal antibody to a protein representing the two immunoglobulin domains of sperad expressed in bacteria (anti-sperad ECD: Ala37–Asn255). This antiserum also recognized the same set of proteins. In addition, antibody microaffinity purified from the guinea pig sperm membrane antiserum with the M, 55,000 sperm membrane protein also recognized all three proteins. The sperm membrane antiserum recognized only the full-length protein and not the truncated immunoglobulin domain protein expressed by bacteria. Thus, based on these observations, the M, 36,000 and 28,000 proteins probably represent degradation products of the M, 55,000 protein.

Deglycosylation of the M, 55,000 protein with N-glycosidase F resulted in a shift to M, 44,000 (Fig. 5). The mobilities of the M, 36,000 and 28,000 components were not affected by N-glycosidase F, suggesting that they do not contain N-linked oligosaccharides. When the full-length clone (Ala37–Val330) was expressed in bacteria, the isolated fusion protein had a M, 44,000 rather than the calculated 32,000. The agreement between the size of the protein expressed in bacteria and the size of the deglycosylated M, 55,000 membrane protein confirmed that the clones were complete.

Indirect immunofluorescence of fixed, permeabilized guinea pig spermatozoa with anti-sperad localized the protein specifically over the acrosome (Fig. 6). No signal was obtained with acrosome intact spermatozoa using preimmune serum, or using the specific antiserum without a permeabilization step. The predominant epitope recognized by the anti-sperad antibody is the proline-rich repeat as determined by immunoblotting of sperad protein fragments expressed in bacteria (data not shown). Therefore, the inability to detect sperad without permeabilization was further evidence that the proline-rich region was intracellular. Following the ionophore-induced acrosome reaction, little or no immunofluorescence remained associated with the sperm cells.

The acrosomal location of sperad suggested that it was associated either with the plasma membrane or the outer acrosomal membrane. Immunoelectron microscopy of caudal epididymal spermatozoa with the anti-sperad antibody demonstrated labeling of the periacrosomal plasma membrane (Fig. 7). There was no difference in labeling pattern between the regions of the...
The results demonstrate haploid cell expression of the protein. Serum sections were labeled with sperad antiserum, which was glycosylated, as determined by immunofluorescence. The expressed adhesion molecule, which is primarily homotypic, and its localization to the spermatozoon interact with other reproductive tract components such as epithelial cells of the epididymis, uterus, and oviduct, as well as the cumulus oophorus and zona pellucida associated with the egg.

Acknowledgments—We gratefully acknowledge the technical assistance of Lynda Doolittle and Deborah Miller. We also thank Dennis Bellotto for advice and technical assistance in the electron microscopy study.

REFERENCES

1. Yanagimachi, R. (1994) in The Physiology of Reproduction (Knobil, E., and Neill, J. D., eds) pp. 189–317, Raven Press, Ltd., New York
2. Eddy, E. A., and O’Brien, D. A. (1994) in The Physiology of Reproduction (Knobil, E., and Neill, J. D., eds) pp. 29–77, Raven Press, Ltd., New York
3. Yanagimachi, R., Okada, A., and Tung, K. S. (1981) Biol. Reprod. 24, 512–518
4. Tung, K. S., Okada, A., and Yanagimachi, R. (1986) Biol. Reprod. 35, 877–886
5. O’Rand, M. G. (1981) Biol. Reprod. 25, 621–628
6. Richardson, R. T., Yamashita, N., and O’Rand, M. G. (1994) Dev. Biol. 165, 688–701
7. Lathrop, W. F., Carmichael, E. P., Myles, D. G., and Primakoff, P. (1990) J. Cell Biol. 111, 2939–2949
8. Blobel, C. P., Wolfsberg, T. G., Turck, C. W., Myles, D. G., Primakoff, P., and White, J. M. (1992) Nature 356, 244–252
9. Thompson, J. A. (1995) Transgen. Biol. 16, 10–16
10. Hardy, D. M., Huang, T. T., Jr., Driscoll, W. J., Tung, K. K., and Wild, G. C. (1988) Biol. Reprod. 38, 423–437
11. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
12. Davis, L. G., Dibner, M. D., and Battey, J. F. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 4239–4249
13. Arboleda, C. E., and Gerton, G. L. (1988) Dev. Biol. 125, 217–225
14. Shiino, Y., Saji, M., and Takahashi, T. (1990) Mol. Reprod. Dev. 30, 279–289
15. Tokuyasu, K. T. (1986) J. Microsc. 143, 139–149
16. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) Anal. Biochem. 150, 76–85
17. Lasnitzki, U. K. (1970) Nature 227, 680–685
18. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
19. Kosztulak, M. (1988) J. Cell Biol. 108, 229–241
20. von Heijne, G. (1986) Nucleic Acids Res. 14, 6683–6690
21. Williams, A. F., and Barclay, A. N. (1993) Annu. Rev. Immunol. 6, 381–405
22. Chou, P.-H., Culie, O., Qiu, Y., Earley, K., Thompson, N., Hixson, D. C., and Lin, S. H. (1993) Biochem. J. 295, 427–435
23. McCuaig, K., Rosenberg, M., Nadelpec, L., Turbide, C., and Beauchemin, N. (1993) Gene (Amst.) 127, 173–183
24. Barnett, T. R., Drake, L., and Pickle, W., II (1993) Mol. Cell. Biol. 13, 1273–1282
25. Prall, F., Nolzau, P., Neumaier, M., Haubec, H. D., Drzeniek, Z., and Henn, H.-D., Lening, T., and Wagener, C. (1996) J. Histochem. Cytochem. 44, 35–41
26. Turbide, C., Rojas, M., Stanners, C. P., and Beauchemin, N. (1993) J. Biol. Chem. 268, 309–315
27. Hino, Y., Neumaier, M., Heffer, S. A., Drzeniek, Z., Wagener, C., and Shively, L., Heffer, L. J., Shively, J. E., and Paxton, J. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 6559–6563
28. Lin, S.-H., and Guidotti, G. (1989) J. Biol. Chem. 264, 14408–14414
29. Cheng, P.-H., Lao, W., Qiu, Y., Zhang, X., Earley, K., Millersons, P., and Lin, S.-H. (1993) J. Biol. Chem. 268, 24303–24310
30. Rojas, M., Fucks, A., and Stanners, C. P. (1990) Cell Growth Diff. 1, 527–533
31. Brummedendorf, T., and Rathjen, F. G. (1995) Protein Profile 2, 963–108
32. Teixeira, A. M., Fawcett, J., Simonsen, L. D., and Watt, S. M. (1994) Blood 84, 211–219
33. Williamson, M. P. (1994) Biochem. J. 297, 249–260
34. Yu, H., Chen, J. K., Feng, S., Dalgarne, D. C., Brauer, A. W., and Schreiber, S. L. (1994) Cell 76, 933–945
35. Chao, D. C., Bedford, M. T., and Leder, P. (1996) EMBO J. 15, 1045–1054
36. Primakoff, P., and Myles, D. G. (1983) Dev. Biol. 88, 417–428
37. Westbrook-Carse, V. A., Winfrey, V. P., and Olsen, G. E. (1994) Mol. Reprod. Dev. 39, 309–321
38. Anakee, O. O., Sharma, S., Hardy, D. M., and Gerton, G. L. (1991) Mol. Reprod. Dev. 29, 172–179
39. Phelps, B. M., and Myles, D. G. (1987) Dev. Biol. 123, 63–72
40. Cowan, A. E., and Myles, D. G. (1993) Dev. Biol. 155, 124–133

What is the function of sperad in spermatozoa? A role for sperad in mature guinea pig spermatozoa is inferred, since it is specifically expressed only late in spermatogenesis and on mature sperm cells. This post-meiotic expression pattern is shared by many other sperm proteins involved in interaction of the mature spermatozoon with the reproductive system (38–40). In addition, the similarity to other cell adhesion proteins strongly suggests that sperad is involved in the binding of spermatozoon to another cell. Since sperad is lost during the acrosome reaction, a role in mediating adhesion at the egg plasma membrane is excluded. In addition to this interaction, spermatozoa interact with other reproductive tract components such as epithelial cells of the epididymis, uterus, and oviduct, as well as the cumulus oophorus and zona pellucida associated with the egg.