Zonadhesin is a mosaic protein in sperm membrane fractions that binds directly and in a species-specific manner to the extracellular matrix (zona pellucida) of the oocyte. The active form of pig zonadhesin from capacitated, epididymal spermatozoa comprises two covalently associated polypeptide chains of \( M_r \) 105,000 (p105) and \( M_r \) 45,000 (p45). Here we report detection and characterization of multiple zonadhesin isoforms in freshly ejaculated cells. Antibodies to the predicted von Willebrand factor (vWF) D0-D1, D1, and D3 domains of pig zonadhesin were distributed over the entire zona pellucida. Anion exchange chromatography resolved active, p105/p45 zonadhesin from the p60–90,000 inactive forms. Without disulfide bond reduction some zonadhesin was spontaneously formed multimers at \(-246 \text{ mV} E_h^{\text{in vitro}}\) in vitro. Double Cys → Ser mutants of the D1 domain formed multimers with the same apparent kinetics as the wild type protein. Zonadhesin localized to the apical head of pig spermatozoa. We conclude that a heterogeneous combination of specific proteolysis and intermolecular disulfide bond formation in the sperm head generates multiple forms of zonadhesin with differing avidities for the zona pellucida.

Adhesion of mammalian spermatozoa to the zona pellucida (ZP) is a complex process mediated by binding of sperm proteins to complementary ligands in the ZP (1, 2). The complexity of this process derives partly from cellular changes that occur during gamete interactions. Spermatozoa undergo physiological changes in the female reproductive tract that are required for fertilization and are collectively called capacitation (1, 3). Although the molecular basis of capacitation is only partly understood, in some if not all species avidity of sperm-ZP adhesion increases as capacitation progresses. After capacitation is completed, the membranes involved in initial adhesion events are lost from the sperm surface during the acrosome reaction, but adhesion is sustained by interaction of newly exposed structures with the ZP (1, 2). Unique adhesion molecule pairs likely function at different times during fertilization, and the activities of these molecules may change as fertilization progresses (2). It is therefore important to assess the biochemical and functional properties of sperm adhesion molecules at each stage in the fertilization process.

Several sperm proteins that may mediate adhesion to the ZP have been identified and characterized (2). Among these molecules zonadhesin is unique in its ability to bind directly and in a species-specific manner to native, particulate ZP (4, 5). Zonadhesin from pig (5), mouse (6), rabbit (7) and human (7) spermatozoa is a mosaic protein with a predicted Type I integral membrane topology. In each of these species, the large extracellular region of the protein comprises primarily three domain types (meprin/A5 antigen/mu receptor tyrosine phosphatase, mucin, and von Willebrand D (VWD)) that are present in other adhesion molecules (8–10). Although the domain structures of zonadhesin from these four mammals have been predicted from cDNA sequences, relatively little is known about the biochemical and functional properties of the proteins.

The active form of pig zonadhesin in membrane fractions of capacitated, epididymal spermatozoa is a two-chain molecule with disulfide-bonded \( M_r \) 105,000 and 45,000 polypeptides, both of which are derived from a predicted 2467-amino acid nascent precursor (4, 5). High \( M_f \) forms of zonadhesin have also been observed, suggesting the possible formation of covalent oligomers (4). This possibility was further implied by the presence in the pig zonadhesin D1, D2, and D3 domains of a conserved CG(L/V)/C motif (5) that is important for the oligomerization and proper function of von Willebrand factor (11) and for the oligomerization of porcine submaxillary mucin (12–14). These observations suggested that the protein at a minimum undergoes limited proteolysis and possibly also oligomerization as occurs in the functional maturation of vWF and other D-domain proteins (10). However, it is unclear when during sperm maturation such post-translational processing...
occurs or whether it is important for the ZP binding activity of zonadhesin.

Here we report that heterogeneous post-translational processing gives rise to multiple isoforms of pig zonadhesin in freshly ejaculated spermatozoa. Among these, only forms comprising the p105 and p45 polypeptides possess ZP binding activity, and the monomeric p105/p45 form binds more avidly than do higher order covalent oligomers. Furthermore, we find that zonadhesin binds uniformly to homologous ZP and localizes to the apical head of pig spermatozoa. These properties further support a function for zonadhesin in sperm adhesion to the extracellular matrix of the egg.

**EXPERIMENTAL PROCEDURES**

**Isolation of Sperm Membrane Fraction**—Boar spermatozoa in extended, freshly ejaculated semen were washed and immediately disrupted by N2 cavitation at 650 p.s.i. (15). Particulate fractions enriched in sperm plasma membranes were isolated from suspensions of disrupted cells by differential centrifugation (15) as for previous studies with cauda epididymal spermatozoa (4, 5). Solutions for sperm fractions were buffered at pH 7.5 and contained EDTA (1 mM) and dithiothreitol (1 mM) to prevent proteolysis by acidic pro tease, Ca2+ dependent metalloproteases, or serine proteases, respectively. In experiments with intact spermatozoa, solutions also contained 1 mM iodoacetamide to inhibit thiol proteases and to prevent thiol oxidation. Isolated membrane fractions in 20 mM NaHEPES, 130 mM NaCl, 1 mM EDTA, pH 7.5 (HNE), were stored at −70 °C.

**Isolation of Zona Pellucida**—Porcine ZP were isolated from ovary slices by stepwise sieving through screens (16) and then further purified by ultracentrifugation through Percoll (Amersham Pharmacia Biotech) gradients (4). Isolated ZP in HNE were stored at −70 °C.

**ZP Binding Assay**—Detergent-solubilized proteins from sperm membrane fractions were mixed with isolated ZP, and zonadhesin that bound directly to the particulate, native ZP was detected either by electroelution. The final hydrolysates contained a mixture of proteins with Ca2+-EGTA, pH 7.5 (HNE), were stored at 4 °C and activated Sepharose 4B. A protein assay of uncoupled protein confirmed protein expression was induced with 0.1 mM isopropylthiogalactoside at 37 °C for 2 h. After washing the bacteria with 10 mM NaPO4, 150 mM NaCl, pH 7.4 (PBS), soluble fusion proteins were sonicated by sonicating cell pellets in PBS containing 0.5 mM diisopropyl fluorophosphate, 1.0 mM EDTA, 10 mM E64, and 0.2% Triton X-100. Cell lysates were applied to a glutathione (GSH)-Sepharose column (15 ml of bed volume) equilibrated at 22 °C in PBS. Nonbinding proteins were washed through with PBS, and fusion proteins were eluted with 5 mM GSH in 50 mM Tris-HCl, pH 8.0. Eluted fusion proteins were present at concentrations of 5–8 mg/ml in the pooled, peak fractions, and with prior disulfide bond reduction mixed as single bands in SDS-PAGE (10% gels). Total yields of purified fusion protein were 40–45 mg/500 ml of culture media.

**Preparation of Domain-specific Antisera**—Four female New Zealand White rabbits were immunized (intramuscular) with 1 mg of purified fusion protein/animal (two with GST-D1 and two with GST-D3) emulsified in 0.5 ml of Freund's complete adjuvant (day 0). Booster injections consisted of 1 mg of purified fusion protein/animal emulsified in 0.5 ml of Freund's incomplete adjuvant (intramuscular) on day 42, and 1 mg of soluble protein/animal in PBS (subcutaneous) on days 49 and 70. Antisera were recovered from blood obtained by terminal exsanguinations on day 81.

**Preparation of GST, GST-D1, and GST-D3 Affinity Columns**—Purified GST (100 mg), GST-D1 (70 mg), and GST-D3 (70 mg) were dialyzed at 4 °C for >16 h in 0.1 M NaPO4, 0.5 mM NaCl, pH 8.3, to remove GSH and exchange into conjugation buffer. Diaxylized proteins were each coupled at 10 mg/ml to swelled gel to CNBr-activated Sepharose 4B (Amersham Pharmacia Biotech). After washing by suction on a glass filter to remove uncoupled proteins, the remaining activated groups were blocked with 1 mM ethanalamine, and the conjugated resins were washed with three cycles of alternating pH (0.1 M acetate, 0.5 mM NaCl, pH 4.0, and 0.2 M Tris-HCl, pH 9.3). The affinity matrices were poured into 1-cm-diameter glass columns, equilibrated in PBS containing 0.02% NaN3 and then stored at 4 °C.

**Affinity Purification of D1 and D3 Antibodies**—Antibodies to GST were removed by passing 20 ml of antiserum through a 10-ml bed volume GST-Sepharose column equilibrated at 22 °C in PBS. Antibodies to zonadhesin D1 or D3 domains were then affinity purified from their antiserum by chromatography on affinity-purified columns, respectively (7 ml of bed volume each, equilibrated in PBS at 22 °C). Elution of bound antibodies with 0.2 M sodium citrate, 0.15 mM NaCl, pH 3.0, was monitored continuously by A280. Peak fractions were pooled and immediately adjusted to pH 7 by addition of 1 M Tris (unbuffered). Antibodies to GST that were removed in the initial depletion steps were similarly eluted from GST-Sepharose and recovered for use as affinity-purified control antibodies. All purified antibodies were stored at −70 °C.

**Preparation of D3 Immunoaffinity Column**—20 mg of affinity-purified antibody to the D3 domain (11.4 mg from rabbit R128 and 8.6 mg from rabbit R129) were desalted into 0.1 M NaHCO3, 0.5 mM NaCl, pH 8.3 (coupling buffer) in two runs on four tandem 5-ml HiTrap desalting columns (Amersham Pharmacia Biotech). Desalted protein (15 mg in 8.8 ml) was coupled to 0.43 g (dry weight) of freshly swollen CNBr-activated Sepharose 4B. A protein assay of uncoupled protein confirmed that more than 95% of the antibody (>14.5 mg) was coupled to the affinity matrix (1.5 ml of packed volume), which after blocking and washing as for the fusion protein affinity matrices was equilibrated in PBS containing 0.02% NaN3 and stored at 4 °C.

**Affinity Purification of D1 and D3 Immunoaffinity Columns**—20 mg of affinity-purified antibody to the D1 domain (11.4 mg from rabbit R126 and 8.6 mg from rabbit R129) were desalted into 0.1 M NaHCO3, 0.5 mM NaCl, pH 8.3 (coupling buffer) in two runs on four tandem 5-ml HiTrap desalting columns (Amersham Pharmacia Biotech). Desalted protein (15 mg in 8.8 ml) was coupled to 0.43 g (dry weight) of freshly swollen CNBr-activated Sepharose 4B. A protein assay of uncoupled protein confirmed that more than 95% of the antibody (>14.5 mg) was coupled to the affinity matrix (1.5 ml of packed volume), which after blocking and washing as for the fusion protein affinity matrices was equilibrated in PBS containing 0.02% NaN3 and stored at 4 °C.
Site-directed Mutagenesis—Mutations in the pGEX-2T construct encoding GST-D3 were introduced with T4 polymerase-based GeneEditor (Promega Corp., Madison, WI), and those in the construct encoding GST-D1 were introduced with polymerase chain reaction-based Quick-Change (Stratagene Inc., La Jolla, CA) without modification of the primer for generating the double Cys adjuvant (intramuscular) on day 45, antisera were recovered from blood obtained by terminal exsanguinations on day 60.

RESULTS AND DISCUSSION

Zonadhesin from membrane fractions of capacitated, epididymal spermatozoa bound directly and with high affinity to intact ZP (Fig. 1). The bound zonadhesin comprised p105 and p45 polypeptides (Fig. 1a) as previously observed (4). Although earlier work established the species specificity of this interaction, the distribution of zonadhesin-binding sites in the pig ZP has not been characterized. We therefore visualized ZP-bound zonadhesin in situ by affinity microscopy (Fig. 1, b and c). Zonadhesin protein was detected on the entire ZP, indicating that its binding sites were not regionalized in the ZP structure. In addition, the relative evenness of the labeling suggested that the binding sites are intrinsic to the ZP and not associated with adherent materials from the cumulus cell matrix or other potential contaminants of the ZP preparation.

The locations of p105 and p45 tryptic peptides in the sequence of the pig zonadhesin precursor indicated that p45 comprises in part the D1 domain and that p105 comprises in part the D2 and D3 domains (ref. (5) and Fig. 2). To detect zonadhesin isoforms in spermatozoa and to characterize their polypeptide compositions, we prepared various domain-directed antisera and affinity-purified antibodies. The deduced sequence of the precursor specified numerous potentially antigenic regions, including segments located in approximately the same positions within the D1 and D3 domains that exhibited

[Image 308x479 to 555x729]

FIG. 1. Direct binding of zonadhesin to intact ZP. a, biotinylated polypeptides in a membrane fraction of capacitated, epididymal spermatozoa that bound to the pig ZP. CHAPS-solubilized sperm proteins were incubated with particulate ZP. The pig ZP with bound proteins were washed sequentially with CHAPS/HNE and mRIPA, and bound, biotinylated proteins were detected on Western blots (10% SDS-PAGE, disulfides reduced) by probing with horseradish peroxidase-streptavidin as described previously (4, 5). Note that the p105 and p45 major polypeptides of zonadhesin remained bound under these conditions. Peptides comprising a minor portion of the bound zonadhesin (4) migrated at the dye front (df). b, epifluorescence image of zonadhesin bound to the pig ZP. Bound, biotinylated zonadhesin on intact ZP from the experiment shown in a was detected in situ by probing with Texas Red-streptavidin. The labeled ZP were then viewed by fluorescence microscopy. Note that the zonadhesin-derived fluorescence is uniform, and associated with all regions of the ZP fragments. c, differential interference contrast image of the ZP shown in b.
Processing and Activity of Zonadhesin

Figure 2. Domain structure, polypeptide composition, and predicted characteristics of the pig zonadhesin precursor. The N-terminal domain (designated N) is composed of one partial and one full meprin/A5 antigen/mut receptor tyrosine phosphatase domain, whereas this region of mouse and human zonadhesins comprises three tandem meprin/A5 antigen/mut receptor tyrosine phosphatase domains. Vertical bars mark the locations in the precursor of tryptic peptides that were previously isolated from p45 and p105 and sequenced (5). Note that p45 must include much of the D1 domain and that p105 includes most or all of the D2 and D3 domains. Horizontal bars denote segments in the tandem VWD domains that were expressed as fusion proteins for production of antisera. Two potentially antigenic segments in the D1 and D3 domains (short horizontal bars) exhibited high predicted hydrophilicity, flexibility, and surface probability.

This purified zonadhesin preparation was used to raise antisera to the fully processed and disulfide-bonded holoprotein and to confirm the reactivity and specificity of antisera and affinity-purified antibodies (Fig. 3b).

Fig. 4 shows the reactivity of the four immunoreagents with zonadhesin isoforms on Western blots of membrane fractions from pig ejaculated spermatozoa. Antisera to the hydrolyzed D0-D1 protein recognized primarily p150 on blots of nonreduced proteins (Fig. 4a). In contrast to this relatively weak interaction with a single, disulfide-bonded form of zonadhesin, the D0-D1 antisera detected several polypeptides of disulfide-reduced zonadhesin, including the p105 and p45 components described previously, as well as an M₆ 300,000 protein (p₃₀₀) and at least two other polypeptides of intermediate size (designated p₆₀–90). The reaction of the D0-D1 antisera with p45 was consistent with the presence of p45 tryptic peptides in the D1 domain (Fig. 2). The reaction of these sera also with p105 indicated that the N terminus of p105 is likely upstream of Ser²²⁴ (the C terminus of the expressed D0-D1 fragment). The absence of the p₆₀–₉₀ polypeptides from the holoprotein purified by D3 immunoaffinity chromatography (compare with Fig. 3) indicates that these D0-D1-reactive polypeptides neither contain nor are covalently associated with a D3 polypeptide.

Like the D0-D1 antisera, the D1- and D3-specific antibodies also detected p₁₅₀ zonadhesin in nonreduced sperm proteins (Fig. 4a). However, in contrast to the complex pattern of disulfide-reduced proteins the D0-D1 antisera recognized, the D1 antibody recognized only p₄₅. Similarly, the D3 antibody bound primarily to p₁₀₅ and more weakly to an M₆ ₆₀,₀₀₀ polypeptide. Affinity-purified control antibodies to GST did not bind significantly to sperm proteins (not shown). The antiserum to the zonadhesin holoprotein detected p₁₅₀ in nonreduced sperm proteins but primarily recognized proteins with M₆ >₃₀₀,₀₀₀. Overall, the D0-D1 antisera reacted much more weakly with nonreduced forms of zonadhesin than it did with the reduced, constituent polypeptides. In contrast, the D1 and D3 antibodies bound similarly to both nonreduced and reduced zonadhesin, and the antiserum to the zonadhesin holoprotein reacted strongly with nonreduced zonadhesin (Figs. 3b and 4a) but did not recognize the protein’s separated, disulfide-reduced polypeptides. The differential binding of our antibodies to re-
Processing and Activity of Zonadhesin

FIG. 3. Production and characterization of zonadhesin antisera and antibodies. a, preparation and specificity of antisera and antibodies to recombinant fusion proteins. Shown are protein stains and Western blots of SDS-PAGE (10% gels, protein disulfides reduced) as indicated. Purified, recombinant zonadhesin fusion proteins (lanes labeled G10-D0D1-H6 (where “H6” indicates a His_6 tag), GST-D1, and GST-D3) each migrated primarily as single, Coomassie Blue-stained bands in overloaded gels. To generate the immunogen for production of antisera to the D0-D1 domains, the purified Gene 10 fusion protein spanning Pro683–Asp1191 (51,500 Da; middle band), the three predominant bands visible in the hydrolyzed preparation (asterisks) corresponded to Pro^{74}–Asp^{1191} (61,500 Da; top band), a mixture of Pro^{107}–Asp^{1107} and Pro^{807}–Asp^{1107} (42,500 and 42,300 Da, respectively; middle band), and Pro^{807}–Asp^{1107} (33,200 Da; bottom band). GST fusion proteins (lanes labeled GST-D1 and GST-D3) were used to prepare affinity-purified antibodies to segments spanning Ser^{923}–Met^{993} of the pig zonadhesin D1 domain and Ile^{1684}–Pro^{1780} of the D3 domain. Specificity of the antisera and antibodies was determined by Western blotting mixtures of the fusion proteins. Arrowheads mark the locations of the three fusion proteins in a mixture of 50 ng of each partially pure protein (lane labeled Silver stain). Note that the anti-D0-D1 antisera and the anti-D1 antibody readily recognized the G10-D0D1-H6 and GST-D1 proteins but not GST-D3 in mixtures of 10 ng of each protein (lanes overlined with Western blots). Similarly, the anti-D3 antibody recognized GST-D3 but not the other fusion protein. Note also that antisera to the purified, disulfide-bonded zonadhesin holoprotein did not recognize the disulfide-reduced fusion proteins under these conditions. All developed blots for a were exposed to film for 40 s. b, composition and immunoreactivity of processed zonadhesin holoprotein purified from membrane fractions of pig ejaculated spermatozoa. Shown are protein stains and Western blots of SDS-PAGE (4–12% linear gradient gels, protein disulfides either reduced (lanes labeled R) or not reduced (lanes labeled NR) as indicated). The different mobilities of the constituent polypeptides of the purified protein when separated without and with prior reduction of disulfide bonds (lanes overlined with Silver stains). Note also that the different antisera and antibodies each recognized a unique subset of the disulfide reduced and nonreduced polypeptides (lanes overlined with Western blots, containing 220 ng zonadhesin/lane). The developed anti-D0-D1, anti-D1, and anti-D3 blots of b were exposed to film for 40 s, whereas the anti-holoprotein blot was exposed for 5 s.

The cross-reaction of the antibodies to the GST-D1 and GST-D3 proteins with nonreduced zonadhesin extracted from spermatozoa, both on blots and in the isolation of the holoprotein by immunoaffinity chromatography, further suggested that the tertiary structures of these soluble fusion proteins are similar to that of the native protein.

Two-dimensional SDS-PAGE (first dimension, disulfides not reduced; second dimension, disulfides reduced) revealed that the p60–90 zonadhesin polypeptides migrated with the same mobility in each dimension (i.e. on the gel diagonal) and are therefore not covalently bound to other polypeptides. In contrast, p105 and p45 were components of M_r 150,000, 300,000, and ≈900,000 complexes stabilized by intermolecular disulfide bonds (Fig. 4b). The presence of both p105 and p45 in the M_r 150,000 nonreduced protein (p150) indicated that this zonadhesin form comprised primarily one each of the two polypeptides, consistent with both polypeptides being derived by proteolysis from a single precursor molecule. Similar compositions of the M_r 300,000 and ≈900,000 nonreduced proteins, in particular the presence of mostly p105 and p45 in a ratio similar to that of p150, suggested that these large complexes are covalent dimer and hexamer respectively of p150 (Fig. 4b). The relative amounts of the M_r 150,000, 300,000, and ≈900,000 zonadhesins did not change substantially when 1 mm iodoacetamide was included in membrane isolation buffers to inhibit po-
The differences in polypeptides recognized depending on whether protein antisera with zonadhesin separated on 4% SDS-PAGE and Western blotted, and zonadhesin forms were detected with antisera or affinity-purified antibodies. Proteins were separated by SDS-PAGE and Western blotted, and zonadhesin forms were detected with antisera or affinity-purified antibodies. a, reaction of D0-D1 antisera, D1 antibody, D3 antibody, and zonadhesin holoprotein antisera with zonadhesin separated on 4–12% linear gradient gels. Note the differences in polypeptides recognized depending on whether protein disulfides were reduced (lanes labeled N) or not reduced (lanes labeled NR), and the Mr >300,000 disulfide nonreduced protein (asterisk) detected by the D3 antibody and the holoprotein antisera. b, reaction of D0-D1 antisera with zonadhesin polypeptides separated by two-dimensional SDS-PAGE. Note the presence of p60–90 on the diagonal and the migration in the first dimension of p105 and p45 as Mr, 150,000, 300,000, and 900,000 complexes.

At high concentrations in storage, the purified D1 and D3 fusion proteins formed viscous gels that liquefied upon the addition of 10 mM DTT. This observation suggested that the fusion proteins, which each contained the CG(L/V)CG sequence motif, had spontaneously formed intermolecular disulfide bonds even though a mild reductant was present (the 5 mM GSH used to elute them from GSH-Sepharose). SDS-PAGE without prior reduction of disulfides revealed that covalent multimers were indeed present in stored preparations of both fusion proteins but not in identically stored GST (Fig. 7a). Including DTT in isolation buffers preserved the proteins in their monomeric forms (Fig. 7b). The addition of oxidized DTT at concentrations up to 100 mM, which raised the effective potentials in solution ($E'_{\text{eff}}$) at pH 7.4 as high as ~292 mV, did not induce formation of covalent multimers in vitro (not shown). However, the addition of 25 mM GSSG to produce a 246 mV redox buffer induced rapid formation of disulfide-bonded multimers of both proteins (D1 shown in Fig. 7b; D3 not shown). Most of the D1 fusion protein was converted to multimers within 30 min, and multimer formation continued until very little monomeric protein remained (Fig. 7b). To determine whether the vicinal cysteines in the CG(L/V)CG sequence motif...
were important for multimer formation, we compared the multimerization kinetics of the Cys → Ser mutants with those of the wild type proteins. Single mutants of the D1 protein (C933S and C936S) formed multimers at the same apparent rates as the wild type proteins (not shown). The double mutant of the D1 protein (C933S,C936S) also formed multimers at $E_h = -246\, \text{mV}$ with the same kinetics as the wild type protein (Fig. 7b).

Furthermore, the multimerization of the wild type and double mutant D1 fusion proteins were indistinguishable at various $E_h$ values ranging from $-269$ to $-246\, \text{mV}$ (Fig. 7c). Neither the wild type nor the mutant protein multimerized significantly at $-269\, \text{mV}$, but multimer formation was clearly evident when $E_h$ was raised to $-259\, \text{mV}$. Double mutation of the 1709CGVCG1713 motif in the D3 fusion protein to SGVSG also did not perturb multimer formation (not shown). Collectively, these results demonstrate that the expressed D1 and D3 fragments of pig zonadhesin spontaneously form intermolecular disulfide bonds and that the reaction is dependent on $E_h$ but not on the vicinal cysteines in the CG(L/V)CG sequence motif known to be important for vWF multimer formation. Our results show that cysteines other than those in the CG(L/V)CG motif can mediate spontaneous multimerization of purified zonadhesin fragments in vitro.
various zonadhesin forms suggests that the heterogeneous processing of this protein is functionally important, just as proteolytic activation and heterogeneous multimerization are important for the proper function of vWF (10). Unlike vWF multimers, however, zonadhesin multimers appear to bind less avidly than the monomer. This observation, together with our detection of potential differences in the way zonadhesin and vWF form multimers, indicates that VWD domains are versatile structures that share certain properties but nonetheless have unique functions in different proteins. Multimerization of zonadhesin could represent a mechanism for storing the protein in a latent form that can be activated when it is required for interaction with the ZP, or it could reflect an additional function of the protein as a scaffold or other structural element of the sperm head. Further studies will be required to determine whether the ZP binding activity of zonadhesin is dynamically regulated during fertilization, for example, as a component of sperm capacitation.

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Fig. 8. Light microscopic localization of zonadhesin in pig epididymal spermatozoa with the D0-D1 antisera. Shown is a phase contrast/epifluorescence (Texas Red) double exposure image of localization by immunofluorescence on methanol-fixed cells. Note the strong labeling on the apical heads of all cells.

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Heterogeneous Processing and Zona Pellucida Binding Activity of Pig Zonadhesin
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