Localized Surface Antigens of Guinea Pig Sperm Migrate to New Regions Prior to Fertilization

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ABSTRACT We have previously defined distinct localizations of antigens on the surface of the guinea pig sperm using monoclonal antibodies. In the present study we have demonstrated that these antigen localizations are dynamic and can be altered during changes in the functional state of the sperm. Before the sperm is capable of fertilizing the egg, it must undergo capacitation and an exocytic event, the acrosome reaction. Prior to capacitation, the antigen recognized by the monoclonal antibody, PT-1, was restricted to the posterior tail region (principal piece and end piece). After incubation in capacitating media at 37°C for 1 h, 100% of the sperm population showed migration of the PT-1 antigen onto the anterior tail. This redistribution of surface antigen resulted from a migration of the surface molecules originally present on the posterior tail. It did not occur in the presence of metabolic poisons or when tail-beating was prevented. It was temperature-dependent, and did not require exogenous Ca²⁺. Since the PT-1 antigen is freely diffusing on the posterior tail before migration, the mechanism of redistribution could involve the alteration of a presumptive membrane barrier.

In addition, we observed the redistribution of a second surface antigen after the acrosome reaction. The antigen recognized by the monoclonal antibody, PH-20, was localized exclusively in the posterior head region of acrosome-intact sperm. Within 7–10 min of induction of the acrosome reaction with Ca²⁺ and A23187, 90–100% of the acrosome-reacted sperm population no longer demonstrated binding of the PH-20 antibody on the posterior head, but showed binding instead on the inner acrosomal membrane. This redistribution of the PH-20 antigen also resulted from the migration of pre-existing surface molecules, but did not appear to require energy. The migration of PH-20 antigen was a selective process; other antigens localized to the posterior head region did not leave the posterior head after the acrosome reaction. These rearrangements of cell surface molecules may act to regulate cell surface function during fertilization.
of incubation with excess MAb in the medium. It was also found that $5 \times 10^6$ sperm bind 728 ng of PH-20 antibody. 36.4 μg of PH-20 antibody, a 50-fold excess, was added to a suspension of $5 \times 10^6$ sperm for the experiment of incubation with excess MAb in the medium.

**Acrosome reaction:** The acrosome reaction was induced by incubation of sperm for 1–3 h at 37°C in the capacitating medium Ca$^{2+}$ MCM or by the addition of 1 μg/ml A23187 to freshly obtained sperm suspended in Ca$^{2+}$ HEPES at 37°C. Sperm were scored as acrosome-intact or acrosome-reacted using phase-contrast optics.

**RESULTS**

Two different antigens that are localized in domains on the surface of guinea pig sperm migrate into new regions of the sperm surface during the steps that lead up to fertilization.

**Migration of the PT-1 Antigen**

The first example of a migrating antigen is a protein characterized as having a sedimentation coefficient of 6.8S on octylglucoside-containing sucrose density gradients (20). The 6.8S protein is recognized by the monoclonal antibody PT-1 and is limited to the surface of the posterior tail (principle piece and end piece) of sperm freshly taken from the cauda epididymal sperm were collected from mature Hartley guinea pigs purchased from Buck-berg Lab Animals, Tomkins Cove, NY.

**Materials:**

Monoclonal antibodies (MAbs) were generated by a fusion between spleen cells from immunized C57 BI/6 female mice and P3-NS1/Ag 4-1 myeloma cells as described (10, 15). Antibody subclasses were determined by Ouchterlony assays: PT-1 is an IgG2b and PH-20 belongs to the subclass IgG1. The second antibodies used were fluorescein isothiocyanate (FITC)-F(ab')2 or FITC-Fab goat anti-mouse IgG from Cappel Laboratories, Cochranville, PA. Erythro-9-2-hydroxy-3-nonyl)adenine (EHNA) was purchased from Biomol Research Laboratories, Inc., Plymouth Meeting, PA. Research Triangle Park, NC. Cauda epididymal sperm were collected from mature Hartley guinea pigs purchased from Buck-berg Lab Animals, Tomkins Cove, NY.

**Media:**

The capacitating medium used was minimum culture medium (MCM) that contained 102.3 mM NaCl, 25.1 mM NaHCO$_3$, 0.25 mM sodium pyruvate, and 21.7 mM sodium lactate. The MCM also contained either 1.7 mM CaCl$_2$ (Ca$^{2+}$ MCM) or 1.0 mM MgCl$_2$ (Mg$^{2+}$ MCM) (16). In some cases 1.0 mM MgCl$_2$ was added to the MCM to increase sperm survival (17). Other media used were Mg$^{2+}$ HEPES (140 mM NaCl, 4 mM KCl, 4 mM HEPES, pH 7.4, 10 mM glucose, 2 mM MgCl$_2$ and 0.1 mM EGTA) and Ca$^{2+}$ HEPES (Mg$^{2+}$ HEPES, with 2 mM CaCl$_2$ instead of MgCl$_2$ and EGTA) (18).

**Immunofluorescence:** All immunofluorescent staining was carried out on live sperm or sperm fixed in 1.5% formaldehyde (made fresh). Fixed cells were washed through 5 ml heat-inactivated horse serum. Incubations in each of the MCM-based capacitating media tested: Mg$^{2+}$ MCM + 1.0 mM KCl (Mg$^{2+}$ K + MCM) or 1.0 mM MgCl$_2$ (Mg$^{2+}$ MCM) (16). In some cases 1.0 mM MgCl$_2$ was added to the MCM to increase sperm survival (17). Other details have been described elsewhere (15). Patterns were observed on live swimming sperm. For photography, live-stained sperm swimming was stopped by the addition of a drop of formaldehyde. Cells were photographed on a Zeiss Universal Microscope equipped with epi-fluorescent optics using Kodak Tri-X film.

**Incubations:**

To test for the migration of the PT-1 antigen, we incubated 5 × 10$^6$ sperm (unwashed or after one wash in 0.9% NaCl) in 0.1 ml of the appropriate media in a temperature-controlled water bath or in a Lab-Line Orbit Environ-Shaker 18 (Lab-Line Instruments, Inc., Melrose Park, IL), rotating at 60 rpm. To test for migration of the PH-20 antigen, we incubated sperm at concentrations ranging from 4 × 10$^5$ to 10$^6$/ml under conditions leading to the acrosome reaction (compare below).

To test whether the PT-1 or PH-20 MAb dissociate and reassociate during the incubations, we incubated cells labeled with the MAb and a goat Fab-FITC anti-mouse IgG in the presence of excess MAb in the medium. The amount of excess MAb added to the medium and the amount of MAb bound to the cells were determined. The concentrations in culture supernatant of PT-1 (50 μg/ml) and PH-20 (150 μg/ml) were measured by single radial immunodiffusion as described by Springer (19) using goat anti-mouse Fab$_2$ (Cappel Laboratories) as the anti-mouse antibody reagent. The amount of PT-1 and PH-20 antibodies bound to 5 × 10$^6$ sperm was estimated by determining (in a solid phase assay) how much antibody remained in dilutions of culture supernatant after absorption with 5 × 10$^6$ sperm. By this method, it was found that 5 × 10$^6$ sperm bind 232 ng of PT-1 antibody. 23 μg of PT-1 antibody, a 100-fold excess, was added to a suspension of 5 × 10$^6$ sperm for the experiment.

**Abbreviations used in this paper:** CCCP, carbonyl cyanide m-chlorophenylhydrazone; EHNA, erythro-9(2-hydroxy-3-nonyl)adenine; FITC, fluorescein isothiocyanate; IAM, inner acrosomal membrane; MAb, monoclonal antibody; MCM, minimum culture medium; PVP, polyvinylpyrrolidone.

**REFERENCES**

FIGURE 1. Binding pattern of PT-1 MAb on non-incubated sperm and sperm incubated in Mg²⁺-MCM. Arrows indicate the junction between the posterior tail and the anterior tail. (A) Antibody binding pattern of PT-1 MAb on fresh cauda epididymal sperm demonstrated by indirect immunofluorescence. Staining is restricted to the posterior tail. (B) Phase-contrast micrograph of the same sperm. (C and D) PT-1 MAb binding after 60-min incubation at 37°C. Sperm are stained by indirect immunofluorescence after fixation in 1.5% formaldehyde. PT-1 antigen has now moved onto the anterior tail region. (E and F) PT-1 MAb binding after 90-min incubation at 37°C. PT-1 antigen has accumulated on the anterior tail region, but the posterior tail region is still stained. Bars, 5 μm. × 900.

However, was observed on the anterior tail of all the sperm. On the basis of these experiments, we concluded that the PT-1 antigen actually migrates onto the anterior tail.

The PT-1 antigen migration was temperature-dependent. After 30 min of incubation at 37°C, over 50% of sperm
showed binding of PT-1 to the anterior tail region (Fig. 2). After 1 h, 95% of the sperm demonstrated migration of the antigen onto the anterior tail region. In the same experiment, the timing of migration was slowed by lowering the incubation temperature to 33°C, and, at 25°C, no antigen migration was detected even after 2 h of incubation (Fig. 2).

PT-1 antigen migration is also an energy-requiring process. When cells were incubated at 37°C in MCM in the presence of metabolic poisons, carbonyl cyanide m-chlorophenylhydrazone (CCCP) or 2,4-dinitrophenol, the PT-1 antigen showed little or no migration into the anterior tail domain (Fig. 3). Since treatment with these compounds resulted in the cessation of sperm motility, it is possible that sperm motility itself is required for antigen migration. To test this possibility we used other conditions to prevent sperm motility. In the first set of experiments, we used EHNA to inhibit the dynein ATPase necessary for flagellar beating (21, 22). When EHNA was added to a suspension of guinea pig sperm, swimming was blocked. As long as EHNA was present, and swimming had stopped, the PT-1 antigen did not migrate onto the anterior tail. If EHNA was added at 30 min, when a proportion of the cells already showed antigen migration onto the anterior tail, migration of the antigen on the surface of the remainder of the cells was prevented (Fig. 4). The effect of EHNA was reversible. If sperm were washed out of EHNA they would resume swimming and the antigen would migrate onto the anterior tail.

Although EHNA is a useful inhibitor for preventing sperm motility, it may affect other cell enzymes. Therefore, we used an alternative method for immobilizing sperm, by suspending them in a medium of high enough viscosity to physically

![Figure 2](https://example.com/figure2.png)

**Figure 2** Temperature-dependence of PT-1 antigen migration. Sperm from one animal were incubated in Mg²⁺-MCM + 1 mM KCl. Percentage of cells showing binding of PT-1 antibody to the anterior tail region after incubation at 37°C (△), 33°C (○), or 25°C (■). After incubation, cells were fixed and stained by indirect immunofluorescence and 50 sperm were scored for the region of fluorescence for each time point. All cells showing fluorescence on the anterior tail are scored as migrated.

![Figure 3](https://example.com/figure3.png)

**Figure 3** Inhibition of PT-1 antigen migration by metabolic poisons. Percentage of cells showing binding of PT-1 antibody to the anterior tail region after incubation in Mg²⁺-MCM + 1 mM KCl in the presence of 1 mM CCCP (○), or 10 mM 2,4-dinitrophenol (DNP) (■), and in both cases 1% dimethyl sulfoxide. Migration was assayed as in Fig. 2. The (no additions) control containing 1% dimethylsulfoxide is an average of two different experiments. (△) Migration of the PT-1 antigen was not affected by the presence of 1% dimethyl sulfoxide.

![Figure 4](https://example.com/figure4.png)

**Figure 4** Inhibition of PT-1 antigen migration by EHNA. Percentage of cells showing binding of PT-1 MAb to the anterior tail during incubation in Mg²⁺-MCM + 1 mM KCl in the presence of 4 mM EHNA (△) compared to a control with no additions (○). EHNA is added to the incubation medium 30 min after putting the cells at 37°C. At 30 min, ~20% of the cells showed PT-1 antigen migration onto the anterior tail; after the addition of EHNA, that percentage remains the same. Migration was assayed as described in Fig. 2. In this experiment the migration of PT-1 antigen in the control was slower than usual (cf. Figs. 2, 3, and 5), representing an observed variability of the kinetics of migration in different sperm samples.
prevent tail-beating. Sperm suspended in 10% polyvinylpyrrolidone 360 (PVP-360) did not swim and the PT-1 antigen did not migrate. As with the EHNA, this effect was reversible; when cells were washed out of PVP-360, both sperm swimming and antigen migration resumed (Fig. 5). Sperm were held in the nonmotile condition in PVP-360 for at least 1.5 h without affecting the subsequent migration of the PT-1 antigen (Fig. 5).

Migration of the PH-20 Antigen

As shown in Fig. 7, the PH-20 monoclonal antibody binds to the posterior head surface of live sperm freshly removed from the cauda epididymis (Fig. 7, A and B). Immunoprecipitation with the PH-20 MAb from detergent extracts of 125I surface-labeled sperm yields three labeled bands with M, 66,000, 48,000, and 41,000 (10). The migration of the PH-20 antigen immediately follows the acrosome reaction. During the acrosome reaction there is a fusion, shown in Fig. 6, of the outer region of acrosomal membrane with the overlying region of the plasma membrane. The most proximal fusion results in a confluency between the acrosomal membrane and the plasma membrane. Distal to this confluence, hybrid vesicles of acrosomal and plasma membrane are released from the sperm along with the acrosomal contents. Therefore acrosome-reacted sperm are surrounded by a cell membrane that is a hybrid between plasma membrane and the remaining acrosomal membrane, composed primarily of the inner region of the acrosomal membrane (Fig. 6).

After the acrosome reaction, the PH-20 antigen migrated from the posterior head region onto the IAM (Fig. 7, A, B, E, and F). The migration to the IAM occurred regardless of whether the acrosome reaction was induced by capacitation in MCM or by the addition of A23187 to Ca2+ HEPES medium without prior capacitation. The kinetics of migration were followed after induction of the acrosome reaction with A23187. In the results shown in Fig. 8, 98% of the sperm were acrosome-reacted within 7 min of the addition of A23187; by 10 min, 100% of the acrosome-reacted sperm showed binding of PH-20 restricted to the IAM. In repeated experiments with this protocol, the kinetics sometimes showed that as early as 7 min, 100% of the acrosome-reacted sperm had PH-20 MAb bound only to the IAM. During the migration, intermediate stages can be detected on the cells, with antigen present on both the posterior head and IAM, as shown in Fig. 8.

The same two experiments, establishing that the PT-1 antigen actually migrates, were done to show that the PH-20 antigen also migrates. If PH-20 MAb was bound to sperm before the acrosome reaction, the PH-20 antigen moved, with the MAb attached, to the IAM on all the cells. If sperm, with both PH-20 MAb and FITC-Fab goat anti-mouse Igs bound to them, were incubated in medium that contained a 50-fold excess of free PH-20 MAb, and then allowed to acrosome react, fluorescence was seen on the IAM of 100% of the sperm. Thus, we concluded that the PH-20 antigen migrates to the IAM.

Other antigens present on the surface of the posterior head did not migrate to the IAM after the acrosome reaction. Antigens that remained on the posterior head of acrosome-reacted sperm included posterior head antigens recognized by the monoclonal antibodies PH-1, PH-10, and PH-30. The binding pattern of PH-10 is shown before (Fig. 7, C and D) and after (Fig. 7, G and H) the acrosome reaction.

Unlike the migration of the PT-1 antigen, PH-20 antigen...
FIGURE 7 Antibody binding patterns of the PH-20 and PH-10 MAbs on acrosome-intact and acrosome-reacted cells. (A) PH-20 indirect immunofluorescence and (B) phase contrast of acrosome-intact sperm, live staining. Bar, 5 μm. ×1,600. (C and D) PH-10 immunofluorescence on acrosome-intact sperm, live staining. Both antibodies bind exclusively to the posterior head. Bar, 5 μm. ×1,600. (E and F) PH-20 immunofluorescence on acrosome-reacted sperm, capacitated in Ca²⁺-MCM, live staining. Bar, 5 μm. ×1,700. (G and H) PH-10 immunofluorescence on acrosome-reacted sperm, capacitated in Ca²⁺-MCM, live staining. Bar, 5 μm. ×1,600.

**DISCUSSION**

Membranes are a dynamic assembly of proteins and lipids that are able to respond to developmental and environmental signals to modify specific cellular functions. One response that they exhibit is a change of membrane topography or the redistribution of cell surface molecules into or out of specific domains. In some cases, surface molecules are redistributed by the actual migration of surface antigens in the plane of the membrane. Lateral migrations may be stimulated by ligand binding or occur in the absence of ligands. Ligand-stimulated migrations are evident in capping phenomena (23) and also include the movement of concanavalin A receptors to the...
cleavage furrow of cells (24) and the movement of several types of cell surface receptors (e.g., receptors for epidermal growth factor and pseudomonas toxin) into coated pits during endocytosis (25). Lateral migrations may also occur in the absence of ligand binding. These migrations are more difficult to distinguish experimentally from other models of molecular redistribution. A lateral migration of a surface component in the absence of bound ligand appears to occur during the clustering of acetylcholine receptors on cultured myotubes (26). Migrations of cell surface molecules into specific domains also apparently occurs during flagellar regeneration of Euglena (11) and during sea urchin zygote cleavage (9). It has also been suggested that lateral migrations are responsible for the segregation of surface components during erythrocyte differentiation (6), the spontaneous clustering of low density lipoprotein receptors into coated pits (1) and the accumulation of wheat germ agglutinin receptors into sites of cell-cell contact (2).

The redistribution of both PT-1 and PH-20 antigens on the sperm surface resulted from a lateral migration of pre-existing surface molecules in the absence of added ligands. However, different mechanisms may have been responsible for the migration of the PT-1 antigen in contrast to the PH-20 antigen. Before migration, the PT-1 antigen was freely diffusing in the posterior tail region, even though it did not leave this surface domain. The diffusion coefficient of the PT-1 antigen ($2.5 \times 10^{-9}$ cm$^2$/s), measured by fluorescence redistribution after photobleaching (20), is near the theoretical limit of a freely diffusing protein in a lipid bilayer. The mechanism that restricts the PT-1 antigen initially to the posterior tail region is not known. In this study, we have shown that PT-1 antigen migration is temperature-dependent, occurs in the absence of exogenous Ca$^{2+}$, requires energy, and is prevented when sperm swimming is prevented. Several models that would explain these results can be envisioned. Since the PT-1 antigen is already freely diffusing on the posterior tail, it may be a barrier in the membrane that initially prevents the migration of the antigen onto the anterior tail region. The antigen may be translocated across this barrier by an energy-requiring process. Alternatively, energy may be used to disrupt the barrier or modify the antigen so as to allow the PT-1 antigen to passively diffuse into the anterior tail region. In this case, accumulation of the PT-1 antigen in the new region could be explained by trapping of the antigen.

No migration of the PT-1 antigen occurred when sperm tail-beating was prevented by incubation with the drug EHNA or by keeping the sperm in a highly viscous solution of PVP-360. Prevention of antigen migration by preventing tail-beating could indicate that tail-beating is somehow involved in providing a force for PT-1 antigen relocation. Alternatively, tail-beating may in some unforeseen way alter a presumptive membrane barrier or maintain some cellular metabolic balance that allows migration to occur.

In contrast to PT-1 antigen migration, PH-20 antigen migration will occur in the presence of CCCP and 2-deoxyglucose to block ATP synthesis. We cannot exclude the possibility that residual ATP is used for the migration. However, as with the relocation of lectin receptors in regions of cell-cell contact (2), the migration of PH-20 antigen onto the IAM does not appear to require cellular energy. Redistribution of the PH-20 antigen in the presence of metabolic inhibitors is consistent with models involving diffusion and trapping (2, 4) or thermodynamic partitioning.

The movement of PH-20 antigen did not result from a mass flow of posterior head membrane components since it is a selective process. Only the PH-20 antigen was shown to move onto the IAM; other antigens remained behind in the posterior head region.

The migrations of the surface antigens PT-1 and PH-20 are each coincident with a change in the functional state of the sperm. Various surface changes have been noted during in vitro capacitation of mammalian sperm (13, 27). In particular, the tail of guinea pig sperm demonstrates changes in intramembrane particle distribution (28-30) and in lectin agglutinability (31). Migration of the PT-1 antigen into a region where it was not previously detected means that the antigen is potentially entering a new environment. Contact between the PT-1 antigen and anterior tail molecules, not present in the posterior tail, could result in the activation of new functions. A precedent for such a model exists in the well-studied activation of the catalytic unit of adenylate cyclase by contact with a receptor protein resulting from lateral mobility of membrane molecules (32). This type of enzyme activation (or a similar type of inhibition) could occur when the PT-1 antigen gains access to the anterior tail region. Alternatively, the migration may simply result in the concentration of a membrane protein in a new surface region where its activity could have a different effect on cellular function.

The migration of the PH-20 antigen alters the composition of both the IAM and the posterior head plasma membrane and occurs immediately after the acrosome reaction. It has been reported that acrosome reaction is required for guinea pig sperm to bind to the zona pellucida (14) and acrosome reaction is known in numerous mammalian species to be required for sperm-egg plasma membrane fusion (14). PH-20 antigen migration might function in one or both of these steps in fertilization. On the IAM, the migrated PH-20 antigen might have a role in zona binding. On the posterior head surface, a fusion activity could be activated after migration, if the PH-20 antigen acts in its initial region as an inhibitor.

Control of specific membrane activities through migration of pre-existing cell surface molecules into or out of cell surface domains may be important in the regulation of cell surface function. These rearrangements have the potential to regulate cell surface function in response to external stimuli as well as in response to an internal program during differentiation.

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