Redistribution of Mouse Sperm Surface Galactosyltransferase after the Acrosome Reaction

Linda C. Lopez and Barry D. Shur

Department of Biochemistry and Molecular Biology, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Abstract. Gamete recognition in the mouse is mediated by galactosyltransferase (GalTase) on the sperm surface, which binds to its appropriate glycoside substrate in the egg zona pellucida (Lopez, L. C., E. M. Bayna, D. Litoff, N. L. Shaper, J. H. Shaper, and B. D. Shur, 1985, *J. Cell Biol.*, 101:1501–1510). GalTase has been localized by indirect immunofluorescence to the dorsal surface of the anterior sperm head overlying the intact acrosome. Sperm binding to the zona pellucida triggers induction of the acrosome reaction, an exocytotic event that results in vesiculation and release of the outer acrosomal and overlying plasma membranes. Consequently, we examined the fate of sperm surface GalTase after the acrosome reaction. Contrary to our expectations, surface GalTase is not lost during the acrosome reaction despite the loss of its membrane domain. Rather, double-label indirect immunofluorescence assays show that GalTase is redistributed to the lateral surface of the sperm, coincident with the acrosome reaction. This apparent redistribution of GalTase was confirmed by direct enzymatic assays, which show that 90% of sperm GalTase activity is retained during the acrosome reaction. No GalTase activity is detectable on plasma membrane vesicles released during the acrosome reaction. In contrast, removal of plasma membranes by nitrogen cavitation releases GalTase activity from the sperm surface, showing that GalTase redistribution requires a physiological acrosome reaction. The selective redistribution of GalTase to a new membrane domain from one that is lost during the acrosome reaction suggests that GalTase is repositioned for some additional function after initial sperm–zona binding.

In a number of species, gamete recognition is mediated by interactions between sperm surface receptors and their complementary ligands on the extracellular coat of the egg. In many systems, the sperm surface receptors have been shown to be carbohydrate-binding proteins that recognize specific oligosaccharide residues on the egg's surface (for review, see Shur, 1987; Shapiro et al., 1981; Monroy and Rosati, 1983).

The egg receptor on the mouse sperm surface has been identified as galactosyltransferase (GalTase),[1] which binds noncatalytically to its glycoside substrate in the zona pellucida (Shur and Hall, 1982*a*, *b*; Lopez et al., 1985). Mouse sperm are unable to bind the zona pellucida before capacitation, due in part to the presence of competitive GalTase substrates occupying the active site of the sperm surface enzyme. During capacitation, these competitive substrates are shed from the sperm surface, exposing the GalTase active site and enabling the enzyme to bind its complementary substrate in the zona pellucida. The nature of the zona pellucida substrate is under investigation, and a likely candidate for this substrate is a glycoprotein of 83 kD whose glycoside chains serve as receptors during sperm–zona binding (Florman and Wassarman, 1985).

In the mouse, only acrosome-intact sperm are able to initiate binding with the zona pellucida (Saling and Storey, 1979; Florman and Storey, 1982; Bleil and Wassarman, 1983). However, this does not exclude the likely possibility that some early aspects of the acrosome reaction occur in the cumulus matrix (Cummins and Yanagimachi, 1986). Results suggest that the selective binding of acrosome-intact sperm to the zona pellucida is due to the fact that GalTase is localized exclusively to a small plasma membrane domain on the dorsal surface of the sperm head overlying the intact acrosome (Lopez et al., 1985). When the acrosome reaction is triggered by binding to the zona pellucida, this plasma membrane domain is shed from the sperm along with the outer acrosomal membrane, releasing the contents of the acrosome and exposing the inner acrosomal membrane. Initially, it seemed obvious that GalTase must also be lost from the sperm surface during the acrosome reaction, coincident with the loss of its plasma membrane domain. In this study, we have examined the fate of sperm surface GalTase after the acrosome reaction.

By the use of double-label indirect immunofluorescence,

1. *Abbreviations used in this paper*: GalTase, galactosyltransferase; medium B, 130 mM NaCl, 5 mM KCl, 20 mM Na Hepes, pH 7.2; PIC, protease inhibitor cocktail.

© The Rockefeller University Press, 0021-9525/87/10/1663/8 $2.00

the distribution of GalTase was determined on individual sperm whose acrosomal status could be defined by an appropriate monoclonal antibody. Contrary to our expectations, GalTase is not lost during the acrosome reaction, but rather is redistributed to a new domain on the lateral sperm surface. This finding was confirmed by direct enzymatic assays, which show that acrosome-reacted sperm have similar levels of GalTase activity to acrosome-intact sperm. GalTase activity is not detectable on the plasma membrane vesicles released during the acrosome reaction. Furthermore, GalTase redistribution requires a physiological acrosome reaction, since removal of the plasma membrane by a physical means, nitrogen cavitation, releases GalTase from the sperm surface.

The selective redistribution of GalTase out of a plasma membrane domain that is lost during the acrosome reaction suggests that GalTase is salvaged for some additional function subsequent to initial sperm binding to the zona pellucida. It is obvious that some mechanism must stabilize the binding of acrosome-reacted sperm to the zona surface during the initial stages of zona penetration. The redistribution of GalTase to the lateral sperm surface after the acrosome reaction suggests that it may facilitate sperm–zona binding after the acrosome reaction, as well as before.

Materials and Methods

Induction of the Acrosome Reaction

Sperm from the caudae epididymides of 10-wk-old CD-1 mice (Charles River Breeders, Wilmington, MA) were dispersed in a modified Krebs-Ringer bicarbonate solution containing sodium pyruvate (1 mM), sodium lactate (25 mM), glucose (5.56 mM), and BSA (20 mg/ml). Particulate material was removed by filtration through Nitex cloth, and the sperm were capacitated for 30 min as described previously (Lopez et al., 1985). After capacitation, the NaCl concentration was increased from 120 to 180 mM. The sperm were incubated an additional 30 min at 37°C, and the acrosome reaction induced by the addition of either the calcium ionophore A23187 (10 μM final) (Sigma Chemical Co., St. Louis, MO) (Florman and Storey, 1982) or neutralized, acid-solubilized zonae-pellucidae (2 zonae/μl final) (Bleil and Wassarman, 1983) for 1 h at 37°C. This procedure resulted in 29–58% acrosome-reacted sperm above background (no ionophore or no zonae added) as determined by indirect immunofluorescence. In some experiments, the acrosome reaction was induced in the presence of a protease inhibitor cocktail (PIC) which includes antipain (2 μg/ml), aprotinin (0.1%), benzamidine (10 μg/ml), chymostatin (1 μg/ml), leupeptin (1 μg/ml), and pepstatin (1 μg/ml). The extent of the acrosome reaction was unaffected by the PIC. The degree of "spontaneous" acrosome reactions was determined by indirect immunofluorescence in aliquots of sperm after dissection and after capacitation and varied ~15%. To directly compare the percent of sperm undergoing the acrosome reaction with the immunofluorescence distribution of GalTase and with the level of GalTase enzymatic activity, a common sperm suspension, either acrosome reacted or control, was used for these assays.

Evaluation of Acrosomal Status and GalTase Distribution by Indirect Immunofluorescence

Correlations of acrosomal status and the distribution of sperm surface GalTase were determined by double-label indirect immunofluorescence. GalTase distribution was defined with a monospecific rabbit anti–GalTase IgG as described previously (Lopez et al., 1985). The anti–GalTase IgG can be shown to recognize GalTase on the mouse sperm surface by indirect immunofluorescence, inhibition of enzyme activity on whole sperm, and immunoprecipitation of detergent-solubilized enzyme activity. Acrosomal status was assayed with the mouse monoclonal antibody HS21, kindly supplied by Dr. Don Wolf which reacts only with acrosome-intact sperm (Wolf et al., 1985). Loss of anti–HS21 reactivity is indicative of acrosome-reacted sperm.

Acrosome-reacted and control sperm (no ionophore or zonae treatment) were pelleted by centrifugation (1,000 *g*, 10 min), washed three times by centrifugation in medium B (130 mM NaCl, 5 mM KCl, 20 mM Na Hepes, pH 7.2), and resuspended in 0.4 ml medium B. To form wells, 7-mm holes were punched into Mylar plastic sheets (Dynatech Laboratories, Inc., Alexandria, VA) and applied to precleaned glass microscope slides. All of the following procedures were conducted at room temperature in a humidified chamber. 20 μl of sperm were added to the wells and allowed to dry. To prevent nonspecific antibody binding, 10 μl of solution A (1% BSA, 0.05% NaN_3 in medium B) were added to the wells. After 10 min, 10 μl of rabbit anti–GalTase IgG/HS21 monoclonal antibody (9:1) or preimmune rabbit IgG/control hybridoma supernatant (9:1) were added to a final concentration of 0.45 mg/ml for the rabbit IgGs. After a 45-min incubation, the sperm were washed twice with solution A and 10 μl of biotinylated goat anti–rabbit IgG (Vector Laboratories, Inc., Burlingame, CA)/rhodamine-conjugated goat anti-mouse IgG (Cappel Laboratories, Inc., Cochranville, PA) (both second antibodies at 7.5 μg/ml in solution A) were added and incubated for 30 min. The sperm were washed three times with solution A, and 10 μl of fluorescein-conjugated DCS-avidin (20 μg/ml in solution A) (Cappel Laboratories, Inc.) were added. After a 20-min incubation, the slides were washed with solution A, mounted, and viewed with epi-illumination using a Leitz Dialux EB22 fluorescence microscope equipped with an H_2 (Leitz) filter cube (390–490 excitation) and GG455 (Leitz) barrier filter for fluorescein and with an N2.1 (Leitz) filter cube (515–560 excitation) for rhodamine. Sperm with a uniform rhodamine fluorescence, specific for HS21 antibody, over the acrosomal cap region were scored as acrosome intact (see Fig. 1). Sperm lacking this fluorescence pattern were considered acrosome reacted. At least 100 sperm in a minimum of four fields were scored for each sample.

GalTase Enzymatic Activity on Acrosome-reacted Sperm

The surface GalTase enzymatic activity of acrosome-reacted and control sperm suspensions was assayed as described below. In some experiments, the sperm were pelleted after the acrosome reaction and washed with medium B plus PIC as described above, and the supernatant and pellet washes were retained for isolation of released membrane vesicles. The supernatant reaction media and washes were pooled and centrifuged at 100,000 *g* (4°C, 1 h). The membrane pellets were resuspended in 0.4 ml medium B. The sperm and their corresponding membrane preparations were then assayed for GalTase enzymatic activity.

Effect of Nitrogen Cavitation on Sperm GalTase Distribution

Cauda epididymal sperm (10^8 cells) were isolated as described above and resuspended in 3 ml medium B containing PIC. The plasma membranes were released from sperm by nitrogen cavitation according to the procedure of Peterson et al. (1980) and Mack et al. (1986). 1.5 ml of the sperm suspension were placed in a Parr bomb and subjected to nitrogen pressure of 800 psi for 10 min. The sperm were extruded and placed on ice. Control sperm (1.5 ml) were retained on ice during the same time period. 1 ml of both the cavitate and control sperm samples was centrifuged at 1,000 *g* (4°C, 10 min), the sperm pellet retained and the supernatant recentrifuged as before. The resulting supernatant, examined by light microscopy and found to be free of intact sperm, was centrifuged at 100,000 *g* (4°C, 1 h) to pellet membrane vesicles.

Integrity of the plasma membrane over the sperm acrosomal cap region was assessed by double-label indirect immunofluorescence as described above.

GalTase enzymatic activities in the sperm pellet, the released membranes collected by high-speed centrifugation, and the total unfractionated cavitates and controls were assayed as described below.

GalTase Enzymatic Activity

Sperm surface GalTase was assayed under optimal enzymatic conditions as previously described (Shur and Hall, 1982*a*, *b*). Briefly, GalTase incubations contained 200 μM UDP-^{3}H-Gal (0.2 Ci/mmole) (New England Nuclear, Boston, MA), 30 mM *N*-acetylglucosamine, 10 mM $MnCl_2$, and sperm (or membrane) in a total volume of 300 μl medium B with or without PIC. (The presence of PIC had no effect on GalTase enzymatic activity.) At 30-min intervals, 50-μl aliquots were removed and the reaction terminated by the addition of 10 μl ice-cold 0.2 M EDTA, pH 7.2. 50 μl of this mixture were applied to the origins of chromatography paper (3 MM; Whatman, Inc., Clifton, NJ) and subjected to high voltage electrophoresis (3,000 V,

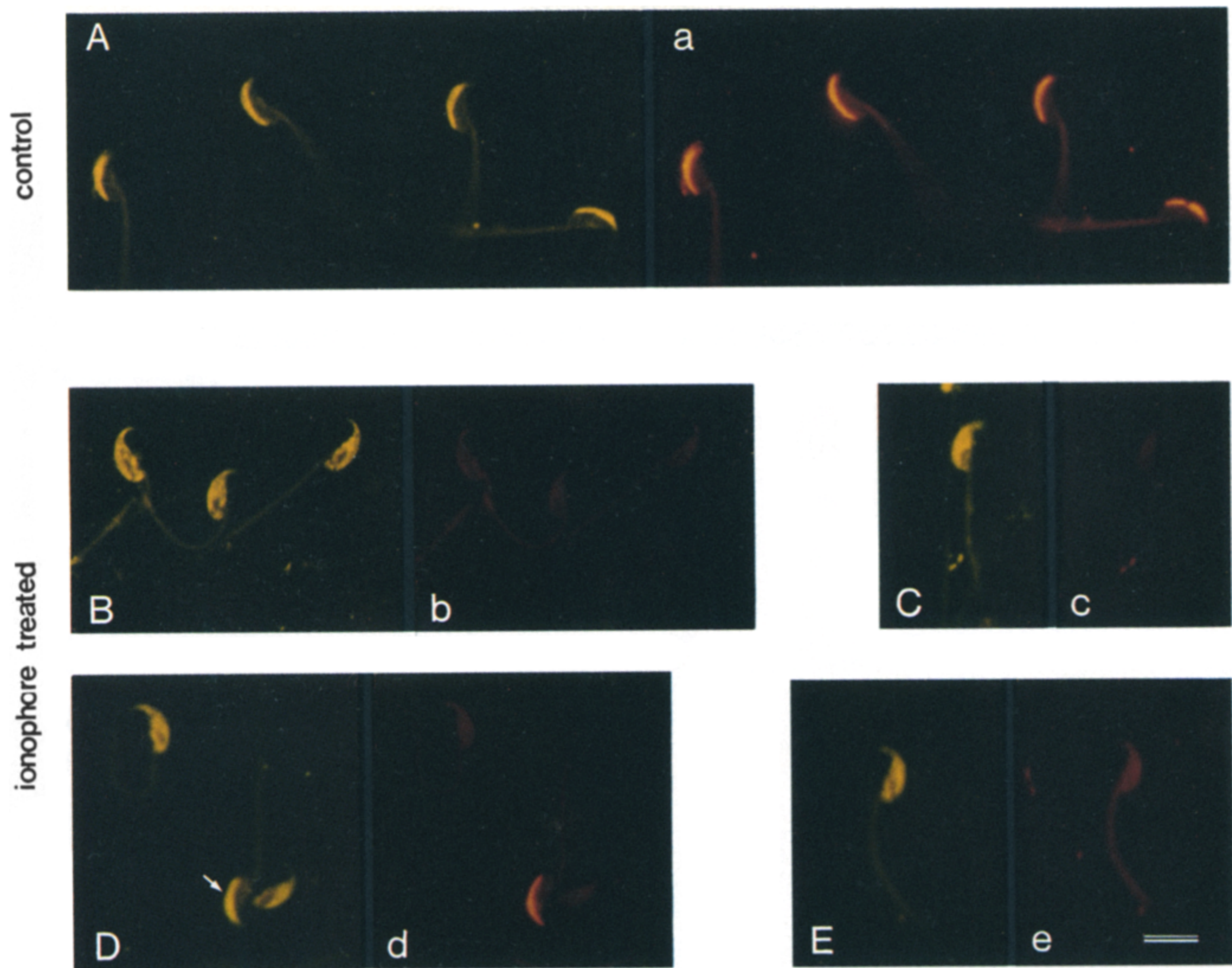

Figure 1. Fluorescence photomicrographs illustrating the redistribution of sperm surface GalTase after the acrosome reaction. The acrosome reaction was induced with ionophore in capacitated sperm as described in Materials and Methods. The sperm were washed, applied to glass slides, and examined by double-label indirect immunofluorescence to monitor the acrosome reaction and distribution of GalTase. The localization of GalTase is shown in *A*, *B*, *C*, *D*, and *E*, while the presence of the acrosomal marker is shown in *a*, *b*, *c*, *d*, and *e*. Before the acrosome reaction, GalTase is localized to a discrete plasma membrane patch on the dorsal surface of the anterior head. After the acrosome reaction, as defined by the loss of HS21 reactivity, GalTase is redistributed to the lateral sperm surface. In *D*, the arrow points to a sperm whose acrosome remained intact. Bar, 10 μm.

275 mA, 45 min) in borate buffer. Under these conditions the ^{3}H-galactosylated product, *N*-acetyllactosamine, remains at the origin, while unused UDP-^{3}H-Gal and its breakdown products migrate away. The dried origins were removed and the incorporated radioactivity determined in a liquid scintillation counter (model 5800; Packard Instrument Co., Inc., Downers Grove, IL). Under these conditions, sperm GalTase activity is linear with time, proportional to enzyme concentration, and saturating for all substrates and cofactors (Shur and Bennett, 1979).

Results

We have shown previously that GalTase is localized to a discrete portion of the sperm plasma membrane overlying the intact acrosome (Lopez et al., 1985). Since the plasma membrane and outer acrosomal membranes are shed by vesiculation during the acrosome reaction, we examined the fate of GalTase after the acrosome reaction.

Distribution of Sperm Surface GalTase after the Acrosome Reaction

The acrosome reaction was induced in capacitated sperm by the addition of ionophore, A23187 (10 μM), or by the addition of solubilized zonae pellucidae (2 zonae/μl). Sperm incubated in parallel, but without the addition of ionophore or zonae, were used to control for the background level of acrosome reaction. After a 1-h incubation, the sperm were washed three times and subjected to double-label indirect immunofluorescence. GalTase was localized using a monospecific rabbit anti-GalTase IgG that was identified with biotinylated goat anti-rabbit IgG and fluoresceinated avidin. The status of the acrosome reaction was monitored with the mouse monoclonal antibody HS21, which labels the acrosome cap and overlying plasma membrane of acrosome-

intact sperm (Wolf et al., 1985). HS21 binding was identified by rhodamine-conjugated goat anti-mouse IgG. This approach allowed us to correlate the distribution of the sperm surface GalTase with the acrosomal status of individual sperm by virtue of the presence or absence of the HS21 antigen.

On acrosome-intact sperm, surface GalTase and HS21 antigen colocalize exclusively to a discrete patch on the dorsal surface of the anterior head overlying the acrosome (Fig. 1, *A* and *a*). However, on acrosome-reacted sperm, as assessed by the loss of HS21 reactivity, the sperm surface GalTase now appears to be redistributed to the lateral sperm surface (Fig. 1, *B* and *b*; *C* and *c*; *D* and *d*; *E* and *e*). Both ionophore and solubilized zonae pellucidae induced similar GalTase redistributions. A higher resolution photomicrograph of acrosome-reacted sperm, induced by solubilized zonae pellucidae, shows that the redistributed GalTase is usually confined to the anterior half of the lateral sperm head (Fig. 2), although a narrow band of immunoreactivity is occasionally seen along the extreme posterior sperm head (as in Fig. 1). The relative surface area possessing GalTase immunoreactivity was calculated using ocular micrometers. As shown in Table I, GalTase on acrosome-reacted sperm was distributed to four times the surface area that originally possessed GalTase activity on acrosome-intact sperm.

The correlation between the acrosome reaction and the redistribution of GalTase is shown in Table I. 99% of HS21-positive, acrosome-intact sperm had GalTase localized over the intact acrosome as described previously (Lopez et al. 1985) and as shown in Fig. 1, *A* and *a*. 97% of the sperm tha showed GalTase redistribution to the lateral surface, as in Fig. 1, had completely lost the HS21 antigen. The remaining 3% showed an irregular pattern of HS21 reactivity.

Effect of the Acrosome Reaction on Sperm Surface GalTase Activity

The indirect immunofluorescence assays suggest that GalTase redistributes during the acrosome reaction to the lateral portion of the sperm head. As an independent approach to confirm the apparent redistribution of GalTase after the acrosome reaction, we compared the level of GalTase activity on acrosome-intact and acrosome-reacted sperm. The acrosome reaction was induced by ionophore in capacitated sperm as described above, after which they were washed three times by centrifugation (1,000 *g*) to leave any released membrane vesicles in the supernatant. Sperm-associated GalTase activity was determined, and the extent of the ionophore-induced acrosome reaction quantitated by indirect immunofluorescence using HS21 antibody.

Fig. 3 is the result of three different experiments in which ionophore induced an average of 42% acrosome reactions above background (ionophore treated, 71%; control, 29%), but sperm-associated GalTase activity remained 90% of control. Consequently, sperm have similar levels of GalTase activity before and after the acrosome reaction, despite the

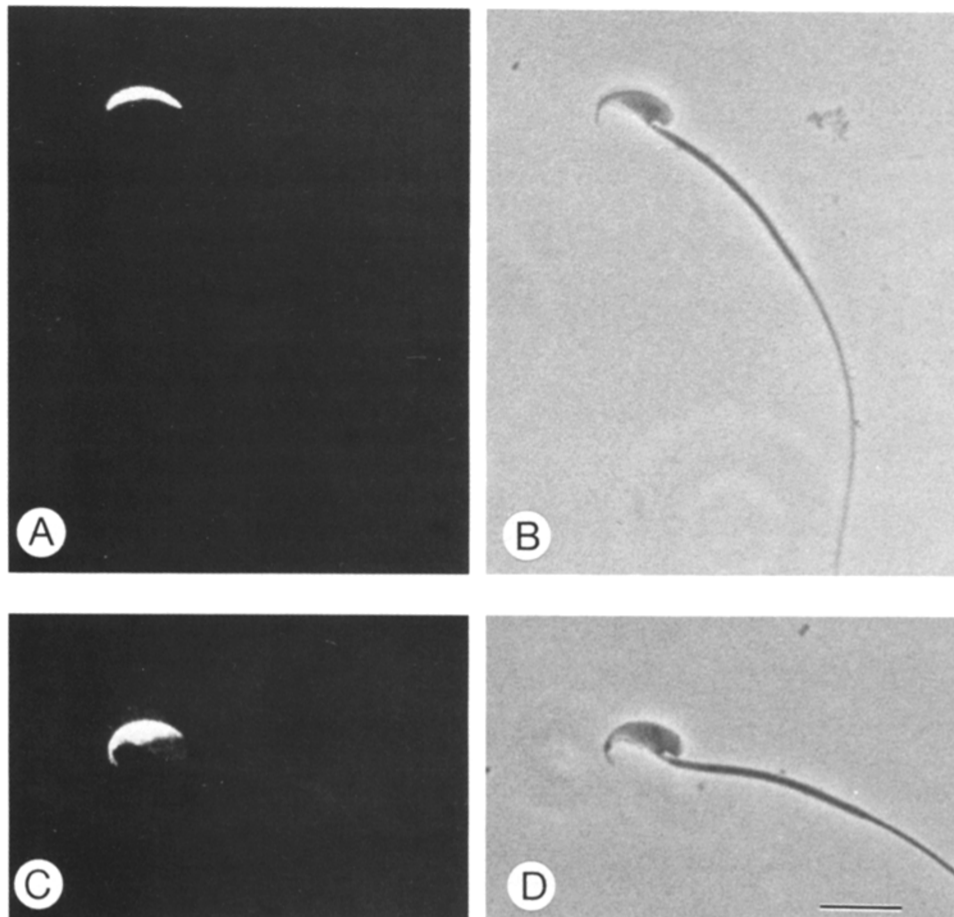

Figure 2. Higher resolution fluorescence photomicrograph of the GalTase distribution on zonae-induced, acrosome-reacted sperm. GalTase is localized to the dorsal aspect of the anterior sperm head on acrosome-intact sperm (*A* and *B*), and is redistributed after the acrosome reaction to the anterior half of the lateral sperm head, possibly reflecting the equatorial segment (*C* and *D*). Bar, 10 μm.

Table I. Redistribution of Sperm Surface GalTase after the Acrosome Reaction

Sperm	GalTase distribution	Acrosome status*	GalTase activity‡	GalTase area§	GalTase activity/ μm²
			pmoles/10⁶ sperm·h	*μm²*	*pmoles/10⁶ sperm·h*
Control	Dorsal	99% intact	23.9 (100)¶	9.27	2.58 (100)
Ionophore treated	Lateral	97% reacted‖	21.5 (90)	37.1	0.580 (22)

Sperm were isolated, washed, treated with ionophore, washed, and assayed for GalTase activity as described in Materials and Methods.
* Acrosomal status was evaluated with the monoclonal antibody, HS21, as described in Materials and Methods. 100 sperm were scored for each group.
‡ Data taken from Fig. 3.
§ Surface area was calculated from the fluorescent fields with ocular micrometers as described in Scully et al., 1987.
‖ The remaining 3% of sperm with redistributed GalTase had an irregular pattern of HS21 reactivity.
¶ Numbers in parentheses indicate the percent of control values.

differences in the enzyme's topographical distribution. Within these three experiments, there was no correlation between the extent of the acrosome reaction, and the residual sperm-associated GalTase activity, and the error bars (Fig. 3) suggest that the 10% loss of GalTase activity is not significant. As shown in Table I, when the GalTase activity per cell is normalized to the relevant surface area possessing GalTase immunoreactivity, the GalTase concentration, per μm^2, decreases by 78% after the acrosome reaction.

Distribution of GalTase Activity between Acrosome-reacted Sperm and Released Membrane Vesicles

Even though the level of sperm GalTase activity is similar before and after the acrosome reaction, we determined whether the GalTase activity present on intact sperm was released with the plasma membrane during the acrosome reaction. This experiment addresses the possibility that a second, similarly active population of GalTase, previously cryptic (e.g., intra-acrosomal) on intact sperm, was exposed after the acrosome reaction. This possibility was considered unlikely for two reasons. First, the level of enzyme activity associated with the redistributed GalTase on acrosome-reacted sperm is similar to the level of GalTase activity present on intact sperm (Fig. 3). Second, we had previously shown that the GalTase distribution is identical on both living, acrosome-intact sperm and on sperm whose plasma membranes were permeabilized by fixation with paraformaldehyde or methanol (Lopez et al., 1985).

The acrosome reaction was induced in capacitated sperm as described above with ionophore, the sperm pelleted by centrifugation and the released membrane vesicles collected from the supernatant at 100,000 *g* (4°C, 1 h). GalTase activity associated with the acrosome-reacted sperm and with the released membrane vesicles was assayed as described above.

Fig. 4 is a representative time course of GalTase activities in the released membrane vesicles and sperm pellet of acrosome-reacted and control (no ionophore) samples. In both the control and acrosome-reacted samples, the distribution of GalTase activity between the sperm pellet (83% of the total activity) and supernatant (17%) was identical, even though the acrosome reaction was induced in ~50% of the treated sperm compared with 19–20% reacted in controls. While the level of GalTase activity released into the supernatant varied between experiments, the ratio of released to sperm-associated activity remained constant between the control and acrosome-reacted samples. Controls show that the presence of ionophore A23187 did not affect GalTase activity (data not shown).

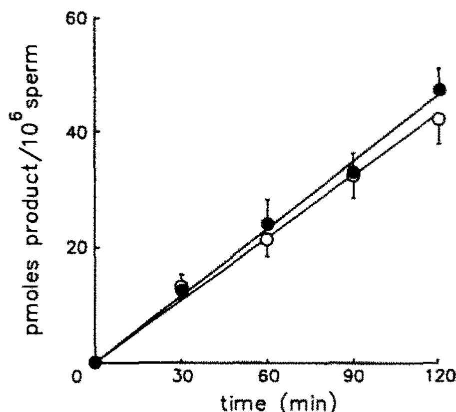

Figure 3. Sperm have similar levels of GalTase activity before and after the acrosome reaction. The acrosome reactions were induced in capacitated sperm, washed, and assayed for residual GalTase activity as described in Materials and Methods. Control sperm (acrosome intact) were incubated in parallel but did not receive ionophore. Acrosome-reacted sperm populations (○) have 90% the activity of acrosome-intact, control sperm populations (●), despite the fact that acrosome reactions were induced from 29% in controls to 71% in ionophore-treated sperm, as judged by the loss of HS21 reactivity. Data are the average of three experiments.

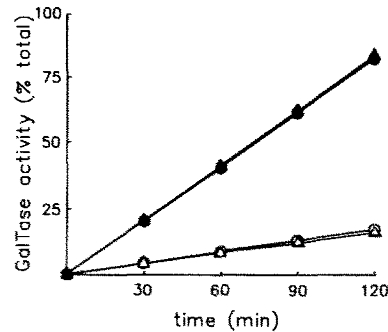

Figure 4. GalTase activity remains associated with acrosome-reacted sperm and is not found on released plasma membrane vesicles. The acrosome reaction was induced in capacitated sperm, which were collected by centrifugation. The released membrane vesicles were collected from the supernatant by high speed centrifugation and GalTase activity remaining with the sperm (*solid symbols*) was compared with that associated with the membrane vesicles (*open symbols*). Controls were incubated in parallel but did not receive ionophore. GalTase activity was consistently found associated with the acrosome-reacted sperm. GalTase activity released into the supernatant was equal in control and ionophore-treated sperm, irrelevant of the extent of acrosome reactions induced above background levels. The experiment was conducted three times; a representative assay is shown. Data are presented as a percentage of the total enzymatic activity associated with either control (●, ○) or ionophore-treated (▲, △) sperm.

Effect of Nitrogen Cavitation on Sperm Surface GalTase Distribution

The above results suggest that sperm surface GalTase redistributes after the acrosome reaction to the lateral surface of the sperm head. The redistributed GalTase does not represent a new population of enzyme activity that is uncovered upon loss of the plasma membrane, but appears to be the same population which has become redistributed during the course of the acrosome reaction. To determine whether this redistribution requires a physiological acrosome reaction, we examined the distribution of GalTase after the plasma membranes were removed physically by nitrogen cavitation.

Cauda epididymal sperm were washed as described in Materials and Methods, and subjected to nitrogen pressures of 800 psi for 10 min as described (Peterson et al., 1980; Mack et al., 1986). The extruded and control sperm (i.e., without cavitation) were centrifuged at 1,000 *g* and the supernatants collected. The sperm pellets and supernatants containing released membranes were examined by light microscopy to ensure that the sperm heads remained in the pellet and that the supernatants were devoid of sperm. The control and cavitate sperm and supernatants were assayed for GalTase activity as described.

In the control, 94% of the GalTase activity remained associated with the sperm, with 6% of the activity released into the supernatant fraction (Fig. 5). On the other hand, only 22% of the GalTase activity remained associated with the cavitated sperm; 78% of the activity had been released into the supernatant fraction (Fig. 5). Indirect immunofluorescence using monospecific anti-GalTase IgG and HS21 IgG showed that ~55% of the sperm had completely lost the GalTase and HS21 antigens. The remaining sperm had membranes with an irregular, ragged appearance (as judged by indirect immunofluorescence), which presumably accounts for some additional loss of GalTase activity below the limits of resolution of indirect immunofluorescence.

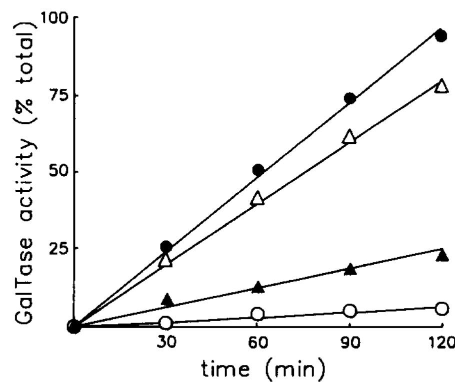

Figure 5. Sperm GalTase activity is released from the surface after physically removing the membranes by nitrogen cavitation. Sperm were washed and subjected to 800 psi of nitrogen in a Parr bomb as described in Materials and Methods. The extruded sperm were pelleted by low speed centrifugation, and the released membranes isolated by high speed centrifugation. Sperm-associated (*solid symbols*) and membrane-associated (*open symbols*) GalTase activities were determined. 78% of the sperm GalTase was released from the sperm surface and could be recovered in the membrane-containing supernatant after nitrogen cavitation (▲, △). Control sperm had released only 6% of their GalTase activity into the supernatant during the same incubation period (●, ○). Data are presented as a percentage of the total enzymatic activity associated with either cavitated or control sperm preparations.

Discussion

In the mouse, acrosome-intact sperm bind to the egg zona pellucida, which induces the acrosome reaction (Saling and Storey, 1979; Florman and Storey, 1982; Bleil and Wassarman, 1983). Past studies have localized the sperm's receptor for the zona pellucida, i.e., GalTase, to a discrete portion of the plasma membrane overlying the intact acrosome, thus explaining the specific binding of acrosome-intact sperm to the zona pellucida (Shur and Hall, 1982*a*, *b*; Lopez et al., 1985). Since this plasma membrane domain is lost as a result of the acrosome reaction, it was expected that the GalTase would be lost as well. Results presented in this paper show this is not the case. Rather, the GalTase is redistributed to the lateral portion of the sperm head, coincident with the acrosome reaction. This redistribution was determined by double-label indirect immunofluorescence, as well as by direct enzyme assay, which showed that sperm had similar levels (i.e., 90%) of GalTase activity before and after the acrosome reaction despite the enzyme's altered topography. No GalTase activity could be recovered on the hybrid membrane vesicles released from sperm during the acrosome reaction; virtually all of the original activity was retained on the sperm. However, physically removing the membranes by nitrogen cavitation did release GalTase from the sperm surface, showing that a physiological acrosome reaction is necessary for GalTase redistribution.

The techniques used in our present studies are not able to define to which membrane compartment the GalTase is being redistributed. In the mouse, only a small piece of plasma membrane is lost from the dorsal sperm surface after the acrosome reaction. Most of the plasma membrane remains on the lateral surface of the sperm head, in association with the underlying outer and inner acrosomal membranes, forming a broad equatorial segment (Saling et al., 1985). After the acrosome reaction, GalTase redistributes predominantly to the anterior, lateral sperm head surface, which may therefore correspond to the equatorial segment.

During the course of this study, we found the plasma membrane overlying the acrosome to be very labile. When sperm were sequentially incubated at 4°C and then at 37°C, they were characterized by a fragmented, ragged distribution of GalTase, often seen as sheets of immunofluorescence extending from the sperm surface (data not shown). This suggests that the integrity of the sperm plasma membrane overlying the acrosome is easily compromised by drastic temperature shifts, and consequently, we avoided exposing sperm to such temperature shifts. However, these observations can account for findings from other workers that show that the sperm's ability to bind to the zona pellucida has a half-life of 15 min at 4°C (Heffner and Storey, 1982).

The redistribution of sperm surface antigens during capacitation and the acrosome reaction has been well-documented in the guinea pig (Myles and Primakoff, 1984), and more recently in the boar (Saxena et al., 1986). Myles, Primakoff, and their co-workers were the first to show that sperm surface antigens, defined by monoclonal antibodies, were confined to plasma membrane domains, and that some of these anti-

gens redistribute to new positions during capacitation and the acrosome reaction (Primakoff and Myles, 1983). Recent studies suggest that one of these guinea pig antigens, PH-20, participates during sperm binding to the zona pellucida (Primakoff et al., 1985). Interestingly, PH-20 redistributes after the acrosome reaction, similar to results reported here for the mouse sperm receptor, GalTase (Myles and Primakoff, 1984). While the patterns of redistribution differ between these two gamete sperm receptors, which may simply reflect species-specific differences in the mechanism of sperm–zona pellucida binding, both receptors demonstrate the principle of acrosome reaction–induced redistribution to new membrane domains.

The redistribution of mouse sperm GalTase after the acrosome reaction is the second observed redistribution that this enzyme undergoes during the life cycle of the male germ cell. We have shown previously that during spermatogenesis the surface GalTase redistributes from an initially diffuse and uniform distribution on spermatogonia and primary spermatocytes to a restricted plasma membrane domain overlying the intact acrosome characteristic of mature sperm (Scully et al., 1987). During spermatogenesis, the GalTase activity per cell remains constant despite the drastic reduction in cell surface area that accompanies sperm differentiation. When normalized to the relevant cell surface area, the GalTase is concentrated 77-fold from primary spermatocytes to cauda epididymal sperm. The redistribution of GalTase during spermatogenesis coincides with the appearance of filamentous actin, suggesting that the cytoskeleton may participate in the redistribution of GalTase during spermatogenesis. Whether the cytoskeleton also participates in the redistribution of GalTase during the acrosome reaction is unknown, but this possibility would explain why an integral membrane glycoprotein is maintained on the sperm cell surface despite the loss of its original plasma membrane domain. It is equally plausible that diffusion barriers maintain the GalTase in its plasma membrane domain overlying the intact acrosome (Myles et al., 1984). The acrosome reaction may break down this putative diffusion barrier, allowing the enzyme to diffuse rapidly along the lateral portions of the sperm head. This possibility would explain why 10% of the GalTase activity appears to be lost after the acrosome reaction, if a similar percentage of plasma membrane surface area was released by vesiculation.

The most interesting issue, however, is why the sperm would salvage its receptor for the zona pellucida, despite the loss of its original plasma membrane domain. This implies that GalTase serves some additional function subsequent to initial sperm–zona binding. At least three possibilities seem obvious.

Sperm initially bind loosely to the zona pellucida, and after the acrosome reaction, the sperm associates tightly to the zona via its lateral surface (Katz et al., 1986), which is where GalTase redistributes to during the acrosome reaction. Since the acrosome-reacted sperm must be retained on the zona surface to initiate penetration, some mechanism must exist to stabilize binding between the acrosome-reacted sperm and the zona pellucida. Since the location of GalTase seems to correlate with the sperm surface mediating attachment to the zona, GalTase may facilitate sperm–zona adhesion both before and after the acrosome reaction. This then raises the question as to why acrosome-reacted sperm do not initially bind to the zona pellucida if GalTase is present on their lateral surfaces. Since the concentration of GalTase, per μm^2, decreases about fivefold from the dorsal to the lateral sperm surfaces after the acrosome reaction, it may generate insufficient affinity to initiate binding between an actively motile sperm and the zona, but may be sufficient to maintain adhesions after initial binding has occurred. Another potential function for the redistributed GalTase is that it may participate during penetration of the zona pellucida by interacting with zona glycoproteins along the path of sperm migration.

After sperm penetrate the zona pellucida, they bind to and fuse with the egg plasma membrane on their lateral surfaces, presumably via the equatorial segment (for review, see Shapiro et al., 1981). Since GalTase has been repositioned to the lateral sperm surface after the acrosome reaction, another possible function for the redistributed GalTase is to mediate sperm attachment to the egg plasma membrane. Experiments are in progress to address these possibilities.

The authors are grateful to Dr. Don Wolf (The University of Texas Health Science Center, Houston, TX) for making HS21 monoclonal antibody available; to Ms. Carol Neely, in our laboratory, for preparing monospecific anti-GalTase IgG; to Dr. Joel H. Shaper (The Johns Hopkins University School of Medicine, Baltimore, MD) for samples of monospecific anti-GalTase IgG for some initial immunofluorescence studies; to Drs. Diana Myles and Paul Primakoff (The University of Connecticut Health Center, Farmington, CT) for fruitful discussions regarding these experiments; and to Ms. Ellen Madson for typing this manuscript.

This work was supported by a grant (PCM 84-00817) from the National Science Foundation to B. D. Shur. L. C. Lopez was a recipient of a National Research Service Award (F32 HD06517) from the National Institutes of Health during these studies.

Received for publication 20 March 1987, and in revised form 18 June 1987.

References

Bleil, J. D., and P. M. Wassarman. 1983. Sperm-egg interactions in the mouse: sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein. *Dev. Biol.* 95:317–324.

Cummins, J. M., and R. Yanagimachi. 1986. Development of ability to penetrate the cumulus oopholus by hamster spermatozoa capacitated in vitro, in relation to the timing of the acrosome reaction. *Gamete Res.* 15:187–212.

Florman, H. M., and B. T. Storey. 1982. Mouse gamete interactions: the zona pellucida is the site of the acrosome reaction leading to fertilization *in vitro*. *Dev. Biol.* 91:121–130.

Florman, H. M., and P. M. Wassarman. 1985. O-linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity. *Cell.* 41:313–324.

Heffner, L. J., and B. T. Storey. 1982. Cold lability of mouse sperm binding to the zona pellucida. *J. Exp. Zool.* 219:155–161.

Katz, D. F., G. N. Cherr, and H. Lambert. 1986. The evolution of hamster sperm motility during capacitation and interaction with the ovum vestments in vitro. *Gamete Res.* 14:333–346.

Lopez, L. C., E. M. Bayna, D. Litoff, N. L. Shaper, J. H. Shaper, and B. D. Shur. 1985. Receptor function of mouse sperm surface galactosyltransferase during fertilization. *J. Cell Biol.* 101:1501–1510.

Mack, S. R., J. Everingham, and L. J. D. Zaneveld. 1986. Isolation and partial characterization of the plasma membrane from human spermatozoa. *J. Exp. Zool.* 240:127–136.

Monroy, A., and F. Rosati. 1983. Review article: a comparative analysis of sperm-egg interaction. *Gamete Res.* 7:85–102.

Myles, D. G., and P. Primakoff. 1984. Localized surface antigens of guinea pig sperm migrate to new regions prior to fertilization. *J. Cell Biol.* 99:1631–1641.

Myles, D. G., P. Primakoff, and D. E. Koppel. 1984. A localized surface protein of guinea pig sperm exhibits free diffusion in its domain. *J. Cell Biol.* 98:1905–1909.

Peterson, R., L. Russell, D. Burdman, and M. Freund. 1980. Evaluation of the purity of boar sperm plasma membranes prepared by nitrogen cavitation. *Biol. Reprod.* 23:637–645.

Primakoff, P., and D. G. Myles. 1983. A map of the guinea pig sperm surface constructed with monoclonal antibodies. *Dev. Biol.* 98:417–428.

Primakoff, P., H. Hyatt, and D. G. Myles. 1985. A role for the migrating sperm

surface antigen PH-20 in guinea pig sperm binding to the egg zona pellucida. *J. Cell Biol.* 101:2239-2244.

Saling, P. M., and B. T. Storey. 1979. Mouse gamete interactions during fertilization in vitro. Chlortetracycline as a fluorescent probe for the mouse sperm acrosome reaction. *J. Cell Biol.* 83:544-555.

Saling, P. M., G. Irons, and R. Waibel. 1985. Mouse sperm antigens that participate in fertilization. I. Inhibition of sperm fusion with the egg plasma membrane using monoclonal antibodies. *Biol. Reprod.* 33:515-526.

Saxena, N. K., L. D. Russell, N. Saxena, and R. N. Peterson. 1986. Immunofluorescence antigen localization on boar sperm plasma membranes: monoclonal antibodies reveal apparent new domains and apparent redistribution of surface antigens during sperm maturation and at ejaculation. *Anat. Rec.* 214:238-252.

Scully, N. F., J. H. Shaper, and B. D. Shur. 1987. Spatial and temporal expression of cell surface galactosyltransferase during mouse spermatogenesis and epididymal maturation. *Dev. Biol.* In press.

Shapiro, B. M., R. W. Schackmann, and C. A. Gabel. 1981. Molecular approaches to the study of fertilization. *Annu. Rev. Biochem.* 50:815-843.

Shur, B. D. 1987. Galactosyltransferase as a recognition molecule during fertilization and development. *In* The Molecular Biology of Fertilization. H. Schatten and G. Schatten, editors. Academic Press, Inc., New York. In press.

Shur, B. D., and D. Bennett. 1979. A specific defect in galactosyltransferase regulation on sperm bearing mutant alleles of the T/t-locus. *Dev. Biol.* 71: 243-259.

Shur, B. D., and N. G. Hall. 1982*a*. Sperm surface galactosyltransferase activities during in vitro capacitation. *J. Cell Biol.* 95:567-573.

Shur, B. D., and N. G. Hall. 1982*b*. A role for mouse sperm surface galactosyltransferase in sperm binding to the egg zona pellucida. *J. Cell Biol.* 95: 574-579.

Wolf, D. P., J. Boldt, W. Byrd, and K. B. Bechtol. 1985. Acrosomal status evaluation in human ejaculated sperm with monoclonal antibodies. *Biol. Reprod.* 32:1157-1162.