

UC Davis

UC Davis Previously Published Works

Title

Characterization of a monoclonal antibody that induces the acrosome reaction of sea urchin sperm.

Permalink

<https://escholarship.org/uc/item/37c933hm>

Journal

Journal of Cell Biology, 105(3)

ISSN

0021-9525

Authors

Trimmer, JS

Ebina, Y

Schackmann, RW

et al.

Publication Date

1987-09-01

DOI

10.1083/jcb.105.3.1121

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial-ShareAlike License, available at <https://creativecommons.org/licenses/by-nc-sa/4.0/>

Peer reviewed

Characterization of a Monoclonal Antibody That Induces the Acrosome Reaction of Sea Urchin Sperm

James S. Trimmer,* Yukiko Ebina,* Robert W. Schackmann,‡ Carl-Georg Meinhof,* and Victor D. Vacquier*

*Marine Biology Research Division A-002, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093; and ‡Department of Biology, University of Utah, Salt Lake City, Utah 84112

Abstract. A monoclonal antibody, J18/29, induces the acrosome reaction (AR) in spermatozoa of the sea urchin *Strongylocentrotus purpuratus*. J18/29 induces increases in both intracellular Ca^{2+} and intracellular pH similar to those occurring upon induction of the AR by the natural inducer, the fucose sulfate-rich glycoconjugate of egg jelly. Lowering the Ca^{2+} concentration or the pH of the seawater inhibits the J18/29-induced AR, as does treatment with Co^{2+} , an inhibitor of Ca^{2+} channels. The J18/29-induced AR is also inhibited by verapamil, tetraethylammonium chloride, and elevated K^+ . All these treatments cause similar inhibition of the egg jelly-induced AR. J18/29 reacts with a group of membrane proteins ranging in molecular mass from

340 to 25 kD, as shown by immunoprecipitation of lysates of ^{125}I -labeled sperm and Western blots. The most prominent reacting proteins are of molecular masses of 320, 240, 170, and 58 kD. The basis of the multiple reactivity appears to reside in the polypeptide chains of these proteins, as J18/29 binding is sensitive to protease digestion but resistant to periodate oxidation. There are $\sim 570,000$ sites per cell for J18/29 binding. J18/29 is the only reagent of known binding specificity that induces the AR; it identifies a subset of sperm membrane proteins whose individual characterization may lead to the isolation of the receptors involved in the triggering of the AR at fertilization.

BEFORE fusion with the egg the sea urchin spermatozoon passes through the egg jelly layer. The major macromolecular component of egg jelly, a large fucose sulfate-rich glycoconjugate (FSG),¹ induces the sperm acrosome reaction (AR) (SeGall and Lennarz, 1979, 1981; Kopf and Garbers, 1980; Garbers et al., 1983). The AR consists of exocytosis of the acrosome granule and extension of the F-actin-containing acrosomal process (Tilney et al., 1978; Tilney, 1985; Lopo, 1983). The AR is required for fertilization as it exposes the sperm membrane which fuses with the egg plasma membrane (Dan, 1967). Induction of the AR by FSG is accompanied by increases in both intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) and pH (pH_i), and depolarization of the potassium-supported membrane potential (Schackmann et al., 1978, 1981, 1984; Lee et al., 1983; Schackmann and Shapiro, 1981; Christen et al., 1983).

Induction of the sea urchin sperm AR by FSG is a model system for studying the transduction of transmembrane sig-

nals. The sperm are a uniform population of cells that can be obtained in vast quantity, and egg jelly can be easily isolated. Induction of the AR occurs in seconds and is readily quantified. In addition to the induction of the AR, the FSG component of egg jelly also causes increases in the activities of adenylate cyclase and protein kinase, elevation of cAMP (reviewed in Garbers and Kopf, 1980), and the phosphorylation of sperm histone H1 (Porter and Vacquier, 1986). However, other components of egg jelly, such as the peptide speract in *Strongylocentrotus purpuratus* egg jelly, also causes changes in cyclic nucleotide metabolism (Garbers and Kopf, 1980; Garbers et al., 1982), $[\text{Ca}^{2+}]_i$ (Schackmann and Chock, 1986), and pH_i (Repaske and Garbers, 1983; Lee and Garbers, 1986). The speract receptor has been identified as a 77-kD membrane protein (Dangott and Garbers, 1984). As specific probes reacting with speract are not yet available, it is difficult to screen for the presence of this or other contaminating peptides in preparations of FSG. Although preliminary characterizations of FSG has been accomplished, (SeGall and Lennarz, 1979; Garbers et al., 1983), detailed structural analyses of the specific ligand or ligands in FSG that induce the AR have not been reported, thus, receptor-ligand binding studies and identification of sperm receptors for FSG by standard receptor methodologies have not been successful.

In an attempt to identify membrane proteins involved in

Ms. Ebina's permanent address is Central Research Laboratory, Fukushima Medical College, Fukushima, Japan.

1. *Abbreviations used in this paper:* AR, acrosome reaction; $[\text{Ca}^{2+}]_e$ and $[\text{Ca}^{2+}]_i$, external and intracellular concentrations of calcium, respectively; FSG, fucose sulfate glycoconjugate of egg jelly; pH_e and pH_i , external and intracellular pH, respectively; HSW, millipore-filtered natural seawater buffered with 10 mM Hepes, pH 8.0.

the mechanism of signal transduction underlying the AR, we have generated mAbs to external domains of membrane proteins of sea urchin spermatozoa, using isolated plasma membrane vesicles or whole cells as the immunogen (Trimmer et al., 1985; Trimmer and Vacquier, 1986). Several mAbs reacting monospecifically with a membrane glycoprotein of approximate molecular mass 210–260 kD (the 210-kD protein) have been isolated. Some of these mAbs inhibit the egg jelly-induced AR (Trimmer et al., 1985). In addition, the binding of these mAbs induces large increases in $[Ca^{2+}]_i$ without causing the elevation of pH_i (Trimmer et al., 1986).

Here we report the isolation of mAb J18/29 which, like FSG, induces the AR and large increases in both $[Ca^{2+}]_i$ and pH_i . The J18/29-induced AR exhibits sensitivities to lowered external Ca^{2+} ($[Ca^{2+}]_e$) and external pH (pH_e), and to Co^{2+} , similar to those of the FSG-induced AR. J18/29 reacts with a group of sperm membrane proteins as determined by both immunoprecipitation and Western blots. Although J18/29 reacts with more than one protein, it is of value because it appears to mimic the effects of FSG on sperm, and it identifies a small subset of sperm membrane proteins upon which future research can be concentrated. Characterization of the individual proteins reacting with J18/29 may lead to the identification of sperm surface proteins involved in FSG recognition and the triggering of the AR.

Materials and Methods

Reagents and Gametes

Millipore-filtered natural seawater, buffered with 10 mM Hepes, pH 8.0 (HSW) was used unless indicated. Ca^{2+} -free seawater was formulated as follows: 454 mM NaCl, 9.7 mM KCl, 26 mM $MgSO_4$, 24.9 mM $MgCl_2$, 4.4 mM $NaHCO_3$, 10 mM Hepes, pH 8.0. HSW (which contains 10 mM Ca^{2+}) and Ca^{2+} -free seawater were mixed to yield seawater containing various concentrations of Ca^{2+} . Seawaters of various pH values were prepared as described (Porter and Vacquier, 1986). Acid-solubilized whole *S. purpuratus* egg jelly and its 70% ethanol-insoluble FSG component were obtained as described (G. E. Ward et al., 1986; Porter and Vacquier, 1986). All chemicals were from Sigma Chemical Co. (St. Louis, MO). Adult *S. purpuratus* were spawned by intracoelomic injection of 0.5 M KCl, spermatozoa were collected from the gonopores with a pipette, and stored without dilution (dry) on ice. Acrosome reactions were scored by light microscopy (Podell and Vacquier, 1984).

Monoclonal Antibodies

Hybridomas producing mAb J18/29 (IgG₂) were obtained from the fusion of spleen cells from a BALB/c mouse immunized with *S. purpuratus* sperm membrane vesicles (Podell et al., 1984) and whole sperm, with the myeloma cell line SP2/0 as described (Trimmer et al., 1986). Screening of hybridoma culture supernatants by ELISA and immunofluorescence was as described (Trimmer et al., 1985, 1986).

Immunoglobulin subclass was determined by ELISA using subclass specific detection antisera (Zymed Labs, San Francisco, CA). J18/29 immunoglobulins were purified from ascites fluid by chromatography on protein A Sepharose (Ey et al., 1978). Fab fragments were prepared and purified as described (Trimmer et al., 1985, 1986). IgG and Fab purification was monitored by SDS-PAGE and silver staining. Purified IgG and Fab fragments were dialyzed versus HSW before use. J10/14 and J18/2 are mAbs reacting with the 210-kD protein (Trimmer et al., 1985, 1986) and T8/40 is a mAb made against the human lymphoma cell line CCRF-CEM and shows no reaction to sperm (Trimmer et al., 1985).

Immunoprecipitation and Western Blots

Immunoprecipitations were performed as in Trimmer et al. (1985) with the following modifications: ^{125}I -labeled sperm were solubilized in lysis buffer containing 1% NP-40 instead of 0.5% NP-40/0.1% SDS. After centrifuga-

tion at 40,000 g for 1 h, the supernatant was divided into two aliquots, and one aliquot was made 0.5% in SDS from a 20% stock. These two sperm lysates were subjected to immunoprecipitation, with either 1% NP-40 or 1% NP-40/0.5% SDS used in all incubation and wash buffers. Immunoprecipitation was also performed on 0.5% NP-40/0.1% SDS lysates of ^{125}I -labeled sperm that had been reduced and alkylated by standard procedures (Allen, 1981). Iodoacetamide was added to 20 mM to the 40,000 g supernatant of the 1% NP-40/0.5% SDS lysate of labeled sperm. Aliquots were removed and made 5, 10, 15, and 20 mM in 2-mercaptoethanol (4°C) 10 min before the addition of J18/29. All immunoprecipitation buffers contained 20 mM iodoacetamide to prevent disulfide formation.

Western blots were performed as in Trimmer et al., 1985. Cavitated sperm membrane vesicles (Ward et al., 1985) were subjected to electrophoresis on 5–15% gradient SDS-PAGE, or solubilized in 2% cholate and clarified by centrifugation at 175,000 g in an airfuge (Beckman Instruments, Inc., Palo Alto, CA). Aliquots (400 μ l at 0.5 mg/ml) were removed and incubated for 3 h at 37°C in the presence of 0.1 mg/ml pronase or 0.1 mg/ml pronase that had been boiled for 30 min to destroy its activity. Pronase was obtained from Calbiochem (La Jolla, CA), and before use was self-digested for 30 min at 60°C to inactivate contaminating enzymes that may be present (Beely, 1985). After incubation in pronase, the samples were boiled in sample buffer and subjected to electrophoresis on 5–15% gradient SDS-PAGE. Protein standards for estimation of relative molecular mass were purchased from Sigma Chemical Co. Estimations of M_r of bands above the 205-kD myosin standard are only rough approximations. After electrophoretic transfer to nitrocellulose, duplicate strips containing lanes of each of the above samples were incubated for 1 h at 23°C in the dark in 50 mM sodium acetate buffer, pH 4.5 with and without freshly opened 20 mM sodium periodate. The strips were washed in PBS, and after a 30-min incubation in 50 mM sodium borohydride in PBS, the strips were washed in PBS and blocked in blotto-PBS (Johnson et al., 1984). The remainder of the Western blot procedure to detect mAb binding was exactly as described in Trimmer et al. (1985), with the exception that 4-chloro-1-naphthol was used as the substrate for detection of bound horseradish peroxidase-conjugated second antibody.

Quantitative Binding Assays

Purified immunoglobulins at 2–10 mg/ml in HSW were radioiodinated using one washed iodobead (Pierce Chemical Co., Rockford, IL) per ml reaction mixture, and 160 μ Ci $Na^{125}I$ (Amersham Corp., Arlington Heights, IL) per mg immunoglobulin. Free iodide was removed by chromatography on Sephadex G-25 (Sigma Chemical Co.) in HSW. For binding assays, 100 μ l of sperm at 2.3×10^8 cells/ml in 5% nonfat dry milk in HSW (HSW-blotto) were added to microtiter wells containing 100 μ l of ^{125}I -mAb diluted in HSW-blotto. After 1-h incubation at 23°C, cells were harvested using a Phd cell harvester (Cambridge Scientific Industries, Cambridge, MA), deposited on filter disks and washed with 100 vol HSW by continuous flow filtration. Filter disks containing cells and bound ^{125}I -mAb were counted in a gamma counter. Data were analyzed on double reciprocal, Scatchard (Beely, 1985), and semi-logarithmic plots (Klotz, 1982). The use of these analyses is valid for these data as a number of the data points occurred above the inflection point of the saturation binding curve (Klotz, 1982).

Determination of $[Ca^{2+}]_i$ and pH_i

$[Ca^{2+}]_i$ was determined using indo-1 and fura-2 as described (Schackmann and Chock, 1986; Trimmer et al., 1986), which were obtained from Molecular Probes, Inc. (Eugene, OR). Values for intracellular calcium are expressed here as $[Ca^{2+}]_i \times K_d^{-1}$, where K_d^{-1} is the dye- Ca^{2+} dissociation constant. For a full discussion of the derivation of these terms, see Trimmer et al., 1986. Relative change in pH_i was determined by 9-aminoacridine fluorescence (Christen et al., 1982).

Results

Biochemical Characterization of the J18/29 Antigen

Monoclonal antibody J18/29 immunoprecipitates a group of membrane proteins from lysates of ^{125}I -labeled *S. purpuratus* sperm, when either 1% NP-40 alone, (Fig. 1, lane B) or 1% NP-40 containing 0.5% SDS (Fig. 1, lane C) are used in all incubation and wash buffers. The immunoprecipitated

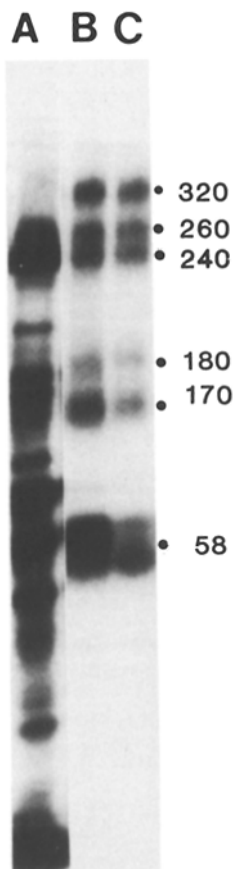


Figure 1. Immunoprecipitation of ^{125}I -labeled sperm analyzed by 5–15% gradient SDS-PAGE under reducing conditions followed by autoradiography. (A) Total ^{125}I -labeled sperm proteins used for immunoprecipitation (20,000 cpm loaded). (B) J18/29 immunoprecipitate in 1% NP-40. (C) J18/29 immunoprecipitate in 1% NP-40 containing 0.5% SDS. In lanes B and C, 5,000 cpm were loaded per lane. Molecular masses in kilodaltons are presented on right.

proteins range in approximate molecular mass from 340 to 25 kD, and include a small amount of the 210-kD protein that is recognized exclusively by mAbs such as J10/14 (Trimmer et al., 1985, 1986). The major bands precipitated are of molecular masses of 320, 240, 170, and 58 kD. The SDS-PAGE profile of the proteins immunoprecipitated by J18/29 is quite different from the immunoprecipitation starting material, which is the 1% NP-40 extract of ^{125}I -labeled sperm (Fig. 1, lane A). The greatest enrichment is seen for the protein of 320 kD, which is barely visible in the starting material. The 210-kD protein, which is the major iodinated protein in sperm extracts, shows little enrichment. On the gel system used in this work, the 210-kD protein (Podell et al., 1984) from the sperm head has a molecular mass of 260 kD, while that of the sperm flagellum is more abundant and has a molecular mass of 240 kD (Trimmer, 1987). The fact that the immunoprecipitation pattern is not altered in the presence of 0.5% SDS indicates that the multiple reactivity is not due to the coprecipitation of members of a surface complex bound by noncovalent interactions (Oettgen et al., 1986).

To determine if these multiple immunoprecipitation products are the result of the coprecipitation of members of a cell surface complex linked by interchain disulfide bonds, immunoprecipitates were performed on sperm extracts which had been reduced and alkylated by the addition of 2-mercaptoethanol (0–20 mM) and 20 mM iodoacetamide (Allen, 1981). Analysis by SDS-PAGE of this immunoprecipitation experiment shows that this treatment has no effect on the proteins immunoprecipitated by either J18/29, or J10/14 (directed against only the 210 kD protein) as compared with the pro-

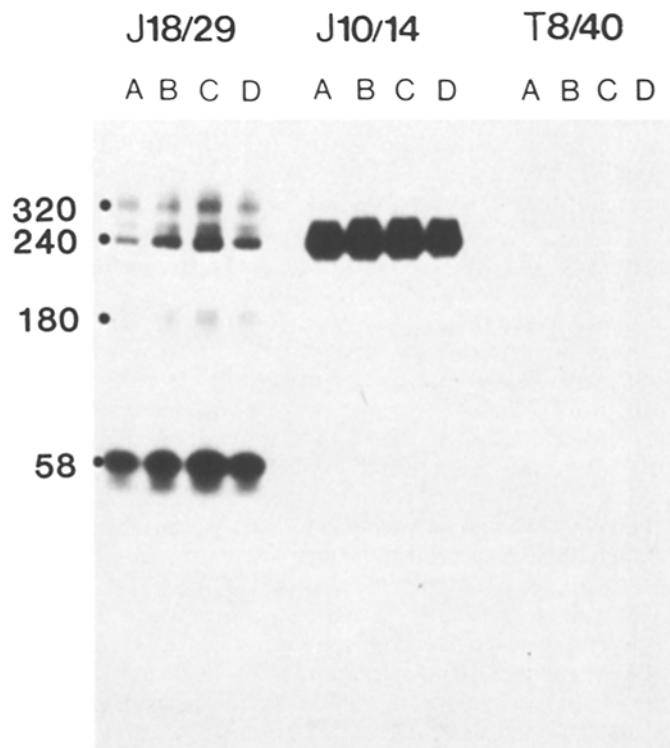


Figure 2. Detergent extracts (0.5% NP-40/0.1% SDS) of ^{125}I -labeled sperm were reduced and alkylated with various concentrations of 2-mercaptoethanol and 20 mM iodoacetamide, and then subjected to immunoprecipitation by either J18/29, J10/14 (anti-210 kD), or T8/40 (negative control ascites). 4,000 cpm were loaded per lane except for the T8/40 control, which immunoprecipitated only 200 cpm. Lane A, no 2-mercaptoethanol; lane B, 5 mM 2-mercaptoethanol; lane C, 10 mM 2-mercaptoethanol; lane D, 20 mM 2-mercaptoethanol. Immunoprecipitates were analyzed by 5–15% gradient SDS-PAGE under reducing conditions followed by autoradiography. Molecular masses in kilodaltons shown on left.

teins immunoprecipitated when no reducing agent is added before immunoprecipitation (Fig. 2). Control mAb T8/40 shows no reaction under any of these conditions. This figure also exemplifies the difference between the immunoprecipitation products of J18/29 and J10/14. In this experiment, J10/14 immunoprecipitated approximately five times as many cpm from the same amount of starting material as did J18/29 (21,900 vs. 4,600). For the SDS-PAGE analysis shown in Fig. 2, equal numbers of cpm were loaded in the J18/29 and J10/14 lanes. It is clear that the amount of 210-kD protein immunoprecipitated by J18/29 is small compared with the amount precipitated by mAb J10/14 to the 210 kD protein.

To analyze further the specificity of J18/29 binding, and to attempt to determine the nature of the antigenic determinant, Western blot analyses of J18/29 binding were performed. Preliminary analysis of J18/29 binding by dot blots had shown that the binding to isolated plasma membranes is destroyed by pretreating the membranes with pronase, whereas pretreatment with periodate had no effect. Western blot analysis of electrophoretically separated sperm membrane proteins (Fig. 3) shows that J18/29, unlike the monospecific reaction of J10/14 with the 210-kD protein (Trimmer et al., 1985), reacts with multiple components of this SDS-denatured plasma membrane preparation, including major bands at

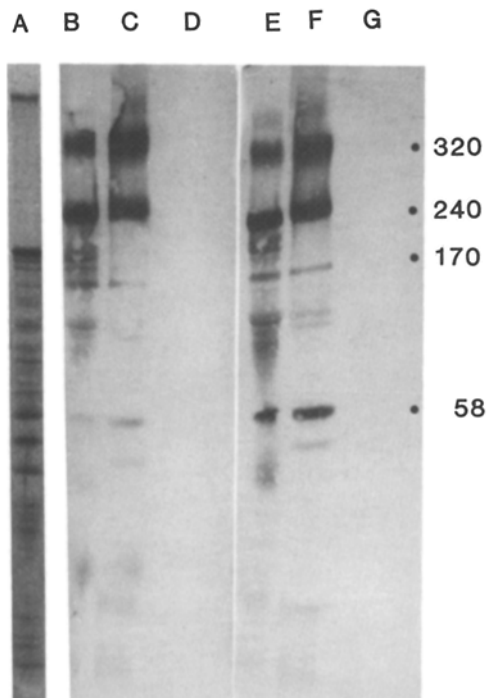


Figure 3. J18/29 binding to pronase and periodate treated proteins. Sperm membranes (as prepared by Ward et al., 1985) were subjected to electrophoresis on 5–15% gradient SDS-PAGE (untreated), followed by staining with Coomassie Blue (lane A), or transfer to nitrocellulose (lanes B and E). Identical samples were solubilized in 2% cholate, clarified by centrifugation, and treated with boiled pronase (lanes C and F), or active pronase (lanes D and G), before electrophoresis and transfer to nitrocellulose. The nitrocellulose strips were incubated in acetate buffer alone (lanes B–D) or acetate buffer plus 20 mM periodate (lanes E–G). The strips were then incubated in 20 $\mu\text{g}/\text{ml}$ J18/29. Bound IgG was visualized with horseradish peroxidase-conjugated goat anti-mouse Ig using 4-chloro-1-naphthol as the substrate. Numbers on the right correspond to mobility of the major bands in relation to molecular mass standards from a duplicate blot stained with Coomassie Blue.

~ 320 , 240, 210, 170, and 58 kD (Fig. 3), which correspond in apparent molecular mass to the major ^{125}I -labeled proteins immunoprecipitated by J18/29 (Fig. 1, lanes B and C). Treatment of these solubilized sperm membranes with pronase destroys the binding of J18/29 (Fig. 3, lanes D and G), indicating that the mAb epitope is protein in nature. Periodate oxidation of the nitrocellulose strip containing the transferred proteins has no effect on the binding of J18/29 to any of these sperm plasma membrane preparations (Fig. 3, lanes E–G). The negative control mAb, T8/40, shows no reaction with any of these membrane proteins in this Western blot analysis, either before or after periodate treatment (not shown). The results of the immunoprecipitation and Western blots together indicate that J18/29 binds to a common protein determinant that is expressed on the extracellular domains of these different sperm membrane proteins.

Analysis of J18/29 Cell Surface Binding

Immunofluorescent localization shows that J18/29 binds the entire sperm surface (Fig. 4). This localization is obtained at all concentrations of mAb. This localization is in contrast

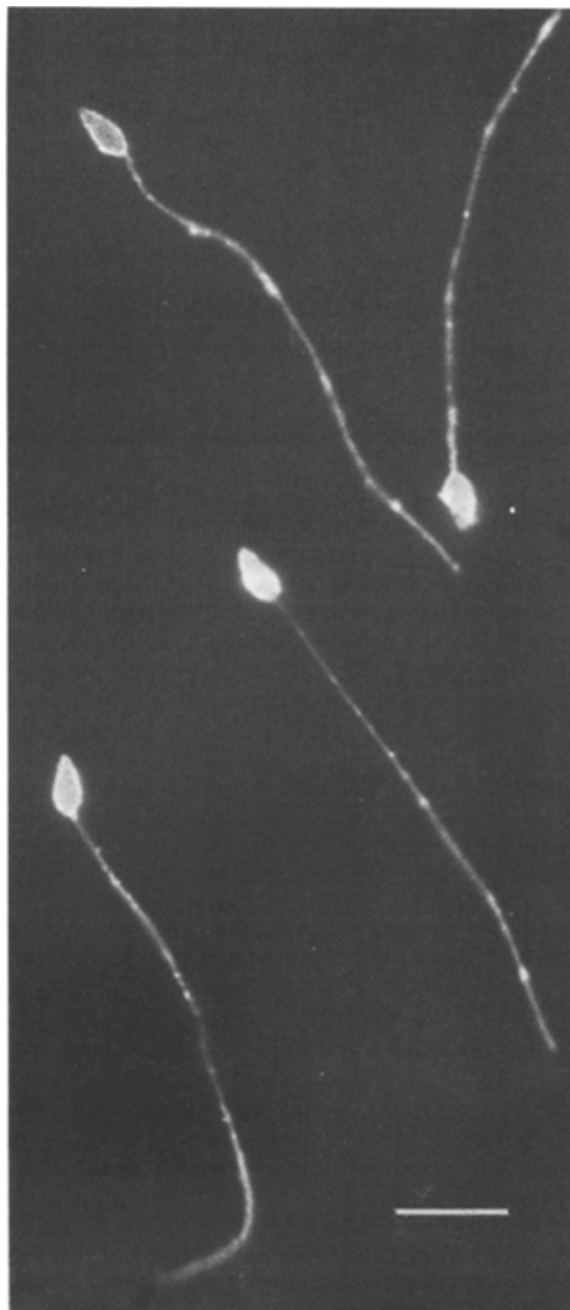


Figure 4. Immunofluorescent localization of J18/29 binding. Cells were reacted with 40 $\mu\text{g}/\text{ml}$ J18/29, followed by the addition of rhodamine-conjugated goat anti-mouse IgG. J18/29 binding is on the entire sperm plasma membrane. Bar, 5 μm .

to the localization seen with mAbs such as J10/14 that react with the 210-kD protein, which bind to the flagellum and to a narrow collar over the anterior nuclear fossa just posterior to the acrosome granule (Trimmer et al., 1985). Quantitative analysis of ^{125}I -J18/29 binding shows that this mAb displays saturating binding to live sperm (Fig. 5 A). At saturation, J18/29 binds $\sim 570,000$ sites per cell, with an apparent $K_d = 1 \times 10^{-8}$. Analysis of the binding data by Scatchard plots indicates that J18/29 binding displays positive cooperativity (Fig. 5 B). A Hill plot of these data with a slope of 2 supports this finding (Fig. 5 C).

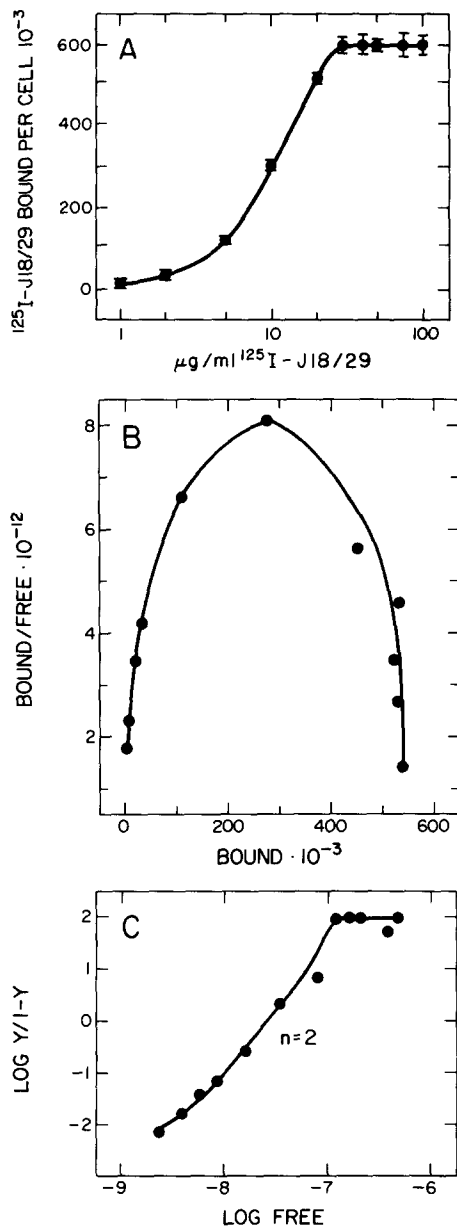


Figure 5. Quantitative binding of J18/29. Quantitative analyses of J18/29 binding. (A) Saturation binding. The number of sites per cell was determined from cell number and specific activity of the ^{125}I -J18/29. (B) Scatchard analysis of the binding data in A. (C) Hill plot of the binding data in A. (Y) The fractional saturation of sites.

Induction of the Acrosome Reaction

Treatment of sperm with mAb J18/29 results in induction of the AR. Maximal induction is at 40 $\mu\text{g/ml}$ and half-maximal at 15 $\mu\text{g/ml}$ (Fig. 6). J18/2, an IgG₃ mAb reacting exclusively with the 210-kD protein (Trimmer et al., 1986), does not induce the AR at similar concentrations (Fig. 6). The J18/29-induced AR is sensitive to both lowered $[\text{Ca}^{2+}]_e$ and pH_e , and to the presence of Co^{2+} , a Ca^{2+} channel inhibitor. The ability of J18/29 to induce the AR decreases dramatically below the normal seawater concentration of 10 mM $[\text{Ca}^{2+}]_e$, with half-maximal induction at 4.75 mM, and no induction below 2 mM $[\text{Ca}^{2+}]_e$ (Fig. 7 A). As shown in Fig. 7 B, the J18/29-induced AR is also sensitive to lowered pH_e . Induc-

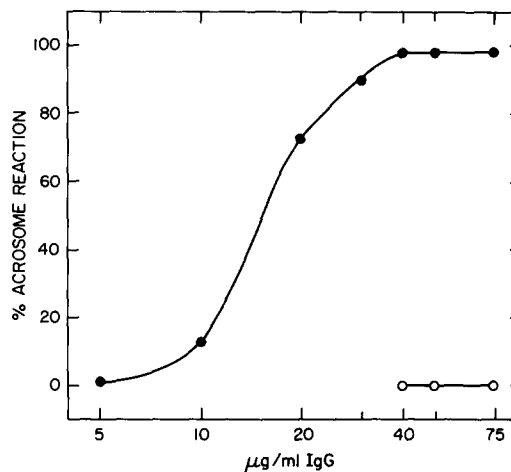


Figure 6. Induction of the acrosome reaction by J18/29. Fresh undiluted sperm were diluted 150-fold in HSW and 100 μl added to 100 μl of IgG in HSW. After 5 min at 15°C, 200 μl of 5% glutaraldehyde in HSW was added and the cells examined by phase contrast microscopy for induction of the AR. A minimum of 150 cells were scored per tube, each point represents the average of two tubes. J18/2 reacts exclusively with the 210-kD protein. (●) J18/29 IgG₃; (○) J18/2 IgG₃.

tion is maximal at pH_e 8.0, it is depressed to half maximal at approximately pH_e 7.7, and does not occur below pH_e 7.2. The J18/29-induced AR is blocked by the presence of Co^{2+} in the external medium (Fig. 7 C). Although Co^{2+} at concentrations up to 10 mM has no effect on sperm viability as judged by microscopic examination of motility, the J18/29-induced AR is completely inhibited at 1 mM, with half-maximal inhibition at 0.35 mM. The J18/29-induced AR is also inhibited by verapamil, tetraethylammonium chloride and high K^+ , which inhibit the FSG-induced AR (Table I).

Although we have had success in generating Fab fragments from other mAbs to sperm membrane proteins (Trimmer et al., 1985, 1986), we have not been able to isolate Fab fragments from J18/29 that retain the ability to bind sperm, even at concentrations of 1 mg/ml. The absence of any detectable cell surface binding by these Fab preparations has not allowed us to determine the possible role of IgG-induced protein-protein cross-linking in the induction of the AR by J18/29.

mAb-induced Increases in $[\text{Ca}^{2+}]_i$ and pH_i

J18/29 induces increases in $[\text{Ca}^{2+}]_i$ in fura-2-loaded sperm which are similar to the increases induced by FSG in that the increases are dependent on millimolar $[\text{Ca}^{2+}]_e$ and do not saturate at $[\text{Ca}^{2+}]_e$ up to 40 mM (Fig. 8). In comparison, ionomycin induces large increases in sperm $[\text{Ca}^{2+}]_i$ at 1 mM $[\text{Ca}^{2+}]_e$. Addition of 60 $\mu\text{g/ml}$ J18/29 ascites fluid to indo-1-loaded sperm in seawater causes a rapid increase in fluorescence (Fig. 8 inset, upper trace), similar to that seen on treatment of sperm with FSG (Trimmer et al., 1986). No such increase occurs when indo-1-loaded sperm are diluted into Ca^{2+} -free seawater before mAb addition (Fig. 8 inset, bottom trace).

J18/29 also induces a large, rapid increase in sperm pH_i , which is similar in magnitude and rate to the pH_i change induced by FSG (Fig. 9). The concentration dependence of the

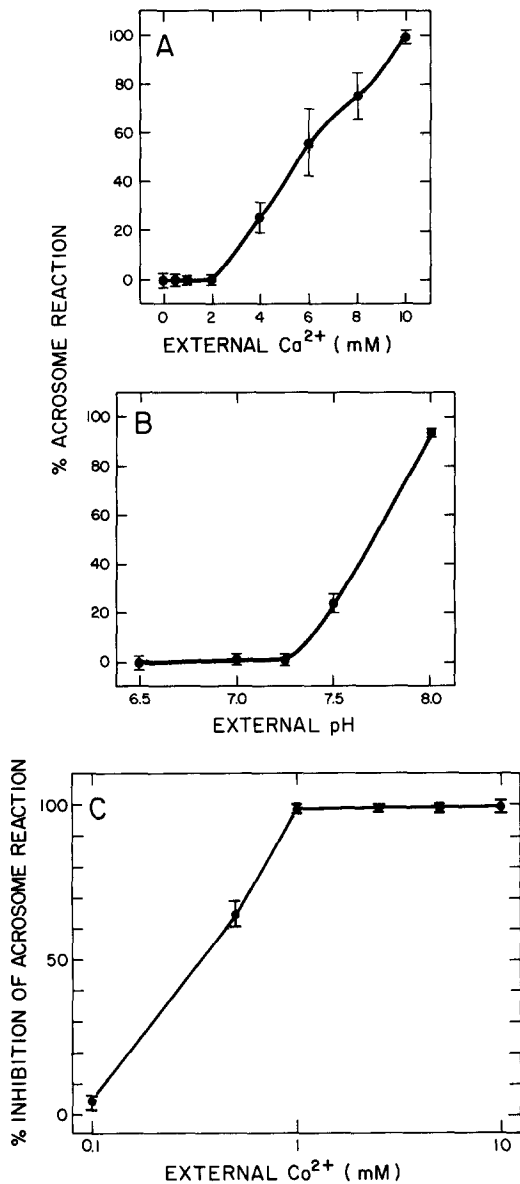


Figure 7. Sensitivity of the J18/29-induced AR to lowered $[Ca^{2+}]_e$, and pH_e , and to Co^{2+} . Acrosome reactions were induced by the addition of 20 $\mu g/ml$ J18/29 as given in the Fig. 6 legend, with (A) all sperm dilutions made into seawaters (pH_e 8.0) of appropriate $[Ca^{2+}]_e$; (B) all sperm dilutions were made into seawaters buffered with 10 mM HEPES, 10 mM Tris, 10 mM 2-(N-morpholino)ethane sulfonic acid at the indicated pH_e ; (C) all sperm dilutions were made into seawater containing the indicated $[CoCl_2]$ concentration.

J18/29-induced AR follows closely its ability to induce increased pH_i (compare Fig. 6 with Fig. 9).

Discussion

In this report we describe mAb J18/29 which defines a novel antigenic determinant on the surface of sea urchin sperm. J18/29 induces large increases in $[Ca^{2+}]_i$ and pH_i , and triggers the AR. Whereas previous studies on the sea urchin sperm AR using polyclonal antisera (Lopo and Vacquier, 1980; Saling et al., 1982; Eckberg and Metz, 1982; Podell and Vacquier, 1984) and mAbs (Trimmer et al., 1985; Trim-

Table I. Effects of Inhibitors of the FSG-induced AR on the J18/29-induced AR*

Treatment	AR %
No treatment	77 \pm
Verapamil (40 $\mu g/ml$)	2
Tetraethylammonium chloride (10 mM)	4
K^+ (50 mM)	0
Ca-free seawater	2 \S

* J18/29 IgG was added to 60 $\mu g/ml$ final concentration after addition of sperm to HSW containing inhibitors.

\dagger FSG at 15 $\mu g/ml$ fucose induced 78% AR, no addition showed <2% AR. Percentages are averages of three different sperm batches.

\S Sperm were diluted into Ca-free seawater before addition of J18/29 IgG to 60 $\mu g/ml$.

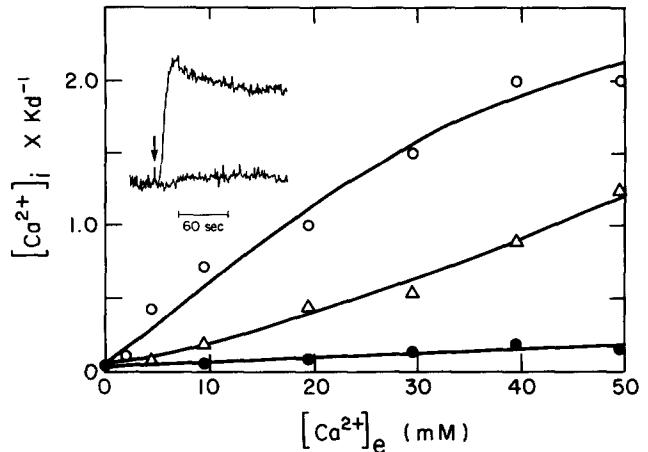


Figure 8. Increase in $[Ca^{2+}]_i$ by J18/29. Sperm were loaded with fura-2 and monitored for fluorescence emission at 500 nm, with excitation at 345 and 380 nm. Sperm were diluted into seawaters containing various amounts of $[Ca^{2+}]_e$ and the maximum increase in $[Ca^{2+}]_i$ was determined by the ratio method (Trimmer et al., 1986) after the addition of either 60 $\mu g/ml$ J18/29 (\circ), 12 $\mu g/ml$ J18/29 (Δ), or no addition (\bullet). (Inset) A tracing of indo-1 fluorescence after the addition of 60 $\mu g/ml$ J18/29 (arrow) after the indo-1-loaded sperm were diluted into either HSW (with 10 mM Ca^{2+} , upper tracing) or Ca-free seawater (lower tracing).

mer and Vacquier, 1986) have reported inhibitory effects on the FSG-induced AR, here we report the isolation of a mAb that induces the AR through cell surface binding.

In contrast to the mAbs we have isolated that react exclusively with the 210-kD protein, J18/29 exhibits multiple reactivity to a subset of sperm membrane proteins. This multiple reactivity appears to be the result of J18/29 binding to a common protein determinant expressed on extracellular domains of the different proteins immunoprecipitated in Fig. 1, as judged by the fact that J18/29 binding is pronase sensitive, and periodate resistant. Although J18/29 exhibits saturation binding to sperm, the large number of binding sites at saturation (570,000 per cell) also indicates that J18/29 is probably binding to more than a single protein on the cell surface. Scatchard plot analysis of these binding data (see Klotz, 1982, for a discussion of the importance of performing these analyses on saturation binding data) is nonlinear, and indicates positive cooperativity in the binding of J18/29 to live sperm. Analysis of this cooperativity by Hill plots yields a

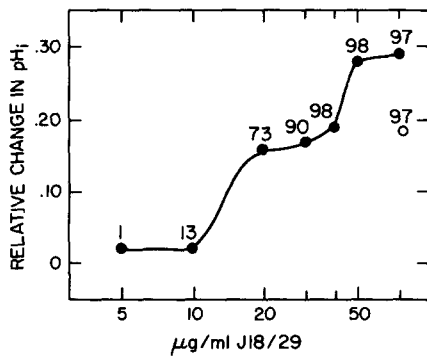


Figure 9. Elevation of pH_i by J18/29. Fresh dry sperm were diluted 1:100 in $2.5 \mu\text{M}$ 9-amino-acridine in HSW, and after dye equilibration, J18/29 IgG (4.8 mg/ml stock in HSW) was added to the indicated final concentrations. Fluorescence was monitored for 10 min after J18/29 addition. 10- μl aliquots were fixed and scored for induction of the AR. Numbers above the data points indicate percent AR for those samples. (●) J18/29 IgG; (○) FSG 5 μg fucose/ml.

slope (Hill coefficient) >2 , indicating more than two binding sites. J18/29 exhibits multiple reactivity by immunoprecipitation under detergent conditions which have been shown to disrupt noncovalent protein interactions (Oettgen et al., 1986). This multiple reactivity is also unaffected by reduction and alkylation, which cleaves disulfide bonds between cysteines, before immunoprecipitation (Allen, 1981). These results argue against the hypothesis that the multiple J18/29 immunoprecipitation products are due to J18/29 binding a single component of a cell surface complex, which results in the immunoprecipitation of the entire complex. These data, combined with the multiple reactivity on Western blots, indicate that J18/29 is binding to a common determinant on the individual components of the immunoprecipitate and not to one component of a single complex composed of these proteins. Two similar examples of mAbs that show multiple reactivities with different proteins have been recently reported. Bloodgood et al. (1986) described a "high molecular weight" antigen on the flagella of *Chlamydomonas reinhardtii*, which is composed of glycoproteins ranging from 350 to 50 kD. S. Ward et al. (1986) reported the isolation of mAbs reacting with a group of *Caenorhabditis elegans* sperm proteins ranging from 215 to 25 kD. In the first example, the basis of the cross-reactivity was shown to reside in the carbohydrate chains of the glycoproteins (Bloodgood et al., 1986); in the latter example mAb reactivity was sensitive to protease digestion, indicating that the multiple reactivity was due to a shared protein determinant (S. Ward et al., 1986). Lane and Koprowski (1982) suggest that such conserved antigenic determinants on different proteins might be recognition sites controlling important interactions in biological processes. It is interesting to speculate that the interaction of FSG with sperm may be through such a multiple reactivity, as FSG exhibits multiple effects on sperm physiology. Further analysis of the biochemical basis of the multiple reactivity exhibited by J18/29 may lead to a deeper understanding of the relationships between the proteins recognized by this mAb, and their roles in the recognition of FSG which induces the AR.

J18/29 binding to sperm results in many of the responses seen when sperm are exposed to FSG. In this report we have shown that J18/29 induces increases in $[\text{Ca}^{2+}]_i$ and pH_i simi-

lar to those induced by FSG, and triggers the AR. The effects of J18/29 on sperm ion fluxes are apparently not due to mAb-induced general membrane leakiness (an ionophoric effect), as the J18/29-induced AR exhibits sensitivities to $[\text{Ca}^{2+}]_e$ and pH_e similar to those of the FSG-induced AR. The inhibition of the J18/29-induced AR by Co^{2+} , verapamil, tetraethylammonium chloride, and high K^+ supports this hypothesis.

In addition to inducing the AR, J18/29 binding activates a pathway which results in the phosphorylation of sperm histone H1 (Porter, D. C., Y. Ebina, J. S. Trimmer, and V. D. Vacquier, manuscript in preparation) on the same serine that is phosphorylated in response to FSG treatment (Porter and Vacquier, 1986). Although the FSG-induced phosphorylation of H1 is dependent on $[\text{Ca}^{2+}]_e$, it is not induced by the $\text{Ca}^{2+}/\text{H}^+$ ionophore A23187 (Porter and Vacquier, 1986), indicating that cell surface binding of either FSG or J18/29 is required to activate the signal transduction mechanism underlying activation of the H1 kinase. These data indicate that J18/29 binding may mimic the interaction of FSG with the cell in more than just the induction of the AR. Unlike FSG, mAb J18/29 has the advantage of being a homogeneous immunoglobulin that exhibits saturation binding to sperm of a defined affinity and specificity, and can therefore be used in studies that are to date not possible with FSG. Numerous attempts to identify the sperm receptor for FSG by a number of standard techniques of receptor biochemistry have to date been unsuccessful. It is interesting to speculate that one or more of the proteins immunoprecipitated by J18/29 may be FSG receptors. The proteins immunoprecipitated by J18/29 consist of a small subset of sperm membrane proteins whose individual characterization may lead to the identification of those surface proteins that are involved in triggering the acrosome reaction.

The authors thank Dr. Ian S. Trowbridge and the members of the Department of Cancer Biology at the Salk Institute for Biological Studies for critical discussions and their invaluable assistance in mAb production, which was partially supported by National Institutes of Health Grant CA 17733 to Dr. Trowbridge. Dr. Ross Allen is thanked for assistance in mAb subtyping, Dr. Michael Luther for assistance with the binding data, and Frank Califano and Sue Stultz for technical assistance.

This work was supported by National Institutes of Health Grants HD 12986 to V. D. Vacquier, and HD 18670 to R. W. Schackmann. Part of this work was performed while R. W. Schackmann was a visitor in the Laboratory of Biochemistry at the National Heart, Lung and Blood Institute.

Received for publication 2 December 1986, and in revised form 11 May 1987.

References

- Allen, G. 1981. *Laboratory Techniques in Biochemistry and Molecular Biology*. Vol. 9. Sequencing of proteins and peptides. Elsevier/North Holland, New York. 28-31.
- Beely, J. G. 1985. *Laboratory Techniques in Biochemistry and Molecular Biology*. Vol. 16. Glycoprotein and proteoglycan techniques. Elsevier, North Holland, Amsterdam. 314-318.
- Bloodgood, R. A., M. P. Woodward, and N. L. Salomonsky. 1986. Redistribution and shedding of flagellar membrane glycoproteins visualized using an anti-carbohydrate monoclonal antibody and concanavalin A. *J. Cell Biol.* 102:1797-1812.
- Christen, R., R. W. Schackmann, and B. M. Shapiro. 1982. Elevation of intracellular pH activates respiration and motility of sperm of the sea urchin, *Strongylocentrotus purpuratus*. *J. Biol. Chem.* 257:14881-14890.
- Christen, R., R. W. Schackmann, and B. M. Shapiro. 1983. Interactions between sperm and sea urchin egg jelly. *Dev. Biol.* 98:1-14.
- Dan, J. C. 1967. The acrosome reaction and lysins. In *Fertilization*. C. B. Metz and A. Monroy, editors. Academic Press, Inc., New York. 1:237-293.
- Dangott, L. J., and D. L. Garbers. 1984. Identification and partial characteriza-

- tion of the receptor for speract. *J. Biol. Chem.* 259:13712-13716.
- Eckberg, W. R., and C. B. Metz. 1982. Isolation of an *Arbacia* sperm fertilization antigen. *J. Exp. Zool.* 221:101-105.
- Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG₁, IgG₂, and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochem.* 15:429-436.
- Garbers, D. L., and G. S. Kopf. 1980. The regulation of spermatozoa by calcium and cyclic nucleotides. *Adv. Cyclic Nucleotide Res.* 13:251-306.
- Garbers, D. L., G. S. Kopf, D. J. Tubb, and G. Olson. 1983. Elevation of sperm adenosine 3'-5'-monophosphate concentrations by a fucose-sulfate-rich complex associated with eggs. I. Structural characterization. *Biol. Reprod.* 29:1211-1220.
- Garbers, D. L., H. D. Watkins, J. R. Hansbrough, A. Smith, and K. S. Misono. 1982. The amino acid sequence and chemical synthesis of speract and of speract analogs. *J. Biol. Chem.* 257:2734-2737.
- Hansbrough, J. R., and D. L. Garbers. 1981. Speract: purification and characterization of a peptide associated with eggs that activates spermatozoa. *J. Biol. Chem.* 256:2235-2241.
- Johnson, D. A., J. W. Gautsch, J. R. Sportsman, and J. H. Elder. 1984. Improved technique utilizing nonfat dry milk for analysis of proteins and nucleic acids transferred to nitrocellulose. *Gene Anal. Tech.* 1:3-8.
- Klotz, I. M. 1982. Numbers of receptor sites from Scatchard graphs: facts and fantasies. *Science (Wash. DC)*. 217:1247-1249.
- Kopf, G. S., and D. L. Garbers. 1980. Calcium and a fucose-sulfate-rich polymer regulate sperm cyclic nucleotide metabolism and the acrosome reaction. *Biol. Reprod.* 22:1118-1126.
- Lane, D., and H. Koprowski. 1982. Molecular recognition and the future of monoclonal antibodies. *Nature (Lond.)*. 296:200-202.
- Lee, H. C., and D. L. Garbers. 1986. Modulation of the voltage-sensitive Na⁺/H⁺ exchange in sea urchin spermatozoa through membrane potential changes induced by the egg peptide speract. *J. Biol. Chem.* 261:16026-16032.
- Lee, H. C., C. Johnson, and D. Epel. 1983. Changes in internal pH associated with the initiation of motility and acrosome reaction of sea urchin sperm. *Dev. Biol.* 95:31-45.
- Lopo, A. C. 1983. Sperm-egg interactions in invertebrates. In *Mechanism and Control of Animal Fertilization*. J. F. Hartmann, editor. Academic Press, Inc. New York. 269-324.
- Lopo, A. C., and V. D. Vacquier. 1980. Antibody to a specific sperm surface glycoprotein inhibits the egg jelly-induced acrosome reaction. *Dev. Biol.* 79:325-333.
- Oettgen, H. C., C. L. Pettey, W. L. Maloy, and C. Terhost. 1986. A T3-like protein complex associated with the antigen receptor on murine T cells. *Nature (Lond.)*. 320:272-275.
- Podell, S. B., and V. D. Vacquier. 1984. Inhibition of sea urchin sperm acrosome reaction by antibodies directed against two sperm membrane proteins. *Exp. Cell Res.* 155:467-476.
- Podell, S. B., G. W. Moy, and V. D. Vacquier. 1984. Isolation and characterization of a plasma membrane fraction from sea urchin sperm exhibiting species specific recognition of the egg surface. *Biochim. Biophys. Acta.* 778:25-37.
- Porter, D. C., and V. D. Vacquier. 1986. Phosphorylation of sperm histone H1 is induced by the egg jelly layer in the sea urchin *Strongylocentrotus purpuratus*. *Dev. Biol.* 116:203-212.
- Repaske, D. R., and D. L. Garbers. 1983. A hydrogen ion flux mediates stimulation of respiratory activity by speract in sea urchin spermatozoa. *J. Biol. Chem.* 258:6025-6029.
- Saling, P. M., W. R. Eckberg, and C. B. Metz. 1982. Mechanism of univalent antisperm antibody inhibition of fertilization in the sea urchin *Arbacia punctulata*. *J. Exp. Zool.* 221:93-99.
- Schackmann, R. W., and P. B. Chock. 1986. Alteration of intracellular [Ca²⁺] in sea urchin sperm by the egg peptide speract. *J. Biol. Chem.* 261:8719-8728.
- Schackmann, R. W., and B. M. Shapiro. 1981. A partial sequence of ionic changes associated with the acrosome reaction of *Strongylocentrotus purpuratus*. *Dev. Biol.* 81:145-154.
- Schackmann, R. W., R. Christen, and B. M. Shapiro. 1981. Membrane potential depolarization and increased intracellular pH accompany the acrosome reaction of sea urchin sperm. *Proc. Natl. Acad. Sci. USA.* 78:6066-6070.
- Schackmann, R. W., R. Christen, and B. M. Shapiro. 1984. Measurement of plasma membrane and mitochondrial potentials in sea urchin sperm. Changes upon activation and induction of the acrosome reaction. *J. Biol. Chem.* 259:13914-13922.
- Schackmann, R. W., E. M. Eddy, and B. M. Shapiro. 1978. The acrosome reaction of *Strongylocentrotus purpuratus* sperm. Ion requirements and movements. *Dev. Biol.* 65:483-495.
- SeGall, G. K., and W. J. Lennarz. 1979. Chemical characterization of the component of the jelly coat from sea urchin eggs responsible for induction of the acrosome reaction. *Dev. Biol.* 71:33-48.
- SeGall, G. K., and W. J. Lennarz. 1981. Jelly coat and induction of the acrosome reaction in echinoid sperm. *Dev. Biol.* 86:87-93.
- Tilney, L. G. 1985. The acrosomal reaction. In *Biology of Fertilization*, C. B. Metz and A. Monroy, editors. Academic Press, Inc., Orlando. 2:157-213.
- Tilney, L. G., D. P. Kiehart, C. Sardet, and M. Tilney. 1978. Polymerization of actin. IV. Role of Ca²⁺ and H⁺ in the assembly of actin and in membrane fusion in the acrosomal reaction of Echinoderm sperm. *J. Cell Biol.* 77:536-550.
- Trimmer, J. S. 1987. Ph.D. Thesis, University of California, San Diego.
- Trimmer, J. S., and V. D. Vacquier. 1986. Activation of sea urchin gametes. *Annu. Rev. Cell Biol.* 2:1-26.
- Trimmer, J. S., R. W. Schackmann, and V. D. Vacquier. 1986. Monoclonal antibodies increase intracellular Ca²⁺ in sea urchin spermatozoa. *Proc. Natl. Acad. Sci. USA.* 83:9055-9059.
- Trimmer, J. S., I. S. Trowbridge, and V. D. Vacquier. 1985. Monoclonal antibody to a membrane glycoprotein inhibits the acrosome reaction and associated Ca²⁺ and H⁺ fluxes of sea urchin sperm. *Cell.* 40:697-703.
- Ward, G. E., D. L. Garbers, and V. D. Vacquier. 1985. Effects of extracellular egg factors on sperm guanylate cyclase. *Science (Wash. DC)*. 227:768-770.
- Ward, G. E., G. W. Moy, and V. D. Vacquier. 1986. Dephosphorylation of sea urchin sperm guanylate cyclase during fertilization. In *Molecular and Cellular Biology of Fertilization*. J. Hedrick, editor. Plenum Publishing Corp. New York. 359-382.
- Ward, S., T. M. Roberts, S. Strome, F. M. Pavalko, and E. Hogan. 1986. Monoclonal antibodies that recognize a polypeptide antigenic determinant shared by multiple *Caenorhabditis elegans* sperm-specific proteins. *J. Cell Biol.* 102:1778-1786.