Monoclonal antibodies to a membrane glycoprotein induce the phosphorylation of histone H1 in sea urchin spermatozoa.

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Abstract. Two groups of mAbs reacting with external domains of a major sea urchin sperm membrane glycoprotein of 210 kD were isolated. Previous studies have shown that group I mAbs inhibit the acrosome reaction induced by egg jelly and also cause large increases in intracellular Ca$^{2+}$ ($[\text{Ca}^{2+}]_i$). Group II mAbs, at comparable levels of cell surface binding, neither inhibit the egg jelly–induced acrosome reaction nor cause increases in $[\text{Ca}^{2+}]_i$. In this paper, we investigate the ability of these mAbs to induce the cAMP-dependent phosphorylation of sperm histone H1. Group I mAbs induce H1 phosphorylation to the same level and on the same peptide, as occurs upon treatment of sperm with egg jelly. These mAbs also activate adenylate cyclase to the same extent as egg jelly. Group II mAbs do not induce H1 phosphorylation and are only poor activators of adenylate cyclase. Group I mAbs compete with each other, but not with group II mAbs, for binding to the cell surface. These data indicate that the activation of adenylate cyclase is an initial event in the pathway leading from the binding of mAbs to a specific domain of the 210-kD protein at the cell surface, to the discrete phosphorylation of histone H1 in highly condensed sperm chromatin. The domain on the 210-kD protein recognized by group I mAbs plays a critical role in signal transduction during the early events of fertilization.

Sea urchin eggs are covered by a jelly layer that can be solubilized by brief exposure to pH 5 seawater. The major macromolecule of egg jelly is a fucose sulfate–rich glycoconjugate (FSG) that induces the acrosome reaction of sperm (Segall and Lennarz, 1979, 1981; Garbers et al., 1983; DeAngelis and Glabe, 1987). This morphological change consists of the exocytosis of the acrosome granule and the polymerization of actin to form the acrosomal process, which is covered by the membrane destined to fuse with the egg (reviewed in Lopo, 1983; Tilney, 1985). FSG has profound physiological effects on sperm, including the opening of ion channels, resulting in the net influx of Ca$^{2+}$ and Na$^+$, the net efflux of K$^+$ and H$^+$, and the depolarization of the K+-supported membrane potential (reviewed by Schackmann, 1986, 1988). In addition to these ionic events, FSG induces the activation of adenylate cyclase and protein kinase, the elevation of cAMP concentrations (reviewed in Garbers and Kopf, 1980), and the elevation of inositol trisphosphate (Domino and Garbers, 1988). These effects of FSG are all dependent on the presence of external Ca$^{2+}$. The major substrate phosphorylated in vivo by the FSG-induced increases in cAMP-dependent protein kinase activity is sperm-specific histone H1 (Porter and Vacquier, 1986), which is phosphorylated on a single seryl site in the peptide RKGS(PO$_4$)SNAR (Porter et al., 1988a). The phosphorylation of H1 on this single site in response to FSG may be a prerequisite for the hyperphosphorylation of H1 by egg kinases and its loss from sperm chromatin within 10 min after the sperm nucleus enters egg cytoplasm (Green and Poccia, 1985; Porter and Vacquier, 1988; Porter et al., 1988b). These effects of FSG on sperm are complex, and the relationships between the changes in ionic permeability and the mobilization of the cyclic nucleotide cascade leading to H1 phosphorylation are not well understood. Although it has never been rigorously demonstrated, these effects of FSG on sperm must involve interaction with extracellular domains of cell surface proteins. In an attempt to identify sperm surface proteins involved in the recognition of FSG and the transduction of this signal across the plasma membrane, mAbs were isolated that react with extracellular domains of sperm membrane proteins (Trimmer, 1987). The major antigenic determinant on the sperm surface is a glycoprotein of 210–260 kD (the 210-kD protein). Two groups of mAbs reacting mono-

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1. Abbreviations used in this paper: FSG, fucose sulfate glycoconjugate of egg jelly; WGA, wheat germ agglutinin.
specifically with this protein were isolated. Group I mAbs inhibit the FSG-induced acrosome reaction (Trimmer et al., 1985; Trimmer and Vacquier, 1986), yet induce large increases in intracellular Ca^{2+} (Trimmer et al., 1986). Group II mAbs do not exhibit these effects on sperm. Here we show that the group I mAbs, but not the group II mAbs, are potent inducers of adenylate cyclase and the phosphorylation of histone H1. This indicates that the 210-kD protein mediates the activation of sperm adenylate cyclase. The domain of the 210-kD protein recognized by group I mAbs must be critical for the interaction of FSG with sperm during fertilization.

**Materials and Methods**

**mAbs**

The production of mouse mAbs reacting with external domains of sea urchin sperm plasma membrane proteins has been described in detail (Trimmer et al., 1985, 1986, 1987; Trimterr, 1987; and Trimterr and Vacquier, 1988). This description includes preparation of the immunogen, screening by ELISA, labeling sperm with ^125I, detergent extraction of labeled sperm, immunoprecipitation of labeled protein, gel electrophoresis of immunoprecipitates and autoradiography, production of ascites fluid, isotyping of immunoglobulins, Fab fragment production, ^125I labeling of antibodies, and competitive binding experiments. In the experiments reported in this paper, mAb preparations used were IgG partially purified from ascites fluid by two cycles of ammonium sulfate precipitation by standard methods (Garvey et al., 1977) followed by dialysis into seawater and determination of protein concentration. mAb TB48/40 reacts with an unknown human lymphocyte antigen and was a generous gift of Dr. I. S. Trowbridge of the Salk Institute, La Jolla, CA.

**Gametes and the Induction of H1 Phosphorylation by Egg Jelly and mAbs**

Gametes of Strongylocentrotus purpuratus were obtained by injection of adults with 0.5 M KC1. We have previously shown that the macromolecular fraction of egg jelly containing FSG, and not the peptide-containing fraction, is the inducer of H1 phosphorylation (Porter and Vacquier, 1986). Preparation and quantitation of egg jelly, collection and storage of sperm at 0°C as undiluted semen (1 μl = 100 μg protein and 4 × 10^7 cells), in vivo labeling of cells with ^33P, dilution of the cells into test substances, preparation of the cells for PAGE, and autoradiography were as described (Porter and Vacquier, 1986). All filtered seawater was buffered at pH 8.0 with 10 mM Heps. Briefly, carrier-free H[^33]PO_4 (ICN Biochemicals, Irvine, CA) was added directly to undiluted semen to 2 mCi/ml and the tube was rotated at 1 rpm (15°C) for 2 h. Seawater was then added to achieve a 1:10 dilution of the semen and 25-μl samples (250 μg protein sperm) were added to 1 ml seawater containing mAb or other test substances (15°C). The final dilution of the semen was 1:400. At 10 min, the sample was made 10% in TCA and then prepared for PAGE in SDS and β-mercaptoethanol (Laemmli, 1970; Porter and Vacquier, 1986).

**Miscellaneous Procedures**

Analysis of the site on H1 phosphorylated by the H1 kinase (Fig. 4) was performed exactly as described (Porter et al., 1986a). The determination of the binding of mAb to fixed cells was a modification of previously described methods (Trimmer et al., 1985, 1987). Sperm were fixed in 3% paraformaldehyde, 0.1% glutaraldehyde in seawater. Fixed cells were collected by centrifugation at 1,000 g for 5 min and washed twice in seawater containing 0.1 M glycine and 1 mg/ml BSA. A 1-ml suspension of fixed cells was prepared in NaCl buffer (150 mM NaCl, 10 mM NaH_2PO_4, 10 mM Tris pH 7.5, 1 mM glycolic acid (NaCl) buffered at 4 × 10^7 cells per ml. 100-μl portions of various antibody dilutions (0-200 μg/ml) were placed in 12 × 75 mm borosilicate tubes, 5 μl stock sperm suspension was added, and slow rotary motion was applied for 45 min. 2 ml NaCl buffer was then added, the tubes were centrifuged at 1,000 g for 5 min and the supernatant was carefully aspirated away. After three such washes, 100 μl of a 1:200 dilution of rhodamine-labeled, FC-specific goat anti-mouse IgG was added to the pellet. The second antibody had been absorbed overnight against an excess of fixed sperm and then centrifuged 30,000 g and the supernatant stored in 100-μl aliquots at −70°C. The cells were gently agitated for 45 min and then washed as given above three times in 2-ml portions of NaCl buffer as before. The cells were quantitatively transferred to a new tube and pelleted. After the supernatant was removed, the cells were resuspended in 3 ml NaCl buffer containing 1% sodium dodecyl sulfate and 1% β-mercaptoethanol and heated at 70°C for 30 min. After centrifugation at 1,000 g for 20 min, 2 ml of the supernatant containing the denatured rhodamine-conjugated antibody was removed and the fluorescence was determined on a double-beam fluorescence spectrophotometer with excitation at 540 nm and emission at 570 nm. The percentage saturation of binding sites on sperm of the mAbs was calculated in arbitrary units of fluorescence. The competition binding experiment (Fig. 5) was done with living cells exactly as described previously (Trimmer et al., 1987). For the sequential treatment of cells with two mAbs (Fig. 6), 25-μl portions of ^32P-labeled sperm (a 1:10 dilution of semen) were added to tubes containing 0.5 ml seawater with 50 μg J18/2 (15°C). At 5 min, 0.5 ml seawater containing either J10/14 or J16/5 IgG at concentrations between 0 and 200 μg/ml was added and the tubes were incubated 10 min before being fixed in TCA. To determine the effects of mAbs on the egg jelly-induced phosphorylation of H1 (Fig. 7), sperm were treated with either seawater or seawater containing mAb J34/4 or J18/2 at 100 μg/ml (the ^32P-labeled semen was diluted 200-fold); after 5 min, 0.5-ml portions were added to 0.5 ml of egg jelly at concentrations ranging from 0 to 200 μg fucose per milliliter. After 10 min, the cells were fixed in trichloroacetic acid. Quantitating the agglutination of fixed sperm by mAb and determination of mAb-induced inhibition of the egg jelly-induced acrosome reaction of living sperm were performed as described (Podell and Vacquier, 1984).

**Adenylate Cyclase**

The method described by Schultz and Jakobs (1983) was modified as follows. Buffers A contained 90 mM Heps, pH 7.5, 6.6 mM cAMP, 3 mM ATP, 15 mM MgCl_2, 60 mM NaF, and 60 mM NaN_3. Buffer B contained 10 mM NaCl, 10 mM KCl, 2 mM Heps (pH 7.5), 20 mM NaN_3, 300 μg/ml creatine kinase, and 45 mM creatine phosphate (pH 7.5). Buffer C contained 10 mM NaCl, 10 mM KCl, 2 mM Heps, 5 μM EDTA, 0.1 mM DTT, 20 mM NaN_3, and 12 mg/ml BSA. 1-ml aliquots of all three buffers were stored at −70°C. One part buffer A was mixed with one part buffer B and the isotopic ([α-32p]ATP, [ICN Biochemicals]) was added (1 μCi/μl, 38 μCi/μl A+B). Usually, 750 μl of this mixture was placed in a 1.5-ml Eppendorf tube in a 23°C water bath. The cell lysate was then prepared exactly as follows. A 1-ml portion of seawater, containing egg jelly (185 μg fucose/ml) or mAb was placed in Eppendorf tubes at 23°C. A fresh 1:10 dilution of semen (dry sperm) was made, 60 μl (600 μg protein) was added, and the tube was capped and inverted. At 120 s, the tube was microfuged for 45 s, the supernatant was immediately removed by aspiration and 1 ml 0°C seawater was added, the cell pellet was resuspended with a 1-ml disposable pipette tip, the cells were resuspended by centrifugation, the supernatant was carefully removed, and 500 μl buffer C (0°C) was added to lyse the cells. The lysate was pulled up several times in the pipette tip, and at exactly 8 min after exposure of the cells to the test medium, 375 μl of lysate was added to 750 μl of reaction mixture. Every 3 min, 120 μl of reaction mixture (~25 μg sperm protein) was removed and added to a test tube containing 480 μl of 0.2 M zinc acetate, followed by 600 μl 0.2 M NaClO_4. The tubes were capped, vortexed, and either stored frozen or centrifuged 5 min at top speed in a microfuge. 1 ml of supernatant was put over 3 ml columns of neutral alumina equilibrated in 100 mM Tris pH 7.5. The columns were washed into scintillation vials with two portions of 3 ml Tris buffer and the Cerenkov radiation was determined in a scintillation counter. [H]cAMP was used to monitor recovery of [32P]cAMP formed; recovery was virtually 100%. No [32P]ATP substrate was detectable in the column eluates. Data points were linear from 3 to 18 min and activity was proportional to added sperm protein. Phosphodiesterase inhibitors were not included in the assay.

**Results**

**Characterization of mAbs**

The group I mAbs, J10/14 (IgG_2a), J18/2 (IgG_3), J16/5 (IgG_3), and J17/10 (IgG_2a); and group II mAbs, J4/4 (IgG_2a) and J18/27 (IgG_2a), react monospecifically with the 210-kD protein, as shown by autoradiograms of SDS-PAGE analysis of immunoprecipitates of detergent extracts of 125I-labeled sperm proteins (Fig. 1). The lane of total detergent extract of radiolabeled sperm used as the starting material for the
imunoprecipitates shows the specificity of the mAbs for the 210-kD protein.

**mAb-induced Phosphorylation of H1**

To investigate the effects of these mAbs on the in vivo phosphorylation of H1, the ATP pools of sperm were labeled with 32P and the cells were diluted into seawater containing egg jelly or mAb. Group I mAbs (J10/14, J16/5, and J17/10) at 100 µg/ml induced H1 phosphorylation to the same extent as did egg jelly (Fig. 2). Group II mAbs (J4/4 and J18/27), and the negative control mAb T8/40, were noninductive at concentrations from 5 to 400 µg/ml (Fig. 2). Both J10/14 IgG and Fab fragments were inductive at concentrations as low as 5 µg/ml. mAb J18/2 (group I) was unique in that at high concentrations (100–200 µg/ml, Fig. 2) it was noninductive, but at lower concentrations it induced H1 phosphorylation to the same extent as seen upon treatment of sperm with egg jelly (Fig. 3). In this same experiment, mAb J17/10 (group I) was inductive to 200 µg/ml, whereas J18/27 (group II) was noninductive at all concentrations tested.

**H1 Site Phosphorylated by mAb Treatment**

Previous chemical analysis for phosphate of purified H1 had
shown that before treatment with egg jelly there is 0.16 mol phosphate/mol protein, and that after egg jelly treatment this number increases to 0.46 (Porter and Vacquier, 1986). Two-dimensional, thin-layer separation of Endo Arg C digests of the NH₂-terminal CNBr-generated fragment of H1 yielded only one labeled peptide (Porter et al., 1988a). This autoradiogram of the thin-layer plate (20 × 20 cm) shows one major labeled peptide. o, origin; E, direction of first dimension electrophoresis; C, direction of second-dimensional chromatography.

Binding of mAbs to the Sperm Surface

To test if the failure of mAbs J4/4 and J18/27 to induce the phosphorylation of H1 was due to a lack of sperm surface binding, binding analyses were performed under the conditions used for the phosphorylation experiments. We previously reported that J4/4 saturates all binding sites at ~125 μg/ml and that after egg jelly treatment this site on the 210-kD protein, whereas the group II mAbs bind a different region(s) of the protein.

From the data presented thus far, it is clear that mAb J18/2 is unique among the group I mAbs in that it displays a narrow concentration dependence in its ability to induce H1 phosphorylation, even though it binds to the 210-kD protein at high levels at the noninductive concentrations (Table 1).

Table I. Relative Binding of Anti–210-kD mAbs

| Group | mAb | A | B |
|-------|-----|---|---|
| II    | J4/4 | 87 | 140 |
| II    | J18/27 | 100 | 620 |
| I     | J18/2 | 80 | 400 |
| I     | J10/14 | 100 | 460 |
| I     | J16/5 | 89 | 630 |
| I     | J17/10 | 96 | 590 |

Column A, relative amounts of mAb bound to fixed cells at 100 μg mAb/ml, expressed as a percentage of the amount bound at 200 μg mAb/ml. Column B, arbitrary units of fluorescent second antibody bound after treatment of cells with 200 μg mAb/ml.

In Table I, we present data on the relative binding of these six anti–210-kD mAbs to sperm under incubation conditions equivalent to those used for the in vivo phosphorylation of H1 by mAbs (0–200 μg/ml). Fixed cells were used in these experiments, as we have never observed a quantitative difference between the binding of mAb to fixed as opposed to living sperm (the hybridomas were selected in an ELISA using fixed cells [Trimmer et al., 1985]). Also, at subsaturating concentrations, mAbs J18/2 and J18/27 cause the 210-kD protein to be shed from the surfaces of living cells (Trimmer and Vacquier, 1988). The surface binding data are presented as the relative amount of mAb bound at 100 μg/ml as a percentage of the amount bound at 200 μg/ml (Table I). Using this cell surface binding assay, it is evident that at 100 μg/ml, mAbs J18/27 and J10/14 are present at saturating concentrations, whereas J4/4, J18/2, J16/5, and J17/10 are slightly below saturation. The relative amount of fluorescent second antibody bound at 200 μg mAb/ml was lowest for J4/4, being about one third to one fourth of the group I mAbs. However, the other group II mAb, J18/27, bound roughly the same amount of second antibody as the group I-treated cells (Table I). The differences in inductive ability exhibited by group I and group II mAbs thus are not due simply to differences in cell surface binding.

To study in more detail the binding of these mAbs and how it relates to the striking differences in their ability to induce H1 phosphorylation, we performed competitive binding analysis using 125I-J10/14. The group I mAbs J16/5 and J18/2 compete as well as J10/14 itself for 125I-J10/14 binding to cells, whereas the group II mAbs (J4/4 and J18/27) are noncompetitive at all concentrations tested, to 250 μg/ml (Fig. 5). It therefore appears as if the group I mAbs bind at or near the same site on the 210-kD protein, whereas the group II mAbs bind a different region(s) of the protein.
Figure 5. Competitive binding using $^{125}$I-10/14. $^{125}$I-10/14 (20 μg) was mixed with the indicated micrograms of each unlabeled antibody and then sperm were added. Bound 10/14 was determined with a gamma counter (Trimmer et al., 1987). Each point is the average of three samples.

Figure 7. Effect of mAbs on the egg jelly-induced phosphorylation of HI. Sperm were diluted into seawater only, or seawater containing 100 μg/ml of either J4/4 or J18/2. After 5 min an equal volume of seawater containing egg jelly was added to yield the final micrograms per milliliter fucose values of FSG shown on top of figure. After 10 min, the cells were processed for autoradiography. J4/4 does not alter the concentration dependence of egg jelly–induced HI phosphorylation, whereas J18/2 inhibits the phosphorylation of HI by egg jelly.

I and Fig. 5). An experiment was performed to determine if J10/14 or J16/5 could override J18/2 to yield the maximum mAb-inducible level of HI phosphorylation. Cells were diluted into J18/2 at 100 μg/ml and after 5 min, either J10/14 or J16/5 was added to concentrations ranging from 0 to 100 μg/ml (5 μg/ml J10/14 is fully inductive). At 100 μg/ml, J18/2 does not induce the translocation and shedding of surface proteins (Trimmer and Vacquier, 1988). The results (Fig. 6) show that J18/2 at 100 μg/ml induced only a small amount of HI phosphorylation that was not increased by later addition of J10/14 or J16/5 (as might be expected from Fig. 5). Treatment of sperm with either 100 μg/ml J10/14 or J16/5 alone gave high levels of HI phosphorylation, as did egg jelly (Fig. 6). These data indicate that the blocking of the common binding site for these three group I mAbs by noninductive concentrations of mAb J18/2 disrupts the ability of both J10/14 and J16/5 to induce HI phosphorylation.

An additional experiment was performed to determine if pretreatment of cells with J18/2 would alter the concentration dependence of FSG-induced HI phosphorylation. As shown in Fig. 7, pretreatment with 100 μg/ml J18/2 completely inhibits the FSG-induced phosphorylation of HI. In contrast, the binding of the group II mAb J4/4 has no effect on the concentration dependence of the FSG-induced phosphorylation, relative to FSG alone. These data indicate that the site recognized by the group I mAb plays a role not only in the mAb-induced phosphorylation, but also in the phosphorylation of HI induced by FSG.

mAb Activation of Adenylate Cyclase

To determine the relationship between the activation of adenylate cyclase and HI phosphorylation, sperm were treated with these six mAbs or egg jelly, washed, lysed, and the adenylate cyclase activity was determined. Six such experiments are presented in Table II, the enzyme rates being calculated from the linear slope of six time points. The fold increase in enzyme activity induced by egg jelly alone ranged from 2.49 to 13.45, with a mean of 6.79. The group I mAbs stimulate adenylate cyclase activity to the same level as does egg jelly, whereas the group II mAbs (and wheat germ agglutinin [WGA]) failed to induce these large increases in adenylate cyclase activity. We have previously shown that the phosphorylation of HI by the endogenous protein kinase is cAMP dependent (Porter et al., 1988a). The ability of group I mAbs, but not group II mAbs, to activate adenylate cyclase is consistent with the idea that increased levels of cAMP activate the kinase phosphorylating histone HI (Porter and Vacquier, 1986; Porter et al., 1988a).

Discussion

Sea urchin spermatozoa are ideal for studying the mechanism of signal transduction. They possess a large membrane
**Table II: Activation of Adenylate Cyclase with Egg Jelly and mAb**

| Exp. No. | Group | mAb or Jelly | Fold increase in rate over no jelly | Percent of rate of jelly-treated sample |
|---------|-------|--------------|-------------------------------------|-----------------------------------------|
| 1       | Jelly | I J18/2      | 3.67                                | 100                                     |
|         |       | I J10/14     | 7.58                                | 102                                     |
| 2       | Jelly | II J4/4      | 1.45                                | 19                                      |
|         |       | II J10/14    | 7.58                                | 102                                     |
| 3       | Jelly | II J4/4      | 0.92                                | 37                                      |
|         |       | I J10/14     | 2.66                                | 107                                     |
| 4       | Jelly | II J18/27    | 0.51                                | 5                                       |
|         |       | I J16/5      | 11.57                               | 118                                     |
|         |       | I J17/10     | 7.30                                | 74                                      |
| 5       | Jelly | WGA 1        | 1.75                                | 13                                      |
|         |       | I J18/2      | 13.36                               | 99                                      |
|         |       | I J18/2*     | 11.57                               | 86                                      |
| 6*      | Jelly | II J4/4      | 3.84                                |                                         |
|         |       | II J18/27    | 1.79                                | 47                                      |
|         |       | I J18/2      | 0.67                                | 17                                      |
|         |       | I J10/14     | 4.07                                | 106                                     |
|         |       | I J10/14     | 4.47                                | 116                                     |
|         |       | I J16/5      | 3.94                                | 102                                     |
|         |       | I J17/10     | 3.94                                | 102                                     |

* mAb concentrations were 100 μg/ml, except in one J18/2 sample in exp. 5 and for all samples of exp. 6, in which the concentrations were 200 μg/ml.
† Egg jelly concentrations were 100 μg fucose/ml.
‡ Basal adenylate cyclase specific activity in lysates of sperm not treated with egg jelly was 0.67 ± 0.38 nmol cAMP formed/min per mg protein (n = 7).
§ Rates were calculated from the linear slopes of six time points over 18 min.
¶ WGA was 60 μg/ml, which yields 100% inhibition of the FSG-induced acrosome reaction (Podell and Vacquier, 1984).

Surface area (31–35 μm²; Cross, 1983) relative to their volume (8 μm³; Christen et al., 1982; Lee et al., 1983), they can be obtained as pure cells with ease and in vast quantity, and several methods exist to isolate the plasma membrane (reviewed in Vacquier, 1987). Egg jelly and its FSG component can be obtained from eggs with ease and its effects on sperm occur synchronously in a time span of seconds. In addition to FSG, egg jelly contains such small peptides as resact (Bentley et al., 1987) and speract (Schackmann and Chock, 1986; reviewed in Garbers et al., 1987) that bind to specific sperm surface receptors and alter cyclic nucleotide metabolism and ion channel activity. G proteins have also been reported in sperm membranes (Kopf et al., 1986; Bentley et al., 1986), and most recently GTP has been shown to control K⁺ channels in the flagellar membrane of sea urchin sperm (Lee, 1988). The FSG component of egg jelly is a very large, complex molecule (SeGallery and Lennarz, 1979, 1981; Garbers et al., 1983; DeAngelis and Glabe, 1987), and the mechanism by which it interacts with the sperm surface remains unknown. Because of the complexity of FSG, classical methods to identify the membrane proteins with which it interacts have been untenable. One approach to the analysis of such complex systems is to use antibodies as probes to identify proteins mediating signal transduction. Using this approach, we have shown that the 210-kD protein plays a critical role in transduction of the FSG response (Trimmer et al., 1985, 1986, 1987).

In this study, we show that the domain of the 210-kD protein defined by the group I mAbs activates adenylate cyclase and induces the cAMP-dependent phosphorylation of H1. We propose that these mAbs use the same pathway as does FSG in inducing these changes when sperm contact eggs. The precise mechanism by which binding of mAb to the group I domain activates adenylate cyclase is of general interest, and most likely involves a conformational change in the 210-kD protein that activates Ca²⁺ channels (Trimmer et al., 1986). The activation of the cyclase is strictly dependent on the presence of external Ca²⁺ (Garbers and Kopf, 1980). However, ionophore A23187 (2–40 μM) does not induce H1 phosphorylation in sperm (Porter and Vacquier, 1986), but such negative data are inconclusive because ionophores act nonspecifically on cellular compartments and thus alter intracellular Ca²⁺ concentrations unphysiologically.

The three group I mAbs tested compete equally well for binding to cells (Fig. 5), and also show the same concentration dependencies for inhibition of the FSG-induced acrosome reaction (Garbers and Vacquier, 1986). That these mAbs activate adenylate cyclase but do not induce the acrosome reaction indicates that the cAMP-dependent protein kinase activity phosphorylating H1 is not directly involved in the acrosome reaction. The group II mAbs (and WGA), although they bind to cells as avidly as group I mAbs, neither activate adenylate cyclase nor induce H1 phosphorylation; they provide the proof that only certain domains of the 210-kD protein mediate signal transduction across the cell membrane. Additional understanding of the inductive and noninductive domains of the 210-kD protein will come from molecular cloning.

A separate topic of discussion is the possible biological significance of the phosphorylation of H1 by the endogenous kinase on only one serine residue in a single peptide (Porter et al., 1988b). Sea urchin sperm-specific histone H1 is a unique type of H1 possessing an extended NH₂-terminal tail with an unusual amino acid sequence (reviewed in Poccia, 1987). Within 10 min after fertilization, sperm H1 becomes hyperphosphorylated (~10 phosphates/mol, Green and Poccia, 1985; Porter and Vacquier, 1988; Porter et al., 1988b), and lost from the sperm pronuclear chromatin, having been replaced by cleavage stage H1 present in the egg cytoplasm (Green and Poccia, 1985; Poccia, 1987). In vitro labeling of H1 in detergent permeabilized sperm shows that the endogenous kinase is completely cAMP dependent. Reactions with purified sperm H1 and rabbit muscle cAMP-dependent protein kinase show a maximum stoichiometry of 1 mol phosphate/mol HI (Porter et al., 1988b). We can conclude from these data that there is only one site for phosphorylation of H1 by the endogenous kinase; may cause a change in chromatin structure that could expose other sites on H1 to be phosphorylated by cAMP-independent kinases of the egg (Porter et al., 1988b), which would result ultimately in loss of sperm HI from the male pronucleus (Green and Poccia, 1985). Regardless of the rea-

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son for its existence, this is the only system in which the phosphorylation of H1 in vivo occurs on a single defined site. Whether it has structural consequences to the chromatin remains to be determined.

Regarding the sperm histone kinase, it could be located outside the nucleus and then move through the nuclear envelope after activation, or it could be resident in the nucleus being packaged with the highly condensed chromatin. One surprising fact about its action is that it is so specific for H1. One would assume that of the many score of different proteins in the nucleus, the preferred sequence (Kemp et al., 1977) for phosphorylation of R/K-X-S (in which X is any amino acid) would be present in many other proteins that would label if the kinase were freely diffusing in the nucleus. The H1 kinase may be resident in the nucleus, tightly associated with H1, and thus inaccessible to other substrates. Regardless of the above speculation, this system is of value in studying how signals at the cell surface are transduced across the cell membrane and through the nuclear envelope to be ultimately expressed by the action of a protein kinase on a protein involved in chromatin structure. As recently discussed (Rauscher et al., 1988), little is yet known about this fundamental process that is central to our understanding of cellular activation.

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