Protein Tyrosine Phosphorylation in Response to Fertilization*

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The sea urchin egg contains one or more protein tyrosine kinases which are active during the response of the egg to fertilization. In the present study, we have used an antibody specific for phosphorytrosine to determine which egg proteins are phosphorylated on tyrosine in response to fertilization. Analysis of immunoblots prepared from fertilized and unfertilized eggs revealed that fertilization results in a major increase in the phosphotyrosine content of a 350-kDa egg protein. Increased phosphorylation of this protein was detected as early as 1 min after fertilization, at which time it represented the most prominent phosphotyrosine-containing protein in the egg. Tyrosine phosphorylation of this protein was transient however, and after 5 min post-insemination, the protein was dephosphorylated or otherwise degraded. Egg membrane proteins of approximately 40, 75, and 145 kDa were also found to act as substrates for protein tyrosine kinases in vitro, but did not exhibit significant changes in phosphotyrosine content during egg activation.

Fertilization results in the execution of a series of preprogrammed biochemical events which serve to stimulate egg metabolism, activate biosynthetic pathways, and initiate cell division. Studies on the role of protein phosphorylation in egg activation have demonstrated that a variety of protein kinases are active during this period of development (1-3). Of particular interest is the observation that the unfertilized egg contains one or more protein tyrosine kinases which are activated as a consequence of fertilization (4-7). Tyrosine-specific protein kinase activity is an intrinsic property of several oncogene-encoded proteins as well as of cell surface receptors for many growth factors or polypeptide hormones (8). Functional studies in a number of systems suggest that protein tyrosine kinases may play an important role in transducing a signal to initiate cell proliferation or in otherwise regulating cell growth (9-12). Our objective has been to identify the protein tyrosine kinases which are activated at fertilization and study their role in egg activation.

To understand the specific enzymatic or cellular functions that are regulated by tyrosine phosphorylation, it is essential to identify the substrate proteins which are phosphorylated on tyrosine during egg activation. Earlier studies detected several egg proteins which act as substrates for endogenous protein tyrosine kinases in vitro (4, 5). Specific changes were found in the tyrosine phosphorylation of egg membrane proteins prepared from embryos at different stages of development, suggesting that developmental changes occur in the activity of certain kinases and/or in the availability of substrate proteins (4).

The objective of the present study is to determine which egg proteins are phosphorylated in vivo by protein tyrosine kinases in response to fertilization. We have used an antibody specific for phosphorytrosine to detect phosphorytrosine-containing proteins in immunoblots prepared from eggs at different points during the egg activation process. These experiments revealed that a 350-kDa egg protein is transiently phosphorylated on tyrosine during the first few minutes after fertilization. During this period, the 350-kDa phosphoprotein is the predominant phosphorytrosine-containing protein detected in eggs by the Western immunoblot technique, suggesting that it may play an important role in egg activation.

EXPERIMENTAL PROCEDURES

Antibody Production—New Zealand White rabbits were immunized with keyhole limpet hemocyanin (Behring Diagnostics) which had been derivatized with the synthetic phosphotyrosine analog p-azobenzyl phosphonate (13). For the initial injections, 850 µg of protein (1.4 µmol of PO4/mg of protein) emulsified in Freund’s complete adjuvant was given intradermally. Later injections of 500 µg were in incomplete adjuvant. Anti-phosphotyrosine activity in serum was monitored by a solid phase enzyme-linked immunoassay in which bovine serum albumin coupled to phosphotyrosine by the carbodiimide procedure (14) was used to coat wells of a microtiter plate and IgG binding was detected with biotinylated goat anti-rabbit IgG followed by avidinated alkaline phosphatase (Vector Laboratories). To purify the antibodies specific for phosphorytrosine, an affinity resin was prepared by derivatizing cyanogen bromide-activated Sepharose (Pharmacia LKB Biotechnology Inc.) with 1-phosphotyrosine (Sigma) (15). Antibodies with a high affinity for phosphorytrosine were allowed to bind to this phosphotyrosine-Sepharose column which was then washed, and the antibodies were eluted with phenyl phosphate (16).

Eggs and Embryos—Gametes were collected from the sea urchin Lytechinus variegatus or Strongylocentrotus purpuratus and the eggs were washed at pH 5.5 to remove the jelly coat. For controlled development, eggs were suspended with constant stirring (1% v/v) in sterile sea water buffered at pH 8.3 with 5 mM TAPS (25 °C for L. variegatus, 7-8 °C for S. purpuratus). Samples to be used in immunoblot experiments were removed before and at various times after fertilization and the eggs were rapidly pelleted in a hand centrifuge. The egg pellet was immediately extracted with 40 volumes of chloroform/methanol (2:1 v/v) to arrest development and extract egg lipids. The protein residue was dried and solubilized in SDS gel sample buffer (17).

Subcellular Fractionation—Unfertilized or fertilized eggs were washed three times in 10 volumes of calcium- and magnesium-free seawater (0.5 mM NaCl, 10 mM KCl, 10 mM Na2HPO4, 25 mM EGTA (18)). The eggs were then suspended in 10 volumes of a homogeni-
zation buffer consisting of calcium- and magnesium-free seawater to which was added 10 mM Na3PO4, 10 mM NaF, 10 μM Na2VO4, 10 μg/ml aprotinin (Sigma), and 10 μg/ml soybean trypsin inhibitor (Sigma). The egg suspension was homogenized in a Potter-Elvehjem homogenizer and layered over a discontinuous sucrose gradient consisting of 10 ml of 30%, 10 ml of 40%, and 5 ml of 78% sucrose dissolved in homogenization buffer and centrifuged at 25,000 rpm for 4 h at 4°C. Material collected at the 0/30%, 30/40%, and 40/78% interfaces was suspended in homogenization buffer and pelleted at 25,000 rpm for 1 h. The pellets were solubilized in SDS gel sample buffer and immediately heated at 95°C for 5 min. In some experiments, the cell surface complex fraction consisting of large sheets of plasma membrane with attached cortical vesicles was purified by a modification of the original method (18). The egg homogenate prepared as above was centrifuged at 1,000 g for 1 min. The pellet was resuspended in homogenization buffer and centrifuge twice more (18), and then centrifuged through a sucrose step gradient as above. The purified cell surface complex was recovered from the 40/78% interface.

Immunoprecipitation and Immunoblot Analysis—For immunoprecipitation experiments, membrane fractions were phosphorylated in vitro by incubation in a phosphorylation buffer containing 10 mM HEPES, 10 mM MnCl2, 10 mM Na2VO4, 10 μg/ml aprotinin, and 0.1 mg/ml Nonidet P-40. The reaction was started by addition of [γ-32P]ATP (43 Ci/mmol) to a final concentration of 3.0 μM and the samples were incubated at 25°C for 2 min. The 32P-labeled proteins were solubilized in an immunoprecipitation buffer containing 150 mM NaCl, 50 mM Tris, 10 mM EDTA, 10 mM Na2PO4, 100 mM NaF, 10 μM Na2VO4, 5 mM phenylmethylsulfonyl fluoride, 1.0% Triton X-100, and 0.1 mg/ml aprotinin (Sigma) and centrifuged at 100,000 × g for 30 min at 4°C. The solubilized proteins were then incubated with affinity-purified anti-phosphotyrosine antibody (0.3 μg/ml) at 4°C for 4 h, after which 25 μl of protein A-Sepharose (Pharmacia) was added for each 0.1 μg of antibody. After 1 h, the immune complexes were collected by centrifugation, washed twice with immunoprecipitation buffer, once with 50 mM Tris, pH 7.5, solubilized in SDS gel sample buffer, and heated at 90°C for 5 min. Immunoprecipitates to be analyzed for phosphoamino acid content were washed once more with distilled water and were solubilized in 6 N HCl containing 50 μg each of phosphoserine, phosphothreonine, and phosphotyrosine as standards. Acid hydrolysis and paper electrophoresis were done as described elsewhere (5); some analyses were performed by two-dimensional thin-layer electrophoresis (19).

Samples for Western immunoblot analysis were electrophoresed on SDS polyacrylamide gels and electrophoretically transferred to 0.45 μM pore nitrocellulose sheets (Schleicher & Schuell). The nitrocellulose sheets were then blocked in 150 mM NaCl, 50 mM Tris (pH 7.5), 0.2% Nonidet P-40, 1% gelatin, and 0.1% bovine serum albumin for 12 h, after which they were incubated in blocking solution containing the affinity-purified antibody (0.3 μg/ml) for 4 h. After several washes in blocking buffer, the blots were incubated with 56 μCi/μg 125I-protein (ICN) at a concentration of 1 μCi/ml for 1 h. The blots were then washed in blocking buffer and dried.

RESULTS

Detection of Egg Proteins Phosphorylated on Tyrosine in Vitro—Previous work in this and other laboratories has used chemical methods to show that membrane fractions from eggs and early embryos contain protein tyrosine kinase activity capable of phosphorylating endogenous membrane proteins in vitro. To further characterize the proteins which act as substrates for these kinases and to test the specificity of the anti-phosphotyrosine antibody in the sea urchin system, we tested its ability to recognize phosphotyrosine-containing proteins from egg membrane fractions which had been phosphorylated in vitro. Egg homogenates prepared before and at different times after fertilization were fractionated over a step gradient prepared from sucrose in calcium- and magnesium-free seawater. Previous studies have shown that the membrane fraction banding at the 30/40% interface has the highest level of protein tyrosine kinase activity (4). When aliquots of this fraction were incubated with [γ-32P]ATP, solubilized in immunoprecipitation buffer, and incubated with the anti-phosphotyrosine antibody, a characteristic set of phosphoproteins were immunoprecipitated upon addition of protein A-Sepharose. Analysis of the immunoprecipitates by SDS gel electrophoresis and autoradiography revealed that phosphoproteins of 40, 57, 75, and 145 kDa were immunoprecipitated by the antibody (Fig. 1). This result correlates well with earlier in vitro studies in which proteins of 45, 57, 74, and 120 kDa were found to contain phosphotyrosine by chemical analysis (4, 5). Samples immunoprecipitated from egg extracts prepared at various times between fertilization and the first cell division contained an identical pattern of phosphoproteins. However, there was a fertilization-dependent increase in the amount of radioactivity incorporated as would be expected from previous measurements of protein tyrosine kinase activity. As seen in Fig. 1, immunoprecipitation of these proteins was inhibited by the presence of phosphotyrosine indicating that the antibody binds through its specific interaction with phosphotyrosine. Antibody specificity was further tested by analysis of the 32P-labeled phosphoamino acids present in the membrane proteins immunoprecipitated by the antibody. Egg membrane proteins phosphorylated in vitro as in Fig. 1 contain phosphoserine and phosphothreonine as the predominant labeled amino acids with phosphotyrosine accounting for about 10% of the radiolabeled species (Fig. 2A). However, in the proteins immunoprecipitated by the antibody, phosphotyrosine accounts for over 95% of the radiolabeled phosphoamino acids (Fig. 2B). Thus it is unlikely that the antibody

FIG. 1. Immunoprecipitation of phosphotyrosine-containing membrane proteins. Membranes (600 μg of protein) prepared from L. variegatus eggs 30 min post-insemination were incubated in phosphorylation buffer containing [γ-32P]ATP for 2 min at 25°C. The membranes were solubilized and divided into two aliquots, and the phosphotyrosine-containing proteins were immunoprecipitated with the anti-phosphotyrosine antibody as described under "Experimental Procedures." The phosphoproteins were then analyzed on a 10% SDS polyacrylamide gel and detected by autoradiography. Each immunoprecipitation contained 150 μg of membrane protein and 0.3 μg/ml affinity-purified antibody (lane A). In some samples, phosphotyrosine (lane B) was included at 5 mM as a competitive inhibitor to demonstrate the specificity of the antibody reaction.
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Fig. 2. Phosphoamino acid analysis of proteins immunoprecipitated by the anti-phosphotyrosine antibody. Egg membranes (1.5 mg of protein), phosphorylated as in Fig. 1, were solubilized and the phosphotyrosine-containing proteins immunoprecipitated as described. After immunoprecipitation, the phosphoamino acid content of the detergent-soluble (A) and immunoprecipitated (B) proteins was determined by two-dimensional paper electrophoresis at pH 3.5 (bottom to top) and pH 1.9 (right to left) (5). P-Ser, phosphoryserine; P-Thr, phosphothreonine; P-Tyr, phosphotyrosine.

has any low affinity, nonspecific interactions with phosphoserine or phosphothreonine.

In addition to the 40-, 57-, 75-, and 145-kDa proteins described above, a high molecular mass protein of approximately 350 kDa was found to be phosphorylated in the cell surface complex fraction collected from the 40/78% interface of the sucrose gradient. This membrane fraction contains sheets of plasma membrane which have retained their association with the cortical secretory vesicles, cytoskeletal elements, and vitelline layer proteins. Detergent-insoluble protein aggregates in this material reduced the specificity of immunoprecipitation; however, when phosphorylated membranes were analyzed directly by SDS gel electrophoresis and treated with alkali prior to autoradiography, the 350-kDa protein became apparent (Fig. 3A). Phosphoamino acid analysis of the 350-kDa band cut from blots of preparative gels demonstrated that this protein is phosphorylated on tyrosine and on threonine residues (Fig. 3B).

Detection of Proteins Phosphorylated on Tyrosine in Vivo— To detect egg proteins which are phosphorylated on tyrosine as part of the response of the egg to fertilization, we prepared Western immunoblots of egg samples taken before and at various times after insemination. This method is particularly well suited for the fertilization system, in which metabolic labeling studies are complicated by the fact that the mature, unfertilized egg exhibits a very low rate of phosphate uptake, approximately 1% of the rate in the fertilized egg (20, reviewed in Ref. 21). In addition, the fact that the fertilization-dependent increase in phosphate transport does not begin until 20–30 min after fertilization (22) makes it difficult to study protein phosphorylation during the first few minutes after sperm egg fusion. In Fig. 4, samples of S. purpuratus eggs were taken before insemination and at different times between fertilization and the first cell division. After electrophoresis on an SDS polyacrylamide gel, the proteins were transferred to nitrocellulose and treated with the anti-phosphotyrosine antibody in the absence (A) or presence (B) of 5 mM phosphotyrosine. The bound antibody was localized with 125I-protein A and detected by autoradiography. Prior to fertilization, phosphotyrosine could be detected in the 350-kDa egg protein; however, the high level of background antibody binding prevented the reproducible detection of the 145-kDa or other proteins of lower molecular mass. Fertilization resulted in a rapid increase in the level of phosphotyrosine detected in the 350-kDa protein. An increase in antibody binding was evident within 1 min after insemination, and reached a max-
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FIG. 4. Effect of fertilization on the phosphotyrosine content of egg proteins. Samples of S. purpuratus eggs (225 μg of protein) prepared before and at various times (in minutes, across the top) after fertilization were analyzed on a 7.5% SDS polyacrylamide gel and transferred to a nitrocellulose sheet. The blots were incubated with the anti-phosphotyrosine antibody (A) or with the antibody plus 5 mM phosphotyrosine as a competitive inhibitor (B). Bound antibody was then localized with 125I-labeled protein A and autoradiography.

FIG. 5. Relative phosphotyrosine content of the 350-kDa protein during egg activation. Samples of S. purpuratus eggs were analyzed by SDS gel electrophoresis and immunoblots were prepared with and without 5 mM phosphotyrosine as a competitive inhibitor. The relative level of antibody binding to the 350-kDa protein was quantitated by densitometric scanning of the autoradiographs under conditions where density was linearly dependent on radioactivity. The phosphotyrosine-specific antibody binding was determined by subtracting the values obtained with antibody + 5 mM phosphotyrosine from the value obtained with antibody alone. Values are the average of four experiments ± S.E. and are expressed relative to the values of the unfertilized egg.

As an additional control to insure that binding of the antibody to the 350-kDa protein was specific for phosphotyrosine, blots of egg samples prepared at 5 min after fertilization were incubated with antibody solutions containing either phosphoserine, phosphothreonine, or phosphotyrosine as competitive inhibitors of antibody binding. As seen in Fig. 6, phosphoserine and phosphothreonine had little effect on binding of the antibody to the 350-kDa egg protein, whereas 5 mM phosphotyrosine almost completely inhibited antibody binding. This demonstrates that antibody binding was specific for phosphotyrosine.

Western blot analysis of eggs and embryos from two other marine invertebrates revealed that a similar 350-kDa phosphotyrosine-containing protein is detectable in L. variegatus and a protein doublet of approximately 350–370-kDa is seen

FIG. 6. Specificity of antibody binding to the 350-kDa egg protein. To ensure that antibody binding to the 350-kDa protein occurred through a specific interaction with phosphotyrosine, immunoblots were prepared from an aliquot of eggs taken 5 min post-insemination. The blots were incubated with the anti-phosphotyrosine antibody (A) or antibody plus 5 mM phosphothreonine (B), phosphoserine (C), or phosphotyrosine (D), as competitive inhibitors.

FIG. 7. Phosphorylation of the 350-kDa protein in eggs of other species. Samples of eggs (200 μg) from the sea urchin L. variegatus (top) and the sand dollar C. rosea (bottom) were analyzed by SDS gel electrophoresis and blots were treated with the anti-phosphotyrosine antibody in the absence (A) or presence (B) of 5 mM phosphotyrosine. The blots were then incubated with 125I-protein A and bound antibody was detected by autoradiography. The time (in minutes post-fertilization) at which the samples were taken is indicated at the top of each panel.
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in eggs of the sea biscuit Clypeaster rosacea (Fig. 7). In L. variegatus, the time course of phosphorylation of P-350 was similar to that in S. purpuratus; however, the level of phosphotyrosine in P-350 from unfertilized L. variegatus eggs was barely detectable, and consequently the relative increase after fertilization was larger than that in S. purpuratus and varied more from experiment to experiment. Unfertilized eggs from C. rosacea consistently had a high level of phosphotyrosine in the 350-kDa protein. Fertilization resulted in a modest (approximately 2-fold) increase in phosphotyrosine content which declined to low levels by 15 min post-insemination. These studies in different species seem to indicate that, although the phosphotyrosine content of P-350 in the unfertilized egg may vary from species to species, fertilization results in the phosphorylation of this protein to some maximal level which is maintained until about 10–15 min post-insemination. As development proceeds, the protein is either dephosphorylated or otherwise degraded in each species.

Subcellular Localization—To determine the subcellular localization of the phosphotyrosine-containing form of the 350-kDa protein, eggs were homogenized in the presence of phosphatase inhibitors and fractionated on a sucrose gradient also containing these inhibitors. The fractions were then subjected to SDS gel electrophoresis and Western blot analysis using phosphotyrosine antibody. As seen in Fig. 8, antibody binding to the 350-kDa protein was detected primarily in the cell surface complex membrane fraction banding at the 40/78% interface. Similar results were seen in fractions prepared from fertilized eggs (not shown).

FIG. 8. Identification of the phosphorylated form of the 350-kDa protein in subcellular fractions. Eggs of the sea urchin S. purpuratus were homogenized and centrifuged through a sucrose step gradient as described under “Experimental Procedures.” The soluble material at the top of the gradient was concentrated by precipitation with 10% trichloroacetic acid. Material banding at the S. purpuratus soluble material at the top of the gradient was concentrated by antibody and "I-protein. Gradient interfaces was washed by centrifugation. Samples from each fraction (100 µg of protein) were analyzed by SDS gel electrophoresis, blotted onto nitrocellulose, and treated with the anti-phosphotyrosine antibody and "I-protein A. Samples shown in this autoradiograph are: egg homogenate (A), 40/78% sucrose gradient interface (B), 30/40% interface (C), 0/30% interface (D), and soluble (cytosol) fraction (E). F is identical to B except that incubation of the blot with anti-phosphotyrosine antibody was carried out in the presence of 5 mM phosphotyrosine.

DISCUSSION

Fertilization resembles many other mitogenic processes in that a resting cell (the egg) is stimulated to divide in response to an external stimulus (sperm fusion). A number of in vitro studies have shown that the protein tyrosine kinase activity in egg homogenates increases in response to fertilization (4–7). In this report we have used an antibody specific for phosphotyrosine to identify the egg proteins which are phosphorylated in vivo during the response to fertilization. We have found that a 350-kDa protein is phosphorylated by one or more protein tyrosine kinases as early as 1 min after fertilization. By 15 min after fertilization, this protein is largely dephosphorylated or otherwise degraded. The 350-kDa protein could not be detected in blots by protein staining techniques such as colloidal gold (Aurodye, Pierce) or in gels by silver staining. Therefore, we were unable to determine whether the changes in the phosphotyrosine content of the protein represent an increase in the phosphorylation of a constant pool of 350-kDa protein, or a change in the amount of the protein which remains phosphorylated at a constant stoichiometry. The 350-kDa protein was by far the major phosphotyrosine-containing protein during the first few minutes after fertilization. Although the 350-kDa and other egg proteins of 40, 57, 75, and 145 kDa could be phosphorylated on tyrosine in vitro, the lower molecular weight proteins were difficult to detect by Western blot of whole eggs because the level of antibody binding to these proteins was similar to the background antibody binding in this region of the gel. Numerous attempts were made to detect phosphorylation of these proteins by immunoprecipitation of eggs metabolically labeled with 32PO4. However, the unfertilized egg has a very low rate of phosphate uptake which does not increase until 20–30 min after fertilization (22). Therefore, even prolonged labeling of large samples of unfertilized eggs (100 mg of egg protein for 48 h) did not produce enough incorporation to demonstrate phosphotyrosine chemically in individual proteins during the first few minutes after fertilization.

The rapid phosphorylation of the 350-kDa egg protein was surprising in view of our earlier in vitro studies which indicated that the protein tyrosine kinase activity in egg homogenates did not increase significantly until 20 min after fertilization (5). However, Satoh and Garbers (7), using a different peptide substrate, were able to detect a 2-fold increase in protein tyrosine kinase activity within the first 3 min post-insemination. Activation of a protein tyrosine kinase that early in the egg activation process would provide a potential mechanism for the increase in phosphorylation of the 350-kDa protein that we have identified here. To evaluate the possibility that the fertilizing sperm could supply the phosphorylated 350-kDa protein at fertilization, we have analyzed sea urchin sperm by Western blot technique and could find no evidence that the 350-kDa protein is phosphorylated in sperm. It is possible that the sperm may contain a kinase, not present in the egg, which may phosphorylate the 350-kDa protein. However, the fact that the 350-kDa protein can be phosphorylated in cell surface complex fractions prepared from unfertilized eggs as well as our preliminary observation that the parthenogenic agent A23187 can stimulate phosphorylation of the 350-kDa protein in vivo suggest that the sperm is not required.

It is useful to consider the above results in the context of the other biochemical changes which are induced by fertilization. Depolarization of the plasma membrane resting potential occurs within the first few seconds after sperm-egg fusion (23) and is followed at 15 s by the phosphorylation and turnover of polyphosphoinositides (24, 25). Accumulation of inositol 1,4,5-triphosphate is thought to trigger the “calcium transient,” the temporary elevation of free calcium concentrations to 2–5 µM (26, 27) beginning 30 s after fertilization and

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continuing until about 3 min post-fertilization. The calcium transient is accompanied by exocytosis of the cortical secretory vesicles and intense endocytotic activity (28, 29). Tyrosine phosphorylation of the 350-kDa protein reported here is first detected at 60 s after fertilization at a time when intracellular calcium levels would be high. Other responses to fertilization such as the stimulation of protein synthesis, beginning 5–10 min after fertilization (30), initiation of DNA synthesis at 25–30 min after fertilization (31), and the first cell division at 60 min, occur subsequent to the phosphorylation of the 350-kDa protein. Although protein tyrosine kinases, in general, do not require calcium and are not stimulated by calcium, the temporal correlation between the calcium transient and the phosphorylation of the 350-kDa egg protein suggests that these two events may be related. Future work with parthenogenic agents such as the calcium ionophore A23187 may clarify the role of the calcium transient in the phosphorylation of the 350-kDa egg protein.

The rapid, often transient phosphorylation of tyrosine residues in cellular proteins has been observed in several other cell types during their response to growth factors or polypeptide hormones. Treatment of fibroblasts with serum (32) or purified growth factors (33) as well as insulin stimulation of hepatocytes and adipocytes (34, 35), resulted in a severalfold increase in the phosphotyrosine content of specific proteins. The receptors for many growth factors and for insulin possess intrinsic protein tyrosine kinase activity, which explains the rapid phosphorylation response (as early as 30 s (35)) in these systems. Interestingly, stimulation of the epidermal growth factor receptor kinase has been shown to result in the transient phosphorylation, on tyrosine, of several cytoskeleton-associated proteins in the 300–350-kDa size range (36). Phosphorylation is initiated by the cell surface interaction between the sperm and egg (37, 38) and it is intriguing to speculate whether the binding of a sperm to the sperm receptor present on the egg surface (39) may activate a protein tyrosine kinase in the egg plasma membrane.

REFERENCES

1. Keller, C., Gundersen, G., and Shapiro, B. M. (1980) Dev. Biol. 74, 86–100
2. Ballinger, D. G., Bray, S. J., and Hunt, T. (1984) Dev. Biol. 101, 192–200
3. Ward, G. E., Vacquier, V. D., and Michel, S. (1983) Dev. Biol. 95, 360–373
4. Dasgupta, J. D., and Garbers, D. L. (1983) J. Biol. Chem. 258, 6174–6178
5. Ribot, H., Eisenman, E., and Kinsey, W. H. (1984) J. Biol. Chem. 259, 5333–5338
6. Kinsey, W. H. (1984) Dev. Biol. 105, 137–143
7. Setoh, N., and Garbers, D. L. (1985) Dev. Biol. 111, 515–519
8. Hunter, T., and Cooper, J. A. (1985) Annu. Rev. Biochem. 54, 897–930
9. Morgan, D. O., Korn, L. J., and Roth, R. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 328–332
10. Setton, B. M., Hunter, T., Beemon, K., and Eckhart, W. (1980) EMBO J. 3, 483–489
11. Haring, H. U., White, M. F., Kahn, C. R., Kasuga, M., Lauris, V., Fleischmann, R., Murray, M., and Pawelek, J. (1984) J. Cell Biol. 99, 900–908
12. Grigorescu, F., Flier, J. S., and Kahn, C. R. (1984) J. Biol. Chem. 259, 15003–15006
13. Landt, M., Boltz, S. T., and Butler, L. G. (1983) Biochemistry 17, 916–919
14. Ek, B., and Heldin, C. (1984) J. Biol. Chem. 259, 11145–11152
15. Comiglio, P. M., DiRenzo, M. F., Tarone, G., Giancotti, M., Naldini, L., and Marchisio, P. C. (1984) EMBO J. 3, 483–489
16. Pike, L. J., Bowen-Pope, D. F., Ross, R., and Krebs, E. G. (1983) J. Biol. Chem. 258, 9383–9390
17. Cleveland, D. W., Fisher, S. G., Kirschner, M. W., and Laemml, U. K. (1977) J. Biol. Chem. 252, 1102–1106
18. Detering, N. K., Decker, G. L., Schmell, E. D., and Lennarz, W. J. (1977) J. Cell Biol. 75, 899–914
19. Cooper, J. A., Setten, B. M., And Hunter, T. (1983) Methods Enzymol. 99, 387–401
20. Brooks, S. C., and Chamber, E. L. (1948) Biol. Bull. 95, 262–263
21. Tyler, A. and Tyler, B. S. (1966) in Physiology of Echinodermata (Booletian, R. A., ed.) pp. 693–741, John Wiley and Sons, New York
22. Chambers, E. L., and White, W. E. (1984) Biol. Bull. 166, 297–307
23. Chambers, E. L., and Arnedo, J. D. (1979) Exp. Cell Res. 122, 203–218
24. Turner, P. R., Sheetz, M. P., and Jaffe, L. A. (1984) Nature 310, 414–415
25. Kamel, L. C., Bailey, J., Schoenbaum, L., and Kinsey, W. H. (1985) Lipids 20, 350–356
26. Whitaker, M., and Irvine, R. F. (1984) Nature 312, 636–639
27. Turner, P. J., Jaffe, L. A., and Fein, A. (1986) J. Cell Biol. 102, 70–76
28. Chandler, D. E., and Heuser, J. (1979) J. Cell Biol. 93, 91–108
29. Fisher, G. W., and Rebhun, L. I. (1983) Dev. Biol. 99, 456–472
30. Winkler, M. M., Steinhardt, R. A., Grainger, J. L., and Minning, L. (1980) Nature 287, 558–560
31. Epel, D. (1975) An. Zool. 15, 507–522
32. Morla, A. O., and Wang, J. Y. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8191–8195
33. Kohono, M. (1985) J. Biol. Chem. 260, 1771–1779
34. White, M. F., Baron, R. and Kahn, C. R. (1985) Nature 318, 183–185
35. Kasuga, M., Zick, Y., Blith, D. L., Karlsson, F. A., Haring, H. U., and Kahn, C. R. (1985) J. Biol. Chem. 257, 9891–9894
36. Landreth, G. L., Williams, W. L., and Rieser, G. D. (1985) J. Cell Biol. 101, 1341–1350
37. Hiramoto, Y. (1962) Exp. Cell Res. 27, 416–426
38. Uehara, T., and Yanagimachi, R. (1976) Biol. Reprod. 15, 467–470
39. Ruiz-Bravo, N., and Lennarz, W. J. (1986) Dev. Biol. 118, 202–208
40. Schuel, H., Wilson, W. L., Chen, K., and Lorand, L. (1973) Dev. Biol. 34, 175–186