Evidence That Fertilization Activates Starfish Eggs by Sequential Activation of a Src-like Kinase and Phospholipase Cγ

Received for publication, February 9, 2000, and in revised form, March 20, 2000
Published, JBC Papers in Press, March 22, 2000, DOI 10.1074/jbc.M001091200

Andrew F. Giusti‡§, Wenqing Xu†, Beth Hinkle‡§, Mark Terasaki‡§, and Laurinda A. Jaffe‡§†

From the ‡Marine Biological Laboratory, Woods Hole, Massachusetts 02543, the §Department of Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032, and the ¶Department of Biological Structure, Biomolecular Structure Center, University of Washington, Seattle, Washington 98195

Recent evidence has indicated a requirement for a Src family kinase in initiating Ca2+ release at fertilization in starfish eggs (Giusti, A. F., Carroll, D. J., Abassi, Y. A., Terasaki, M., Foltz, K. R., and Jaffe, L. A. (1999) J. Biol. Chem. 274, 29318–29322). We now show that injection of Src protein into starfish eggs initiates Ca2+ release and DNA synthesis, as occur at fertilization. These responses depend on the phosphorylation state of the Src protein; only the kinase active form is effective. Like Ca2+ release at fertilization, the Ca2+ release in response to Src protein injection is inhibited by prior injection of the SH2 domains of phospholipase Cγ. These findings support the conclusion that in starfish, sperm-egg interaction causes egg activation by sequential activation of a Src-like kinase and phospholipase Cγ. Injection of the SH2 domain of Src, which inhibits Ca2+ release at fertilization, does not inhibit Ca2+ release caused by Src protein injection. This indicates that the requirement for a Src SH2 domain interaction is upstream of Src activation in the pathway leading to Ca2+ release at fertilization.

At fertilization, signals at the site of sperm-egg interaction cause a rise in cytosolic Ca2+ (1, 2). This opens ion channels and stimulates exocytosis of cortical granules, resulting in blocks to polyspermy, and also stimulates the resumption of the cell cycle (3–5). The Ca2+ rise results, at least in large part, from Ca2+ release from the endoplasmic reticulum, mediated by inositol 1,4,5-trisphosphate (IP3)1 (6–10). Much recent work on fertilization has focused on the signal transduction pathways that lead to IP3 production.

The phospholipase C family of enzymes produces IP3 and diacylglycerol from the membrane lipid phosphatidylinositol 4,5-bisphosphate (11). In echinoderm eggs, it is the γ isofrom of phospholipase C (PLCγ) that functions at fertilization. PLCγ enzyme activity increases by 30 s post-fertilization in sea urchin eggs (12), and inhibition of PLCγ activation inhibits Ca2+ release at fertilization in both sea urchin and starfish eggs (13–15). In these experiments, PLCγ activity was inhibited by injection of eggs with excess Src homology 2 (SH2) domains of PLCγ. SH2 domains are found in many signaling proteins, and provide a site for specific interaction of a particular protein with a particular phosphorylated tyrosine on another protein (16). Excess SH2 domains, introduced into cells by microinjection, function as specific dominant negative inhibitors of such interactions.

PLCγ can be activated by phosphorylation of a regulatory tyrosine, although other factors may also be significant (17–20). In sea urchin eggs, attempts to determine if PLCγ is tyrosine phosphorylated at fertilization have been inconclusive, since the phosphotyrosine in PLCγ immunoprecipitates was barely detectable either before or after fertilization (12, 21). As discussed by these authors, a local increase at the site of sperm-egg interaction might have been too small to detect by the methods used. Nevertheless, tyrosine kinase activity increases within 15 s after fertilization (22), and the tyrosine kinase inhibitor genistein delays Ca2+ release at fertilization (23). One group of tyrosine kinases that participates, directly or indirectly, in activation of PLCγ is the Src family (20, 24–26), and in vitro experiments with starfish eggs have shown a fertilization-dependent association of a Src-like kinase with the SH2 domains of PLCγ (27). Further evidence that a Src family kinase functions to activate PLCγ at fertilization comes from findings that in both starfish and sea urchin eggs, injection of excess SH2 domains of Src family kinases inhibits Ca2+ release at fertilization (28, 29). In addition, in sea urchin eggs, the Src family kinase inhibitor PP1 delays Ca2+ release at fertilization, and the activity of a Src-like kinase increases by 30 s post-insemination (29).

These findings indicate that a Src-like kinase may, directly or through intermediate molecules, activate PLCγ at fertilization, leading to Ca2+ release and egg activation. In this report, we examine four questions related to this model. 1) Is the kinase activity of the Src-like protein required for Ca2+ release? 2) Does a Src-like protein initiate DNA synthesis as well as Ca2+ release? 3) Does the Src-like protein act upstream of PLCγ? 4) Do the SH2 domains of the Src-like protein interact with an upstream or downstream component of the pathway? We approached these questions by injecting starfish eggs with human cSrc protein that was produced in insect cells. The activity of Src family kinases is regulated by phosphorylation of two tyrosines: phosphorylation of an internal tyrosine (Tyr-419 in human cSrc) is stimulatory, while phosphorylation of the COOH-terminal tyrosine (Tyr-530) is inhibitory (30). A third tyrosine phosphorylation site has also been identified, but its functional significance is not known (31). We injected starfish eggs with Src protein that was either completely unphosphorylated, or phosphorylated on Tyr-530 only. Unphosphorylated Src protein will, in the presence of ATP in the cytoplasm, rapidly autophosphorylate on Tyr-419, resulting in a fully ac-
tive protein (Ref. 32 and see “Discussion”). Phosphorylation of Tyr-530 locks Src in a closed conformation in which intramolecular interactions among the SH3, SH2, and kinase domains down-regulate the kinase activity (33–36).

We found that injection of starfish eggs with unphosphorylated Src protein causes Ca^{2+} release and DNA synthesis. We then injected eggs with the SH2 domains of PLCγ, followed by unphosphorylated Src protein, to investigate whether Src releases Ca^{2+} by way of PLCγ. We also injected eggs with the SH2 domains of Src, followed by unphosphorylated Src protein, to determine whether, in the pathway leading to PLCγ activation at fertilization, the requirement for the Src SH2 domain is upstream or downstream of the activation of the Src-like kinase.

EXPERIMENTAL PROCEDURES

Recombinant Proteins—An NH2-terminal deletion mutant of human cSrc (N-85-srcTK, amino acids 86–536) was expressed in baculovirus-infected Sf9 cells (31). The Src fraction that was phosphorylated on Tyr-530, and not on the other two tyrosine phosphorylation sites, was purified as described previously (33). Unphosphorylated Src was purified with a combination of ATP-Sepharose, phosphotyrosine, and anion-exchange chromatography. The phosphorylation state of the protein fractions was confirmed by matrix-assisted laser desorption/ionization mass spectrometry after trypsin digestion, two-dimensional phosphopeptide mapping, kinase assay, and immunoblotting using a Tyr-530 phosphospecific Src antibody (44-662, 0.25 μg/ml; BIOSOURCE International, Camarillo, CA). A non-phosphospecific Src antibody (SC-18, 0.4 μg/ml; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used as a control. The blots were developed using a horseradish peroxidase-conjugated antibody against rabbit IgG (SC-2030, Santa Cruz Biotechnology, and ECL reagents (Amersham Pharmacia Biotech). Src protein solutions used for injection contained 7.5–8.0 mg/ml protein in 20 mM Hepes (pH 7.5), 0.1 M NaCl, and 5 mM dithiothreitol. Protein concentrations were determined using a BCA assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard.

SH2 domain glutathione S-transferase fusion proteins were made in bacteria and purified as described previously (13, 28). SH2 domain protein solutions for injection contained 33 mg/ml protein and 330 mM calcium green dextran in 137 mM NaCl, 2.7 mM KCl, 8.1 mM NaHPO4, and 1.5 mM KH2PO4 (pH 7.2).

Microinjection—Starfish (Asterina miniata) were obtained from Marinus, Inc. (Long Beach, CA) and from Will Borgeson (Bodega Marine Lab, Bodega Bay, CA). Oocytes were collected as described previously (13). Quantitative microinjection was performed using mercury-filled micropipettes (37, 38). Calcium green dextran, Oregon green dUTP, and SH2 domain proteins were injected into immature oocytes; 1 μM 1-methyladenosine (Sigma) was then applied to cause oocyte maturation. In one set of experiments, we confirmed that PLCγ SH2 domains have the same effect on Ca^{2+} release, whether they are injected before or after oocyte maturation (see also Ref. 13). Src proteins were injected into mature eggs at first meiotic metaphase, 40–160 min after the initial injection. Injection volumes were 1–5% of the egg volume (3100 picoliters), and concentrations given in the text indicate the final values in the egg cytoplasm. IP3 (Culbiochem, San Diego, CA) was used as a 10 μM stock, and 5% of the egg volume was injected. All experiments were performed at 16–18 °C, with the eggs in natural sea water.

Calcium Measurements—Intracellular free Ca^{2+} measurements were made in eggs injected with 10 μM calcium green-10-kDa dextran (Molecular Probes, Eugene, OR), and calcium green fluorescence was detected with a photodiode (71182; Oriel Instruments, Stratford, CT) with bovine serum albumin as the standard.

Values were measured using a BCA assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard.

RESULTS

Injection of Starfish Eggs with Kinase Active Src Protein Causes Ca^{2+} Release—Human e-Src protein (amino acid residues 86–536) was produced in insect cells, and two major phosphorylation forms were purified: unphosphorylated (P0 Src) and monophosphorylated at Tyr-530 (PY530Src). Both phosphorylation forms contain the catalytic, SH2, and SH3 domains, but lack the NH2-terminal hydrophobic domain by which Src associates with membranes (Fig. 1). In the presence of P0 Src in the cytoplasm, P0 Src will rapidly autophosphorylate on Tyr-419 and acquire high enzymatic activity (see “Discussion”).

Injection of P0 Src into starfish eggs (370 μg/ml; 7.2 μM final concentration) caused Ca^{2+} release in all eggs tested (n = 31), beginning −1.6 min after injection (Fig. 2B, Table I). Ca^{2+} was detected using calcium green dextran; the fluorescence
reached a peak that was 74 ± 18% greater than the baseline level (S.D., n = 31). This Ca\textsuperscript{2+} increase was somewhat smaller than that seen at fertilization (Fig. 2A), where the peak fluorescence increase was 102 ± 15% (n = 5), using the same optical measurement conditions. The Ca\textsuperscript{2+} rise caused by Src protein injection usually lasted for several minutes, although the duration was generally somewhat shorter than at fertilization (compare Fig. 2, A and B). Src protein injection also caused partial or complete elevation of the fertilization envelope, indicating the occurrence of partial or complete cortical granule exocytosis. Ca\textsuperscript{2+} release in response to injection of Src protein was concentration dependent, and occurred in only 50% of eggs injected with P\textsuperscript{y}Src at a final concentration of 220 \mu g/ml (4.3 \mu M). Ca\textsuperscript{2+} release was not detected when the Src protein concentration was reduced to 75 \mu g/ml (1.5 \mu M) (Table I).

Confocal imaging of calcium green dextran fluorescence following Src injection (370 \mu g/ml) showed that the Ca\textsuperscript{2+} rise occurred in a wave that started after the characteristic delay described above, from a site near the plasma membrane toward which the solution had been expelled from the micropipette (Fig. 3) (n = 6). The Ca\textsuperscript{2+} wave closely resembled that seen at fertilization (see Refs. 13 and 28). It differed from the pattern of Ca\textsuperscript{2+} release seen when eggs were injected with IP\textsubscript{3}, where the Ca\textsuperscript{2+} release began immediately, and from the site of injection rather than from the egg surface (Fig. 3) (n = 4).

To examine whether the kinase activity of the Src protein was required for the Ca\textsuperscript{2+} release in response to injection of the protein, we purified, from the mixture of Src forms produced by the insect cells, Src protein that was monophosphorylated on the inhibitory tyrosine at the COOH terminus (PY530Src) (Fig. 3). In this phosphorylation state, the Src tyrosine kinase activity is down-regulated, even in the presence of ATP (30, 32, 39). Upon injection into starfish eggs (400 \mu g/ml = 7.8 \mu M), PY530Src caused little or no Ca\textsuperscript{2+} release; any calcium green dextran fluorescence increase that occurred was always less than 10% of the baseline fluorescence (Fig. 2C, Table I). These results indicate that kinase activity is required for Ca\textsuperscript{2+} release in response to Src injection.

**Src Protein Injection Causes DNA Synthesis**—The resumption of the cell cycle is a common feature of fertilization in all species; in the starfish *A. miniata*, this is marked by the occurrence of DNA synthesis at about 2 h after fertilization (40, 41). We examined whether injection of starfish eggs with P\textsuperscript{y}Src protein caused DNA synthesis.

DNA synthesis was detected by preinjecting the eggs with a fluorescent nucleotide analog, Oregon green dUTP (see Ref. 14, and "Experimental Procedures"). 2.5–5 h after injection of active Src protein (P\textsuperscript{y}Src), we examined the eggs using confocal microscopy. In 15 of 18 eggs, a condensed cluster of Oregon green dUTP-labeled chromatin was visible (Fig. 4). In 4 of these eggs, we confirmed that DNA synthesis had occurred by photobleaching to remove unincorporated Oregon green dUTP. Photobleaching in a region of the egg cytoplasm away from the chromatin did not remove the chromatin fluorescence; this showed that the fluorescent nucleotides in the chromatin region were no longer diffusible, indicating that they had been incorporated into DNA (see Ref. 14, and "Experimental Procedures"). In contrast, a parallel set of experiments showed that eggs injected with catalytically inhibited Src protein (PY530Src) did not undergo DNA synthesis (n = 3). In the PY530Src injected eggs, no Oregon green dUTP fluorescence remained in the egg cytoplasm after photobleaching. These
results showed that elevating Src kinase activity in starfish eggs stimulates DNA synthesis, as occurs at fertilization. Although some eggs subsequently showed multiple nuclei or irregular cleavage, no further development was observed. The failure of the Src-injected eggs to undergo normal cell division and development might be due to the absence of the sperm centriole that normally provides the mitosis organizing center in the fertilized egg (42).

Src Acts Upstream of PLCγ—To examine whether the Ca\(^{2+}\) release in response to injection of P'Src occurred by the same pathway as at fertilization, we investigated whether the P'Src response was inhibited by preinjection of the SH2 domains of PLCγ. As described previously (13), injection of PLCγ SH2 domains (1 mg/ml) delays and reduces Ca\(^{2+}\) release at fertilization (Fig. 5A). Injection of PLCγ SH2 domains (1 mg/ml) also had an inhibitory effect on Ca\(^{2+}\) release following injection of P'Src (Fig. 5B, Table I). Two of the 10 eggs tested showed no Ca\(^{2+}\) release in response to P'Src injection. Eight of the 10 eggs eventually released Ca\(^{2+}\), but the delay between injection of the P'Src and Ca\(^{2+}\) release was significantly longer than in eggs containing the SH2 domains of a control protein, the phosphatase SHP2 (Fig. 5C, Table I). Tests of PLCγ and SHP2 SH2 domains were carried out alternately in each set of experiments. Although the peak amplitude of the calcium green fluorescence in P'Src injected eggs containing PLCγ SH2 domains was not significantly different from that in eggs containing control SH2 domains, the Ca\(^{2+}\) elevation in the PLCγ SH2-injected eggs was usually shorter in duration and often consisted of several brief peaks instead of the sustained rise observed in the control-injected eggs (compare Fig. 5, B and C). The increase in the delay between P'Src injection and Ca\(^{2+}\) release, caused by PLCγ SH2 domains, indicates that both fertilization and P'Src initiate Ca\(^{2+}\) release by way of PLCγ.

The SH2 Domain of Src Interacts with an Upstream Regulator in the Pathway Leading to Ca\(^{2+}\) Release—Injection of starfish eggs with the SH2 domain of Src also delays and inhibits Ca\(^{2+}\) release at fertilization (Ref. 28; Fig. 6A). This could result from an inhibition of the interaction of a Src-like kinase either with an upstream regulator of Src activation, or with a downstream target of the activated kinase. To examine these alternatives, we investigated whether injecting eggs with Src SH2 domains inhibited Ca\(^{2+}\) release in response to subsequent injection of P’Src. We found that Src SH2 domains did not prevent the Ca\(^{2+}\) rise in response to P’Src, and did not significantly increase the delay between P’Src injection and the Ca\(^{2+}\) rise (Fig. 6B Table I), or reduce the amplitude of the Ca\(^{2+}\) rise relative to that in eggs preinjected with SH2 domains of a control protein, the tyrosine kinase Abl. These results support

**Fig. 3. Microinjection of unphosphorylated Src initiates Ca\(^{2+}\) release in a wave starting near the plasma membrane, while microinjection of IP\(_3\) initiates Ca\(^{2+}\) release starting at the injection site.** Starfish eggs injected with 10 \(\mu\)M calcium green dextran were injected with P’Src (370 \(\mu\)g/ml = 7.2 \(\mu\)M) or IP\(_3\) (0.5 \(\mu\)M), while recording confocal images at 0.5-s intervals. Concentrations indicate final values in the egg cytoplasm. Time post-injection (s) is indicated on each panel. Each image pair shows a scanning transmission image (left) and calcium green fluorescence (right). The injection pipette enters the egg from the left. The first image pair in each sequence is before injection. The second image pair shows the moment of injection as indicated by the oil droplet at the tip of the micropipette. In the P’Src sequence, a small Ca\(^{2+}\) transient at the injection site was followed by a rapid return to baseline. No further Ca\(^{2+}\) increase was observed until 70 s post-injection, when a wave of Ca\(^{2+}\) release began from the right side of the egg. In the IP\(_3\) sequence, Ca\(^{2+}\) release began at the injection site, immediately after injection, and spread out to the plasma membrane by 2 s post-injection. Scale bar = 100 \(\mu\)M. Quicktime movies showing these sequences are available online. Each movie is composed of confocal images taken at 2 frames/s, and played back at 10 frames/s (5 \(\times\) real time).
Fig. 4. Microinjection of unphosphorylated Src initiates DNA synthesis. A starfish egg preinjected with 1 μg Oregon green dUTP was injected with P–Src (370 μg/ml final concentration) and incubated for 2.7 h at 18 °C before imaging by confocal microscopy. The bright structure within the egg is fluorescently labeled chromatin, and is positioned near the site where the meiotic divisions produced polar bodies. The small round structure outside the egg surface is a polar body. Scale bar = 10 μm.

the conclusion that in the pathway leading to PLCγ activation and Ca2+ release at fertilization, the requirement for a Src SH2 domain interaction is upstream of the activation of the endogenous Src-like kinase.

DISCUSSION

Src Tyrosine Phosphorylation and Kinase Activity—By injecting starfish eggs with the tyrosine kinase Src, we have demonstrated that Src kinase activity is sufficient to initiate Ca2+ release quite similar to that at fertilization, and to initiate DNA synthesis as occurs at fertilization. Two forms of the Src protein were used, P–Src, in which none of the tyrosines are phosphorylated, and PY530Src, which is phosphorylated on Tyr-530 only. Upon exposure to ATP in the egg cytoplasm, >80% of P–Src is expected to rapidly autophosphorylate on Tyr-419, leading to activation of enzymatic activity (32). In vitro at 25 °C, in the presence of 1 mM ATP (comparable to that in the egg cytoplasm; Ref. 45), Tyr-419 phosphorylation occurs within <2 min (32). Thus the time required for autophosphorylation could account for a part of the ~1.6 min delay that we observe between injection of the Src protein and the Ca2+ rise. Diffusion of the Src protein in the egg cytoplasm, and the time required for steps leading to PLCγ activation and IP3-induced Ca2+ release, could also contribute to the delay. The Ca2+ rise in response to P–Src injection, while similar to that at fertilization, is not identical, being somewhat smaller in amplitude and duration. This could reflect the fact that in the presence of ATP, Src protein gradually autophosphorylates on Tyr-530 and autodephosphorylates on Tyr-419, such that the maximally active form of the protein is transient (32). Furthermore, our injection conditions do not precisely mimic the conditions under which a Src family kinase may be activated at fertilization; for example, in the continuing presence of an activator of Src, the PY419 state might be maintained for a longer time. Decreased membrane binding of the injected Src protein, due to its lack of a myristoylation site at the amino terminus, could be another significant factor.

PY530Src is also expected to undergo some autophosphorylation on Tyr-419 when exposed to ATP in the egg cytoplasm, but the activity of Src that is phosphorylated on both Tyr-419 and Tyr-530 is ~20% of that for Src phosphorylated on PY419 only (32). Therefore, injection of PY530Src should introduce much less kinase activity in the egg cytoplasm compared with injection of P–Src. Correspondingly, PY530Src did not cause Ca2+ release.

In previous studies, Src family kinases have been introduced into cells by viral infection, transfection of DNA, and injection of RNA. These studies have shown that overexpression of constitutively active Src mutants causes various downstream cellular responses, including unregulated cell division and cytoskeletal rearrangements (e.g. Refs. 44 and 45). Here we introduced Src into a cell directly as a purified recombinant protein, allowing us to study a rapid response (Ca2+ release) to a step increase in the amount of Src protein in the cell, and to test the differential effects of two distinct phosphorylated forms of Src. Only the kinase active form of Src results in Ca2+ release and initiation of DNA synthesis.

Signaling Pathways at Fertilization—As summarized in the Introduction, recent evidence indicates requirements for both PLCγ and a Src family kinase in the signaling pathway leading to Ca2+ release at fertilization. The findings reported in this paper establish that the Src-like kinase acts upstream of PLCγ, since injection of PLCγ SH2 domains prevents or delays Ca2+ release in response to injection of active Src protein. What intermediate molecules may function in this pathway are unknown, but studies of immune cells and platelets suggest that intermediate kinases such as Syk and ZAP-70, and/or linker proteins such as LAT or SLP-76, may be involved (25, 26, 46). Injection of starfish eggs with SH2 domains of mammalian Syk and ZAP-70 does not inhibit Ca2+ release at fertilization (28), but there may be different intermediate kinases in the starfish egg. A kinase cascade, if it included a positive feedback loop, could serve to amplify a local signal at the site of sperm-egg interaction (see Ref. 47).

PLCγ activation at fertilization leads to IP3 production and Ca2+ release from the endoplasmic reticulum (see Introduction). Consequences of the Ca2+ rise include exocytosis of cortical granules, which establishes a block to polyspermy (3), and inactivation of mitogen-activated protein kinase, which leads to the initiation of DNA synthesis (5, 14, 41, 48). PLCγ activation also results in production of diacylglycerol, which may stimulate other egg activation events (see 49).

This model for echinoderm egg activation at fertilization applies to vertebrate eggs in some but not all aspects. At fertilization, vertebrate eggs also produce IP3 and release Ca2+, leading to cortical granule exocytosis and resumption of the cell cycle (4, 9, 50, 51). However, Ca2+ release at fertilization in frog and mouse eggs is not inhibited by excess PLCγ SH2 domains, indicating that if PLCγ is activated, it is not by an SH2 domain-dependent mechanism (52, 53). Nevertheless, in frog eggs, a Src-like kinase becomes tyrosine phosphorylated within 1 min of insemination (54), and tyrosine kinase inhibitors inhibit Ca2+ release and other activation events at fertilization (55–57). Likewise, both PLC and tyrosine kinase inhibitors show some inhibitory effects on Ca2+ release at fertilization in mouse eggs (58). These findings indicate that tyrosine kinases function in vertebrate fertilization, but the connection between these kinases and IP3 production is not understood.

Initiation of Src Family Kinase Activation at Fertilization—Injection of starfish eggs with excess SH2 domains of Src, which inhibits Ca2+ release at fertilization, does not inhibit Ca2+ release caused by Src protein injection. This indicates that the Src SH2 domain functions upstream of the activation of the Src family kinase in the pathway leading to Ca2+ release at fertilization. Therefore, whatever is directly upstream of the

2 W. Xu, unpublished results.
Src family kinase should have a binding site for the Src SH2 domain. Activation of Src family kinases in cells is hypothesized to occur by at least four different means (30, 33, 35, 59–61). 1) Tyrosine 419 might be phosphorylated, causing a conformational change that activates the kinase. 2) Dephosphorylation of the COOH-terminal tyrosine (Tyr-530) could release this tyrosine from its intramolecular association with Src’s SH2 domain, and thus disrupt the inhibitory closed conformation of Src and result in an active tyrosine kinase. 3) Interaction of Src’s SH2 domain with a phosphorylated tyrosine on another protein could outcompete the binding of the SH2 domain to the COOH-terminal tyrosine, and thus disrupt the closed conformation. 4) Interaction of Src’s SH3 domain with a high affinity proline-rich ligand might also open up Src’s protein structure. Our findings support the third possibility, since this model would account for the inhibition of Ca^{2+} release at fertilization by excess SH2 domains, and the lack of effect of excess SH3 domains (28).

Proteins that activate Src by binding to Src’s SH2 domain include the platelet-derived growth factor receptor (see Ref. 59), antigen receptors, by way of their “immune receptor tyrosine activation motifs” (62), and the focal adhesion kinase FAK (59), antigen receptors, by way of their “immune receptor tyrosine activation motifs” (62), and the focal adhesion kinase FAK

REFERENCES

1. Ridgway, E. B., Gilkey, J. C., and Jaffe, L. F. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 623–627
2. Stricker, S. A. (1999) Dev. Biol. 211, 157–176
3. Zucker, R. S., and Steinhardt, R. A. (1978) Biochim. Biophys. Acta 541, 459–465
4. Kline, D. (1988) Dev. Biol. 126, 346–361
5. Carroll, D. J., Albay, D. T., Hoang, K. M., O’Neill, F. J., Kumano, M., and Foltz, K. R. (2000) Dev. Biol. 217, 179–191
6. Whitaker, M., and Irvine, R. F. (1984) Nature 312, 636–639
7. Ciapa, B., and Whitaker, M. (1996) FEBS Lett. 395, 347–351
8. Terasaki, M., and Sardet, C. (1991) J. Cell Biol. 115, 1031–1037
9. Miyazaki, S., Yuzaki, M., Nakada, K., Shirakawa, H., Nakanishi, S., Nakade, S., and Mikoshiba, K. (1999) Science 285, 251–255
10. Mohri, T., Ise, A. I., and Chambers, E. L. (1995) Dev. Biol. 172, 139–157
11. Rhee, S. G., and Bae, T. S. (1997) J. Biol. Chem. 272, 15455–15458
12. Rongish, B. J., Wu, W., and Kinsey, W. H. (1999) Dev. Biol. 215, 147–154
13. Carroll, D. J., Harper, C. S., Mehlmann, L. M., Roche, S., Terasaki, M., and Jaffe, L. A. (1997) J. Cell Biol. 138, 1303–1311
14. Carroll, D. J., Albay, D. T., Terasaki, M., Jaffe, L. A., and Foltz, K. R. (1999) Dev. Biol. 216, 232–247
15. Shearer, J., De Nadai, C., Emily-Fenouil, F., Gache, C., Whitaker, M., and Ciapa, B. (1999) Development 126, 2273–2284
16. Pawson, T. (1995) Nature 372, 573–580
17. Kamat, A., and Carpenter, G. (1997) Cytokine Growth Factor Rev. 8, 109–117
18. Singer, W. D., Brown, H. A., and Sternweis, P. C. (1997) Annu. Rev. Biochem. 66, 475–509
19. Irvine, R. F. (1998) Curr. Biol. 8, R557–R559
20. Zhang, X., Chopp, R. H., Akiyama, T., Owen, J. D., Ruest, P. J., Carpenter, G., and Hancock, S. B. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9021–9026
21. De Nadai, C., Ciapa, B., and Ciapa, B. (1998) Dev. Neurobiol. 29, 669–676
22. Ciapa, B., and Edep, D. (1991) FEBS Lett. 295, 167–170
23. Shen, S. S., Kinsey, W. H., and Lee, J.-J. (1999) Dev. Growth Differ. 41, 345–355
24. Arkinstall, S., Payton, M., and Maundrell, K. (1995) Mol. Cell. Biol. 15, 1431–1438
25. Melford, S. K., Turner, M., Bridgen, S. J., Tybulewicz, V. L. J., and Watson, S. P. (1997) J. Biol. Chem. 272, 27539–27542
26. Clements, J. L., and Carpenter, G. (1999) J. Clin. Invest. 103, 925–929
27. Giusti, A. F., Carroll, D. J., Abassi, Y. A., and Foltz, K. R. (1999) Dev. Biol. 216, 189–199
28. Giusti, A. F., Carroll, D. J., Abassi, Y. A., Terasaki, M., Foltz, K. R., and Jaffe, L. A. (1999) J. Biol. Chem. 274, 28316–28322
29. Abassi, Y. A., Carroll, D. J., Giusti, A. F., Belton, R. J., and Foltz, K. R. (2000) *Dev. Biol.* 218, 206–219
30. Brown, M. T., and Cooper, J. A. (1996) *Biochim. Biophys. Acta* 1287, 121–149
31. Barker, S. C., Kassel, D. B., Weigl, D., Huang, X., Luther, M. A., and Knight, W. B. (1995) *Biochemistry* 34, 14843–14851
32. Boerner, R. J., Kassel, D. B., Barker, S. C., Ellis, B., DeLacy, P., and Knight, W. B. (1996) *Biochemistry* 35, 9519–9525
33. Xu, W., Harrison, S. C., and Eck, M. J. (1997) *Nature* 385, 595–602
34. Xu, W., Doshi, A., Lei, M., Eck, M. J., and Harrison, S. C. (1999) *Mol. Cell* 3, 629–638
35. Sicheri, F., Moarefi, I., and Kuriyan, J. (1997) *Nature* 385, 602–609
36. Williams, J. C., Weijland, A., Gonfloni, S., Thompson, A., Courtneidge, S. A., Superti-Furga, G., and Wierenga, R. K. (1997) *J. Mol. Biol.* 274, 757–775
37. Hiramoto, Y. (1962) *Exp. Cell Res.* 27, 416–426
38. Kiehart, D. P. (1982) *Methods Cell Biol.* 25, 13–31
39. Moarefi, I., LaFevre-Bernt, M., Sicheri, F., Huse, M., Lee, C.-H., Kuriyan, J., and Miller, W. T. (1997) *Nature* 385, 650–656
40. Carroll, D. J., and Jaffe, L. A. (1995) *Dev. Biol.* 170, 690–700
41. Sadler, K. C., and Ruderman, J. V. (1998) *Dev. Biol.* 197, 25–38
42. Wachstani-Nemoto, S., Saitoh, C., and Nemoto, S. (1994) *Dev. Biol.* 163, 293–301
43. Epel, D. (1969) *Exp. Cell Res.* 58, 312–319
44. Unger, T. F., and Steele, R. E. (1992) *Mol. Cell. Biol.* 12, 5485–5498
45. Thorn, J. F., Armstrong, N. A., Cantrell, L. A., and Kay, R. K. (1999) *Zygote* 7, 113–122
46. Law, C.-L., Chandran, K. A., Sidorenko, S. P., and Clark, E. A. (1996) *Mol. Cell. Biol.* 16, 1305–1315
47. Ferrell, J. E. (1999) *BioEssays* 21, 866–870
48. Tachibana, K., Machida, T., Nomura, Y., and Kishimoto, T. (1997) *EMBO J.* 16, 4333–4339
49. Shen, S. S., and Buck, W. R. (1990) *Dev. Biol.* 140, 272–280
50. Kline, D. J., and Kline, J. T. (1992) *Dev. Biol.* 149, 80–89
51. Stith, B. J., Gaolstone, M., Silva, S., and Jaynes, C. (1993) *Mol. Biol. Cell* 4, 435–443
52. Mehmann, L. M., Carpenter, G., Rhee, S. G., and Jaffe, L. A. (1998) *Dev. Biol.* 203, 221–232
53. Runfö, L. L., Watras, J., and Jaffe, L. A. (1999) *Dev. Biol.* 214, 399–411
54. Sato, K., Aoto, M., Morii, K., Akasofu, S., Tokmakov, A. A., Sahara, S., and Fukami, Y. (1996) *J. Biol. Chem.* 271, 13250–13257
55. Sato, K., Iwao, Y., Fujimura, T., Tamaki, I., Aoto, M., Tokmakov, A. A., and Fukami, Y. (1998) *FEBS Lett.* 424, 113–118
56. Sato, K., Iwao, Y., Fujimura, T., Tamaki, I., Ogawa, K., Iwasaki, T., Tokmakov, A. A., Hatano, O., and Fukami, Y. (1999) *Dev. Biol.* 209, 308–320
57. Glahn, D., Mark, S. D., Behr, R. K., and Nuccitelli, R. (1999) *Dev. Biol.* 205, 171–180
58. Dupont, G., McGuinness, O. M., Johnsen, M. H., Berridge, M. J., and Bergese, F. (1996) *Biochem. J.* 316, 583–591
59. Erpel, T., and Courtneidge, S. (1995) *Curr. Opin. Cell Biol.* 7, 176–182
60. Hardwick, J. S., and Sefton, B. M. (1997) *J. Biol. Chem.* 272, 25429–25432
61. Thomas, M. L. (1999) *Curr. Opin. Immunol.* 11, 270–276
62. Johnson, S. A., Pleiman, C. M., Pao, L., Schneringer, J., Hippen, K., and Cambier, J. C. (1995) *J. Immunol.* 155, 4596–4603
63. Thomas, J. W., Ellis, B., Boerner, R. J., Knight, W. B., White, G. C., II, and Schaller, M. D. (1998) *J. Biol. Chem.* 273, 577–583
64. Schaller, M. D., Hildebrand, J. D., and Parsons, J. T. (1999) *Mol. Biol. Cell* 10, 3489–3505
65. Miyazaki, S., Ohmori, H., and Sasaki, S. (1975) *J. Physiol.* 246, 37–54
66. Miyazaki, S., and Hirai, S. (1979) *Dev. Biol.* 70, 327–340
67. McCulloh, D. H., and Chambers, E. L. (1992) *J. Gen. Physiol.* 99, 137–175
Evidence That Fertilization Activates Starfish Eggs by Sequential Activation of a Src-like Kinase and Phospholipase Cγ
Andrew F. Giusti, Wenqing Xu, Beth Hinkle, Mark Terasaki and Laurinda A. Jaffe

J. Biol. Chem. 2000, 275:16788-16794. doi: 10.1074/jbc.M001091200 originally published online March 22, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M001091200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 67 references, 21 of which can be accessed free at http://www.jbc.org/content/275/22/16788.full.html#ref-list-1