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Biochemistry

A protein kinase from *Xenopus* eggs specific for ribosomal protein S6

(ribosomal protein S6 protein kinase/growth control/S6 phosphopeptide mapping)

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ABSTRACT **A protein kinase specific for ribosomal protein S6 has been purified from eggs of *Xenopus laevis*. As visualized on a silver-stained polyacrylamide gel, the major protein in the preparation migrated with a M_r of 90,000. Incubation of the enzyme preparation with [γ-^{32}P]ATP led to phosphorylation of this protein on serine residues. Upon glycerol gradient centrifugation, the S6 kinase activity and the M_r 90,000 protein both sedimented with a M_r of 50,000–55,000. Two-dimensional gel electrophoresis demonstrated that up to 4–5 phosphate groups per S6 molecule could be incorporated with this enzyme *in vitro*, and two-dimensional peptide mapping demonstrated that the phosphopeptides from S6 labeled *in vitro* with the enzyme comigrated with those from highly phosphorylated S6 labeled *in vivo* in response to progesterone treatment. The purified S6 protein kinase did not phosphorylate at a significant rate ribosomal protein S10, histone H1, histone H4, mixed histones, casein, or phosvitin, indicating a high degree of substrate specificity. These results indicate that activation of a single S6 protein kinase may be sufficient to account for increased S6 phosphorylation after a growth stimulus.**

Phosphorylation of proteins is believed to play a major role in the control of cell proliferation. Part of the evidence supporting this belief has been the finding that a number of viral oncogenes encode tyrosine-specific protein kinases, and the amount of phosphotyrosine in protein increases during viral transformation (1–9). Moreover, the receptors for several growth factors, including epidermal growth factor, platelet-derived growth factor, insulin-like growth factor 1, and insulin, also express a tyrosine protein kinase activity (10–14). In addition to an increase in phosphorylation of protein on tyrosine residues, several of these growth factors and viral oncogenes have been reported to cause an increase in phosphorylation of proteins on serine residues (15–19). One protein in which phosphorylation on serine is associated with the action of all of these growth factors and with several viral oncogenes is ribosomal protein S6 (15, 16, 20–23).

The step(s) that S6 phosphorylation affects in protein synthesis has not been established, but several lines of evidence suggest a role in recruitment of specific mRNAs. In 3T3 cells, ribosomes containing maximally phosphorylated S6 molecules (4–5 mol of phosphate per mol of S6) are incorporated preferentially into new polysomes after serum stimulation of growth (16, 24), and the synthesis of many of the new proteins after stimulation is under translational control (25). In ribosomes from heat-shocked *Drosophila* cells, S6 is dephosphorylated, and these ribosomes exhibit a reduced ability to translate certain species of mRNA (26, 27). An increase in S6 phosphorylation correlates with increased protein synthesis in *Xenopus* oocytes induced to mature with progesterone or insulin, and the mechanism of increased protein synthesis has been reported to occur largely as a result of mRNA recruitment (28–30). Increased S6 phosphorylation is also associated with the response of oocytes to the maturation-promoting factor, a cytoplasmic factor that causes an immediate increase in protein phosphorylation and rapid maturation of oocytes after microinjection (28, 31). Recent studies indicate an increase in the binding and translation of poly(AUG) in a reticulocyte lysate supplemented with phosphorylated 40S subunits (32). These results suggest that phosphorylation of S6 may provide a means to recruit specific mRNA species associated with the activation of growth.

The fact that growth-promoting agents associated with tyrosine protein kinase activity cause an increase in serine phosphorylation of S6 suggests that the activity of a serine kinase or phosphatase may be under the direct or indirect control of a tyrosine protein kinase. This concept has been supported by studies with *Xenopus* oocytes, which exhibit a significant increase in S6 phosphorylation on serine after microinjection of the transforming protein of Rous sarcoma virus, pp60$^{v\text{-}src}$, or of the tyrosine kinase encoded by Abelson murine leukemia virus (23, 33). These effects of tyrosine protein kinases could reflect the direct phosphorylation of an S6 serine kinase or phosphatase, or there could be a number of steps between the expression of tyrosine kinase activity and altered activity of S6 enzymes. To study the interaction between tyrosine kinases and S6 serine kinases, we have undertaken the purification of S6 serine protein kinases from *Xenopus* eggs. A number of protein kinases have been described from various sources that are able to phosphorylate S6 (35–40). Many of these have been purified initially using substrates other than S6, and none of the enzymes were homogeneous except for cAMP-dependent kinase, which is able to phosphorylate only some of the sites in S6. In this paper, we describe an S6 serine protein kinase from *Xenopus* eggs that is able to phosphorylate all the sites in S6 associated with growth-promoting stimuli. The relationship of this protein kinase to other S6 kinases is discussed.

MATERIALS AND METHODS

Female *Xenopus laevis* were obtained from Xenopus I (Ann Arbor, MI). For production of eggs, animals were primed with 70 international units of pregnant mare's serum gonadotropin (Calbiochem) and 36 hr later were injected with 700 international units of human chorionic gonadotropin (Sigma). Eggs were collected in tap water containing 0.1 M NaCl and dejellied with 2% cysteine (pH 8). Mixed histones, phosvitin, and casein were from Sigma and bovine serum albumin (Pentex, fraction V) was from Miles. Calf thymus histone H1 was a gift from T. A. Langan (Department of Pharmacology) and histone H4 was a gift from R. A. Masaracchia (North

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Abbreviation: PAK, protease-activated kinase.
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Texas State University, Denton, TX). Ultrapure urea and sucrose were from Schwarz/Mann. [γ-^{32}P]ATP was synthesized by the method of Johnson and Walseth (41). The catalytic subunit of type II bovine heart cAMP-dependent protein kinase was purified by the triple-column procedure described by Beavo *et al.* (42), and protein kinase inhibitor was purified from rabbit skeletal muscle by the method of Walsh *et al.* (43). The specific activity of the catalytic subunit was 10.5 μmol/min per mg with Kemptide as substrate and 36 nmol/min per mg with *Xenopus* oocyte 40S subunits as substrate. The value with 40S subunits was not measured under V_{max} conditions.

Purification of *Xenopus* Egg S6 Protein Kinase. Eggs were homogenized in a buffer similar to that designed for stabilization of maturation-promoting factor activity (44), consisting of 55 mM β-glycerophosphate, pH 6.8/5 mM EGTA/5 mM $MgCl_2$/50 mM NaF/100 mM $Na_4P_2O_7$/5 mM phosphotyrosine/2 mM dithiothreitol/0.1 mM phenylmethylsulfonyl fluoride. Briefly, the procedure, which will be described in detail elsewhere, involved ammonium sulfate precipitation and successive chromatography on DEAE-Sephacel, phosphocellulose, Sephacryl S200, and the Mono S and Mono Q columns of a Pharmacia fast protein liquid chromatography system. The peak of this enzyme activity on DEAE-Sephacel accounted for >80% of the S6 kinase activity recovered from the column, and this activity fractionated as a single peak in all subsequent steps. The Mono Q enzyme (Fig. 1*A*) was purified >400-fold with a yield of ≈3% and had a specific activity of 41 nmol/min per mg with *Xenopus* 40S ribosomal subunits. The enzyme was stable when stored at −20°C in 55 mM β-glycerophosphate/5 mM EGTA/2 mM

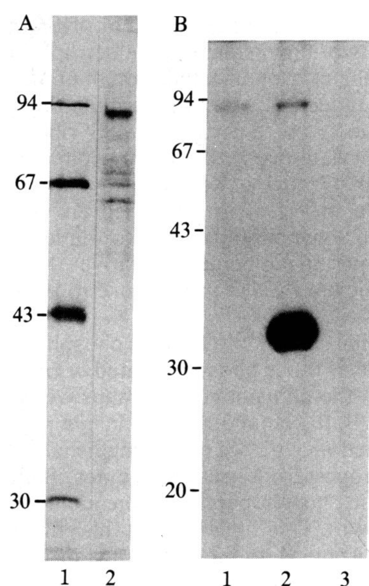

FIG. 1. (*A*) Silver-stained gel of the *Xenopus* S6 kinase preparation. The S6 kinase was purified through chromatography on a Mono Q column. A sample of the preparation was electrophoresed through a 10% $NaDodSO_4$ gel and silver-stained by the procedure of Oakley *et al.* (48). Lane 1, molecular weight markers; lane 2, *Xenopus* S6 kinase preparation. (*B*) *In vitro* phosphorylation of ribosomal protein S6. Protein kinase reactions using the preparation shown in *A* were carried out in the presence and absence of 40S ribosomal subunits as described. The reactions were stopped by addition of 5× sample buffer and the products of the reaction were resolved by electrophoresis through a 12.5% $NaDodSO_4$/polyacrylamide gel. Lanes: 1, *Xenopus* S6 kinase alone; 2, *Xenopus* S6 kinase with 40S ribosomal subunits; 3, 40S ribosomal subunits alone. Other experiments have shown that the phosphorylated band in lane 1 comigrated with the major silver-stained band in *A* (lane 2). The positions of the molecular weight markers ($\times 10^{-3}$) are indicated on the left.

dithiothreitol/5 mM $MgCl_2$/50% (vol/vol) ethylene glycol, pH 6.8.

Purification of 40S Ribosomal Subunits. Ribosomes were prepared from *X. laevis* ovaries according to the procedure of Cox *et al.* (45). The S6 in these ribosomes is almost all in the dephosphorylated form (28). The ribosomes were resuspended in buffer containing 700 mM KCl, incubated at 30°C for 15 min, aggregates were removed by centrifugation at 2000 × *g* for 5 min, and the ribosomal subunits were fractionated by sucrose gradient centrifugation as described by Zasloff and Ochoa (46). The 40S ribosomal subunits were collected by centrifugation and resuspended in 50 mM Tris·HCl, pH 7.8/200 mM KCl/10 mM Mg acetate/0.1 mM EDTA/20 mM 2-mercaptoethanol/250 mM sucrose (46), mixed with an equal volume of ethylene glycol and stored at −20°C.

Protein Kinase Assay. Reactions were carried out for 15 min at 30°C in a final vol of 30 μl containing 20 mM Hepes, pH 7.0/5 mM 2-mercaptoethanol/3.3 mM $MgCl_2$/100 μM ATP/1 × 10^7 cpm of [γ-^{32}P]ATP. When 40S ribosomal subunits were used as substrate, 4 μl of the subunit preparation was added, yielding a final concentration of 0.67 mg of 40S subunits per ml. Under these conditions, incorporation of radiolabel into S6 was linear with time to at least 45 min and with amount of enzyme. Preliminary experiments indicate that this concentration of 40S subunits is below the K_m for the enzyme, and it was not technically possible to prepare 40S subunits at a concentration sufficient to saturate the enzyme. Reactions were terminated by the addition of 1/4th vol of 5× concentrated electrophoresis sample buffer [1× sample buffer = 0.07 M Tris·HCl, pH 6.8/11% (vol/vol) glycerol/3% $NaDodSO_4$/0.01% bromophenol blue/5% 2-mercaptoethanol], followed by incubation at 95°C for 1 min. The products of the reaction were resolved by $NaDodSO_4$/polyacrylamide gel electrophoresis through 12.5% gels prepared as described by Laemmli (47). Gels were either stained with Coomassie blue or silver-stained by the procedure of Oakley *et al.* (48). The S6 band was identified by staining and autoradiography, and radioactivity in S6 was quantified by liquid scintillation counting of excised gel bands. Proteins used as molecular weight standards were phosphorylase b, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; and soybean trypsin inhibitor, 20,100 (all from Pharmacia).

Glycerol Gradient Sedimentation Analysis. Samples were centrifuged in an SW55 rotor at 54,000 rpm for 20 hr at 3°C through linear glycerol gradients (10%–30%) containing 50 mM β-glycerophosphate, pH 6.8/5 mM $MgCl_2$/5 mM EGTA/25 mM NaCl/2 mM dithiothreitol/0.01% Brij 35. Proteins used as molecular weight standards were alcohol dehydrogenase (yeast; M_r, 143,000) and peroxidase (horseradish; M_r, 43,000) from Boehringer Mannheim, or ovalbumin and aldolase (M_r, 158,000) from Pharmacia.

Two-Dimensional Gel Electrophoresis. Two-dimensional acid and alkaline urea gel electrophoresis of ribosomal proteins was carried out by a modification of the procedure of Thomas *et al.* (16) as described (23). Prior to electrophoresis, the sample was mixed with carrier ribosomal protein from *Xenopus* eggs and *Xenopus* ovary. Gels were stained with Coomassie blue, dried, and autoradiographed on Kodak XRP film at −70°C with the aid of a Lightning Plus (Dupont) intensifying screen.

Two-Dimensional Phosphopeptide Mapping. Radiolabeled S6 was eluted from a preparative gel, 100 μg of bovine serum albumin was added, and the proteins were precipitated with trichloroacetic acid and washed as described by Collett *et al.* (49). The proteins were digested in 100 μl of 0.05 M NH_4HCO_3 (pH 7.8) with 20 μg of chymotrypsin (α-chymotrypsin; Worthington) for 1 hr at 37°C, then an additional 20 μg of chymotrypsin was added and digestion was continued for another 3 hr. After three lyophilizations from 1 ml of

H_2O, the phosphopeptides were dissolved in 0.2 M NH_4OH and spotted onto a plastic-backed cellulose sheet (Kodak). Phosphopeptides were resolved by electrophoresis at 760 V for 45 min at pH 1.5 [formic acid/acetic acid/water (1:3:16)] in the first dimension and at 760 V for 50 min at pH 3.5 [pyridine/acetic acid/water (1:10:89)] in the second dimension (50). The sheets were dried overnight and then autoradiographed.

RESULTS

Preliminary studies indicated that the S6 kinase specific activity was 5- to 10-fold higher in extracts of eggs than of oocytes. This difference was also evident in the major peak of S6 protein kinase activity eluted from DEAE-Sephacel, which was used for further purification steps. Fig. 1 presents the silver-staining pattern of the enzyme preparation used in the studies presented here. Upon NaDodSO$_4$/polyacrylamide gel electrophoresis, the major protein in the preparation migrated with a M_r of 90,000 (Fig. 1*A*). Incubation of the preparation with [γ-^{32}P]ATP revealed that this protein underwent phosphorylation (Fig. 1*B*). Moreover, incubation with 40S ribosomal subunits demonstrated that the enzyme was highly specific for S6, with no phosphorylation of other ribosomal proteins detectable (Fig. 1*B*). Phospho amino acid analysis of the M_r 90,000 protein and of S6 revealed exclusively phosphoserine (data not shown). When fractionated by centrifugation on a 10%–30% glycerol gradient, the S6 kinase activity sedimented with a M_r of 50,000–55,000 for the peak tube, using the markers described in *Materials and Methods*. In addition, the M_r 90,000 protein visualized by NaDodSO$_4$ gel electrophoresis and autoradiography cosedimented with the peak of enzyme activity (Fig. 2).

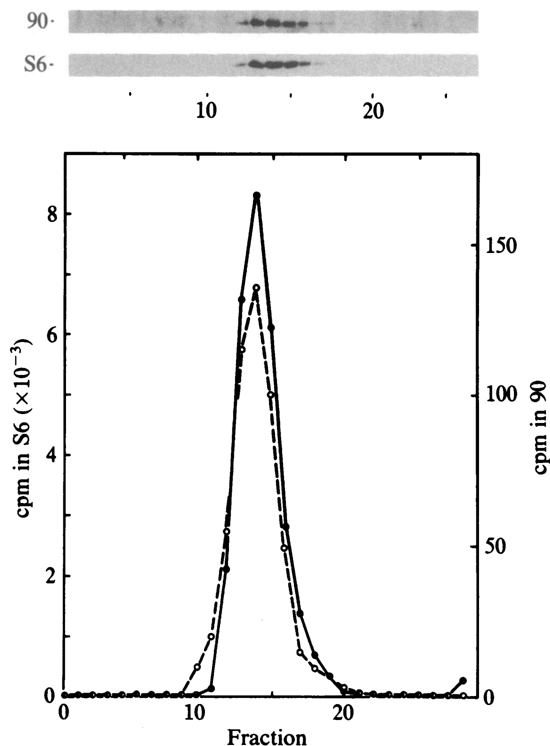

FIG. 2. (*Lower*) Sedimentation analysis of *Xenopus* S6 kinase activity. A sample of the S6 kinase preparation shown in Fig. 1*A* was centrifuged through a glycerol gradient. Fractions were collected and samples of each fraction were assayed for S6 kinase activity as described in *Materials and Methods*, except the specific activity of the ATP was increased 3-fold. In addition, samples of each fraction were incubated with [γ-^{32}P]ATP in the absence of 40S subunits to locate the M_r 90,000 protein. The products of the reaction were resolved by polyacrylamide gel electrophoresis, and the M_r 90,000 (90; ○) and S6 (●) regions were excised and counted. All of the S6 kinase and the autophosphorylated M_r 90,000 protein were recovered in fractions 12 to 18. Sedimentation was from right to left. (*Upper*) Autoradiograms of the M_r 90,000 (90) and S6 regions of the gels are shown.

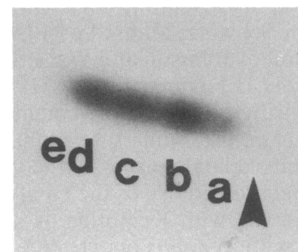

FIG. 3. Two-dimensional gel analysis of 40S ribosomal proteins phosphorylated by *Xenopus* S6 kinase. 40S ribosomal subunits were phosphorylated as described in the legend to Fig. 1 except the concentration of subunits was 0.1 mg/ml, 3 μg of the heat-stable inhibitor of cAMP-dependent protein kinase was added, and the reaction was carried out for 2 hr. The reaction was terminated by the addition of 100 μg of unlabeled 40S ribosomal subunits, 1/10th vol of 1 M Mg acetate, and 2 vol of glacial acetic acid. The ribosomal proteins were extracted, precipitated with acetone, and subjected to two-dimensional electrophoresis in the presence of carrier ribosomal proteins from *Xenopus* unfertilized eggs (phosphorylated S6) and *Xenopus* ovary (unphosphorylated S6) (28). Derivatives a–e (containing 1 to 5 mol of phosphate per mol of S6, respectively) are indicated, based on the Coomassie blue staining pattern of the carrier proteins. Arrow indicates position of unphosphorylated S6. Only the pertinent region of the autoradiogram is shown.

In vivo S6 exists in multiply phosphorylated forms, containing 0–5 mol of phosphate per mol of S6, which can be resolved by two-dimensional gel electrophoresis (16). To confirm that the phosphorylated protein of M_r 34,000 shown in Fig. 1 was indeed S6 and to determine the distribution of radiolabel among the derivatives, 40S ribosomal subunits were phosphorylated *in vitro*, and ribosomal protein was extracted and analyzed by two-dimensional gel electrophoresis. As shown in Fig. 3, the purified enzyme was able to phosphorylate some S6 molecules to the level of 4–5 phosphate groups per S6 molecule (derivatives d and e), the maximal level achieved *in vivo* in response to growth-promoting stimuli.

To determine whether the sites phosphorylated *in vitro* were the same as those phosphorylated *in vivo* in response to progesterone, the phosphopeptide maps were compared. As shown in Fig. 4, the chymotryptic phosphopeptide map of S6 phosphorylated *in vitro* was indistinguishable from the map of S6 from progesterone-treated oocytes. Similarly, the patterns of tryptic phosphopeptides were identical to each other (data not shown). This suggests that this enzyme preparation is able to phosphorylate the same sites *in vitro* as are phosphorylated *in vivo*. The presence of a complete set of phosphopeptides is consistent with the fact that all derivatives of S6 were radiolabeled with this enzyme *in vitro* (Fig. 3) and with the production of maximally phosphorylated S6 in response to progesterone treatment (28).

Previous reports of partially purified S6 protein kinases have indicated that a wide variety of substrates can be phosphorylated by these preparations. To better define the relationship of the *Xenopus* egg enzyme to previously described preparations, the substrate specificity of this enzyme was investigated and compared to that of the catalytic subunit of cAMP-dependent protein kinase. The specific activity of the purified egg enzyme for S6 (41 nmol/min per mg) was comparable to the specific activity of catalytic subunit for S6 (36 nmol/min per mg) under the assay conditions used in these experiments. However, as shown in Fig. 5, the *Xenopus* en-

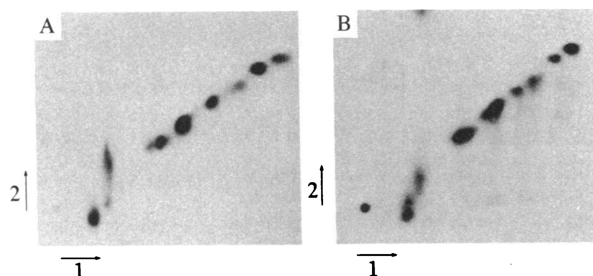

FIG. 4. Phosphopeptide maps of ribosomal protein S6 phosphorylated *in vitro* or isolated from progesterone-treated oocytes. (*A*) 40S ribosomal subunits were phosphorylated as described in the legend to Fig. 3, and the products of the reaction were resolved by $NaDodSO_4$/polyacrylamide gel electrophoresis. S6 was eluted, digested, and the phosphopeptides were resolved by two-dimensional thin layer electrophoresis followed by autoradiography. (*B*) Ribosomes were isolated from ^{32}P-labeled oocytes that had undergone germinal vesicle breakdown in response to progesterone. Such oocytes contain S6 in the highly phosphorylated derivatives (28). Ribosomal proteins were resolved by $NaDodSO_4$/polyacrylamide gel electrophoresis, and S6 was eluted, digested, and mapped as described in *A*.

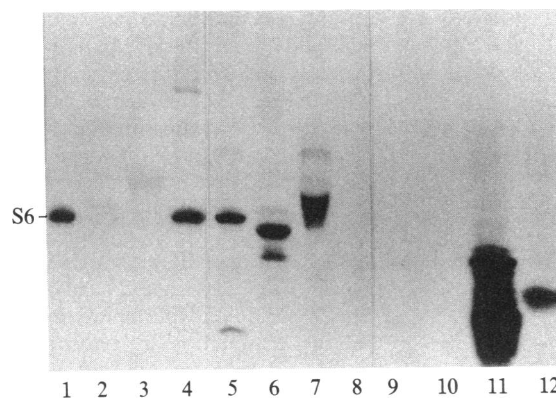

FIG. 5. Substrate specificity of the *Xenopus* egg S6 kinase. Various protein substrates were incubated with *Xenopus* S6 kinase or with the catalytic subunit of cAMP-dependent protein kinase. In these comparative studies, the amount of catalytic subunit of cAMP-dependent protein kinase used was chosen to give equivalent incorporation of radiolabel into S6 as was obtained with the amount of *Xenopus* egg S6 kinase in the reaction. Reactions were terminated by addition of 5× sample buffer and the products were resolved by $NaDodSO_4$ gel electrophoresis and autoradiography as described in *Materials and Methods*, except that 15% gels were used in lanes 9–12. Lanes: 1–4, 9, and 10, *Xenopus* S6 kinase; 5–8, 11, and 12, catalytic subunit; 1 and 5, 40S ribosomal subunits; 2 and 6, casein (830 μg/ml); 3 and 7, phosvitin (1 mg/ml); 4 and 8, 40S ribosomal subunits plus 3 μg of the heat-stable inhibitor of cAMP-dependent protein kinase; 9 and 11, mixed histones (2.5 mg/ml); 10 and 12, H4 histone (1.3 mg/ml).

zyme was unable to phosphorylate at a significant rate mixed histone, histone H4, phosvitin, or casein. Similarly, other experiments have shown that it was unable to phosphorylate histone H1 (data not shown). The enzyme was clearly much more specific for S6 than cAMP-dependent protein kinase (Fig. 5) or other previously characterized S6 protein kinases. In addition, the inability of the heat-stable inhibitor of cAMP-dependent protein kinase to block phosphorylation by the *Xenopus* enzyme (lanes 1 and 4) further confirms that the preparation is free of catalytic subunit activity.

DISCUSSION

The results presented in this paper describe a purified S6 protein kinase that is able to phosphorylate all the physiologically relevant sites observed in the maximally phosphorylated derivatives of S6 (4–5 mol of phosphate per mol of S6). The radiolabeled S6 phosphopeptides after *in vitro* phosphorylation comigrated with those from 3T3 cells after serum stimulation and from oocytes after microinjection of Abelson murine leukemia virus tyrosine-specific protein kinase (23). Previous studies by Martin-Perez and Thomas (50) have indicated that phosphorylation of S6 is ordered and that each derivative of S6 contains all the phosphopeptides of the preceding derivatives, plus one or more additional phosphopeptides. The phosphorylation of all the sites in S6 is stimulated after microinjection of the Abelson murine leukemia virus transforming kinase into oocytes, and the increase is consistent with an ordered mechanism (23). It remains to be determined whether phosphorylation of S6 *in vitro* by the *Xenopus* egg enzyme occurs in an ordered manner. Regardless of whether phosphorylation is ordered, it is possible that as many as five different protein kinases would be required to produce maximally phosphorylated S6. However, the results presented here suggest that a single protein kinase whose activity may be altered as a consequence of progesterone treatment, growth factor action, or oncogene expression might be sufficient to account fully for a maximal level of S6 phosphorylation.

The major silver-staining protein in the preparation, accounting for most of the protein present, had a M_r of 90,000 and was the only protein in the preparation to undergo phosphorylation upon incubation with [γ-^{32}P]ATP. The fact that most protein kinases undergo autophosphorylation reactions upon incubation with [γ-^{32}P]ATP (51) suggests that this M_r 90,000 protein may be the S6 protein kinase. More evidence in this regard is the glycerol gradient analysis, which demonstrated that essentially all the M_r 90,000 protein sedimented with the enzyme activity at a M_r of 50,000–55,000 (Fig. 2).

It is important to compare the properties of this S6 protein kinase with the properties of other S6 kinases described by various investigators. A significant feature of this enzyme is its high degree of specificity for S6 relative to other substrates frequently used for protein kinases *in vitro*. The enzyme did not utilize S10, casein, phosvitin, histones H1 or H4 as substrate, and only a slight phosphorylation of an unidentified protein in a mixed histone preparation was observed. The only S6 kinase that is homogeneous is the catalytic subunit of cAMP-dependent protein kinase. Under the assay conditions used here, the egg S6 kinase had a specific activity for *Xenopus* oocyte 40S subunits similar to that of catalytic subunit, a known S6 kinase *in vivo* (36). However, the egg S6 kinase is clearly distinct from cAMP-dependent kinase because it is unaffected by the specific heat-stable inhibitor of cAMP-dependent protein kinase (Fig. 5), because it is more specific for S6 (Fig. 5), and because it can phosphorylate all the sites in S6 (Figs. 3 and 4), whereas cAMP-dependent protein kinase can phosphorylate only two sites (52).

Lubben and Traugh have reported that a partially purified preparation of protease-activated kinase II (PAK II) is an S6 protein kinase (37). The PAK II proenzyme migrates on gel filtration with a M_r of 80,000, and the protease-activated species has a molecular weight of 45,000–55,000 (37). The possibility that the *Xenopus* egg enzyme described here is the proenzyme form of PAK II is remote because protease treatment under the conditions described by Traugh inactivated the *Xenopus* enzyme (data not shown). In addition, unlike PAK II, the *Xenopus* egg enzyme did not phosphorylate S10 or histone H1, and was not activated by phospholipids (data not shown). Perisic and Traugh have reported that partially purified PAK II preparations phosphorylate S6 *in vitro* at the same sites that are phosphorylated in 3T3 cells in response to insulin (34, 37). On this basis, they concluded that PAK II is the target S6 enzyme activated by insulin. However, the re-

sults presented here demonstrate that an S6 kinase with different properties can also phosphorylate all the sites in S6. Many phosphoproteins can be phosphorylated at the same site(s) by a variety of protein kinases, and clearly more data are needed to establish which S6 enzymes are regulated by hormones and growth factors.

Cobb and Rosen (38) reported that casein kinase I could phosphorylate S6 *in vitro* and suggested that it was the target for activation by the insulin receptor. This enzyme is clearly different from the enzyme described here, which did not phosphorylate casein (Fig. 5). Protein kinase C has also been reported to phosphorylate S6 in a phospholipid-stimulated fashion (35). In contrast, no stimulation by phospholipid, calcium, or diacylglycerol (in any combination) was observed with the egg enzyme (data not shown). In addition, protein kinase C has the ability to phosphorylate histones, and has a M_r of 77,000 on gel electrophoresis. Donahue and Masaracchia (39) reported purification of a histone H4 protein kinase that is also able to phosphorylate S6. The major protein in that preparation had a M_r of 82,000 but eluted on gel filtration with a M_r of 52,000 (39). Although that chromatographic behavior is similar to that of the enzyme described here, the *Xenopus* egg enzyme did not phosphorylate histone H4 (Fig. 5), indicating a fundamentally different substrate specificity.

Previous studies with *Xenopus* oocytes have shown that treatment with either progesterone or insulin, or microinjection of the tyrosine-specific protein kinases of Rous sarcoma virus or Abelson murine leukemia virus leads to phosphorylation of S6 on serine residues (23, 28, 30, 33). The possibility exists that this increased phosphorylation is achieved by activation or synthesis of the purified S6 enzyme described in this paper. The idea that the level of enzyme activity can be regulated is supported by the higher specific activity of the kinase in eggs as compared to oocytes. In addition to causing an increase in S6 phosphorylation on serine, $pp60^{v-src}$ also accelerates the rate of progesterone-induced meiotic maturation (33), suggesting that the pathway of regulation used by progesterone is related to the pathway utilized by tyrosine kinases. Regulation could involve a direct interaction of the S6 kinase with a tyrosine-specific protein kinase, or there could be one or more intermediate steps between expression of tyrosine kinase activity and increased S6 kinase activity. These possibilities are currently under investigation as part of the analysis of regulation of the *Xenopus* egg S6 enzyme during oocyte maturation.

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