A Purified S6 Kinase Kinase from *Xenopus* Eggs Activates S6 Kinase II and Autophosphorylates on Serine, Threonine, and Tyrosine Residues*

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S6 kinases I and II have been purified previously from *Xenopus* eggs and shown to be activated by phosphorylation on serine and threonine residues. An S6 kinase clone, closely related to S6 kinase II, was subsequently identified and the protein product was expressed in a baculovirus system. Using this protein, termed "rsk" for Ribosomal Protein S6 Kinase, as a substrate, we have purified to homogeneity from unfertilized *Xenopus* eggs a 41-kDa serine/threonine kinase termed rsk kinase. Both microtubule-associated protein-2 and myelin basic protein are good substrates for rsk kinase, whereas a-casein, histone H1, protamine, and phosvitin are not. rsk kinase is inhibited by low concentrations of heparin as well as by β-glycerophosphate and calcium. Activation of rsk kinase during *Xenopus* oocyte maturation is correlated with phosphorylation on threonine and tyrosine residues. However, in *vitro*, rsk kinase undergoes autophosphorylation on serine, threonine, and tyrosine residues, identifying it as a "dual specificity" enzyme. Purified rsk kinase can be inactivated in *vitro* by either a 37-kDa T-cell protein-tyrosine phosphatase or the serine/threonine protein phosphatase 2A. Phosphatase-treated S6KII can be reactivated by rsk kinase, and S6 kinase activity in resting oocyte extracts increases significantly when purified rsk kinase is added. The availability of purified rsk kinase will enhance study of the signal transduction pathway(s) regulating phosphorylation of ribosomal protein S6 in *Xenopus* oocytes.

Changes in the phosphorylation state of various proteins are a conserved response of cells to mitogenic stimuli. Phosphorylation of the 40 S ribosomal subunit protein S6 is an ubiquitous example of this phenomenon in higher eucaryotes (1–5). We have studied this event in *Xenopus laevis* oocytes, where S6 is phosphorylated during mitogen-induced meiotic maturation as part of a large burst of phosphorylation events that accompany activation of cdc2 kinase (6).

Two chromatographically and immunologically distinct kinases specific for S6, termed S6 kinase I and S6 kinase II, account for S6 phosphorylation in the maturing oocyte, and each has been purified and characterized from *Xenopus* eggs (1, 7). Both activated kinases contain phosphoserine and phosphothreonine residues when labeled *in vivo* (1, 8), and phosphorylation coincides with increased kinase activity during maturation (8). That direct phosphorylation underlies the mechanism of S6 kinase activation is also evident in the subsequent dephosphorylation and deactivation of S6 kinase upon fertilization or parthenogenetic activation of eggs (8).

An S6 kinase clone, isolated using oligonucleotide probes based on peptide sequences in purified S6 kinase II, has been expressed in both *Escherichia coli* and Sf9 cells infected with recombinant baculovirus (9, 10). The predicted amino acid sequence, however, does not contain the exact sequence of some of the peptides isolated from S6 kinase II, and therefore the recombinant protein product is termed rsk (Ribosomal protein S6 Kinase). When rsk is expressed in Sf9 cells it has very little S6 kinase activity, but rsk can be activated when Sf9 cells are coincubated with baculovirus expressing both rsk and pp60*src* (10). This increased activity appears to be dependent on phosphorylation of rsk (10).

The regulation of purified S6 kinase I and II by phosphorylation and dephosphorylation has also been studied in *vitro*. Treatment with phosphatase 2A results in a decrease in S6 kinase activity with either enzyme (1, 11). Significantly, S6 kinase II activity can be partially restored by addition of a 42-kDa protein kinase partially purified from 3T3-L1 cells (11). This kinase, termed MAP kinase for its original substrate (MAP-2) or for Mitogen-Activated Protein kinase, is activated just prior to the increase in S6 kinase activity when 3T3-L1 cells are treated with insulin (12). One form of MAP kinase has also been shown to become activated and phosphorylated in *Xenopus* oocytes and eggs in a cell cycle-related manner (13). Moreover, MAP kinase appears to be related to pp42, a phosphotyrosyl protein whose phosphorylation is correlated with the growth-promoting actions of many growth factors and transforming gene products (14, 15).

In addition to S6 kinase I and II and MAP-2, the 3T3 cell MAP kinase phosphorylates myelin basic protein (16) but not 40 S ribosomal subunits or other common protein kinase substrates such as histone H1 or casein (17). MAP kinase is phosphorylated on threonine and tyrosine residues (18), and its activity can be abolished in *vitro* by treatment with phosphatase 2A or CD45, which remove phosphate groups from either serine/threonine or tyrosine residues, respectively (19). MAP kinase is thus an upstream element in a protein kinase

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1 The abbreviations used are: rsk, ribosomal protein S6 kinase; MAP-2, microtubule-associated protein-2; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin S6 KII; S6 kinase II; GVBV, germinal vesicle breakdown.
cascade regulating S6 phosphorylation and is the best current example of a serine/threonine kinase activated by direct phosphorylation on tyrosine residues.

Recently, a number of kinases related to MAP kinase have been identified in a variety of cell types including: Swiss 3T3 cells (20), sea star oocytes (21), rat 1 HIRE B cells (22), rat liver cells (23), PC-12 cells (24, 25), and Xenopus eggs (26–28). The Mₙ of all of these kinases, with one exception (23), varies between 40,000 and 45,000. All share similar substrate specificity with the 3T3-L1 cell MAP kinase and, of those examined, all are inactivated by treatment with phosphatases. In addition, several partially purified MAP kinases have been shown to activate S6 kinases (29–31). A 70-kDa S6 kinase from rabbit liver, which is distinct from the rsk family of S6 kinases, has been reported to be activated by a fibroblast MAP kinase (5), but purified 70-kDa S6 kinase cannot be activated by MAP kinase preparations from several other laboratories (32, 33).

We are interested in elucidating the mechanism of S6 kinase activation by mitogens using the Xenopus oocyte maturation system as a model. Using rsk isolated from baculovirus-infected S9F cells as a substrate, we have purified a kinase that phosphorylates and activates a truncated, 37-kDa T-cell form (39) were gifts of Dr. N. K. Tonks.

Experimental Procedures

Materials—Female X. laevis were obtained from Xenopus 1 (Ann Arbor, MI). The RSK-α baculovirus constructs were a gift from Dr. R. L. Erikson (Harvard University), and the S9F cells were grown and infected in the cell culture facilities at the University of Colorado Cancer Center. [γ-32P]ATP was either prepared by the method of Johnson and Waileth (34) or obtained from ICN (Irvine, CA). Bovine serum albumin (Pentax, fraction V) used in densitometry was from Miles. Fast flow S-Phasophorae, DEAE-Sephacel, and G-25 PD-10 disposable columns were obtained from Pharmacia LKB Biotechnol-

ogy Inc., and hydroxyapatite was from Bio-Rad. The heat stable inhibitor protein of the CAMP-dependent protein kinase was prepared in this laboratory as described (36–38). The protein tyrosine phosphatases, CD45 and the spontaneously active, COOH terminally truncated, 37-kDa T-cell form (39) were gifts of Dr. N. K. Tonks (Cold Spring Harbor Laboratory). Bovine brain MAP-2 and 3T3-L1 cell MAP kinase were gifts of Dr. T. W. Sturgill (University of Virginia). Myelin basic protein, protinin, heparin (grade 1 from porcine intestinal mucosa), p-nitrophenyl phosphate, α-casein, and protamine and pregnant mare's serum gonadotropin were purchased from Calbiochem (La Jolla, CA).

rsk Purification—rsk protein was purified from insect cells infected with recombinant baculovirus. S9F cells were grown at 8 × 10⁶ cells/100-mm dish (six dishes total) and allowed to attach for 1 h. Recombinant baculovirus constructs containing the RSK-α clone were added to a multiplicity of infection of 10, and the cells were harvested after a 40-h incubation. The cells were washed off the dishes with phosphate-buffered saline, centrifuged at 1000 rpm for 10 min, resuspended in phosphate-buffered saline, pelleted again, and frozen at –70 °C. The cells were lysed in 15 ml of lysis buffer (10 mM potassium phosphate, 1 mM EDTA, 5 mM EGTA, 10 mM MgCl₂, 50 mM β-glycerophosphate, 1 mM Na₃VO₄, 2 mM dithiothreitol, 40 μg of phenylmethylsulfonyl fluoride/ml, 10 μg of leupeptin/ml, 10 μg of pepstatin/ml, pH 7.05) and clarified by centrifugation at 90,000 g for 30 min at 4 °C. The supernatant was loaded onto a 5-ml Fast Flow S column pre-equilibrated in buffer C (10 mM potassium phosphate, 1 mM EDTA, 5 mM EGTA, 10 mM MgCl₂, 50 mM β-glycerophosphate, 1 mM Na₃VO₄, 2 mM dithiothreitol, 0.05% Brij 35, 10% glycerol, pH 7.2) and eluted with 120 ml of 0–1 M NaCl in buffer C. Aliquots of selected fractions were electrophoresed in a 8% Laemml polyacrylamide gel (40), stained with Coomassie Blue, and fractions containing rsk were pooled and loaded onto a 4-ml hydroxyapatite (Bio-Gel HTP) column pre-equilibrated in buffer C1. Flow-through and wash fractions with protein were pooled and dialyzed overnight against C1 buffer. The protein was loaded on a 1-ml Fast S column and eluting with a 0.4 M NaCl step gradient. The fractions were analyzed by polyacrylamide gel electrophoresis and Coomassie Blue staining, and fractions with rsk were pooled and dialyzed overnight against C1 with 50% ethylene glycol. This protocol yielded 110 μg of rsk that had an apparent Mₙ of 83,000. The rsk from the phosphorylated by purified rsk kinase to a stoichiometry of approximately 0.72 mol of phosphate/mol of rsk. Phosphorylation of rsk by rsk kinase increased the apparent molecular weight of rsk in a polyacrylamide gel when compared with autophosphorylated rsk. This shift in apparent molecular weight has been noted previously (10, 30).

Kinase Assays—Unless otherwise indicated, all kinase assays were performed in: 20 mM HEPES, pH 7.0, 5 mM β-mercaptoethanol, 5 mM MgCl₂, 0.1 mg/ml BSA, 100 μM [γ-32P]ATP (1–5 cpm/nmol), 1.4 μg/ml rsk. Reactions were usually incubated for 10–15 min at 30 °C, terminated by the addition of the 0.25 volume of 5 × sample buffer (1× sample buffer contains 70 mM Tris- HCl, pH 6.8, 11% glycerol, 3% SDS, 0.01% bromphenol blue, 5% 2-mercaptoethanol), immersed in boiling water for 2–3 min, and electrophoresed in a 10% SDS-polyacrylamide gel. The labeled bands were identified by autoradiography, excised, and quantified by liquid scintillation spectrometry. Autoradiographs were analyzed using a Molecular Dynamics densitometer, and the density of the sample was compared with the densities of known amounts of BSA stained on the same gel. The sample buffer contains 70 mM Tris-HCl, pH 7.0, 5 mM β-mercaptoethanol, 11% glycerol, 3% SDS, 0.01% bromphenol blue, 5% 2-mercaptoethanol), immersed in boiling water for 2–3 min, and electrophoresed in a 10% SDS-polyacrylamide gel. The labeled bands were identified by autoradiography, excised, and quantified by liquid scintillation spectrometry. Autoradiographs were analyzed using a Molecular Dynamics densitometer, and the density of the sample was compared with the densities of known amounts of BSA stained on the same gel.

The reaction was stopped by the addition of 5 × sample buffer, and the samples were resolved on a 10% SDS-polyacrylamide gel. Protein phosphorylations and is the best current example of a serine/threonine kinase activated by direct phosphorylation on tyrosine residues.

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RESULTS

rsk Kinase Purification—Unfertilized Xenopus eggs arrested at metaphase of meiosis II exhibit maximal S6 kinase activity (8) and were used as a source of rsk kinase. Following homogenization and a low speed centrifugation, the homogenate was loaded onto a DEAE-Sephacel column and eluted with increasing NaCl (Fig. 1). As found for 3T3 cell MAP kinase previously (17), it is important to inhibit phosphatase activity at this stage of the purification by addition of p-nitrophenyl phosphate to the collection tubes prior to elution (final concentration 40 mM). When the phosphatase inhibitor was not used, the yield of rsk kinase activity obtained was decreased about 4-fold. In all preparations to date, one peak of activity eluting at 190 mM NaCl was consistently present, and a second, much smaller, peak of activity at 120 mM NaCl was occasionally seen. The shoulder on the 190 mM peak does not appear to represent a distinct kinase since the activity elutes as one peak on all subsequent columns.

Peak fractions of the 190 mM DEAE-Sephacel peak (usually six to seven 2.5-ml fractions) were pooled and concentrated by precipitation with 40% ammonium sulfate prior to gel filtration chromatography on a TSK G3000SW column. The sample was gel filtered in two consecutive runs due to the amount of protein present. The activity eluted as a very broad peak, well after the protein markers and the p-nitrophenyl phosphate (Fig. 2). The unusual retention of the rsk kinase activity was probably due to adsorption to the silica gel matrix.

The sample was then chromatographed on a phenyl-TSK column (Fig. 3) with a gradient of increasing ethylene glycol and decreasing NaCl (17). The activity eluted in a single peak at 60% ethylene glycol. A silver-stained polyacrylamide gel of the pooled peak fractions (Fig. 4) shows that the TSK G3000SW column resolved most contaminating proteins from the rsk kinase, and only a 41-42-kDa doublet was observed following chromatography on the phenyl column. This doublet probably represents two isoforms of the same protein since

rsk kinase without the addition of p-nitrophenyl phosphate to the DEAE fractions shows an increased proportion of protein in the faster migrating form. A summary of the purification protocol (Table I) shows that rsk kinase was purified approximately 800-fold with a 4% recovery.

Inhibitors and Substrate Specificity—In order to characterize the purified rsk kinase, the assay conditions were optimized, and the effect of various inhibitors on phosphotransferase activity was examined. The optimal MgCl2 concentration was 5 mM, and changes in pH between 6 and 8 had little effect on kinase activity (data not shown). rsk kinase was inhibited by NaCl, NaF, several divalent cations, and by β-glycerophosphate and heparin (Table II). The most potent
**Summary of rsk kinase purification**

Protein concentration was determined using Bradford analysis with 1 x or 2 x (GFC pool) Bradford reagent or densitometry of a silver-stained SDS-polyacrylamide gel (phenyl pool). rsk kinase activity was determined using multiple dilutions of each pool where activity was in the linear range.

| Pool          | Total protein | Total activity | Specific activity | Yield | Fold purification |
|---------------|---------------|----------------|-------------------|-------|------------------|
| Supernatant   | 103.6         | 0.214          | 0.012             | 100.0 | 1.0              |
| DEAE         | 9.3           | 0.49           | 0.053             | 40.7  | 4.5              |
| (NH₄)₂SO₄   | 4.1           | 0.25           | 0.062             | 21.0  | 5.3              |
| TSK G3000SW | 0.053         | 0.13           | 2.45              | 21.0  | 5.3              |
| Phenyl       | 0.0054        | 0.049          | 9.26              | 4.0   | 791              |

**Inhibitors of rsk kinase activity**

All of the inhibitors were examined at five different concentrations. Inhibitors were added to kinase assays so that the lowest concentrations tested did not markedly inhibit kinase activity and the highest concentrations inhibited activity to the maximal extent. The IC50 is the extrapolated concentration at which 50% of control kinase activity is inhibited.

| Inhibitor         | IC50 (± S.E.) |
|-------------------|---------------|
| Heparin           | 0.18 ± 0.05 µg/ml |
| Mn(NO3)2         | 1.4 ± 0.1 mM  |
| CaCl2            | 2.5 ± 0.14 mM |
| NaF              | 25.0 ± 3.7 mM |
| β-Glycerophosphate | 28.0 ± 2.0 mM |
| NaCl             | 118.0 ± 17.3 mM |

**Substrate specificity of rsk kinase**

The substrates were incubated with purified rsk kinase for 3–10 min using standard kinase assay conditions described under "Experimental Procedures." Incorporation of 32P was linear with time with 0.04 pmol/min of phosphate incorporated into rsk. An asterisk (*) indicates no detectable phosphorylation.

| Substrate | Conc. | % of rsk |
|-----------|-------|---------|
| rsk       | 1.4 µg/ml | 100     |
| MAP-2     | 100 µg/ml | 3300    |
| MBP       | 100 µg/ml | 3500    |
| α-Casein  | 100 µg/ml | 62      |
| Histone H1| 100 µg/ml | 52      |
| Phosvitin | 100 µg/ml | *       |
| Protamine | 100 µg/ml | *       |
| 40 S subunits | 667 µg/ml | 1.7     |

**Time course of activation of rsk kinase**

Oocytes were incubated in modified oocyte Ringer solution and stimulated to mature with 10 µM progesterone. Maturation was scored based on white spot formation (○) and H1 kinase activity (not shown). At selected times, 10 oocytes were homogenized in 25 mM Tris, pH 7.5, 50 mM NaF, 2 mM EGTA, 2 mM dithiothreitol, 100 µM phenylmethylsulfonyl fluoride, 10 µg/ml leupeptin, 1 mM NaVO₃, centrifuged for 5 min at 13,000 x g, and the supernatant was frozen. Aliquots of the homogenate (0.3 µl) were assayed for rsk kinase activity (●) or S6 kinase activity (▪) as described under "Experimental Procedures."
The effects of the serine/threonine protein phosphatases 1 and 2A, the protein tyrosine phosphatase CD45, and the truncated 37-kDa T-cell phosphatase on rsk kinase activity were then examined. rsk kinase activity was not reduced in the presence of 10 units/ml of phosphatase 1, whereas the same concentration of phosphatase 1 decreased S6 kinase II activity by more than 50% (data not shown). Phosphatase 2A (10 units/ml), however, effectively inactivated rsk kinase (Fig. 7). Only 5% of rsk kinase activity remained after 60 min, whereas almost 80% of the activity remained when rsk kinase was treated with phosphatase 2A inactivated with NaF. Both CD45 (data not shown) and the T-cell protein tyrosine phosphatase (Fig. 7) also inactivated rsk kinase. After 20 min of incubation in the presence of the T-cell protein tyrosine phosphatase (50 units/ml), only 10% of the original activity remained. Since all of the protein tyrosine phosphatases examined thus far have had an absolute specificity for phosphotyrosine (44), it is likely that the protein tyrosine phosphatase is essential for activity. In contrast, phosphatase 2A can remove phosphate groups from phosphotyrosine as well as phosphoserine and phosphothreonine (45); therefore, it cannot be determined from the data shown here whether phosphotyrosine is essential for activity. In a similar experiment, however, with 3T3 L1 cell MAP kinase phosphorylated on both serine/threonine and tyrosine residues, Anderson et al. (19) showed that phosphatase 2A hydrolyzed only the phosphothreonine and not the phosphotyrosine in MAP kinase.

Phosphorylation of rsk and S6KII—MAP kinase from 3T3 L1 cells has been shown to phosphorylate both S6 kinase I and S6 kinase II in vitro and to activate S6KII (11). rsk kinase also phosphorylated both S6 kinases (data not shown), and in addition, caused an 11-fold increase in the activity of phosphate-treated S6KII (Fig. 8). Consistent with this result, rsk kinase activated S6 kinase activity 15-fold when added to unstimulated oocyte extracts (Fig. 8).

3T3-L1 cell MAP kinase has already been shown to phos-
phosphorylate and activate S6KII. In order to compare the phosphorylation sites of rsk kinase and MAP kinase, two-dimensional-phosphopeptide maps of rsk phosphorylated by each kinase were prepared (Fig. 9). rsk phosphorylated by rsk kinase yielded five phosphopeptides whereas phosphorylation by MAP kinase produced seven phosphopeptides. Based on the results of the mixing experiment, it appears the five phosphopeptides resulting from phosphorylation by MAP kinase are the same as five of the seven phosphopeptides resulting from phosphorylation by MAP kinase. Both kinases phosphorylated rsk on serine and threonine residues (Fig. 9D).

**DISCUSSION**

In this paper we report the purification of a 41-kDa Xenopus rsk kinase to apparent homogeneity and characterize the kinase with respect to inhibitors, substrate specificity, phosphorylation, in vitro autophosphorylation, and inactivation by phosphatases. Active rsk kinase is a monomeric phosphoprotein inactivated by both a serine/threonine phosphatase and a protein-tyrosine phosphatase, and it phosphorylates MAP-2 and myelin basic protein in addition to rsk and S6 kinase II.

In comparing the characteristics of rsk kinase with other kinases, rsk kinase is most similar to the MAP kinases. The MAP kinases generally have a Mr between 40,000 and 45,000, phosphorylate MAP-2 and myelin basic protein, bind tightly to hydrophobic columns, and are phosphorylated in vivo on both tyrosine and serine or threonine residues. Based on these criteria, rsk kinase appears to be a member of this "family" of enzymes, but a number of important differences distinguish it from previously described members of the family.

While this work was in progress, Gotoh et al. (26) reported purification of a 42-kDa MAP kinase from unfertilized Xenopus eggs. Several differences between this enzyme and rsk kinase are apparent, however. Both CaCl2 and β-glycerophosphate inhibit rsk kinase (IC50 = 2.5 and 28 mM, respectively) whereas the Xenopus egg MAP kinase purified by Gotoh et al. is not inhibited by CaCl2 at concentrations up to 10 mM, and the IC50 for β-glycerophosphate is 150 mM. More importantly, the MAP kinase purified by Gotoh et al. (26) is phosphorylated during activation in oocytes on both serine and tyrosine residues, whereas rsk kinase is phosphorylated on threonine and tyrosine residues (Fig. 5).

**rsk kinase autophosphorylates in vitro on serine, threonine, and tyrosine residues.** In contrast to this result, the MAP kinase studied by Sturgill does not undergo autophosphorylation in vitro and MAP kinase from sea star oocytes auto-phosphorylates only on serine residues (46). Recently, two mammalian MAP kinases have been cloned (designated ERK1 (47) and ERK2 (48)) and it has been reported that both ERK1 and recombinant ERK2 autophosphorylate on threonine and tyrosine residues (49). This autophosphorylation correlates with an increase in recombinant ERK2 kinase activity. In contrast to ERK1 and ERK2, rsk kinase is extensively autophosphorylated on serine residue(s) as well as on tyrosine and threonine sites (Fig. 6). Seger et al. (49) have suggested that a partially purified activator of MAP kinase may act by stimulating autophosphorylation of MAP kinase. Since the rate of autophosphorylation of purified rsk kinase is very low and since rsk kinase is not phosphorylated on serine during activation in vitro, the effect of autophosphorylation on rsk kinase activity in vivo is unknown. We have observed no activation after autophosphorylation in vitro. However, the results here clearly establish that rsk kinase, a member of the newly emerging ERK/MAP kinase family, exhibits dual specificity for autophosphorylation in vitro. Whether rsk kinase can phosphorylate exogenous substrate proteins on tyrosine residues remains to be seen, although we detected no phosphorylation by purified rsk kinase of enolase, lactate dehydrogenase, or poly (Glu, Tyr, 1:4) in vitro. In addition, phosphoamino acid analysis of rsk and MBP phosphorylated by rsk kinase showed no incorporation into phospho-tyrosine residues. Recently, several newly cloned yeast and mammalian cell protein kinases distinct from MAP kinase have also been reported to autophosphorylate on serine, threonine, and tyrosine residues (50–53).

**Fig. 9. Phosphopeptide maps and phosphoamino acid analysis of rsk phosphorylated in vitro by MAP kinase and rsk kinase.** rsk was phosphorylated in vitro by MAP kinase or rsk kinase. Equal counts of rsk were loaded onto an SDS-polyacrylamide gel, eluted, precipitated with trichloroacetic acid and digested with trypsin (58). Peptides were fractionated on Kodak Chromagram cellulose sheets by electrophoresis at pH 1.9 (formic acid/acetic acid/H2O, 25:37:88) in the first dimension (right to left), followed by ascending chromatography (butanol/pyridine/acetic acid/H2O, 15:10:3:12) in the second dimension (bottom to top). Panel A, rsk phosphorylated by rsk kinase; panel B, rsk phosphorylated by MAP kinase; panel C, mixture of A and B. Samples of peptides from both samples were analyzed for phosphoamino acids. The peptides were hydrolyzed in HCl at 100 °C for 3–4 h, spotted onto a Polygram 300 sheet, and electrophoresed at pH 5.5. Panel D, rsk phosphorylated by rsk kinase.
purified S6KII with a single purified rsk kinase in an homologous system.

The ability of purified rsk kinase to reactivate phosphatase-treated S6KII in vitro supports the supposition that rsk kinase phosphorylates and activates S6KII in vivo. Since fewer phosphopeptides are evident in rsk phosphorylated by rsk kinase as compared to MAP kinase (Fig. 9), rsk kinase may have a more restricted substrate specificity than the 3T3 cell kinase from Sturgill's laboratory. This is additional evidence that rsk kinase is a distinct member of the MAP kinase family.

At present little is known about the sites in rsk phosphorylated by MAP kinase or rsk kinase. Two groups (16, 54) have reported that the MAP kinase phosphorylation site in myelin basic protein has the sequence .TPRT**PPP... and Takishima et al. (55) have shown that rsk kinase phosphorylates a synthetic peptide corresponding to the sequence in the epidermal growth factor receptor around T669 (.QPLT*PSG...). Recently, we found that Xenopus cyclin B1 was an excellent substrate for rsk kinase at either 594 or S96 (.EPPS*PS*PME...) (56). Serine 94 has a sequence motif similar to that found in myelin basic protein and the epidermal growth factor receptor, and therefore it seems likely that MAP kinase prefers serine or threonine residues NH2-terminal to a proline with a consensus PX/S/Y. However, examination of the sequence of rsk (9) does not reveal the presence of a site with this consensus. Selectivity for sites NH2-terminal to proline is also evident in the specificity of cdc2 kinase (57), although in that case basic residues are usually NH2- or COOH-terminal to the phosphorylated residue, unlike the known MAP kinase phosphorylation sites.

In Xenopus, MAP kinase and rsk kinase appear to be involved in several physiologically important steps. Gotth et al. (26) have shown that MAP kinase promotes an interphase-to-metaphase transition in microtubule arrays in Xenopus egg extracts. We have shown that rsk kinase phosphorylates and activates purified S6 kinase II in vitro as well as in oocyte extracts, and Izumi and Maller (56) have shown that rsk kinase phosphorylates cyclin B1. Considerable evidence supports the view that these events occur in vivo, and the MAP kinase family is clearly a large and important group of regulatory enzymes that mediate transduction of signals from the cell membrane to internal biochemical systems.

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