Cloning and Characterization of Xenopus Rsk2, the Predominant p90 Rsk Isozyme in Oocytes and Eggs*

Received for publication, July 18, 2000, and in revised form, August 6, 2000 Published, JBC Papers in Press, August 8, 2000, DOI 10.1074/jbc.M006386200

Ramesh R. Bhatt‡ and James E. Ferrrell, Jr.§
From the Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, California 94305-5174

The 90-kDa ribosomal S6 kinases, the p90 Rsk, are a family of intracellular serine/threonine protein kinases distinguished by two distinct kinase domains. Rsk2 are activated downstream of the ERK1 (p44) and ERK2 (p42) mitogen-activated protein (MAP) kinases in diverse biological contexts, including progression through meiotic and mitotic M phases in Xenopus oocytes and cyclic Xenopus egg extracts, and are critical for the M phase functions of Xenopus p42 MAPK. Here we report the cloning and biochemical characterization of Xenopus Rsk2. Xenopus Rsk1 and Rsk2 are specifically recognized by commercially available RSK1 and RSK2 antisera on immunoblots, but both Rsk1 and Rsk2 are immunoprecipitated by RSK1, RSK2, and RSK3 sera. Rsk2 is about 20-fold more abundant than the previously described Xenopus Rsk1 protein; their concentrations are approximately 120 and 5 nM, respectively. Rsk2, like Rsk1, forms a heteromeric complex with p42 MAP kinase. This interaction depends on sequences at the extreme C terminus of Rsk2 and can be disrupted by a synthetic peptide derived from the C-terminal 20 amino acids of Rsk2. Finally, we demonstrate that p42 MAP kinase can activate recombinant Rsk2 in vitro to a specific activity comparable to that found in Rsk2 that has been activated maximally in vivo. These findings underscore the importance of the Rsk2 isozyme in the M phase functions of p42 MAP kinase and provide tools for further examining Rsk2 function.

The mitogen-activated protein kinase (MAPK)1 cascade is a three-kinase module comprising a MAPK, a MAP kinase kinase (MAPKKK) (1–3).

One of the best-studied MAPK cascades consists of the p42 and p44 (or ERK2 and ERK1) MAPKs, the MEK1 and MEK2 MAPKKs, and the Raf and Mos MAPKKKs. Raf and Mos can phosphorylate MEK1 and MEK2 on two serine residues in MEK's T-loop, thereby activating the MEK. Active MEK in turn can phosphorylate p42 or p44 at threonine and tyrosine residues within the sequence Thr-Glu-Tyr in MAPKs' T-loop, thereby activating the MAPK.

p42 and p44 MAPK are probably best-known for their roles in mitogenesis and cell fate induction (4). However, these proteins have also been implicated in the regulation of meiotic and mitotic M phase. Much of the work on the M phase roles of MAPKs has been carried out in Xenopus laevis oocytes, eggs, and cyclic egg extracts, where the relevant MAPK is p42 (ERK2) MAPK (5–8).

The first indication that p42 MAPK might play a role in M phase regulation came from studies of Xenopus oocyte maturation. Maturation is a key step in the production of a fertilizable egg, and its study has a long history of providing important insights into the biochemistry of M phase regulation. Fully grown Xenopus oocytes are arrested in a G2-like state with an intact germinal vesicle (nucleus) and inactive Cdc2/cyclin B and p42 MAPK. Progesterone releases oocytes from this arrest state and brings about a resumption of meiosis I. Progesterone-treated oocytes undergo germinal vesicle breakdown, complete the first meiotic division, progress through interkinesis, enter meiosis II, and then spontaneously arrest in metaphase of meiosis II. Germinal vesicle breakdown is immediately preceded by the activation of the Mos/MEK1/p42 MAPK cascade and Cdc2/cyclin B. Interfering with the activation of any member of the MAPK cascade can delay or inhibit Cdc2 activation and oocyte maturation (9–13). The MAPK requirement appears not to be absolute; many batches of oocytes ultimately do activate their Cdc2 and mature even when MAPK activation has been suppressed (14–16). Nevertheless, it does appear that MAPK activation can promote Cdc2 activation (17–20).

The p90 Rsk family of protein kinases (21, 22) may be an important link between the activation of MAPK and the activation of Cdc2 (23). Rsk(s) have been found to be responsible for phosphorylating S6 in Xenopus oocytes (27); however, in many other cell types, the p70 S6 kinase appears to be the more important regulator of S6 (28–30). Like p42 MAPK, Rsk(s) are activated in tissue culture cells in response to diverse mitogens and in oocytes in response to progesterone. Rsk activation depends on a series of phosphorylations carried out by MAPK, PDK1, and Rsk itself (22, 31–33). Rsk(s) have been implicated in transcriptional regulation (34–39), in cell survival (40–42), and, as described below, in the M phase functions of p42 MAPK.

In immature oocytes, cyclin B is present and complexes with Cdc2; nevertheless, the Cdc2 is inactive as a result of inhibitory phosphorylation. The main Cdc2-inhibitory kinase present in oocytes is Myt1 (43, 44). Xenopus Rsk(s) have been found to...
associate with the C terminus of Myt1 and can phosphorylate and inactivate Myt1 in vitro (23). Rsk5 in turn can be phosphorylated and activated by p42 MAPK (45). Thus, p42 MAPK can release Cdc2 from the negative effects of Myt1, through the intermediary of Rsk.

The Mos/MEK/p42 MAPK cascade remains active throughout the remainder of maturation and in mature, unfertilized eggs. It plays a critical role in suppressing DNA replication and promoting Cdc2 activation between meiosis I and meiosis II (46, 47). p90 Rsk may mediate this effect of p42 MAPK; expression of a constitutively active form of p90 Rsk restores many aspects of a normal meiosis I/II transition to oocytes treated with a pharmacological inhibitor of the MAPK cascade (15).

The MAPK cascade is also required for the establishment of the cytoskeletal factor arrest in unfertilized eggs. The cascade suppresses cyclin destruction and keeps unfertilized eggs arrested in metaphase of meiosis II until fertilization occurs (48–50). Again, p90 Rsk appears to be a critical mediator of p42 MAPK. Depleting Rsk from a cycling Xenopus egg extract prevents the extract from undergoing mitotic arrest in response to p42 MAPK activation (51), and expression of constitutively active p90 Rsk causes embryos to arrest in M phase (52).

Given the importance of Rsk in the function of the MAPK cascade in Xenopus oocytes and eggs, we set out to determine what forms of Rsk were present in these systems. Four closely related Rsk cDNAs, RSK1, RSK2, RSK3, and RSK4, plus the more distantly related RSK-B and RLPK/MSK1 cDNAs, have been cloned from humans (53–56). The only Xenopus Rsk cDNAs reported to date have been Rsk1 homologs (57). However, there is clear biochemical evidence for at least one more Rsk-like kinase in Xenopus oocytes; Xenopus Rsk-like kinase activities fractionate into two chromatographically discrete peaks, designated S6 kinase I and S6 kinase II, which copurify with related but distinct 90- to 92-kDa proteins (24, 58, 59).

Therefore, we have carried out a degenerate PCR screen for Xenopus Rsk-like cDNAs. Here we present the isolation and characterization of a new Xenopus Rsk cDNA, the Xenopus homolog of Rsk2, which represents the major Rsk isozyme in Xenopus oocytes and eggs.

**EXPERIMENTAL PROCEDURES**

**RT-PCR and Subcloning**—RNA was typically obtained from 10 to 20 frozen Stage VI oocytes using RNAeasy kits (Qiagen) according to the manufacturer’s instructions. RT-PCR was accomplished using the Superscript 2.0 kit according to the manufacturer’s instructions (Life Technologies, Inc.). 5’ and 3’ RACE reactions were performed as recommended by the manufacturer’s instructions (Life Technologies, Inc.). All PCR-amplified fragments were gel-purified and subcloned into pGEM-T according to the manufacturer’s instructions (Promega).

Hemidigenerate RT-PCR was used to extend the open reading frame of Xenopus Rsk2. Hemidigenerate RT-PCR amplifies cDNA fragments by using a degenerate oligonucleotide derived from a family of sequences and specific oligonucleotide derived from a novel family member. In our case, a Rsk-derived degenerate oligonucleotide ensured that the amplified cDNA was a Rsk family member and a specific Rsk oligonucleotide ensured that the amplified cDNA was derived from our novel Rsk cDNA sequence. Sequencing—DNA sequencing was performed either by cycle sequencing with Thermosequenase or by Sequenase 2.0 dyeoxide chain termination (Amersham Pharmacia Biotech).

**Recombinant Proteins**—Hexahistidine-tagged MEK and MAPK proteins were expressed in Escherichia coli as described previously (60), purified, dialyzed, and stored at −80 °C in 20 mM Tris, pH 7.6, 50 mM NaCl, 1 mM dithiothreitol, 0.25% Triton X-100, 0.25% Tween 20, and 10% glycerol. A hexahistidine epitope sequence (AHHHHHHHASH) was inserted directly after the putative translation initiation methionine codon in the Xenopus Rsk2 cDNA (GenBank™ accession number AF165162). The resulting cDNA was cloned into pFastBac1 (Life Technologies, Inc.) to generate baculovirus for protein expression in SF9 cells. A cDNA for Rsk2 with an inactive N-terminal kinase domain (His6-Rsk2 R97R) was generated by site-directed mutagenesis (QuickChange; Stratagene). Both Rsk2 proteins were purified, dialyzed, and stored at −80 °C in XB + detergents (10 mM KCl, 2.5 mM sucrose, 1 mM HEPES, pH 7.7, 100 mM CaCl2, 1 mM MgCl2, 10% glycerol, 0.25% Triton X-100, and 0.25% Tween 20). A cDNA for Rsk1 was constructed with the terminal 43 amino acids replaced with a hexahistidine epitope sequence (AHHHHHHHASH); Δ43-His6-Rsk1 and expressed in SF9 cells as above. All proteins were purified to near homogeneity by nickel chelate chromatography.

**Determining Rsk1 and Rsk2 Abundances**—Concentrations of purified recombinant Δ43-His6-Rsk1 and Δ43-His6-Rsk2 were determined by Bradford assay and verified by comparing the Coomassie Blue staining observed on SDS-PAGE to a series of albumin standards. The purified recombinant Rsk proteins were then used as standards on Rac1 and Rsk2 immunoblots of oocyte lysates. Rsk amounts were quantified by phosphorimaging (Rsk1) or densitometry (Rsk2).

**Heteromeric Complex Formation in SF9 Lysates**—Previously frozen SF9 cell pellets expressing His6-Rsk2 or GST-MAPK were lysed by thawing at 4 °C in 50 mM Tris-HCl (pH 8.5), 10 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonl fluoride, 1% Nonidet P-40. Lysates were incubated at 4 °C for 10 min and clarified by centrifugation for 5 min at 4 °C in a microcentrifuge. Crude lysates were then mixed and incubated at 4 °C for 15 min. Either protein G-agarose beads and anti-His6 antibodies (CLONTECH, Palo Alto CA) or glutathione-coated agarse beads were added to the lysates and allowed to incubate at 4 °C for an additional 75 min at 4 °C. The beads were collected by brief centrifugation and washed two times with 20 volumes of XB (100 mM KCl, 10 mM HEPES, pH 7.7, 0.1 mM CaCl2, 1 mM MgCl2, and 50 mM sucrose) and resuspended in SDS-PAGE sample buffer. 75 min at 4 °C. The beads were then washed twice with XB and then resuspended in SDS-PAGE sample buffer. Peptide inhibition was performed by incubating diluted extracts and peptide for 10 min at room temperature prior to the addition of His6-Rsk2 protein.

**Rsk2 Activation and S6 Kinase Assay**—Rsk2 activity was determined by using S6 peptide as phosphoacceptor (Santa Cruz Biotechnology). Briefly, recombinant proteins (1.5 μg of His6-Rsk2, 0.3 μg of His6-MEK R4F, 0.2 μg of His6-MAPK) were mixed and incubated in kinase buffer (20 mM HEPES, pH 7.6, 20 mM MgCl2, 0.1 mg/ml bovine serum albumin, and 1 mM ATP), final volume 25 μl, for 60 min at 30 °C. Next, 1% of the protein from above was combined with 10 μg of S6 peptide and kinase buffer supplemented with [γ-32P]ATP (67 Ci/mM; final volume 30 μl). Samples were incubated for 10 min at 30 °C, and the reactions were rapidly terminated on ice by the addition of 10 μl of 0.5 M EDTA. Two equal portions of the S6 kinase assay were spotted onto P81 cellulose paper (Whatman) and then dried. The dry P81 cellulose paper was washed two times with 1% acetic acid, three times with water, and then allowed to dry. Radioactive incorporation was determined by Cerenkov counting of the individual samples.

**Antibodies and Immunoblotting**—Samples were separated on 10.5% low bio polyacrylamide SDS gels (acrylamide:bisacrylamide ratio, 100:1) and transferred to Immobilon P blotting membrane (Millipore). Antibodies were detected with Rsk2 antibodies (Santa Cruz Biotechnology), anti-phospho-Rsk antibodies (New England BioLabs), or MAPK antisera (DC3 or X15) (62), followed by incubation with the species-appropriate alkaline phosphatase-conjugated secondary antibodies (Sigma). Visualization of bands was accomplished by either colorimetric (nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate, Promega) or chemiluminescent (CDP-Star, Tropix) methods. Anti-His6 antibodies were obtained from CLONTECH.

**RESULTS**

Cloning Xenopus Rsk2—We obtained RNA from stage VI Xenopus oocytes and performed RT-PCR with two sets of degenerate oligonucleotides derived from regions in the C-terminal kinase region of Rsk that are well conserved in Xenopus.
Rsk1 and human RSKs 1–4. The RT-PCR reactions yielded three distinct groups of DNA sequences. The first two groups of sequences were derived from *Xenopus Rsk1a* and *Rsk1b*, two closely related Rsk1-like cDNAs that probably reflect the tetraploid ancestry of *X. laevis* (57). The third group of sequences were derived from a cDNA that was distinct from *Rsk1a* and *Rsk1b* but still clearly Rsk-related. We therefore completed the cDNA sequence of this new Rsk isozyme.

Through hemidegenerate RT-PCR we extended the coding sequence upstream and downstream, obtaining nearly 2 kb of what proved to be a 2.2-kb open reading frame. We completed the cloning of a cDNA of ~3 kb, similar in size to other Rsk cDNAs, through 5' and 3' RACE. To ensure that our final cDNA was derived from a single message, we re-isolated the entire cDNA by RT-PCR twice, using two pairs of specific oligonucleotides that hybridized to different sequences in the 5'- and 3'-untranslated regions. We sequenced these two independent clones and found that they both contained the sequences of the hemidegenerate RT-PCR and RACE fragments. We arrived at a consensus sequence by comparing the two long PCR products with each other and with the RT-PCR, hemidegenerate PCR, and RACE products.

The new Rsk-like sequence was identified as *Xenopus Rsk2*. The predicted amino acid sequence of *Xenopus Rsk2* is 92% identical to human Rsk2, overall, and only 77–81% identical to human RSKs 1, 3, and 4, and *Xenopus Rsk1α* (Fig. 1, A and B). *Xenopus Rsk2* has a predicted molecular mass of 83.3 kDa and a predicted isoelectric point of 6.3. *Xenopus Rsk2* contains two protein kinase domains (Fig. 1, A and B, boxed and highlighted in gray), as do all of the Rsk proteins characterized to date. Each of the six phosphorylation sites identified in rat Rsk1 (31) is conserved between *Xenopus Rsk2* and the other Rsk family members (Fig. 1A, asterisks), consistent with the observation that these kinases are regulated similarly. *Xenopus Rsk2* lacks the N-terminal nuclear localization sequence that is characteristic of RSK3 (54).

We constructed phylogram trees for the Rsk N-terminal and C-terminal kinase domains (Fig. 2). Rsk1 and Rsk2 appear to have diverged from each other subsequently to the divergence of nematodes and insects from vertebrates.

Relationship of Xenopus Rsk2 to S6 Kinase II—Erikson and Maller (24, 58, 59) identified, purified, and characterized two S6 kinase activities from *Xenopus* egg extracts. They designated these activities S6 kinase I and S6 kinase II and obtained the sequences of seven tryptic peptides from S6 kinase II. As shown in Table I, six of the seven S6 kinase peptides are found in *Xenopus Rsk2*, and only three of the seven peptides are found in *Xenopus Rsk1α* or Rsk1β. This indicates that S6
kinase II most likely represents Rsk2 rather than Rsk1. The Erikson and Maller S6 kinase II also apparently contains a second protein that gives rise to the 99-6 peptide, ADPSQFELK, whose closest match in Rsk2 is ADPSQFELLK. This second protein might be a closely related Rsk2β protein or a more distantly related Rsk family member.

Phosphorylation of Rsk1 and Rsk2 during Oocyte Maturation—The extraordinarily close similarity between human and Xenopus Rsk2, and, to a lesser extent between human and Xenopus Rsk1, suggested that antibodies specific for human RSK1 and RSK2 might also be specific for Xenopus Rsk1 and Rsk2. We therefore subjected lysates from immature and mature oocytes to immunoblotting with commercially available Rsk1 and Rsk2 protein and Rsk activity (Fig. 5A), with the activities much higher in M phase lysates than G2 phase lysates (Fig. 5B).

We used the results from each of the three Rsk sera to calculate the total Rsk activity present in oocytes. We found an average of 11 pmol·min⁻¹ of anti-Rsk immunoprecipitable S6 kinase activity per M phase oocyte or unfertilized egg (Fig. 5B and data not shown). Given the estimated total amount of Rsk1 and Rsk2 protein (5.2 ng per oocyte) we calculate Rsk's specific S6 kinase activity to be 2.2 pmol·min⁻¹·mg⁻¹ or, in molar terms, 179 mol of substrate phosphorylated per mol of Rsk per min. This specific activity is similar to that reported for purified rabbit ISPK (Rsk2) (2.0 pmol·min⁻¹·mg⁻¹) and for Xenopus S6 kinase II (1.4 pmol·min⁻¹·mg⁻¹), measured using Leu-Arg-Ala-Ser-Leu-Gly (Kemptide) as substrate (58, 64, 65). Thus the total Rsk activity present in Rsk immunoprecipitates can be accounted for by the estimated abundances of Rsk1 and Rsk2.

Rsk2 Forms a Heteromeric Complex with MAPK—We and others have previously reported that Rsk1 and p42 MAPK are present in complex in G2 phase oocytes (62, 66, 67). This finding suggests that Rsk1 has a special role in MAPK signal transduction. We set out to determine whether Rsk2, like Rsk1, can form complexes with p42 MAPK. We expressed GST-tagged p42 MAPK and Hisα-Rsk2 separately in Sf9 cells, then mixed the lysates and carried out precipitations with glutathione beads or anti-His6 antibodies. The anti-His6 antibodies pulled down p42 MAPK when Hisα-Rsk2 was present, but not when Hisα-Rsk2 was absent (Fig. 6A). Conversely, the glutathione beads pulled down Rsk2 when GST-MAPK was present, but not when GST-MAPK was absent (Fig. 6B). Thus Rsk2 and p42 MAPK can form a stable complex, like Rsk1 and p42 MAPK.

Next we examined whether purified Hisα-Rsk2 protein was capable of forming a complex with endogenous oocyte MAPK. We added Hisα-Rsk2 to G2 phase oocytes and immunoprecipitated the lysates with anti-Hisα-coated protein G beads. Endogenous MAPK was efficiently brought down with Hisα-Rsk2 but
Rsks are typically insoluble when expressed in insect cell kinases during its expression in Sf9 cells (Fig. 7A). The basal activity of His6-Rsk2 was approximately 0.2 μmol/min/mg (Fig. 7B). Incubation with activated MAPK caused the activity to increase to 1.8 μmol/min/mg, similar to the specific activity found for Rsk1 immunoprecipitated from phase oocytes (2.2 μmol/min/mg; see above). Despite the fact that Rsk2 and PKAs have similar substrate specificities, His6-Rsk2 kinase activity was insensitive to the PKA pseudosubstrate inhibitor PKI (Fig. 7B), in agreement with a previous report for purified S6 kinase II (69).

**DISCUSSION**

We have cloned the *Xenopus* homolog of human RSK2. *Xenopus* Rsk2 appears to be the main Rsk isozyme in *Xenopus* oocytes and eggs; its concentration is approximately 120 nM, compared with approximately 5 nM for Rsk1. The predicted localization sequence in this region. There is a stretch of sequence in the interkinase linker region (amino acids 387–405) that is well conserved between the two Rsk2s but differs in sequence from Rsk1, 3, and 4; if there are Rsk2-specific regulators or substrates, this region may be involved. Otherwise, Rsk1–4 are very closely related to each other. The extreme C terminus of Rsk1 has been shown to be important for its interaction with MAPK (66–68). Consistent with these observations, we found that a synthetic 20-amino acid peptide derived from the C terminus of Rsk2 could inhibit the association of Rsk2 and p42 MAPK, with inhibition being half-maximal at a peptide concentration of about 8 μM (Fig. 6D). Note that both phosphorylated and non-phosphorylated p42 MAPK were pulled down with Rsk2 and that the C-terminal peptide was equally effective at inhibiting Rsk2 interaction with both forms of p42 MAPK (Fig. 6D). This finding is consistent with the observation that the phosphorylation of Rsk1, not the phosphorylation of p42 MAPK, allows Rsk1-p42 MAPK complexes to dissociate (66).

**Activation of Rsk2 in Vitro**—Next we investigated the requirements for the enzymatic activation of recombinant Rsk. Rsks are typically insoluble when expressed in *E. coli*, and efforts to extract and renature Rsk proteins from inclusion bodies result in low yields of enzymatically inactive protein. Therefore, we expressed and purified His6-Rsk2 protein from Sf9 cells. Purified His6-Rsk2 could be resolved into two or three ~90-kDa bands on immunoblot analysis, suggesting that it had undergone some autophosphorylation or phosphorylation by insect cell kinases during its expression in Sf9 cells (Fig. 7A and data not shown). However, the recombinant His6-Rsk2 migrated faster than fully active Rsk2, and was low in activity (Fig. 7, A and B, and data not shown), indicating that it was not maximally phosphorylated. We incubated His6-Rsk2 with activated MEK R4F and p42 MAPK in a linked kinase reaction to determine whether His6-Rsk2 could become phosphorylated and activated by these kinases in vitro. MEK R4F plus p42 MAPK caused His6-Rsk2 to become hyperphosphorylated (Fig. 7A, top panel) and activated (Fig. 7B). Neither MEK R4F nor p42 MAPK alone caused either hyperphosphorylation or activation (Fig. 7A and data not shown). MEK R4F/p42 MAPK-induced Rsk2 phosphorylation was undiminished in His6-Rsk2 K97R, whose N-terminal kinase domain is non-functional (Fig. 7A, top panel). This observation is consistent with the hypothesis that Rsk phosphorylations are carried out by MAPK and the C-terminal Rsk kinase domain.

Next we examined the status of Ser-383 phosphorylation in His6-Rsk2. Ser-383 is situated in the sequence Phe-Ser-Phe within the linker that connects N-terminal and C-terminal kinase domains (Fig. 1). The Ser-383 site is notably conserved among the AGC protein kinases, which include protein kinase A (PKA), Akt/protein kinase B, protein kinase C, and p70 S6k (31, 32). In some AGC kinases, the site corresponding to Ser-383 appears to be phosphorylated by PDK1 or PDK2; in others, it appears to be autophosphorylated. We found that purified His6-Rsk2 had a low level of Ser-383 phosphorylation, as detected by a phospho-Ser-383 antibody, and underwent an increase in its Ser-383 phosphorylation in response to MEK R4F and p42 MAPK (Fig. 7A). This indicates that PDK1 and PDK2 are dispensable for Ser-383 phosphorylation and suggests that Ser-383 undergoes MAPK-stimulated autophosphorylation. MAPK-stimulated Ser-383 phosphorylation was undiminished in His6-Rsk2 K97R (Fig. 7A). Thus, Ser-383 phosphorylation appears to be carried out by the C-terminal kinase domain.

The basal activity of His6-Rsk2 was approximately 0.2 μmol/min/mg (Fig. 7B). Incubation with activated MAPK caused the activity to increase to 1.8 μmol/min/mg, similar to the specific activity found for Rsk1 immunoprecipitated from phase oocytes (2.2 μmol/min/mg; see above). Despite the fact that Rsk2 and PKAs have similar substrate specificities, His6-Rsk2 kinase activity was insensitive to the PKA pseudosubstrate inhibitor PKI (Fig. 7B), in agreement with a previous report for purified S6 kinase II (69).
kinase domain than of the C-terminal kinase domain. This is particularly apparent from the phylograms shown in Fig. 2. Current evidence suggests that the N-terminal kinase domain of Rsk interacts with multiple downstream substrates, whereas the C-terminal kinase domain interacts only with the ERK MAPKs and with Rsk itself (70, 71). Thus the N-terminal kinase domain may be under more stringent pressure to remain unchanged than the C-terminal kinase domain.

Like Xenopus Rsk1 and mammalian Rskks, Xenopus Rsk2 can form a complex with phosphorylated or non-phosphorylated p42 MAPK. This finding suggests that Rskks play particularly important roles in MAPK signal transduction, and, indeed, Rskks have now been implicated in MAPK M phase functions (15, 23, 51, 52), cell survival functions (40, 41), and transcriptional changes (34, 36, 37, 39, 72). The Rsk2-p42 MAPK complex can be disrupted by peptides from the Rsk2 C terminus. These findings suggest a potential way of specifically interfer-
Ser-383 phosphorylation of His6-Rsk2 in response to MEK R4F and p42 MAPK. Purified recombinant His6-Rsk2 (wild type or K97R mutant) was incubated with constitutively active MEK R4F with or without p42 MAPK, as indicated, for 60 min at 30 °C. The reaction products were subjected to SDS-PAGE followed by immunoblotting with RSK2 antibodies (top blot) or phospho-RSK antibodies (bottom blot) that detected Rsk2 when it was phosphorylated at Ser-383. B, activation of His6-Rsk2 by MEK R4F and p42 MAPK. His6-Rsk2 was incubated as described in A and tested for S6 kinase activity as described in Fig. 5.

Purified recombinant Xenopus Rsk2 can be activated to high specific activity by incubation with purified recombinant MEK R4F and p42 MAPK. This activation process involves phosphorylation by p42 MAPK as well as MAPK-induced autophosphorylation of Ser-383 by the C-terminal Rsk kinase domain. In addition, the protein kinase PDK1 has been implicated in Rsk activation, and PDK1→/−/embryonic stem cells are defective in activating p90 Rsk (32). There are two candidate PDK1 sites for our observation that purified Rsk2 can be fully activated by MEK R4F and p42 MAPK, and Dalby’s observation that Ser-222 is partially phosphorylated when Rsk1 is inactive. The hypothesis remains to be tested directly.

Acknowledgments—We thank Natalie Ahn for providing the MEK R4F expression plasmid, Ray Erikson for providing a Xenopus Rsk1 cDNA, Chyi-Yung Huang for kinase assay advice, Sarah Walter and Michael Sosahsky for essential discussion, and Michael Sosahsky and Jason Myers for help with Fig. 4. We thank Carsten Andersen and members of the Ferrell laboratory for helpful comments on the manuscript.

REFERENCES

1. Lewis, T. S., Shapiro, P. S., and Ahn, N. G. (1998) Adv. Cancer Res. 74, 49–139
2. Engleb, J. P., Pearson, G., Isbacher, J., Szarzynski, J., Kardamich, M., Xu, S., and Cobb, M. H. (1999) Exp. Cell Res. 253, 255–270
3. Marshall, C. J. (1994) Curr. Opin. Genet. Dev. 4, 82–89
4. Ferrell, J. E., Jr. (1996) Curr. Top. Dev. Biol. 33, 1–60
5. Ferrell, J. E., Jr. (1999) Bioessays 21, 833–842
6. Ferrell, J. E., Jr. (1999) Bioessays 21, 866–870
7. Sagata, N. (1997) Bioessays 19, 13–21
8. Gotth, Y., and Nishida, E. (1995) Proc. Cell Cycle Res. 1, 287–297
9. Huang, C. Y. F., and Ferrell, J. E., Jr. (1996) EMBO J. 15, 2169–2173
10. Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J., and Vande Woude, G. F. (1996) Nature 383, 519–525
11. Kosako, H., Gotth, Y., and Nishida, E. (1994) EMBO J. 13, 2131–2138
12. Cross, D. A., and Smythe, C. (1998) Exp. Cell Res. 241, 12–22
13. Muslin, A. J., MacNicol, A. M., and Williams, L. T. (1993) Mol. Cell Biol. 13, 4972–4977
14. Fabian, J. R., Morrison, D. K., and Daar, I. O. (1993) J. Cell Biol. 122, 645–652
15. Gross, S. D., Schwab, M. S., Taieb, F. E., Lewellyn, A. L., Qian, Y. W., and Muller, J. L. (2000) Curr. Biol. 10, 430–438
16. Fisher, D. L., Brassae, T., Galas, S., and Doree, M. (1999) Development 126, 4537–4546
17. Yew, N., Mellini, M. L., and Vande Woude, G. F. (1992) Nature 353, 649–652
18. Gotth, Y., Masuyama, N., Nishida, E., and Shirakabe, K., and Nishida, E. (1995) J. Biol. Chem. 270, 25898–25904
19. Haccard, O., Lewellyn, A., Hartley, R. S., Erikson, E., and Muller, J. L. (1995) Dev. Biol. 186, 677–682
20. Huang, W., Kessler, D. S., and Erikson, R. L. (1995) Mol. Biol. Cell 6, 235–245
21. Bilenis, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 5889–5892
22. Bilenis, J., and Gammeltoft, S. (1999) Mol. Cell. Endocrinol. 151, 65–77
23. Palmer, A., Gavin, A. C., and Nebreda, A. R. (1998) EMBO J. 17, 5037–5047
24. Erikson, E., and Muller, J. L. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 742–746
25. Bilenis, J., and Erikson, R. L. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 7621–7625
26. Tabarin, D., Heinrich, J., and Rosen, O. M. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4389–4373
27. Schwab, M. S., Kim, S., Terada, N., Edjlaff, C., Kozma, S. C., Thomas, G., and Muller, J. L. (1999) Mol. Cell. Biol. 19, 2485–2494
28. Chung, J., Kuo, C. C., and Nishida, E. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 742–746
29. Kuo, C. C., Chung, J., Fiorentino, D. F., Flanagan, W. M., Blenis, J., and Crabtree, G. R. (1992) Nature 358, 70–73
30. Price, D. J., Grove, J. R. C., Alvaro, Y., and Bier, E. B. (1992) Science 257, 973–977
31. Dalby, K. N., Morrice, N., Caudwell, F. B., Avruch, J., and Cohen, P. (1998) J. Biol. Chem. 273, 1496–1505
32. Williams, M. R., Arthur, J. S., Balendra, A., van der Kaay, J., Poli, V., Cohen, P., and Alessi, D. R. (2000) Curr. Biol. 10, 439–448
33. Ferrell, J. E., Jr. (1999) J. Biol. Chem. 274, 1929–1934
34. Bruning, J. C., Gillette, J. A., Zhao, Y., Bjorbaek, C., Kotzka, J., Krone, W., Kahn, C., and Muller-Wieland, D. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 2462–2467
35. Buck, M., Poli, V., van der Geer, P., Chojkier, M., and Hunter, T. (1999) Mol. Cell 4, 1067–1092
36. Xing, J., Ginty, D. D., and Greenberg, M. E. (1996) Science 273, 959–963
37. De Cesare, D., Jaquiery, J., and Sasseone, C. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12202–12207
38. Rivera, V., Miranti, C. C., Reynolds, D., Ginty, D. T., Chen, R. H., Blenis, J., and Greenberg, M. E. (1999) Mol. Cell 3, 6260–6273
39. Chen, R. H., Abate, C., and Blenis, J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 10952–10956
40. Bonni, A., Brunet, A., West, A. E., Datta, S. R., Takasu, M. A., and Greenberg, M. E. (1999) Science 286, 1358–1362
41. Tan, Y., Ruan, H., Demeter, M. R., and Comb, J. J. (1999) J. Biol. Chem. 274, 5385–5398
42. Lizzano, J. M., Morrice, N., and Cohen, P. (2000) Biochem. J. 349, 547–557
43. Murakami, M. S., and Vande Woude, G. F. (1998) Development 125, 237–248
44. Muller, R. P., Coleman, T. R., Kamagai, A., and Dunphy, W. G. (1995) Science 269, 438–447
Xenopus Rsk2

270, 86–90

45. Sturgill, T. W., Ray, L. B., Erikson, E., and Maller, J. L. (1988) Nature 334, 715–718
46. Furuno, N., Nishizawa, M., Okazaki, K., Tanaka, H., Iwashita, J., Nakajo, N., Ogawa, Y., and Sagata, N. (1994) EMBO J. 13, 2399–2410
47. Verlhac, M. H., Kubiak, J. Z., Weber, M., Geraud, G., Collinge, W. H., Evans, M. J., and Maro, B. (1996) Development 122, 815–822
48. Sagata, N., Watanabe, N., Vande Woude, G. F., and Iwakawa, Y. (1989) Nature 342, 512–518
49. Kosako, H., Gotoh, Y., and Nishida, E. (1994) J. Biol. Chem. 269, 28354–28358
50. Haccard, O., Sarcevic, B., Lewellyn, A., Hartley, R., Roy, L., Izumi, T., Erikson, E., and Maller, J. L. (1993) Science 262, 1262–1265
51. Bhatt, R. R., and Ferrell, J. E., Jr. (1999) Science 286, 1362–1365
52. Gross, S. D., Schwab, M. S., Lewellyn, A. L., and Maller, J. L. (1999) Science 286, 1365–1367
53. Moller, D. E., Xia, C. H., Tang, W., Zhu, A. X., and M, J. (1994) Am. J. Physiol. 269(2 Pt 1), C351–C359
54. Zhao, Y., Bjorbaek, C., Weremowicz, S., Morton, C. C., and Moller, D. E. (1995) Mol. Cell. Biol. 15, 4353–4363
55. Pierrat, B., Correia, J. S., Tomas-Zuber, M., and Lesslauer, W. (1998) J. Biol. Chem. 273, 29661–29671
56. Deak, M., Clifton, A. D., Luccoq, L. M., and Alessi, D. R. (1998) EMBO J. 17, 4426–4441
57. Jones, S. W., Erikson, E., Blenis, J., Maller, J. L., and Erikson, R. L. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3377–3381
58. Erikson, E., Maller, J. L., and Erikson, R. (1991) Methods Enzymol. 206, 252–268
59. Erikson, E., and Maller, J. L. (1991) J. Biol. Chem. 266, 5249–5255
60. Ferrell, J. E., Jr., and Bhatt, R. R. (1997) J. Biol. Chem. 272, 19068–19076
61. Murray, A. W. (1991) Methods Cell Biol. 36, 581–605
62. Hsiao, K.-M., Chou, S.-y., Shih, S.-J., and Ferrell, J. E., Jr. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5480–5484
63. Ferrell, J. E., Jr. (1996) Trends Biochem. Sci. 21, 460–466
64. Lavoine, A., Erikson, E., Maller, J. L., Price, D. J., Avruch, J., and Cohen, P. (1991) Eur. J. Biochem. 199, 725–728
65. Grove, J. R., Price, D. J., Banerjee, P., Balasubramanyam, A., Ahmad, M. F., and Avruch, J. (1993) Biochemistry 32, 7727–7738
66. Gavin, A. C., and Nehreda, A. R. (1999) Curr. Biol. 9, 281–284
67. Gavin, A. C., Ni Ainle, A., Chierici, E., Jones, M., and Nehreda, A. R. (1999) Mol. Biol. Cell 10, 2971–2986
68. Smith, J. A., Potent-Smith, C. E., Malarkey, K., and Sturgill, T. W. (1999) J. Biol. Chem. 274, 2883–2898
69. Erikson, E., and Maller, J. L. (1986) J. Biol. Chem. 261, 350–355
70. Bjorbaek, C., Zhao, Y., and Moller, D. E. (1995) J. Biol. Chem. 270, 18848–18852
71. Fisher, T. L., and Blenis, J. (1996) Mol. Cell. Biol. 16, 1212–1219
72. Joel, P. B., Smith, J., Sturgill, T. W., Fisher, T. L., Blenis, J., and Lannigan, D. A. (1998) Mol. Cell. Biol. 18, 1978–1984
73. Zhao, Y., Bjorbaek, C., and Moller, D. E. (1996) J. Biol. Chem. 271, 29773–29779