Rapamycin, Wortmannin, and the Methylxanthine SQ20006
Inactivate p70s6k by Inducing Dephosphorylation of the Same
Subset of Sites*

(Received for publication, May 9, 1995, and in revised form, July 10, 1995)

Jeung-Whan Han, Richard B. Pearsont, Patrick B. Dennis§, and George Thomas¶
From the Friedrich Miescher Institut, PO 2543, CH4002 Basel, Switzerland

Activation of p70s6k in cells stimulated with serum correlates with the phosphorylation of seven sites. Pre-
treatment of Swiss 3T3 cells with the immunosuppres-
sant rapamycin blocks phosphorylation of four of these
sites (Thr229, Thr389, Ser404, and Ser411), whereas phos-
phorylation proceeds in the remaining three sites (Ser418, Thr421, and Ser424). If rapamycin is added post-
serum stimulation, the pattern of phosphorylation is qualitatively similar except that Ser421 is still highly
phosphorylated. The inhibitory effect of rapamycin on serum-induced p70s6k activation and the phosphoryla-
tion of Thr229, Thr389, Ser404, and Ser411 is rescued by
FK506, providing further evidence that the inhibitory effect is exerted through a complex of rapamycin-
FKBP12. Wortmannin treatment pre- or post-serum stimulation inhibits phosphorylation of the same set of
sites as rapamycin, supporting the argument that both agents act on the same pathway. Likewise, methylxan-
thine phosphodiesterase inhibitors block p70s6k activation and phosphorylation of the same set of sites as
wortmannin and rapamycin. However, other agents that raise intracellular cAMP levels have no inhibitory ef-
fect, leading to the hypothesis that the inhibitory actions of methylxanthines on p70s6k activity are not
through activating protein kinase A but through inhibition of an upstream kinase. Together the results indi-
cate that there are two kinase signaling pathways that must converge to activate p70s6k and that only one of
these pathways is sensitive to rapamycin, wortmannin, and methylxanthine inhibition.

p70s6k and p85s6k represent two isoforms of the same kinase that are encoded by a common gene and are identical except for a 23-amino acid extension at the amino terminus of p85s6k (see Refs 1 and 2). Furthermore, both isoforms lie on a p21ras,
p42mapk/p44mapk independent signal transduction pathway (3, 4), which bifurcates at the level of the receptor (4). Whereas
p70s6k seems to be restricted to the cytoplasm (5), the amino-
terminal extension of p85s6k harbors a nuclear localization
signal that constitutively targets it to the nucleus (5). The
major substrate of the kinase in both compartments of the cell appears to be the 40S ribosomal protein S6 (see Ref. 6), whose
multiple phosphorylation in the cytoplasm has been implicated
in the selective translational up-regulation of a family of es-
sential gene products (7, 8). Consistent with this finding, either
microinjection of neutralizing antibodies into cells (9) or treat-
ment of cells with the immunosuppressant rapamycin, which
selectively blocks p70s6k/p85s6k phosphorylation and activation
(10–12), severely impedes cell cycle progression.

Activation of p70s6k/p85s6k is associated with multiple phos-
phorylation of the enzyme, which can be monitored as the slower migration of a family of bands on SDS-PAGE, 1 which
collapse into a single band following treatment with phospho-
tase or rapamycin (10, 13). Initially, phosphorylation at four
major sites, Ser411, Ser418, Thr421, and Ser424, which are
coupled in a putative autoinhibitory domain, was found to paral-
el p70s6k activation (14). Substitution of these four residues
with acidic amino acids mimicked phosphorylation at these
sites (15). Unexpectedly, the acidic form of the kinase was still
inactivated by rapamycin, and more surprisingly, inactivation
was shown to be associated with the dephosphorylation of a
distinct set of sites, with no effect observed on the phosphoryla-
tion of the four identified sites (15). Recently, these novel
rapamycin-sensitive sites have been identified as Thr229 in the
catalytic domain and Thr389 and Ser404 located in a linker
domain that couples the catalytic and autoinhibitory domains. 2

Each of the sites was found to reside in an atypical trypsin
cleavage product, which largely explained the difficulty in their
identification. Earlier rapamycin studies (15) led to the hypo-
thesis that at least two sets of phosphorylation events regulate
kinase activity, one of which was controlled by mitogens and a
second that appeared to be constitutively activated. However,
in re-examining this data, it was noted that in the presence of
serum, the parent and the acidic mutant exhibited a similar
mobility shift when analyzed by Western blots of SDS-PAGE
(15). Furthermore, in both cases, rapamycin treatment caused
a similar increase in electrophoretic mobility of the protein.
These data suggested that one or more of the rapamycin-sen-
sitive phosphorylation sites was involved in the p70s6k mobility
shift (15). Since a similar mobility shift is observed during the
rapid activation of p70s6k by mitogens (4, 10), which is blocked
by rapamycin (10), this finding raises the question of whether
the rapamycin-sensitive phosphorylation sites are also regu-
lated by mitogens.

Since p70s6k activation has not yielded to in vitro reconsti-
tution (3, 16), a number of indirect approaches have been
exploited in an attempt to identify key regulatory points in this

*The costs of publication of this article were defrayed in part by the
payment of page charges. This article must therefore be hereby marked
"advertisement" in accordance with 18 U.S.C. Section 1734 solely to
indicate this fact.
†Recipient of a Human Frontier Science Program Organization long
term postdoctoral fellowship.
‡Recipient of a European Molecular Biology Organization long term
postdoctoral fellowship.
§To whom correspondence should be addressed. Tel.: 0041 61 697
3012; Fax: 0041 61 697 6681.

1 The abbreviations used are: PAGE, polyacrylamide gel elec-
drophoresis; TPA, 12-O-tetradecanoylphorbol-13-acetate; IBMX, iso-butyl-
methylxanthine; DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal
calf serum; IgG, immunoglobulin G; FKBP12, FK506 binding
protein 12; PDGF, platelet-derived growth factor; MAP, mitogen-acti-
vated protein.

2 R. B. Pearson, P. B. Dennis, J.-W. Han, N. A. Williamson, S. C.
Kazma, R. E. H. Wettenhall, and G. Thomas, submitted for publication.
Inhibitors of p70S6K Phosphorylation and Activation

424) and Thr. The cellswere washed twice with ice-cold buffer A (120 mM NaCl, 20 mM MOPS, pH 7.0) and then extracted in the same buffer containing 1% Nonidet P-40. Cell extracts were centrifuged at 12,000  

Though the role of phosphatidylinositol 3-OH kinase in regulating this pathway has been questioned (4, 20), wortmannin clearly inhibits a key step in the p70S6K pathway. In contrast to rapamycin, wortmannin does not inhibit TPA activation of p70S6K, leading to a model in which the wortmannin block has been placed upstream of the site of rapamycin action (18). Exploiting a similar line, Monfar et al. (21) have recently demonstrated that raising intracellular levels of cAMP in T cells either blocks interleukin 2-induced p70S6K activation or causes the immediate inactivation of the kinase in post-interleukin 2-stimulated cells. These findings have led to an expansion of the model above in which activation of protein kinase A acts as a key negative regulator of p70S6K as well as phosphatidylinositol 3-OH kinase, whose activation is also blocked by raising intracellular cAMP levels (21). To raise cAMP levels, Monfar et al. (21) co-treated cells with forskolin, an activator of adenylate cyclase, and IBMX, a phosphodiesterase inhibitor. However, methylxanthines, such as IBMX, have been shown to also act as protein kinase inhibitors (22). Indeed, previous studies have demonstrated that treatment of cells with methylxanthines alone is sufficient to block S6 phosphorylation, whereas raising intracellular cAMP with prostaglandin E1, a potent adenylate cyclase agonist, had no inhibitory effect on this response (23). These latter findings raise the possibility that methylxanthines themselves may block p70S6K activation and, if selective, could be potential tools for identifying upstream kinases in this pathway.

The mapping of individual phosphorylation sites involved in regulating p70S6K enables the assessment of these models and allows for the determination of whether the newly identified sites of phosphorylation are also implicated in p70S6K activation. Here we have employed p70S6K mutants as well as two-dimensional phosphopeptide analysis of endogenous p70S6K to establish whether any of the newly identified rapamycin-sensitive sites of phosphorylation are also involved in mitogenic activation of p70S6K. Next we have assessed whether rapamycin and wortmannin inhibition of p70S6K activation is paralleled by the dephosphorylation of the same or a distinct set of sites. Finally, we have examined whether methylxanthines are capable of blocking p70S6K activation, as opposed to other agents that raise intracellular cAMP levels, and have examined the sites of p70S6K phosphorylation affected by such treatment.

EXPERIMENTAL PROCEDURES

Plasmid Constructs and Mutagenesis—Site-directed mutagenesis resulting in the mutations of Ser114 to Asp or Ala (residues 411, 418, and 424) and Thr to Glu or Ala (residue 421) was performed as described previously (15). To epitope-tag the p70S6K constructs, we introduced by polymerase chain reaction the sequence GAGGAGGACCTG, corresponding to the Myc9E10 epitope (24), after the initiation codon of the wild type p70S6K (p2B4) (25). Exchanging XbaI-BglII fragments (nucleotides 1–911) between wild type p70S6K and p70S6K mutants allowed the introduction of the Myc tag in the mutant constructs.

Cell Culture, DNA Transfection, and Radioactive Labeling—Swiss mouse 3T3 cells were grown, maintained, and arrested in G0, as described previously (26). For in vivo 32P labeling, the medium was changed on day 7 for 15 ml of phosphate-free DMEM containing 0.1% bovine serum albumin (for experiments employing wortmannin, bovine serum albumin was omitted from the medium), after 6 h 1 mCi of 32P, was added, and cells were incubated overnight in the presence of the radioactive label before the various treatments were initiated. For preparation of extracts, the medium was removed from the plate, and the cells were washed twice with ice-cold buffer A (120 mM NaCl, 20 mM NaF, 10 mM pyrophosphate, 5 mM EGTA, 1 mM EDTA, 1 mM benzamidine, 0.1 mM phenylmethylsulfonfyl fluoride, 30 mM 4-nitrophenyl phosphate, 50 mM Tris-HCl, pH 8.0) and then extracted in the same buffer containing 1% Nonidet P-40. Cell extracts were centrifuged at 12,000  

For increased accuracy, the text has been formatted to improve readability, and the metadata has been removed. This method ensures a clear and coherent representation of the document's content. The focus is on maintaining the integrity of the information presented, adhering to the guidelines specified.
each construct was measured before and after serum stimulation. Even though the basal activity of p70s6kD3E is higher than p70s6k and that of p70s6kA4 is lower, both constructs are activated by serum (Fig. 1B). Activation was associated in all three cases with a decrease in mobility of the kinase on SDS-PAGE (data not shown). These results indicate that kinase activation may be dependent on phosphorylation of other sites.

Serum-induced Phosphorylation Sites—Consistent with the above data, in initial phosphopeptide analysis studies other phosphopeptides were detected in some preparations (14), and more recent studies have demonstrated that other phosphorylation sites play a critical role in regulating kinase activity (15). To determine whether additional phosphorylation sites were associated with p70s6k activation, quiescent Swiss 3T3 cells were stimulated with serum for 15 min in the presence of 32Pi. Serum treatment leads to a rapid activation of p70s6k as measured by its ability to phosphorylate 40 S ribosomal protein S6 in vitro (Fig. 2A, lanes 1 and 2) or its slower migration on Western blots of one-dimensional SDS-PAGE (Fig. 2A, lanes 3 and 4). The shift in electrophoretic mobility corresponds to an increase in the incorporation of phosphate into the protein (13). In quiescent cells, two forms of p70s6k can be clearly distinguished on Western blots, designated i and ii, which, upon serum stimulation, exhibit decreased mobility, designated iii and iv (Fig. 2A, compare lanes 3 and 4). Analysis of two-dimensional tryptic phosphopeptide maps of p70s6k from quiescent cells revealed the presence of phosphate in Ser411, Ser418, Thr421, and Ser424 (Fig. 2B). The four sites residing in the autoinhibitory domain (14). Serum stimulation leads to a rapid increase in the amount of 32P incorporated into these four sites, and the appearance of three additional phosphopeptides (Fig. 2C), which have been shown to be singly phosphorylated at Thr229, Thr389, and Ser404. 2 Thr229 is situated in the catalytic domain, whereas Thr389 and Ser404 are located in the linker domain, which couples the catalytic and autoinhibitory domains (Fig. 1A). The appearance of Thr229, Thr389, and Ser404 was unexpected, in that they were not detected in 3T3 cells in earlier studies (14), but it is consistent with the results described in Fig. 1B and the observation that one or more of these sites appear to be involved in the mobility shifts detected in 293 cells transiently overexpressing p70s6k (15). In the initial study, the failure to detect these phosphopeptides in 3T3 cells was probably due to the fact that none of these sites reside in a predicted tryptic peptide, 2 suggesting the presence of a contaminating protease. Taken together the data demonstrate that all seven phosphorylation sites are rapidly induced upon mitogenic stimulation.

Effect of Rapamycin—The three peptides phosphorylated at
The antibiotic wortmannin has been shown to specifically block activation of both phosphatidylinositol 3-OH kinase (18) and p70\textsuperscript{S6k} (17, 18), though its specificity for the p70\textsuperscript{S6k} pathway versus the p42\textsuperscript{MAPK}/p44\textsuperscript{MAPK} pathway has been recently challenged (33, 34). Furthermore, it has been hypothesized that the inhibitory effect is exerted upstream of the rapamycin block as TPA-induced activation of p70\textsuperscript{S6k}, presumably through protein kinase C, is insensitive to wortmannin but is still blocked by rapamycin (18). However, wortmannin may also act on a rapamycin-independent signaling pathway, exerting its inhibitory effect on p70\textsuperscript{S6k} through a rapamycin-insensitive phosphorylation site. To test the role of wortmannin, we first assessed its ability to induce inactivation of p70\textsuperscript{S6k} in cells pretreated with serum. Under these conditions, the IC\textsubscript{50} for p70\textsuperscript{S6k} inactivation is between 50 and 100 nM wortmannin (Fig. 5A, inset). In cells either pretreated with 200 nM wortmannin or treated with the same concentration of the antibiotic 15 min post-serum stimulation, p70\textsuperscript{S6k} activation is either blocked or returns to basal levels within 30 min (Fig. 5A). The inhibitory effect on kinase activity is paralleled by an increase in the mobility of the kinase analyzed on Western blots of SDS-PAGE (Fig. 5B). To examine the effect of wortmannin on the phosphorylation pattern of p70\textsuperscript{S6k}, cells were either pretreated with wortmannin and then stimulated with serum or stimulated with serum followed by subsequent addition of the antibiotic. The results are very similar to those obtained with rapamycin (Fig. 3, C and D); wortmannin pretreatment severely suppressed the phosphorylation of Thr\textsuperscript{229}, Thr\textsuperscript{389}, Ser\textsuperscript{404}, and Ser\textsuperscript{411}, with phosphorylation proceeding in the rapamycin-insensitive sites, whereas treatment following serum stimulation leads to dephosphorylation of Thr\textsuperscript{389} and Ser\textsuperscript{404} with less of an effect on Thr\textsuperscript{229} and Ser\textsuperscript{411} (Fig. 5, C and D, respectively). These data support a model in which both agents are acting on the same signaling pathway.

Effects of SQ20006—Recent studies have shown that raising cAMP levels in T cells by applying forskolin, an adenylyl cyclase agonist, together with IBMX, an inhibitor of cAMP-dependent phosphorylase, blocks p70\textsuperscript{S6k} activation (21). However, previous studies in Swiss 3T3 cells had shown that phosphorylase inhibitors alone, but not other agents that raise cAMP levels, are responsible for blocking mitogen-induced S6 phosphorylation.
Inhibitors of p70^S6K Phosphorylation and Activation

phosphorylation, the target of p70^S6K (23). The results in Fig. 6 demonstrate that neither 8-bromo-cyclic AMP nor forskolin has an effect on serum-induced p70^S6K activation, whereas IBMX had a small but significant inhibitory effect. In contrast, the methylxanthine SQ20006, a more potent inhibitor of phosphodiesterase and S6 phosphorylation (23, 35), severely suppresses p70^S6K activation. If SQ20006 is added post-serum stimulation, it also induces p70^S6K inactivation as measured by its ability to phosphorylate S6 in vitro (Fig. 7A) or by its increased electrophoretic mobility on SDS-PAGE (Fig. 7B). Under these conditions SQ20006 has no effect on p42s6k/p44s6k activation (Fig. 7A, inset). The effect on p70^S6K activity and mobility shift suggests that SQ20006 is operating on the same phosphorylation sites as wortmannin and rapamycin. To examine this possibility the effect of SQ20006 on the pattern of p70^S6K phosphorylation was analyzed. The rapamycin-sensitive sites of phosphorylation are also sensitive to SQ20006, exhibiting approximately the same qualitative pattern if added before or after serum stimulation (Fig. 7, C and D). These results support earlier conclusions that the inhibitory effect of phosphodiesterase inhibitors is not through raising cAMP levels but instead through blocking an upstream kinase (23).

As wortmannin does not block TPA activation of p70^S6K, whereas rapamycin does, each agent has been argued to attack a unique target in the p70^S6K signaling pathway. If both agents operate on the same pathway, the wortmannin target would be situated more proximal to the cell surface receptor, with the rapamycin target situated downstream. To determine where SQ20006 acts in this pathway, cells were stimulated with TPA in the presence or absence of all three agents. The results show that wortmannin has no effect on TPA activation of p70^S6K, as shown by others (17), whereas rapamycin and SQ20006 block kinase activity (Fig. 8). In contrast to p70^S6K activation, all three agents have no effect on p42s6k activation (Fig. 8). These results suggest that SQ20006 operates very similarly to rapamycin, possibly inhibiting a common target.

DISCUSSION

From the data presented here, it is evident that the phosphorylation of Thr229, Thr389, and Ser404 are largely responsible for the mobility shifts observed on Western blots following mitogenic stimulation. The most likely reason these sites were not detected in the initial analysis (14) was that all three reside in atypically cleaved tryptic peptides. This problem is further compounded by the fact that the cleavage efficiency of these peptides is poor and varies between batches of trypsin. The ability of the macrolide to induce an equivalent increase in mobility of the wild type p70^S6K and the p70^S6K_D-E mutant on SDS-PAGE (15) is consistent with the conclusion that the mobility shifts in p70^S6K are due to the rapamycin-sensitive sites. Furthermore, the phosphorylation of the rapamycin-insensitive sites, like the rapamycin-sensitive sites, appears to have a large impact on kinase activity (Fig. 1). Thus, as has been described for other kinases (36), only a subset of sites cause changes in electrophoretic mobility, and therefore, interpretations concerning the extent of p70^S6K phosphorylation and activation by mobility shift should be treated cautiously.

Recently Chung et al. (17), employing specific point mutants of the PDGF receptor, provided evidence for two separate signaling pathways leading to p70^S6K activation. One pathway was regulated through tyrosines 740 and 751 and hypothesized to be mediated through activation of phosphatidylinositol 3-OH kinase, whereas the second pathway was regulated by phosphorylation of tyrosines 1009 and 1021, apparently signaling through protein lipase Cγ. Interestingly, the phosphatidylinositol 3-OH kinase inhibitor wortmannin only blocked signaling from tyrosines 740 and 751 and not tyrosines 1009 and 1021, while rapamycin blocked p70^S6K activation through both pathways (17). These results led to the hypothesis that, in the pathway mediated by tyrosines 740 and 751, the rapamycin block lies downstream of the wortmannin block (18). The hypothesis that both agents inhibit p70^S6K activation through different components, which are located on the same signaling pathway, is consistent with their ability to block the same set of phosphorylation sites in the kinase (Figs. 3–5). Indeed, this same set of phosphorylation sites is also sensitive to SQ20006 treatment (Fig. 7).

Earlier studies had shown that phosphodiesterase inhibitors can inhibit or ablate serum-induced S6 phosphorylation (23, 37). Recent results from Monfar et al. (21) employing the cAMP elevating agents forskolin and IBMX, demonstrated that the two agents together prevent interleukin 2-induced p70^S6K activation in a T cell line, leading them to conclude that this
Inhibitors of p70^{65k} Phosphorylation and Activation

SQ20006 has a very similar p70^{65k} inhibitory profile to that of rapamycin. However, earlier studies showed that SQ20006, at concentrations that completely inhibit p70^{65k} activation, ablate serum-induced up-regulation of protein synthesis (23). In contrast, rapamycin only has a marginal effect on global protein synthesis (7). Although having only a small effect on general protein synthesis, rapamycin selectively suppresses the translational up-regulation of a family of mRNA that are characterized by having a polypyrimidine tract at their 5’ transcriptional start site (7). Taken together this suggests that SQ20006 is inhibiting the function of at least one other cell component that is involved in the up-regulation of translation. Since the inhibitory effect of SQ20006 appears to be exerted at initiation (23), this component may be one of the specific factors involved in initiation of translation (39).

It is clear from the data presented here that the signaling events leading to p70^{65k} activation are not all converging through a rapamycin/wortmannin/SQ20006-sensitive pathway. Instead, there appear to be at least two independent pathways required for p70^{65k} activation, one of which is blocked by the inhibitors employed here. The sites targeted by this pathway have been recently identified as Thr^{229}, Thr^{389} and Ser^{404} (Fig. 2C). In this study, the primary target of rapamycin was Thr^{389}, despite the fact that phosphorylation of residues equivalent to Thr^{229} play a key role in regulating the activity of a number of kinases (40). Thr^{389} is situated in an unusual uncharged sequence flanked by aromatic residues: VFLGFT^{389}YVAPS. Comparison of the sequence surrounding Thr^{389} with sequences found to be substrates for known kinases reveals no obvious candidate for the Thr^{389} kinase (41). The motifs surrounding Thr^{229} and Ser^{404} are similar, suggesting that all three sites may be regulated by a novel kinase. In contrast to the rapamycin-sensitive phosphorylation sites, the sites located in the autoinhibitory sequence all exhibit (S/T)P motifs. The most likely candidate for regulating these sites initially appeared to be the p42^{mapk}/p44^{mapk} (42). However, a number of studies have strongly argued against this possibility (3, 4, 43). It may be instead that these sites are regulated by another member of this family such as J un kinase (44) or stress-activated protein kinase (45), or possibly p38 MAP ki-

FIG. 5. Effect of wortmannin on p70^{65k}. A (inset), Swiss 3T3 cells stimulated with 10% FCS for 15 min were then incubated with increasing concentrations of wortmannin for an additional 30 min. Cell extracts were prepared and assayed as described under “Experimental Procedures.” A and B, cells were preincubated for 30 min with 200 nM wortmannin and then stimulated with serum for 15 min (lane 1) or first stimulated with 10% FCS for 15 min (lane 2) and then treated with 200 nM wortmannin for 15 min (lane 3) or 30 min (lane 4). Cell extracts were prepared and either assayed for (A) S6 kinase activity or (B) the mobility of p70^{65k} on Western blots as described under “Experimental Procedures.” C, two-dimensional tryptic phosphopeptide maps of p70^{65k} from cells pretreated with wortmannin for 30 min prior to stimulation with 10% FCS for 15 min; D, maps of p70^{65k} from cells first stimulated with 10% FCS for 15 min followed by the addition of wortmannin for 30 min. Cells were prelabeled with 32P, and extracts were prepared as in A and B. Two-dimensional phosphopeptide analysis was carried out as described in Fig. 2.

FIG. 6. Effect of cAMP or phosphodiesterase inhibitors on p70^{65k} activity. Swiss 3T3 cells were preincubated for 30 min without (lane 1) or with 500 μM 8-bromo-cAMP (lane 2), 50 μM forskolin (lane 3), 500 μM IBMX (lane 4), or 1.2 mM SQ20006 (lane 5) and then stimulated with 10% FCS for 15 min. Whole cell lysates were assayed for kinase activity as described under “Experimental Procedures.”

A inhibitory effect was exerted through raising intracellular levels of cAMP. The results presented here demonstrate that, in Swiss 3T3 cells, raising cAMP levels either by use of the non-hydrolyzable analogue 8-bromo-cAMP or an adenylate cyclase agonist has no effect on serum-induced p70^{65k} activation, ablate serum-induced up-regulation of protein synthesis (23). However, IBMX alone had a 25% inhibitory effect on kinase activation, whereas the more potent phosphodiesterase inhibitor SQ20006 (35) had a more pronounced effect. Recent studies have shown that the structurally related 2-aminopurine analogue, olomoucine, selectively inhibits a number of cell cycle-regulated kinases both in vitro and in vivo (38). Olomoucine however, had no effect on serum-induced p70^{65k} activation while blocking p42^{mapk}/p44^{mapk} activation (data not shown), suggesting that SQ20006 or similar structural analogues may be useful tools in specifically analyzing the p70^{65k} signal transduction pathway.
Inhibitors of p70<sup>56k</sup> Phosphorylation and Activation

Recent findings have indicated that p70<sup>56k</sup>/p85<sup>56k</sup> plays a critical role in cell cycle progression (9, 10, 47). Furthermore, it appears to mediate this effect through the phosphorylation of 40 S ribosomal protein S6 and the subsequent translational up-regulation of a family of mRNA transcripts that encode for components of the protein synthetic machinery (7, 8). Up-regulation of specific translational components (48, 49) or obstruction of gene products that regulate their function (50) can transform cells or increase their susceptibility to transformation. These observations may explain why p70<sup>56k</sup> is among the most highly conserved mammalian enzymes, having the identical sequence in man, mouse, rat, and rabbit. A highly regulated mechanism of p70<sup>56k</sup> control would be consistent with the loss of this control leading to a constitutive growth state. The use of specific inhibitors should provide invaluable tools in probing regulatory pathways that govern p70<sup>56k</sup>/p85<sup>56k</sup> activation and mechanisms that link the kinase to translational control.

Acknowledgments—We are indebted to Drs. B. A. Hemmings, H. B. J. j efferies, S. C. Kozma, and A. Matus for critical reading of the manuscript. We also thank D. Schofield for her typing expertise and M. Rothnie for preparing the figures.

REFERENCES

1. Kozma, S. C., and Thomas, G. (1994) Semin. Cancer Biol. 5, 255–266
2. Ferrari, S., and Thomas, G. (1994) CRC Crit. Rev. Biochem. Mol. Biol. 29, 385–413
3. Ballou, L. M., Luther, H., and Thomas, G. (1991) Nature 349, 348–350
4. Ming, X. F., Burgering, B. M. T., Wennström, S., Claesson-Welsh, L., Heldin, C. H., Bos, J. L., Kozma, S. C., and Thomas, G. (1994) Nature 371, 426–429
5. Reinhard, C., Fernandez, A., Lamb, N. J. C., and Thomas, G. (1994) EMBO J. 13, 1557–1565
6. Stewart, M. J., and Thomas, G. (1994) BioEssays 16, 1–7
7. j efferies, H. B. J., Reinhard, C., Kozma, S. C., and Thomas, G. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4441–4445
8. j efferies, H. B. J., Thomas, G., and Thomas, G. (1994) J. Biol. Chem. 269, 4367–4372
9. Lane, H. A., Fernandez, A., Lamb, N. J. C., and Thomas, G. (1993) Nature 363, 170–172
10. Chung, J., Kuo, C. J., Crabtree, G. R., and Blenis, J. (1992) Cell 69, 1227–1236
11. Kuo, C. J., Chung, J., Floretino, D. F., Flanagan, W. M., Blenis, J., and Crabtree, G. R. (1992) Nature 358, 70–73
12. Price, D. J., Grove, J. R., Calvo, V., Avruch, J., and Bierer, B. E. (1992) Science 257, 973–977
13. Ballou, L. M., j eno, P., and Thomas, G. (1988) J. Biol. Chem. 263, 1188–1194
14. Ferrari, S., Bannwarth, W., Morley, S. J., Totty, N. F., and Thomas, G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7282–7285
15. Ferrari, S., Pearson, R. B., Siegmann, M., Kozma, S. C., and Thomas, G. (1993) J. Biol. Chem. 268, 16091–16094
16. Mukhopadhyay, N. K., Price, D. J., Kyriakis, J. M., Pelech, S. L., Sanghera, J., and Avruch, J. (1992) J. Biol. Chem. 267, 3325–3335
17. Chung, J., Grammer, T. C., Lemon, K. P., Kazlauskas, A., and Blenis, J. (1994) Nature 370, 71–75
18. Cheatham, B., Vlahos, C. J., Cheatham, L., Wang, L., Blenis, J., and Kahn, C. R. (1994) Mol. Cell. Biol. 14, 4902–4911
19. Myers, M. G., Jr., Grammer, T. C., Wang, L. M., Sun, X. J., Pierce, J. H., Blenis, J., and White, M. F. (1994) J. Biol. Chem. 269, 28783–28789
20. Monfar, M., Lemon, K. P., Grammer, T. C., Cheatham, L., Chung, J., Vlahos, C. J., and Blenis, J. (1995) Mol. Cell. Biol. 15, 326–337
21. Farrell, P. J., Balkow, K., Hunt, T., Jackson, R. J., and Trachsel, H. (1977) Cell 11, 187–200
22. Thomas, G., Siegmann, M., Kubler, A., Gordon, J., and Jimenez de Asua, L. (1980) Cell 15, 1015–1023
23. Lane, H. A., Morley, S. J., Doree, M., Kozma, S. C., and Thomas, G. (1992) EMBO J. 11, 1743–1749
24. Derijard, B., Hibi, M., Wu, I., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994) Cell 76, 1025–1037
25. Sanchez, I., Hughes, R. T., Mayer, B. J., Yee, K., Woodgett, J. R., Avruch, J., Kyriakis, J. M., and Zon, L. I. (1994) Nature 372, 794–798
26. Han, J., Lee, J.-D., Bibbs, L., and Ulevitch, R. J. (1994) Science 265, 808–811
27. Lazaris-Karatzas, A., Montine, K. S., and Sonenberg, N. (1990) Nature 345, 544–547
28. Koromilas, A. E., Lazaris-Karatzas, A., and Sonenberg, N. (1992) EMBO J. 11, 4153–4158
29. Welsh, G. I., Foulstone, E. J., Young, S. W., Tavare, S. W., and Proud, C. G. (1994) Biochem. J. 303, 15–20
30. Cross, D. A., Alesci, D. R., Vandenheede, J. R., McDowell, H. E., Hundal, H. S., and Cohen, P. (1994) Biochem. J. 303, 21–26
31. Chasin, M., Harris, D. N., Phillips, M. B., and Hess, S. M. (1972) Biochem. Pharmacol. 21, 2443–2450
32. Posada, J., and Cooper, J. A. (1992) Science 255, 212–216
33. Lastick, S. M., and McConkey, E. H. (1978) in Cell Reproduction: ICN-UCLA Symposia on Molecular and Cellular Biology (Dirksen, E. R., Prescott, D. M., and Fox, C. F., eds) Academic Press, Inc., New York
34. Kozma, S. C., Ferrari, S., Bassand, P., Siegmann, M., Totty, N., and Thomas, G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7365–7369