Structural requirement of leucine for activation of p70 S6 kinase

Kaori Shigemitsu[a], Yosuke Tsujishita[a], Hiroshi Miyake[b], Sujuti Hidayat[a], Noriaki Tanaka[c], Kenta Hara[a], Kazuyoshi Yonezawa[a,*]

[a] *Biosignal Research Center, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan*
[b] *Fujisawa Pharmaceutical Co., Ltd., 3-4-7 Doshomachi, Chuo-ku, Osaka 541-8514, Japan*
[c] *The First Department of Surgery, Okayama University School of Medicine, 2-5-1 Shikata-cho, Okayama 700-0914, Japan*

Received 22 January 1999; received in revised form 22 February 1999

Abstract The addition of leucine induced activation of $p70^{S6k}$ in amino acid-depleted H4IIE cells. Whereas the activation of $p70^{S6k}$ by leucine was transient, the complete amino acid stimulated $p70^{S6k}$ more persistently. The effect of leucine on $p70^{S6k}$ was sensitive to rapamycin, but less sensitive to wortmannin. Using various amino acids and derivatives of leucine, we found that the chirality, the structure of the four branched hydrocarbons, and the primary amine are required for the ability of leucine to stimulate $p70^{S6k}$, indicating that the structural requirement of leucine to induce $p70^{S6k}$ activation is very strict and precise. In addition, some leucine derivatives exhibited the ability to stimulate $p70^{S6k}$ and the other derivatives acted as inhibitors against the leucine-induced activation of $p70^{S6k}$.

Key words: Leucine; p70 S6 kinase; Amino acid; Translational control

1. Introduction

Amino acids represent a large group of mutually analogous nutrients. They compose proteins, which perform a wide variety of important functions, such as serving as structural components of cells and tissues, an energy source at the skeletal muscles, and acting as protein hormones, antibodies, and enzymes [1]. Another critical role of amino acids is the regulation of translational effectors, including p70 S6 kinase ($p70^{S6k}$), and eIF-4E binding protein1/PHAS1 (4E-BP1) [2–8].

$p70^{S6k}$ is activated through multisite phosphorylation in response to insulin/mitogen and phosphorylates 40S ribosomal protein S6 in vivo [9]. This protein kinase has been shown to play a critical role in regulating selective translation of mRNAs that contain a polypyrimidine tract at their 5′ end [10,11]. 4E-BP1 binds to the 7-methylguanosine cap-binding protein eIF-4E, and prevents eIF-4E from binding to p220/eIF-4G [12]. Mitogens stimulate the phosphorylation of 4E-BP1, resulting in its dissociation from eIF-4E [13], and this makes eIF-4E available for incorporation into the translational initiation complex and restoring translation [14].

$p70^{S6k}$ and 4E-BP1 are both dephosphorylated in vivo in response to the macrolide immunosuppressant rapamycin [15,16]. This compound binds to FKBP12 and FKBP12-rapamycin forms complex with the target of rapamycin (designated mTOR, also named FRAP, RAFT-1, and RAPT-1) [15,17]. The protein kinase activity of mTOR is inhibited by the binding of FKBP12-rapamycin [17]. Recent studies have revealed that mTOR is an upstream regulator of $p70^{S6k}$ and 4E-BP1 [17–19].

More recently, the phosphorylation and the activation of $p70^{S6k}$ and 4E-BP1 in vivo has been reported to be governed by the availability of amino acids [2–8]. Our previous study resulted in the following observations [3]: (i) while amino acid withdrawal diminishes $p70^{S6k}$ activity and phosphorylation of 4E-BP1 in CHO-IR cells and HEK293 cells, amino acid repletion restores these responses; (ii) these effects of amino acid repletion are blocked by rapamycin; (iii) the $p70^{S6k}$ mutant, p70Δ2–46/ΔCT104, which is resistant to rapamycin, is also insensitive to inhibition by amino acid withdrawal. These results suggest that amino acids control $p70^{S6k}$ and 4E-BP1 through a mechanism involving the mTOR signaling pathway.

In the present study, we examined the ability of individual amino acids to stimulate $p70^{S6k}$ activation using H4IIE rat hepatoma cells, in which we have previously shown that the addition of amino acids activates $p70^{S6k}$ and 4E-BP1 and that amino acid depletion from the culture medium can cause the generation of amino acids endogenously through autophagy, a major mechanism of facultative protein degradation in the liver [8]. We showed that the addition of leucine alone after withdrawal of medium amino acid stimulates $p70^{S6k}$ activity. Then using various derivatives of leucine, we further explored which structural characteristics are required to stimulate $p70^{S6k}$ activity.

2. Materials and methods

2.1. Reagents and antibodies

Minimal essential medium α (αMEM) and fetal calf serum (FCS) were purchased from Gibco BRL. Amino acid-deprived medium was prepared by mixing all ingredients contained in Dulbecco's modified Eagle's medium (DMEM) except for amino acids. Protein G-Sepharose 4FF was from Pharmacia. [γ-^{32}P]ATP was purchased from Amersham. Wortmannin was purchased from Sigma. Rapamycin was purchased from Calbiochem. Amino acids were purchased from Wako. Derivatives of leucine were from Bachem. The polyclonal antiserum against the C-terminal 104 amino acids of $p70^{S6k}$ ($p70^{S6k}$CT Ab) used for immunoprecipitation of endogenous $p70^{S6k}$ was a generous gift from Dr. Joseph Avruch (The Diabetes Unit, Massachusetts General Hospital).

2.2. Cell culture and treatments

Rat hepatoma H4IIE cells were grown in αMEM with 10% FCS. Cells were first incubated in αMEM without FCS for 24 h, washed once with amino acid-deprived medium and incubated in the same medium for 2 h. Then, the cells were incubated with the media containing various amino acids or amino acid derivatives as indicated in

*Corresponding author. Fax: (81) (78) 803-1259.
E-mail: yonezawa@kobe-u.ac.jp

Abbreviations: CHO-IR, Chinese hamster ovary cells overexpressing human insulin receptors; DMEM, Dulbecco's modified Eagle's medium; αMEM, minimal essential medium α; MOPS, 4-morpholinepropanesulfonic acid; PAGE, polyacrylamide gel electrophoresis

PII: S0014-5793(99)00304-X

each experiment. The concentration of each amino acid except for tryptophan designated 1× is equivalent to that found in perfusate basal plasma of rat [20] as follows (in μM): arginine, 220; cystine, 34; glutamine, 716; histidine, 92; isoleucine, 114; leucine, 204; lysine, 408; methionine, 60; phenylalanine, 96; proline, 437; serine, 657; threonine, 329; tyrosine, 98; valine, 250. The concentration of tryptophan in DMEM (78 μM) is employed as 1× concentration. A mixture of all these amino acids, each at this concentration, is designated the 'complete amino acid mixture at 1× concentration'. As a reference, the concentration of each amino acid contained in αMEM was as follows (in μM); alanine, 281; arginine, 603; asparagine, 333; aspartic acid, 225; cystine, 99; cysteine, 569; glutamic acid, 510; glutamine, 1998; glycine, 666; histidine, 200; isoleucine, 396; leucine, 396; lysine,400; methionine, 101; phenylalanine, 194; proline, 347; serine, 238; threonine, 403; tryptophan, 49; tyrosine, 287; valine, 393. Treatment of cells was terminated by removal of the medium, followed by freezing with liquid nitrogen; then cells were stored at −80°C until use. Cells were extracted into ice-cold buffer A (50 mM Tris-HCl at pH 8.0, 1% Nonidet P-40, 120 mM NaCl, 20 mM NaF, 1 mM EDTA, 6 mM EGTA, 20 mM β-glycerophosphate, 0.5 mM dithiothreitol, 50 μM *p*-amidinophenylmethylsulfonyl fluoride hydrochloride, 1 μg/ml aprotinin, 1 μg/ml leupeptin), and the extracts were centrifuged at 10 000×*g* for 20 min at 4°C prior to analysis.

2.3. Immunoprecipitation and p70 S6 kinase assays

The $p70^{S6k}$ activity was determined in the immunoprecipitates using 40S ribosomal subunits as substrate. Cells were lysed in ice-cold buffer A, and the extracts were centrifuged at 10 000×*g* for 20 min at 4°C. Aliquots of the supernatants were subjected to immunoprecipitation with $p70^{S6k}$CT Ab for 2 h, absorbed to protein G Sepharose 4FF, washed twice with buffer A containing 0.5 M NaCl, and twice with wash buffer (20 mM MOPS at pH 7.2, 10 mM β-glycerophosphate). The 40S S6 protein kinase assay was started by adding the reaction mixture (50 mM MOPS at pH 7.2, 12 mM $MgCl_2$, 2 mM EGTA, 1 mM dithiothreitol, 60 μM ATP, 10 mM β-glycerophosphate, 0.5 μM protein kinase inhibitor, 0.5 A_{260} units of 40S ribosomal subunits, 5 μCi of [γ-^{32}P]ATP), incubated for 20 min at 30°C, and terminated by adding the SDS sample buffer. The samples were separated by SDS-PAGE on 12% acrylamide gel, and the radioactivity incorporated into 40S S6 protein of each sample was quantitated with BAS-2000 Bioimaging analyzer (Fuji).

Table 1
Stimulation of $p70^{S6k}$ activity by individual amino acids and leucine derivatives in amino acid-depleted cells

Amino acid	$p70^{S6k}$ activity (%)
None	18 ± 5.6
(A)	
Complete amino acids	100
L-Leucine	120 ± 28
L-Methionine	47 ± 2.2
L-Histidine	27 ± 3.0
L-Glutamine	24 ± 8.7
L-Valine	21 ± 0.34
L-Arginine	20 ± 9.8
L-Tyrosine	20 ± 5.6
L-Proline	20 ± 1.6
L-Isoleucine	17 ± 6.1
L-Threonine	16 ± 2.3
L-Lysine	14 ± 0.13
L-Tryptophan	15 ± 2.5
L-Phenylalanine	13 ± 1.3
L-Cystine	12 ± 3.2
(B)	
L-Leucine	100
D-Leucine	20 ± 1.2
Ac-Leu-OH	19 ± 3.2
N-Me-Leu-OH	13 ± 4.9
H-Leu-NH_2•HCl	250 ± 4.6
Ac-Leu-NH_2	17 ± 4.2
Ac-Leu-NHMe	11 ± 0.76
H-α-Me-DL-Leu-OH	140 ± 6.3

After serum starvation for 24 h, H4IIE cells were incubated with amino acid-deprived medium for 2 h. The cells were incubated for another 20 min with amino acid-depleted medium (None), the medium containing the complete amino acid mixture at 4× concentration (Complete amino acids), each amino acid at 4× concentration (A) or various leucine derivatives at 4× concentration (B). $p70^{S6k}$ activity was determined as described in Section 2. The activity in the cells treated with complete amino acids at 4× concentration (A) or L-leucine at 4× concentration (B) was used as 100%. Data are means ± S.D. of triplicates.

3. Results and discussion

3.1. Leucine stimulates $p70^{S6k}$ activity in amino acid-depleted cells

The ability of individual amino acids to stimulate $p70^{S6k}$ activity following amino acid withdrawal was examined using H4IIE cells. As shown in Table 1A, the readdition of the complete amino acid mixture at 4× concentration to the amino acid-depleted cells stimulated $p70^{S6k}$ activity up to 5-fold compared to control. Among the amino acids, leucine and methionine at 4× concentration enhanced $p70^{S6k}$ activity 6- and 2.4-fold, respectively. However, the addition of the other amino acids did not stimulate $p70^{S6k}$ activity significantly. As with $p70^{S6k}$, leucine at 4× concentration stimulated 4E-BP1 phosphorylation (data not shown). Consistent with our observations, the other reports have demonstrated that the readdition of leucine alone after amino acid withdrawal activates $p70^{S6k}$ activity in FAO hepatoma cells [5] and 4E-BP1 phosphorylation in isolated adipocytes [21] and pancreatic β-cells [22]. Xu et al. has shown that valine and isoleucine, as well as leucine, are capable of stimulating the phosphorylation of 4E-BP1 in pancreatic β-cells [22]. The concentrations of branched amino acids they employed were 4–10 mM, which is 10–20 times higher than those used in the present study. The concentrations of amino acids we used are much closer to those found in physiological conditions [20]. Stimulation of $p70^{S6k}$ activity by leucine was observed in a time- and dose-dependent manner, as shown in Figs. 1A and 1B. The $p70^{S6k}$ activity reached a maximum at 20 min after the addition of leucine at 4× concentration and then gradually decreased. On the other

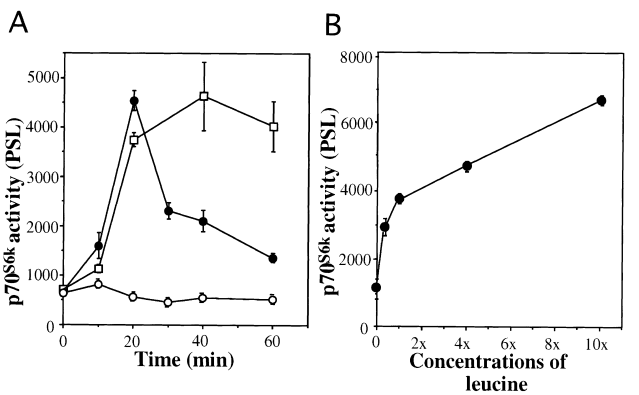

Fig. 1. Stimulation of $p70^{S6k}$ activity by the addition of leucine in amino acid-depleted cells. After serum starvation for 24 h, H4IIE cells were incubated with amino acid-deprived medium for 2 h. A: The cells were then incubated with amino acid-deprived medium in the presence (closed circles) or absence (open circles) of leucine at 4× concentration (1× = 204 μM), or complete medium at 4× concentration (open squares) for the indicated times. B: The cells were incubated with amino acid-deprived medium containing various concentrations of leucine for 20 min. $p70^{S6k}$ activity was determined as described in Section 2, and expressed in arbitrary units (photostimulated luminescence (PSL) units). Data are the means ± S.D. of triplicates.

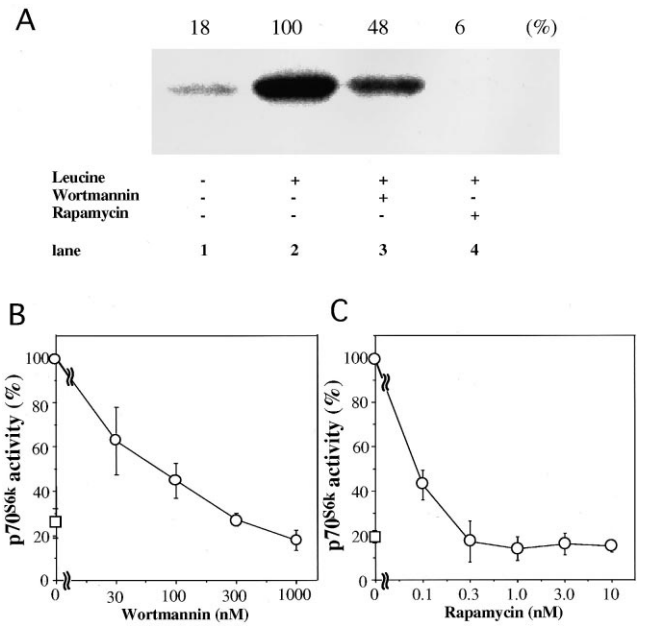

Fig. 2. Effects of wortmannin and rapamycin on the leucine-induced $p70^{S6k}$ activation in amino acid-depleted cells. A: After serum starvation for 24 h, H4IIE cells were incubated with amino acid-deprived medium for 2 h. The cells were then incubated for another 20 min with either amino acid-deprived medium (lane 1) or medium containing leucine at 4× concentration (lanes 2–4). Prior to the addition of leucine, the cells were pretreated for 30 min with vehicle (lane 2), 100 nM wortmannin (lane 3), or 100 nM rapamycin (lane 4). $p70^{S6k}$ activity was determined as described in Section 2. Numbers at the top of each lane represent ^{32}P incorporated into S6 expressed as a percentage of that catalyzed by $p70^{S6k}$ immunoprecipitated from cell extracts treated with leucine at 4× concentration following amino acid withdrawal (lane 2). B and C: After serum starvation for 24 h, H4IIE cells were incubated with amino acid-deprived medium for 2 h (open squares). The cells were then incubated for another 20 min with amino acid-deprived medium containing leucine at 4× concentration (open circles). Prior to the addition of leucine, cells were pretreated with the indicated concentrations of wortmannin (B) or rapamycin (C) for 30 min. $p70^{S6k}$ activity was determined as described in Section 2. $p70^{S6k}$ activity in the cells without pretreatment was used as 100%. Data are the means ± S.D. of triplicates.

hand, the complete amino acid at 4× concentration stimulated the $p70^{S6k}$ activation more persistently and kept stimulating up to 30–60 min. Thus, some other amino acids appear to play a role in inducing persistent $p70^{S6k}$ activation in concert with leucine.

3.2. *Effects of wortmannin and rapamycin on leucine-induced $p70^{S6k}$ activation*

We next examined the upstream inputs required for the leucine-induced $p70^{S6k}$ activation. H4IIE cells were subjected to amino acid withdrawal for 2 h, and pretreated with either the phosphoinositide 3-kinase (PI3-k) inhibitor, wortmannin or the mTOR kinase inhibitor, rapamycin prior to stimulation with leucine (Fig. 2A, lanes 3 and 4). The activation of $p70^{S6k}$ by leucine was strongly inhibited by 100 nM rapamycin, and moderately altered by 100 nM wortmannin. The leucine-induced increase in $p70^{S6k}$ activity was inhibited completely at 1000 nM wortmannin (Fig. 2B) and at 0.3–1.0 nM rapamycin (Fig. 2C). The sensitivities of leucine-activated $p70^{S6k}$ to wortmannin and rapamycin were similar to those of amino acid-activated $p70^{S6k}$ to these agents as described previously in H4IIE cells [9]. As with $p70^{S6k}$, the ability of leucine at 4× concentration to stimulate 4E-BP1 phosphorylation was sensitive to rapamycin, but much less sensitive to wortmannin (data not shown). Thus, these observations suggest that leucine induces $p70^{S6k}$ activation and 4E-BP1 phosphorylation in H4IIE cells through amino acid-mediated signaling, and leucine plays a critical role in amino acid-induced $p70^{S6k}$ activation and 4E-BP1 phosphorylation.

3.3. *The structural requirement of leucine for activation of $p70^{S6k}$*

The present data shown in Table 1A clearly indicate that hydrophilic side chains (such as glutamine, arginine, and lysine) and aromatic side chains (such as histidine, tyrosine, tryptophan and phenylalanine) are not required to activate $p70^{S6k}$. Among aliphatic side chains, the structure of the four branched hydrocarbons of leucine is critical for the ability of leucine to activate $p70^{S6k}$. However, the other branched-chain amino acids, such as valine and isoleucine, failed to activate $p70^{S6k}$ in H4IIE cells.

In order to further analyze the structural requirement of leucine for the activation of $p70^{S6k}$, we employed the following leucine derivatives (Fig. 3): (a) the optical isomer of L-leucine, D-leucine; (b) leucine with its modified amino group, *N*-acetylleucine (Ac-Leu-OH) and *N*-methylleucine (N-Me-Leu-OH); (c) leucine with its modified carboxyl group, leucine amide hydrochloride (H-Leu-NH_2•HCl); (d) leucine with its modified amino and carboxyl groups, *N*-acetylleucine amide (Ac-Leu-NH_2) and *N*-acetylleucine *N*-methylamide (Ac-Leu-NHMe); (e) leucine with its modified α-hydrogen atom: α-methyl-DL-leucine (H-α-Me-DL-Leu-OH), which is a mixture of D- and L-isomers (1:1 molar ratio). After amino acid withdrawal for 2 h, H4IIE cells were treated with each of these leucine derivatives at 4× concentration. As shown in Table 1B, (i) D-leucine, the optical isomer of L-leucine, did not activate $p70^{S6k}$; (ii) modification of the amino group (Ac-Leu-OH, N-Me-Leu-OH, Ac-Leu-NH_2, and Ac-Leu-NHMe) abolished the ability of leucine to activate $p70^{S6k}$; (iii) modification of the carboxyl group (H-Leu-NH_2•HCl) conserved the ability of leucine to activate $p70^{S6k}$; (iv) modification of the α-hydro-

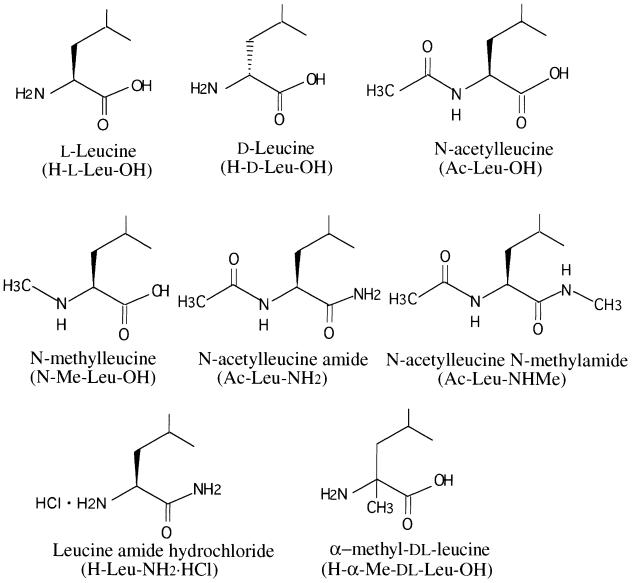

Fig. 3. Structures of leucine derivatives. Formal nomenclatures and abbreviations (in parentheses) are shown.

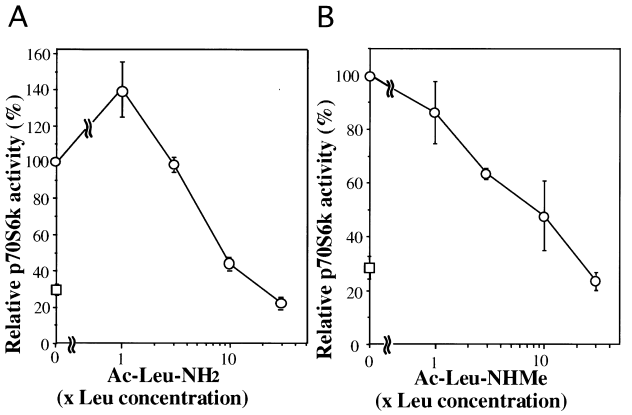

Fig. 4. Effects of leucine derivatives on the leucine-induced $p70^{S6k}$ activation in the amino acid-depleted cells. After serum starvation for 24 h, H4IIE cells were incubated with amino acid-deprived medium for 2 h (open squares). The cells were then incubated for 20 min with amino acid-deprived medium containing leucine at 4× concentration in the presence of Ac-Leu-NH_2 (A) or Ac-Leu-NHMe (B) at the indicated concentrations relative to the concentration of leucine (open circles). $p70^{S6k}$ activity was determined as described in Section 2. $p70^{S6k}$ activity stimulated with leucine at 4× concentration in the absence of leucine derivatives was used as 100%. Data are the means ± S.D. of triplicates.

gen (H-α-Me-DL-Leu-OH) also did not abolish the ability of leucine to activate $p70^{S6k}$. Thus, these results suggest the following points: (i) the chirality (stereoselectivity) is critical; (ii) the primary amine of leucine is also quite important, which is based on the results of the diminished ability of N-Me-Leu-OH; (iii) the carboxyl group and the α-hydrogen of leucine are not critical.

3.4. Inhibitory effects of Ac-Leu-NH_2 and Ac-Leu-NHMe on the leucine-induced $p70^{S6k}$ activation

The effects of leucine derivatives, Ac-Leu-NH_2 and Ac-Leu-NHMe, that do not stimulate $p70^{S6k}$ activity were examined on the leucine-induced $p70^{S6k}$ activation. Among these derivatives, Ac-Leu-NH_2 exhibited slightly stimulatory effects at the lower concentration and inhibitory at the higher concentrations on the leucine-induced $p70^{S6k}$ activation (Fig. 4A). Ac-Leu-NHMe strongly inhibited the leucine-induced $p70^{S6k}$ activation in a dose-dependent manner (Fig. 4B). Both leucine derivatives exhibited maximum inhibition of the leucine-induced $p70^{S6k}$ activation at an approximately 30 times higher concentration than that of leucine used to activate $p70^{S6k}$. These derivatives would be a good tool to control various cellular events, which are known to be regulated by amino acids and/or leucine.

3.5. Conclusion

Using various amino acids and derivatives of leucine, we found that the chirality (stereo-activity), the structure of the four branched hydrocarbons of leucine, and the primary amine of leucine are required to stimulate $p70^{S6k}$ activity. In addition, some leucine derivatives exhibited the ability to stimulate $p70^{S6k}$, and the other derivatives lacked this ability, rather acting as inhibitors against the leucine-induced activation of $p70^{S6k}$.

The mechanism of the leucine-induced $p70^{S6k}$ activation remains to be elucidated. If there does exist an acceptor molecule(s) specific to leucine, the recognition of this acceptor by leucine should be very strict and precise. The system L amino acid transporter has been known to incorporate isoleucine, valine, methionine, phenylalanine, histidine, and 2-(−)-endo-amino-bicycloheptane-2-carboxylic acid from the medium to the intracellular space as well as leucine [23]. Because the structural requirement of these amino acids by the system L transporter is not strict, the system L transporter might not be a candidate for the acceptor protein for leucine to induce the activation of $p70^{S6k}$.

Further analysis of the leucine-induced activation of translational effectors will reveal the mechanism of cellular events mediated by the mTOR signaling pathway, such as mRNA translation, autophagy, and proliferation of T cells.

Acknowledgements: This work was supported in part by research grants from the Ministry of Education, Science, Sports and Culture of Japan and the Sankyo Foundation of Life Science. We are grateful to Dr. Y. Nishizuka for encouragement and Drs. J. Avruch and U. Kikkawa for valuable advice. The skilful secretarial assistance of M. Kusu is cordially acknowledged.

References

[1] Cooper, G.M. (1997) in: The Cell: A Molecular Approach, pp. 39–85, American Society for Microbiology, Washington, DC.
[2] Svanberg, E., Jefferson, L.S., Lundholm, K. and Kimball, S.R. (1997) Am. J. Physiol. 272, E841–847.
[3] Hara, K., Yonezawa, K., Weng, Q.P., Kozlowski, M.T., Belham, C. and Avruch, J. (1998) J. Biol. Chem. 273, 14484–14494.
[4] Xu, G., Marshall, C.A., Lin, T.A., Kwon, G., Munivenkatappa, R.B., Hill, J.R., Lawrence Jr., J.C. and McDaniel, M.L. (1998) J. Biol. Chem. 273, 4485–4491.
[5] Patti, M.E., Brambilla, E., Luzi, L., Landaker, E.J. and Kahn, C.R. (1998) J. Clin. Invest. 101, 1519–1529.
[6] Fox, H., Kimball, S., Jefferson, L. and Lynch, C. (1998) Am. J. Physiol. 274, C206–213.
[7] Wang, X., Campbell, L., Miller, C. and Proud, C. (1998) Biochem. J. 334, 261–267.
[8] Shigemitsu, K., Tsujishita, Y., Hara, K., Nanahoshi, M., Avruch, J. and Yonezawa, K. (1999) J. Biol. Chem. 274, 1058–1065.
[9] Avruch, J. (1998) Mol. Cell. Biochem. 182, 31–48.
[10] Jefferies, H.B., Fumagalli, S., Dennis, P.B., Reinhard, C., Pearson, R.B. and Thomas, G. (1997) EMBO J. 16, 3693–3704.
[11] Terada, N., Patel, H.R., Takase, K., Kohno, K., Nairn, A.C. and Gelfand, E.W. (1994) Proc. Natl. Acad. Sci. USA 91, 11477–11481.
[12] Haghighat, A., Mader, S., Pause, A. and Sonenberg, N. (1995) EMBO J. 14, 5701–5709.
[13] Pause, A., Belsham, G.J., Gingras, A.C., Donze, O., Lin, T.A., Lawrence Jr., J.C. and Sonenberg, N. (1994) Nature 371, 762–767.
[14] Sonenberg, N. (1996) in: Translational Control (Hershey, J.W.B., Mathews, M.B. and Sonenberg, N., Eds), pp. 245–270, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[15] Abraham, R.T. and Wiederrecht, G.J. (1996) Annu. Rev. Immunol. 14, 483–510.
[16] Hall, M.N. (1996) Biochem. Soc. Trans. 24, 234–239.
[17] Brown, E.J., Beal, P.A., Keith, C.T., Chen, J., Shin, T.B. and Schreiber, S.L. (1995) Nature 377, 441–446.
[18] Hara, K., Yonezawa, K., Kozlowski, M.T., Sugimoto, T., Andrabi, K., Weng, Q.P., Kasuga, M., Nishimoto, I. and Avruch, J. (1997) J. Biol. Chem. 272, 26457–26463.
[19] Brunn, G.J., Hudson, C.C., Sekulic, A., Williams, J.M., Hosoi, H., Houghton, P.J., Lawrence Jr., J.C. and Abraham, R.T. (1997) Science 277, 99–101.
[20] Mortimore, G.E., Poso, A.R., Kadowaki, M. and Wert, J.J.J. (1987) J. Biol. Chem. 262, 16322–16327.
[21] Fox, H.L., Pham, P.T., Kimball, S.R., Jefferson, L.S. and Lynch, C.J. (1998) Am. J. Physiol. 275, C1232–1238.
[22] Xu, G., Kwon, G., Marshall, C.A., Lin, T.A., Lawrence Jr., J.C. and McDaniel, M.L. (1998) J. Biol. Chem. 273, 28178–28184.
[23] Christiansen, H.N. (1990) Physiol. Rev. 70, 43–71.