Amino Acids as Regulators of Proteolysis[1,2]

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ABSTRACT Proteolysis, as well as protein synthesis, is a major process that contributes to the body protein turnover. Despite the huge variety of proteases in the body, there are very few proteolytic systems contributing to the complete hydrolysis of proteins to amino acids. The autophagic-lysosomal pathway is responsible for bulk proteolysis, whereas the ubiquitin-proteasome pathway plays a significant role in the fine control of the degradation of specific proteins. Both systems can produce free amino acids as a final product, but only the autophagy system is physiologically controlled by plasma amino acids. Recently, the study of amino acids as regulators of macromolecular turnover has been focused on for their signal transduction mechanism. In autophagic proteolysis, several amino acids have a direct regulatory potential: Leu, Gln, Tyr, Phe, Pro, Met, Trp and His in the liver, and Leu in the skeletal muscle. These amino acids are recognized at the plasma membrane, indicating the possible existence of an amino acid receptor/sensor for their recognition and subsequent intracellular signaling. Another line of evidence has emerged that protein kinase cascades such as mTOR, Erk, eIF2α etc. may be involved in the regulation of autophagy, and that amino acids, in combination with insulin, may exert their effects through these pathways. From the viewpoint of amino acid safety, the contribution of proteolysis to possible adverse effects caused by excessive amino acid intake is not clear. At present, there is one report that excess glutamine at 10-fold the plasma level has an abnormal inhibitory effect on hepatic proteolysis, due to a lysosomotropic toxicity of ammonia derived from glutamine degradation. Whether this may lead to an adverse effect in humans remains to be clarified. J. Nutr. 133: 2052S–2056S, 2003.

KEY WORDS: • *autophagy* • *proteasome* • *amino acid signaling* • *mTOR* • *glutamine*

For the evaluation of the adequate intake of dietary amino acids and the assessment of their safety, basic knowledge of the protein metabolism in the body is essential. Protein synthesis and proteolysis are the major two fluxes. Both are coordinately regulated in the physiological state, but their mechanisms are entirely independent. The rate of proteolysis, as well as that of protein synthesis, is continually controlled to maintain homeostasis of body protein. However, it is hard to determine a priori which is desirable, a high rate or a low one, in a given physiological state. In its accelerated condition beyond a normal range, suppression of proteolysis to the normal level is definitely of benefit to the body. However, within a normal range, maintenance of a higher rate of proteolysis with increasing age might be advantageous to our health (1). When proteolysis is accelerated due to a physiological need, e.g., in a postabsorptive period or early starvation, the compulsory suppression of proteolysis by amino acids below the level beyond physiological adaptation may theoretically be harmful to the body, although such an adverse effect has not been reported before. Amino acids, in combination with hormones, are well known to be primary regulators of body protein turnover (2). In this article, recent findings on the physiological role of bulk proteolysis, major proteolytic pathways, the role of amino acids and their regulatory mechanism and the possibility of inducing an adverse effect by excess intakes of amino acids, will be discussed.

Physiological significance of proteolysis in protein metabolism

Protein synthesis and degradation are equally important processes for living organisms including humans. However, the mechanism of proteolysis is much less understood than that of protein synthesis. The regulatory mechanisms of these two processes are likewise not well understood, although enormous efforts have been undertaken to elucidate them. Nevertheless, in a review article on protein turnover in humans published in 1995, Waterlow (3), looking back over the past two decades, commented as follows:

"Ever since Schoenheimer's discovery of the dynamic state of body constituents, we have been faced with the

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question of how to maintain a steady state. ….. Amino acids and hormones are effectors that can stimulate these processes, but we do not know how they are coordinated, i.e., who conducts the orchestra. The evidence reviewed above suggests that protein degradation is more sensitive than protein synthesis. In Young's summary of the effects of insulin and amino acids on protein turnover, it is remarkable in how many studies the main effect was on protein breakdown."

Recently, it was also reported in a human study that the efficiency of protein utilization in individuals is determined by the variation in the sensitivity of proteolysis to amino acid supply, rather than that of protein synthesis (4). Thus, an understanding of proteolysis is very important for understanding protein turnover. The major reason for the lack of our knowledge about proteolysis is that we do not yet have a simple and accurate method to study proteolysis in vivo yet, compared with that for the study of protein synthesis. Thus, if such a critical in vivo method could be developed, it would be a major breakthrough in this field.

Intracellular proteolytic pathways: autophagy and proteasome

Proteolysis in the body is extremely versatile and complicated processes, which are controlled as delicately as protein synthesis. There are a huge number of specific and nonspecific proteases in the body, but there are only a few complete proteolytic systems hydrolyzing proteins to free amino acids and also contributing to bulk proteolysis. The most famous and classic pathway is a lysosomal system called autophagy, which results in endogenous protein degradation (2, 5). Autophagy is an intracellular membrane-mediated process and can be regulated by physiological effectors such as insulin, glucagon and amino acids. It should be emphasized that autophagy is basically a nonselective process and degrades not only proteins but also all the other cellular constituents, RNA, sugars, lipids, phospholipids, etc. It is a kind of cell restructuring apparatus.

Another major pathway is a ubiquitin-proteasome system, which is also ubiquitous throughout the body, ATP dependent and degrades ubiquitin-conjugated proteins via the 26 S proteasome (6). It is involved in many biologically important processes, such as transcriptional regulation, cell cycle control, antigen processing, apoptosis, DNA repair, etc. The proteins degraded by this system turn over quite rapidly. Both the autophagic and proteasome systems have been thought to have totally different mechanisms and to play their physiological roles in an entirely independent manner or even in a compensatory manner in the cells. However, recent discoveries have revealed that autophagy includes ubiquitin-like conjugation systems in its molecular mechanism, such as Apg5-Apg12 (7) and Apg8-phosphatidylethanolamine (8) for its initiation step, implying that living organisms employ very similar chemical reactions for the proteolytic systems which had seemed to be physiologically unrelated.

In most visceral tissues like the liver, there is no doubt that autophagy operates bulk proteolysis (2). In contrast, in peripheral tissues, mainly skeletal muscle, the proteasome system is regarded to be the main proteolytic pathway for bulk proteolysis (9). However, the relative contributions of both systems in skeletal muscle have not yet been evaluated accurately. A great deal of the molecular mechanism of the proteasome system has been elucidated, but its physiological regulation remains to be solved. Despite a lot of evidence showing parallel control of muscle proteolysis and expression of the components of the ubiquitin-proteasome pathway, the causal relationship using a specific inhibitor, e.g., lactacystin, seems not to be enough, because most inhibitors used in cell studies, e.g., peptide aldehydes, are not strictly specific for the proteasome (10). In the case of myofibrillar proteins, especially intact myofibrils, the proteasome does not work directly and the rate-limiting step in their degradation seems their dissociation from the myofibril (11). Moreover, amino acids have a regulatory effect on proteolysis not only in the liver but also in skeletal muscle, but their effect on the muscle proteasome pathway has not yet been reported. In regard to this, the recent findings that protein feeding (12) and even feeding leucine alone (13) rapidly reduced the plasma level of 3-methylhistidine, a specific marker of myofibrillar proteolysis in vivo, together with its release from muscle incubation in vitro, strongly indicate that amino acids control myofibrillar proteins. This may require reconsideration of the well-cited ideas that amino acids and thus lysosomes are not involved in the proteolysis of myofibrillar proteins (14,15).

One of the major reasons for considering that the autophagic-lysosomal system has a minor role in skeletal muscle has been the morphological evidence that typical and spherical lysosome structures have rarely been seen in normal muscle tissues electronmicroscopically. Nevertheless, it was reported recently that LC3[4], a mammalian homolog of yeast Apg8p, which is defined as one of the autophagy-specific proteins associated with the autophagosomal membranes (16), was also identified in skeletal muscle (17,18). LC3 in muscle was as abundant as in the liver and induced by starvation. This clearly demonstrates the existence of the autophagic apparatus in skeletal muscle. Although there remain controversies on the contribution of these proteolytic pathways in skeletal muscle, amino acids as a regulator of proteolysis have been reported only in connection with the autophagic pathway (2), even in skeletal muscle (19). Thus, we will focus on autophagy to describe the regulatory mechanism of amino acids on the proteolytic system.

Regulatory mechanism of autophagy by amino acids

In mammals, autophagy is most actively expressed in the liver and most sensitive to amino acid regulation. Because it has been extensively studied in the liver, we will describe the details in the liver. Hepatic autophagy is best characterized as being controlled by plasma amino acids, especially, leucine, tyrosine, (phenylalanine), glutamine, histidine, tryptophan, proline and methionine, which are called regulatory amino acids, with alanine as a coregulatory amino acid (2). This amino acid-responsive form is defined as macroautophagy from its morphological characteristics. Amino acids regulate it in a suppressive manner. This is quite reasonable because autophagic proteolysis is thought to be a major source of amino acids during starvation. It is regarded as a feedback product inhibition mechanism.

Although the regulatory effect of amino acids on protein turnover, its synthesis and degradation, was discovered a long time ago (20,21), the mechanism of their regulation has not captured the interest of researchers until recently. Tischler et al. (22) proposed that the effect of leucine on protein degradation in skeletal muscle is mediated via the conversion to its keto

[4] Abbreviations used: LC3, (microtubule-associated protein)-light chain 3; Leu-MAP, leucyl-multiple antigen peptide; mTOR, mammalian target of rapamycin.

acid, α-ketoisocaproate. In contrast, based on the evidence that leucine, an active regulator in the liver which has no substantial transamination activity, Mortimore and his colleagues hypothesized that amino acids, including leucine, control proteolysis not after being metabolized, but directly from extracellular sites in the liver (2). This was finally demonstrated in an elegant but indirect experiment using a nontransportable leucine analog, Leu_8-MAP (23). This model inevitably requires the existence of an amino acid receptor or sensor at the plasma membrane and of the subsequent signal transduction mechanism down to the intracellular site of proteolysis, i.e., the autophagic apparatus (**Fig. 1**). This kind of amino acid receptor(s) has yet to be discovered in mammalian tissues, although a putative amino acid sensor molecule, Ssy1p, was proposed in yeast (24,25). In hepatocytes, a possible candidate for this amino acid receptor, a leucine binding protein of the plasma membrane, was reported (26). Furthermore, this leucine binding protein was detected more specifically by using a photoaffinity-labeled derivative of Leu_7-MAP (27).

Another part of the regulatory mechanism of amino acids is an intracellular signaling pathway. A unique idea came from the data showing the loss of amino acid responsiveness in hepatocytes permeabilized by *S. aureus* α-toxin, which makes small molecules freely permeable. The preliminary finding that a liver extract stimulated with regulatory amino acids mimicked their proteolytic effect in the permeabilized hepatocytes strongly suggest the possible existence of a low molecular weight effector(s) for this signaling pathway (M. Kadowaki and R. Akaishi, unpublished results, 2002). The proteolytic activity of this extract was extractable with ethyl acetate, which could be separated from the amino acids remaining in the extract. Independently, another line of evidence for the contribution of protein phosphorylation signaling for amino acid regulation has been developed. A pioneering study was reported by Meijer and his group that amino acids regulate proteolysis in hepatocytes by stimulating ribosomal protein S6, which leads to the involvement of the mTOR signaling pathway (28). Similar findings that the regulation of protein translation by amino acids employs mTOR signaling (29,30) strongly suggested this possibility. However, new evidence that mTOR kinase is involved in the signaling of insulin, but not of amino acids, was reported recently (31), which makes the amino acid signaling pathway more complicated.

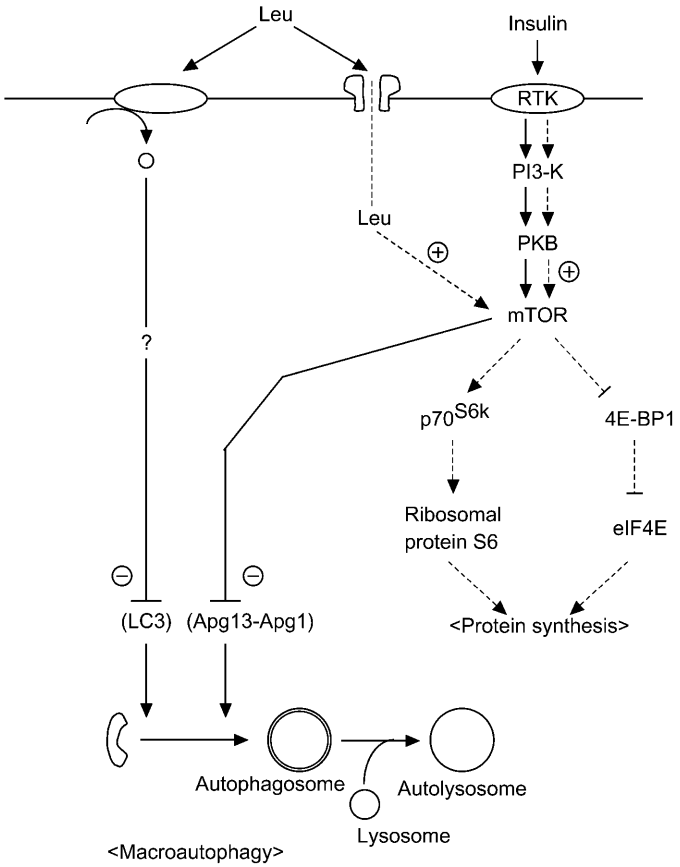

FIGURE 1 Hypothetical signaling pathways of amino acids and insulin for macroautophagy and protein synthesis. A route of amino acid signaling, in this case leucine, for macroautophagy is independent from that for protein synthesis. A route of insulin signaling for macroautophagy shares the route for protein synthesis down to mTOR kinase, although further downstream signaling is not known. Solid line, a route for macroautophagy; dashed line, a route for protein synthesis.

In addition, the contribution of extracellular ion concentrations, e.g., K^+ omission, on the antiproteolytic effect of amino acids has been reported (32), which led to the role of cell hydration. Häussinger et al. (33) proposed that certain amino acids which employ Na-dependent transport, e.g., glutamine, cause their antiproteolytic effect through osmotic swelling. They further showed the involvement of $p38^{MAPK}$ in the signaling by these amino acids (34). However, they commented on the possible existence of some other mechanisms with amino acids, such as leucine, which utilize Na-independent transport systems. Ogier-Denis et al. (35) reported that Erk1/2 signaling is involved in the amino acid control of macroautophagy in colon cancer HT-29 cells. Another possibility was suggested that the eIF2α kinase signaling pathway plays a role in the amino acid control of autophagy in murine embryonic fibroblasts (36). How all of these pieces of evidence fit into the amino acid signaling pathway awaits future studies.

In the past few years, there has been remarkable progress in the understanding of the molecular mechanism of macroautophagy in yeast and mammals (5) and possible molecular targets of amino acid regulation have been identified. Entirely new protein conjugation systems, e.g., Apg5-Apg12 (7), Apg8-phosphatidylethanolamine (8), Apg13-Apg1 (37) and a modification system of LC3 (16) have been discovered. It is highly probable that some of these proteins are candidates for the molecular target of autophagic machinery in amino acid signaling.

Possible adverse effects of amino acids on proteolysis

At the individual organ and cellular levels, there is no doubt that amino acids have a significant regulatory function on proteolysis as described above. However, when we turn our attention to the whole body level, it is quite difficult to obtain a definitive conclusion and especially to answer the question of whether or not their effect becomes untoward beyond the normal and homeostatic range. With regard to autophagic proteolysis, a line of evidence was reported with glutamine (38,39). In the perfused rat liver, glutamine at 10 times the plasma concentration (~7 mM) inhibited proteolysis to below the basal level fully suppressed by regulatory amino acids, possibly due to a lysosomotropic effect caused by ammonia released from the glutamine.

In the assessment of the safety of glutamine, Garlick (40) made a comment that, despite the large number of publications in which glutamine has been administered to patients or healthy subjects, no serious adverse effects have been reported. However, because there are still uncertainties about the safety of glutamine, it is appropriate to continue the investigation of

potentially adverse effects, especially for chronic treatment. Thus, the potentially adverse effects of excess amino acids, e.g., glutamine in this case, on autophagic proteolysis would be worthwhile considering, for the following reasons.

1. Dietary supplements usually consist of a single or a small number of amino acids. A single or a small number of amino acids are enough to affect proteolysis. In contrast, a possible adverse effect of specific amino acids on protein synthesis has not been reported and would not be expected, because a small number of amino acids would not stimulate protein synthesis.
2. If an excessive amount of a single amino acid is ingested in the fed state, in which amino acids are fully supplied from foods and proteolysis is suppressed, there would be no problem. However, if it occurs in the fasted state, in which there is no exogenous amino acid supply and proteolysis is maximized as the only endogenous source of amino acids for the body needs, a compulsory inhibition of autophagic proteolysis might lead to a deficient supply of all amino acids for protein synthesis or other specific metabolic pathways.
3. Because autophagy is a mechanism for degrading all the macromolecules in the cell, to produce not only amino acids but also nucleotides, lipids, sugars, etc., the building blocks for macromolecules in the next cycle, the abnormal inhibition of this mechanism in the fasted condition should be considered seriously, especially in the chronic aspect. In addition, a delay in the degradation of macromolecules or organelles, which need rapid removal, e.g., damaged/oxidized proteins or mitochondria, or lipofuscin accumulated in the ageing process (41, 42), may also cause a long-term adverse effect on the body.

Finally, it should be added that, on the contrary, amino acid effect on proteolysis in the short-term treatment is undoubtedly beneficial in most cases, such as the improvement of liver storage for preventing liver graft dysfunction (43).

Proteolysis is a major flux of protein turnover and affects the nitrogen balance together with protein synthesis. It is quite sensitive to physiological regulation by amino acids as well as hormones. The major proteolytic pathways in the cell include the autophagic/lysosome pathway and the ubiquitin-proteasome pathway, but the contributions of these pathways in the cell are still elusive. The details of the molecular mechanism of amino acid signaling for autophagic proteolysis have just started to be elucidated. It is possible that this proteolytic pathway is under the influence of excess amounts of amino acids, which may lead to potentially adverse effects. Although there have been no reports of adverse effects of high intakes of amino acids in relation to their anti-proteolytic effect, sufficient attention should be paid to the possibility.

LITERATURE CITED

1. Cavallini, G., Donati, A., Gori, Z., Pollera, M. & Bergamini, E. (2001) The protection of rat liver autophagic proteolysis from age-related decline co-varies with the duration of anti-aging food restriction. Exp. Gerontol. 36: 497–506.

2. Mortimore, G. E. & Kadowaki, M. (2001) Regulation of Protein Metabolism in Liver, In: The Endocrine Pancreas and Regulation of Metabolism (Jefferson,L.S. & Sherrington,A.D., eds.), Handbook of Physiology, Section 7, The Endocrine System, Volume II, pp.553–577, American Physiological Society, Oxford University Press.

3. Waterlow, J. C. (1995) Whole-body protein turnover in humans—past, present, and future. Annu. Rev. Nutr. 15: 57–92.

4. Fereday, A., Gibson, N. R., Cox, M., Pacy, P. J. & Millward, D. J. (1998) Variation in the apparent sensitivity of the insulin-mediated inhibition of proteolysis to amino acid supply determines the efficiency of protein utilization. Clin. Sci. 95: 725–733.

5. Klionsky, D. J. & Emr, S. D. (2000) Autophagy as a regulated pathway of cellular degradation. Science 290: 1717–1721.

6. Lecker, S. H., Solomon, V., Mitch, W. E. & Goldberg, A. L. (1999) Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. J. Nutr. 129: 227S–237S.

7. Mizushima, N., Noda, T., Yoshimori, T., Tanaka, Y., Ishii, T., George, M. D., Klionsky, D. J., Ohsumi, M. & Ohsumi, Y. (1998) A protein conjugation system essential for autophagy. Nature 395: 395–398.

8. Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Kominami, E., Noda, T. & Ohsumi, Y. (2000) Ubiquitination-like system mediates novel protein lipidation. Nature 408: 488–492.

9. Rock, K. L., Gramm, C., Rothstein, L., Clark, K., Stein, R., Dick, L., Hwang, D. & Goldberg, A. L. (1994) Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell 78: 761–771.

10. Lee, D. H. & Goldberg, A. L. (1998) Proteasome inhibitors: valuable new tools for cell biologists. Trends Cell Biol. 8: 397–403.

11. Solomon, V. & Goldberg, A. L. (1996) Importance of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. J. Biol. Chem. 271: 26690–26697.

12. Nagasawa, T., Hirano, J., Yoshizawa, F. & Nishizawa, N. (1998) Myofibrillar protein catabolism is rapidly suppressed following protein feeding. Biosci. Biotechnol. Biochem. 62: 1932–1937.

13. Nagasawa, T., Kido, T., Yoshizawa, F., Ito, Y. & Nishizawa, N. (2002) Rapid suppression of protein degradation in skeletal muscle after oral feeding of leucine in rats. J. Nutr. Biochem. 13: 121–127.

14. Lowell, B. B., Ruderman, N. B. & Goodman, M. N. (1986) Regulation of myofibrillar protein degradation in rat skeletal muscle during brief and prolonged starvation. Metabolism 35: 1121–1127.

15. Lowell, B. B., Ruderman, N. B. & Goodman, M. N. (1986) Evidence that lysosomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle. Biochem. J. 234: 237–240.

16. Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y. & Yoshimori, T. (2000) LC3, a mammalian homologue of yeast Apg8p is processed and localized in autophagosome membrane. EMBO J. 19: 5720–5728.

17. Kanazawa, T., Miura, S., Yoshimori, T., Fujimura, S. & Kadowaki, M. (2002) Involvement of LC3, a mammalian homologue of Apg8p, on physiological control of macroautophagy in the rat liver and skeletal muscle. Proceedings of 3rd International Symposium on Autophagy, P-16 (abst), Osaka, Japan.

18. Mizushima, N. (2002) Mice with a fluorescent marker for autophagy. Proceedings of 3rd International Symposium on Autophagy, S5–4 (abst), Osaka, Japan.

19. Mordier, S., Deval, C., Béchet, D., Tassa, A. & Ferrara, M. (2000) Leucine limitation induces autophagy and activation of lysosome-dependent proteolysis in C2C12 myotubes through a mammalian target of rapamycin-independent signaling pathway. J. Biol. Chem. 275: 29900–29906.

20. Fulks, R. M., Li, J. B. & Goldberg, A. L. (1975) Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. J. Biol. Chem. 250: 290–298.

21. Buse, M. G. & Reid, S. S. (1975) A possible regulator of protein turnover in muscle. J. Clin. Invest. 56: 1250–1261.

22. Tischler, M. E., Desautels, M. & Goldberg, A. L. (1982) Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in rat skeletal and cardiac muscle? J. Biol. Chem. 257: 1613–1621.

23. Miotto, G., Venerando, R., Marin, O., Siliprandi, N. & Mortimore, G. E. (1994) Inhibition of macroautophagy and proteolysis in the isolated rat hepatocyte by a nontransportable derivative of the multiple antigen peptide Leu_8-Lys_4-Lys_2-Lys-βAla. J. Biol. Chem. 269: 25348–25353.

24. Iraqui, I., Vissers, S., Bernard, F., de Craene, J.-O., Boles, E., Urrestarazu, A. & André, B. (1999) Amino acid signaling in *Saccharomyces cerevisiae*: a permease-like sensor of external amino acids and F-box protein Grr1p are required for transcriptional induction of the AGP gene, which encodes a broad-specificity amino acid permease. Mol. Cell. Biol. 19: 989–1001.

25. Forsberg, H. & Ljungdahl, P. O. (2001) Genetic and biochemical analysis of the yeast plasma membrane Ssy1p-Ptr3p-Ssy5p sensor of extracellular amino acids. Mol. Cell. Biol. 21: 814–826.

26. Mortimore, G. E., Wert, J. J., Jr., Miotto, G., Venerando, R. & Kadowaki, M. (1994) Leucine-specific binding of photoreactive Leu_7-MAP to a high molecular weight protein on the plasma membrane of the isolated rat hepatocytes. Biochem. Biophys. Res. Commun. 203: 200–208.

27. Miotto, G., Carampin, P., Venerando, R., Kadowaki, M. & Marin, O. (2002) Photoreactive-Leu_7-MAP-biotin: a strategic tool for the isolation of leucine receptor involved in regulation of autophagy in isolated hepatocytes. Proceedings of 3rd International Symposium on Autophagy, P-22 (abst), Osaka, Japan.

28. Blommaart, E. F. C., Luiken, J. J., Blommaart, P. J., Van Woerkom, G. M. & Meijer, A. J. (1995) Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. J. Biol. Chem. 270: 2320–2326.

29. Patti, M.-E., Brambilla, E., Luzi, L., Landaker, E. J. & Kahn, C. R. (1998) Bidirectional modulation of insulin action by amino acids. J. Clin. Invest. 101: 1519–1529.

30. Hara, K., Yonezawa, K., Weng, Q. P., Kozlowski, M. T., Belham, C. & Avruch, J. (1998) Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. J. Biol. Chem. 273: 14484–14494.

31. Kadowaki, M., Kanazawa, T., Akaishi, R., Kamimura, S. & Fujimura, S. (2002) Riddles in amino acid signaling of hepatic autophagy. Proceedings of 3rd International Symposium on Autophagy, S7–6 (abst), Osaka, Japan.

32. Leverve, X. M., Caro, L. H. P., Plomp, P. J. A. M. & Meijer, A. J. (1987) Control of proteolysis in perifused rat hepatocytes. FEBS Lett. 219: 455–458.

33. Häussinger, D., Graf, D. & Weiergräber, O. H. (2001) Glutamine and cell signaling in liver. J. Nutr. 131: 2509S–2514S.

34. Häussinger, D., Schliess, F., Dombrowski, F. & Vom Dahl, S. (1999) Involvement of $p38^{MAPK}$ in the regulation of proteolysis by liver cell hydration. Gastroenterology 116: 921–935.

35. Ogier-Denis, E., Pattingre, S., Benna, J. E. & Codogno, P. (2000) Erk1/2-dependent phosphorylation of Gα-interacting protein stimulates its GTPase accelerating activity and autophagy in human colon cancer cells. J. Biol. Chem. 275: 39090–39095.

36. Tallóczy, Z., Jiang, W., Virgin, H. W., Iv., Leib, D. A., Scheuner, D., Kaufman, R. J., Eskelinen, E.-L. & Levine, B. (2002) Regulation of starvation- and virus-induced autophagy by the eIF2α kinase signaling pathway. Proc. Natl. Acad. Sci. USA 99: 190–195.

37. Kamada, Y., Funakoshi, T., Shintani, T., Nagano, K., Ohsumi, M. & Ohsumi, Y. (2000) Tor-mediated induction of autophagy via an Apg1 protein kinase complex. J. Cell Biol. 150: 1507–1513.

38. Pösö, A. R., Schworer, C. M. & Mortimore, G. E. (1982) Acceleration of proteolysis in perfused rat liver by deletion of glucogenic amino acids: Regulatory role of glutamine. Biochem. Biophys. Res. Commun. 107: 1433–1439.

39. Pösö, A. R., Wert, J. J., Jr. & Mortimore, G. E. (1982) Multifunctional control by amino acids of deprivation-induced proteolysis in liver: Role of leucine. J. Biol. Chem. 257: 12114–12120.

40. Garlick, P. J. (2001) Assessment of the safety of glutamine and other amino acids. J. Nutr. 131: 2556S–2561S.

41. Vittorini, S., Paradiso, C., Donati, A., Cavallini, G., Masini, M., Gori, Z., Pollera, M. & Bergamini, E. (1999) The age-related accumulation of protein carbonyl in rat liver correlates with the age-related decline in liver proteolytic activities. J. Gerontol. A Biol. Sci. Med. Sci. 54A: B318–B323.

42. Brunk, U. T. & Terman, A. (2002) The mitochondrial-lysosomal axis theory of aging. Accumulation of damaged mitochondria as a result of imperfect autophagocytosis. Eur. J. Biochem. 269: 1996–2002.

43. Charrueau, C., Blondé-Cynober, F., Coudray-Lucas, C., Poupon, R., Chaumeil, J.-C., Hannoun, L. & Cynober, L. (2000) Prevention of proteolysis in cold-stored rat liver by addition of amino acids to the preservation solution. J. Gastroenterol. Hepatol. 15: 1199–1204.