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REVIEWS: CURRENT TOPICS

Mechanisms through which sulfur amino acids control protein metabolism and oxidative status

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Abstract

Amino acids regulate protein synthesis and breakdown (i.e., protein turnover) and consequently protein deposition, which corresponds to the balance between the two processes. Elucidating the mechanisms involved in such regulation is important from fundamental and applied points of view since it can provide a basis to optimize amino acid requirements and to control protein mass, body composition and so forth. Amino acids, which have long been considered simply as precursors of protein synthesis, are now recognized to exert other significant influences; that is, they are precursors of essential molecules, act as mediators or signal molecules and affect numerous functions. For example, amino acids act as mediators of metabolic pathways in the same manner as certain hormones. Thus, they modulate the activity of intracellular protein kinases involved in the regulation of metabolic pathways such as mRNA translation. We provide here an overview of the roles of amino acids as regulators of protein metabolism, by focusing particularly on sulfur amino acids. The potential importance of methionine as a "nutrient signal" is discussed in the light of recent findings. Emphasis is also placed on mechanisms controlling oxidative status since sulfur amino acids are involved in the synthesis of intracellular antioxidants (glutathione, taurine etc.) and in the methionine sulfoxide reductase antioxidant system.

Keywords: Protein turnover; Methionine; Cysteine; Oxidative stress; Amino acid signaling

1. Introduction

Amino acids are known as anabolic factors, which induce protein gain by stimulating protein synthesis while inhibiting proteolysis. These effects on protein turnover have been clearly demonstrated in vitro and in vivo (see Refs. [1–8] for reviews). Moreover, studies conducted during the last 10 years indicate that amino acids act as regulators of metabolic pathways (i.e., the concept of "nutrient signal"), with, for instance, an effect targeted on mRNA translation.

Methionine and cysteine hold very significant places among amino acids by playing numerous roles in protein metabolism. Like other amino acids, they are the components of tissue proteins and, therefore, serve as substrates for protein synthesis. They are also precursors of important molecules. For example, methionine participates in methyl group metabolism and synthesis of other sulfur amino acids, notably cysteine (Fig. 1). Cysteine is required for the synthesis of glutathione (GSH) and taurine, which are essential compounds for host defense against oxidative stress. Recent reviews illustrating this role of sulfur amino acids in stress

Abbreviations: DL-HMTBA, DL-2-hydroxy-(4-methylthio) butanoic acid; 4E-BP1, eukaryotic initiation factor 4E binding protein; eIF2α, eukaryotic initiation factor 2α; eIF4B, eukaryotic initiation factor 4B; FOXO, forkhead box-O transcription factor; GβL, G protein β-subunit-like protein; IGF, insulin-like growth factor; mGCN2, mammalian general control nondepressible 2 protein kinase; mTOR, mammalian target of rapamycin; p70S6K, 70-kDa ribosomal protein S6 kinase; PI3K, phosphatidylinositol-3′ kinase; PKB/AKT, protein kinase B (also called AKT); Raptor, regulatory associated protein of mTOR; Rheb, Ras homologue enriched in brain; rpS6, ribosomal protein S6; TSC1/2, tuberous sclerosis 1/2; Ub, ubiquitin.

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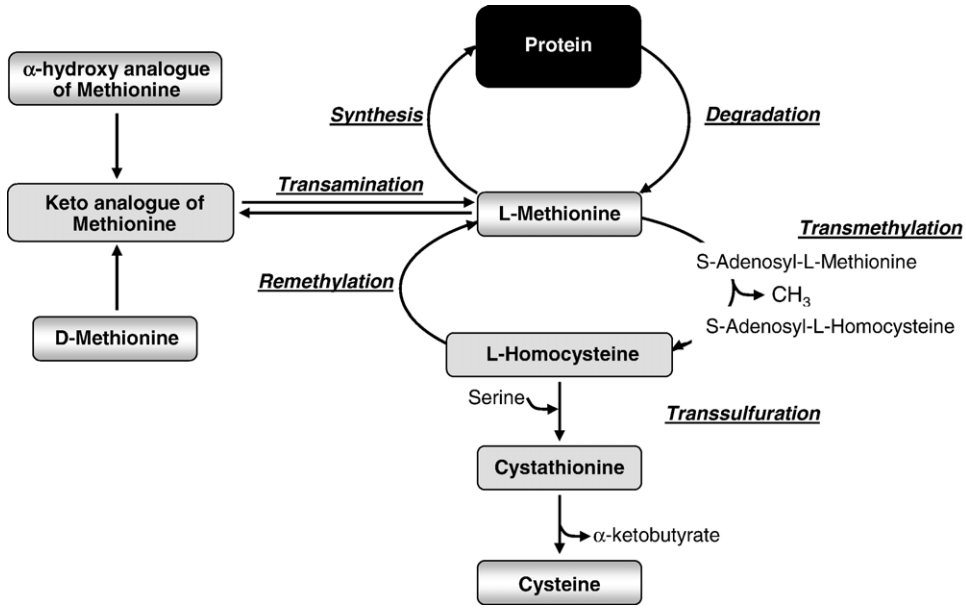

Fig. 1. Methionine–cysteine metabolic pathways.

conditions are available. There are also numerous reviews devoted to the signal role of amino acids, which highlight the key role of leucine in these processes, but an overview on the role of sulfur amino acids is missing. In this review, we will firstly describe the general effects of sulfur amino acids. Emphasis will then be placed on mechanisms through which sulfur amino acids control protein metabolism and oxidative status.

2. General effects of sulfur amino acids

Among sulfur amino acids, methionine is an essential amino acid, which has to be provided in an animal's diet, and it is, for example, the first limiting factor in classical diets used for growing chickens [9]. In this species, the decreased rates of body weight gain and protein accretion induced by methionine and cystine deficiency are more pronounced than those recorded with diets deficient in lysine or histidine [10]. Decreased growth in chicks fed methionine- and cystine-free diets was primarily caused by lower rates of whole-body protein synthesis associated with lower RNA efficiency, suggesting translational regulation. A positive effect of methionine supplementation on muscle growth has also been reported, since the addition of methionine to a methionine-deficient diet, otherwise balanced in terms of other amino acids, increases accretion and synthesis of protein in the gastrocnemius and pectoralis major muscles of chicks [11]. Dietary methionine supplementation is usually provided using chemically synthesized DL-methionine (DL-Met) or one of its hydroxy analogues, for example, DL-2-hydroxy-(4-methylthio) butanoic acid (DL-HMTBA). Research in this field has mainly concerned the respective use of these products, giving relatively controversial results [9,12–17]. This is, in part, due to differences in the experimental conditions used, but differences in intake, intestinal absorption and metabolic conversion to L-methionine have been evoked as confounding factors. However, despite the fact that HMTBA contains a significant proportion of nonmonomeric forms, which have been considered poorly absorbed by the chicken intestine [18], the presence of nonmonomeric forms is not a limiting factor in DL-HMTBA absorption [19]. In addition, no difference was observed in the utilization of DL-Met and DL-HMTBA for protein synthesis in isolated chick hepatocytes [20] or in chick skeletal muscles [11].

Cysteine as methionine is used for protein synthesis, but it is produced through the metabolism of methionine, being consequently considered as a dispensable amino acid. Although the sulfur amino acid requirement can be met from methionine alone, it is more often achieved through a combination of methionine and cyst(e)ine (cysteine and cystine). In growing animals, dietary cyst(e)ine can replace part of the methionine requirement (reported by Shoveller et al. [21]), suggesting the importance of both sources, that is, methionine and cyst(e)ine, in the supply of sulfur amino acids for protein accretion. Interestingly, cysteine is considered as "conditionally indispensable" in particular situations such as stress conditions or inflammatory states. In such situations, cysteine requirement is increased, and this demand for cysteine thus exceeds the capacity of the body to synthesize it (see Refs. [22–24] for reviews). To provide a better understanding of sulfur amino acid requirements, their metabolic flux and interconversion rates have been analyzed. For instance, acute infection in the rat increases the synthesis of a major antioxidative compound named GSH in various tissues (liver, spleen, lung, muscle etc.), with such synthesis

accounting for at least 40% of the enhanced cysteine utilization during infection [25]. Cysteine catabolism via sulfate production is thus dramatically lower, also suggesting that cysteine is spared in order to synthesize GSH [26]. Furthermore, cysteine is extensively used for the synthesis of taurine and acute-phase proteins involved in host defense in the liver of septic rats [23,26]. Methionine metabolism was also affected in normal volunteers subjected to a mild inflammatory challenge (i.e., vaccination; [27]), in agreement with results obtained in acute diseases. Preferential methionine metabolism toward cysteine synthesis has thus been observed, confirming increased requirement for sulfur amino acids in these situations. DL-HMTBA is apparently more efficiently converted to cysteine and taurine than L-methionine, suggesting that this hydroxy analogue of methionine might have a different role in detoxification processes than methionine itself [28].

3. Recent advances in amino acid signaling

Amino acids have recently been shown to act as regulators of metabolic pathways. An increasing number of reviews illustrate these new functions for amino acids [7,29–36]. For example, amino acids act on intracellular proteins involved in the control of mRNA translation, including (a) eukaryotic initiation factor 2 (eIF2), (b) eukaryotic initiation factor 4E binding protein (4E-BP1) and (c) 70-kDa ribosomal protein S6 kinase (p70S6K, also called S6K1). eIF2 is bound to the 40S ribosomal subunit as a ternary complex with GTP and methionyl-tRNA (Met-tRNAi) and is, therefore, involved in the first step of the initiation of mRNA translation [37,38]. 4E-BP1 corresponds to the binding protein of the initiation factor eIF4E and acts as a competitive inhibitor of eIF4F complex formation, consequently limiting the formation of the 48S preinitiation complex [30]. p70S6K is a serine/threonine kinase of the S6 kinase family. This kinase catalyzes phosphorylation of the ribosomal protein S6 (rpS6), a component of the 40S subunit of the eukaryotic ribosome, which was thought to induce the selective translation of 5′ TOP mRNAs, a subset of mRNAs that possess a 5′ terminal oligopyrimidine tract (TOP) [39,40]. The 5′TOP mRNAs encode components of the translational machinery such as eukaryotic elongation factors and ribosomal proteins, and up-regulating their translation probably leads to increased capacity of the cell to synthesize protein. Moreover, p70S6K phosphorylates other proteins such as eukaryotic initiation factor 4B (eIF4B) and induces dephosphorylation of the eukaryotic elongation factor 2, thus affecting both the initiation and elongation stages of mRNA translation [31,39,41].

Signaling pathways that regulate protein metabolism have been intensively studied in the last 10 years. One of the most explored is the mammalian target of rapamycin (mTOR) pathway, and there is strong evidence of amino acid control of mRNA translation through a mechanism involving the TOR signaling pathway from mammals to drosophila [36,42–46]. TOR directly phosphorylates 4E-BP1 and p70S6K (Fig. 2). Several laboratories have identified different mTOR-associated proteins, such as regulatory associated protein of mTOR (raptor) and mLST8 [also known as G protein β-subunit-like protein (GβL)] [47–50]. There is general agreement that raptor is important for TOR signaling in different organisms (yeast, mammals). Although there are different models for the influence of raptor on mTOR (reported in Ref. [51]), it seems that amino acids activate the mTOR pathway through a mechanism that involves destabilization of the interaction between raptor and mTOR. More recently, a novel partner of mTOR has been discovered, referred to as rapamycin insensitive companion of mTOR (rictor), which exists in a protein complex containing rictor, mTOR and GβL but not raptor [52]. Whereas the raptor–mTOR complex phosphorylates the mTOR effector p70S6K, therefore promoting mRNA translation, the rictor–mTOR complex does not but is involved in protein kinase B (PKB, also called AKT) regulation [53,54].

The mechanisms through which mTOR activity is regulated were undefined until recently and are still a matter of debate. There is evidence that TOR integrates signals from nutrients such as amino acids and mitogenic and growth factors, that is, insulin/insulin-like growth factors (IGFs). The signaling cascade initiated by growth factors is mediated by a complex pathway initiated by the activation of p85/p110 phosphatidylinositol-3′ kinase (PI3K) and involves the intracellular AKT, tuberous sclerosis 1/2 (TSC1/2) and the small GTPase Ras homologue enriched in brain protein (Rheb) kinases (Fig. 2) [34,36,45]. Several reviews have reported that TSC1/2 and Rheb could also be involved in transducing signals from amino acids through mTOR [33,51,55]. However, recent findings support a model in which amino acids act on mTOR independently of TSC1/2 [56,57]. The role of Rheb in mediating the amino acid signal to mTOR is still questionable. Roccio et al. [58] show that amino acid depletion completely blocks insulin-induced Rheb activation, suggesting that amino acid sensing occurs upstream of Rheb. Despite this, amino acid depletion can inhibit mTOR kinase signaling in fibroblasts lacking TSC2. Since Rheb activity remains high under these conditions, a second level of amino acid sensing probably exists, affecting mTOR activity in a Rheb-independent fashion.

Several studies have recently reported the identification of a new amino acid signal transducer to mTOR, class 3 PI3K Vps34 [56,59]. These findings indicate that the early steps by which amino acids control mTOR signaling are distinct from those of insulin/IGF: insulin/IGF regulates mTOR function by a pathway including p85/p110 PI3K, referred to as class 1 PI3K, whereas amino acids mediate mTOR activation by signaling through class 3 PI3K Vps34. With regard to proteolysis, amino acid deprivation in C2C12 myotubes stimulates autophagic sequestration by mechanisms that also involve the class 3 PI3K signaling cascade [60]. To date, it

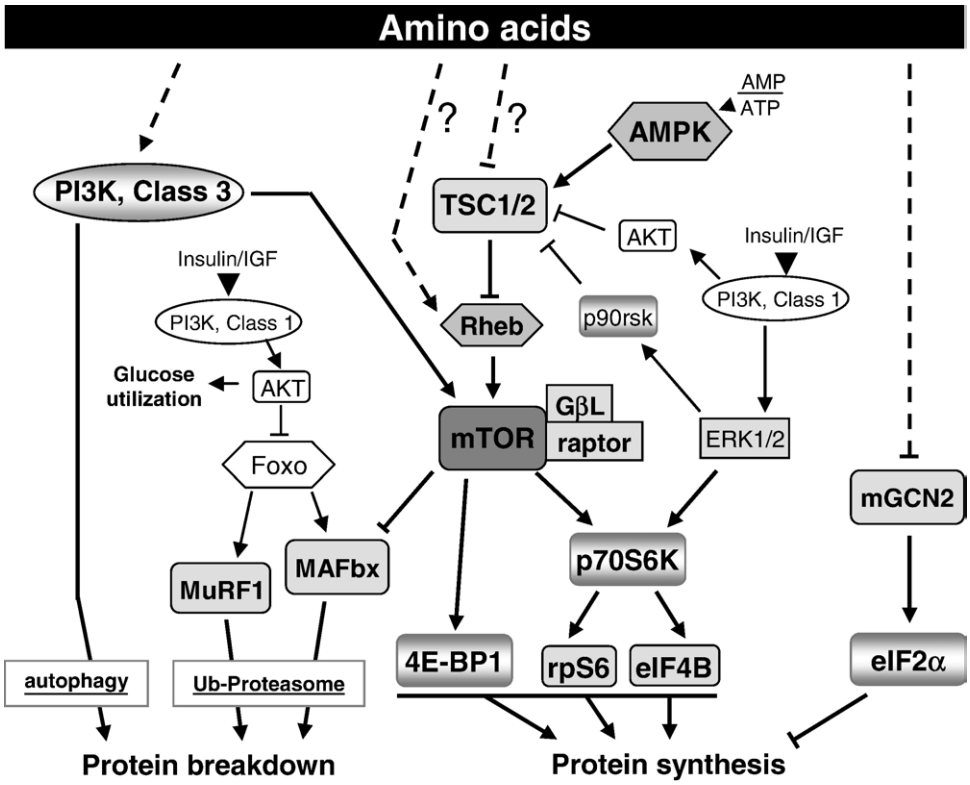

Fig. 2. Amino acid signaling and control of protein turnover. Amino acids regulate protein synthesis and breakdown through mTOR-dependent and -independent pathways. Amino acid and insulin/IGF signaling pathways present similarities or at least common kinases such as mTOR and p70S6K but are mediated by independent PI3K signals. It should be noted that AMP-activated protein kinase (AMPK; an energy sensor) may signal through TSC2 to down-regulate the activity of mTOR and its downstream effectors (4E-BP1, p70S6K). AKT, also called PKB; ERK, extracellular signal-regulated protein kinase; MAFbx, muscle atrophy F box; MurRF-1, muscle ring finger-1; p90rsk, 90-kDa ribosomal S6 kinase.

has not been established whether mTOR is involved in the amino-acid-related inhibition of the ubiquitin (Ub)–proteasome-dependent proteolysis or not. This pathway is initiated by the covalent attachment of Ub molecule chains through the successive actions of the Ub-activating enzyme (El), Ub-conjugating enzymes (E2) and Ub–protein ligases (E3) [61,62]. Ubiquitinated proteins are then targeted for degradation to the 26S proteasome, which is a large proteolytic complex that hydrolyzes protein conjugates. Interestingly, new evidence has demonstrated that AKT, a kinase upstream from mTOR, regulates the expression of two important genes controlling the Ub–proteasome pathway, that is, muscle atrophy F box (also called atrogin-1) and muscle ring finger-1 E3 Ub ligases, via the inhibition of forkhead box-O transcription factors (FOXO) and via a second mechanism downstream from mTOR itself (see Refs. [63–65] for reviews).

The regulation of protein synthesis by amino acids also occurs via mTOR-independent mechanisms (see Ref. [34] for a review). For example, phosphorylation of the rpS6 can occur through rapamycin-insensitive mechanisms. Moreover, amino acid availability regulates phosphorylation of eukaryotic initiation factor 2α (eIF2α) via mammalian general control nondepressible 2 protein kinase (mGCN2), therefore affecting mRNA translation. Interestingly, the mGCN2 pathway is also involved in the regulation of gene expression by amino acids. In the case of amino acid starvation, eIF2α phosphorylation by mGCN2 has been reported to increase the expression of activating transcription factor 4 (ATF4), which is involved in the amino acid control of gene expression [66]. It should be noted that, although ATF4 is necessary for the induction of gene expression in response to amino acid starvation, it is not sufficient and seems to need specific partners and/or a second signal to give it its specificity for amino acids.

4. Potential function for sulfur amino acids in amino acid signaling

Among amino acids, the branched-chain amino acid leucine is clearly recognized as a nutrient regulator of mRNA translation and proteolysis [67–71]. This is certainly the most effective amino acid with regard to the regulation of these processes. One remaining key question relates to the possibility for other amino acids, including methionine, to act as a nutrient signal. Although information is insufficient to allow a definitive conclusion thus far, the potential importance of sulfur amino acids in the control of metabolic functions is discussed here.

It has been shown for a long time that methionine plays a specific role in mRNA translation. Indeed, the first step of the initiation of mRNA translation consists of the binding of initiator Met-tRNAi to the 40S ribosomal subunit to form the 43S preinitiation complex. This initiation step may be inhibited by methionine deficiency. With regard to the potential effect of methionine on intracellular kinases, studies are sparse but indicate that this sulfur amino acid may exert a "signal" function by inducing p70S6K activation in mammals [72–73]. Similar data have been found in an avian myoblast cell line (QM7) of quail origin [46], in which the availability of individual amino acids (i.e., methionine or leucine) regulates S6K1 phosphorylation and protein synthesis.

Several studies have demonstrated that the mGCN2 pathway plays a major role in the regulation of genes by amino acids. These experiments do not support a unique role of leucine in modulating this signaling pathway. First, the use of mice lacking mGCN2 reveals that this kinase is involved in the physiological response (i.e., food aversion) induced by a diet deficient in leucine as in threonine [74]. Moreover, there is evidence that C/EBP homologous protein (CHOP), where C/EBP is CCAAT/enhancer binding protein, and asparagine synthetase (AS) gene expression are up-regulated in response to leucine as well as methionine limitation in vitro (see Refs. [75–76] for reviews). Amino acid response elements (AAREs) have been characterized in the promoter of CHOP and AS genes. It is noteworthy that although these two AAREs present nucleotide sequence similarities, the induction of CHOP and AS following amino acid starvation apparently does not occur through a single common mechanism. The transcription factors involved in the amino-acid-dependent regulation of CHOP and AS expression are also different. For example, ATF2 plays a pivotal role in CHOP expression in response to amino acid starvation but is not able to bind to the AARE of the AS gene, whereas ATF4 can bind to both site promoters. Taken together, these data suggest complex mechanisms requiring further investigation. Finally, some data indicate that sulfur amino acids regulate gene expression. For example, methionine supply affects growth-hormone-induced IGF-I gene expression in ovine hepatocytes [73]. It has also been shown that methionine is able to modulate the expression of the Ub ligase atrogin-1 in avian fibroblasts through the TOR pathway [77]. In addition, cysteine can modulate the activity of transcription factor NFκB [78], which induces the expression of many genes that are involved in cell survival and proliferation and in the regulation of immune and inflammatory responses. No such in vivo responses have been documented, but the potential applications of these properties of amino acids could emerge in the future.

In addition to these roles, sulfur amino acids exert other important functions. For example, methionine is a source of methyl groups that are used to methylate DNA, a process that influences chromatin structure and gene expression (see Ref. [79] for a review). From a nutritional point of view, one interesting application concerns perinatal nutrition since aberrant methyl metabolism in utero is linked with disorders (epigenetic regulation). Finally, sulfur amino acids are involved in controlling oxidative status.

5. Role of sulfur amino acids in controlling oxidative status

Sulfur compounds, including cysteine, taurine and GSH, have a crucial role in oxidative stress conditions since they have the capacity to affect cellular redox status [24,80,81] (Fig. 3). In particular, GSH, which is a tripeptide (L-glutamyl–L-cysteinyl–glycine), is the most important intracellular antioxidant of the body. GSH and cysteine can function as direct scavengers of reactive oxygen species

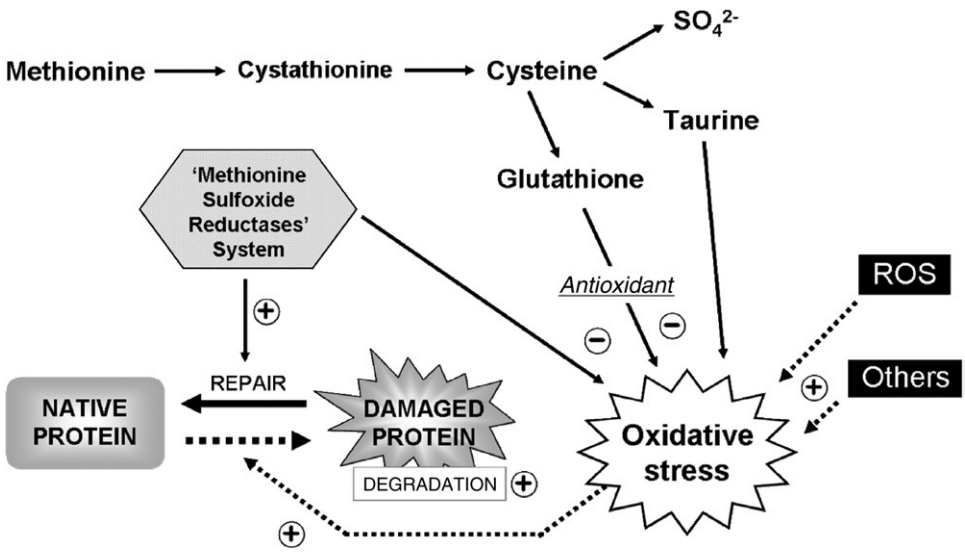

Fig. 3. Sulfur amino acids: their role in the control of oxidative status.

(ROS). ROS and hydrogen peroxide are formed at the mitochondrial level and may produce deleterious effects, such as lipid and protein oxidation and DNA strand-break damage, and affect metabolic processes. Protein oxidation particularly results in damaged proteins and may contribute to changes in protein metabolism and function [82,83]. As detailed by Obled et al. [24], GSH peroxidase catalyzes GSH-dependent reduction of hydrogen peroxide. GSH is thus oxidized to a GSH dimmer (GSSG), which is either reduced back to GSH by GSSG reductase or eliminated from the cell. The GSH/GSSG ratio and the level of GSH within the cell determine the thiol redox status of the cell, which, in turn, can regulate metabolic pathways by activating or inhibiting enzymes and cellular processes such as gene transcription. For example, the DNA binding activity of members of the PAX protein family may be under the control of GSH/GSSG ratios through two cysteine residues present in a paired domain (DNA-binding domain) [84].

GSH and cysteine can also protect proteins from irreversible oxidative damage through interactions between these thiols and proteins and the formation of mixed disulfides, such as glutathiolated proteins (protein-SSG) [85]. In addition, it has been discovered that methionine residues in proteins can act as catalytic antioxidants via the "methionine sulfoxide reductase" (MSR) system (Fig. 3; see Refs. [82,83,86,87] for reviews). Methionine residues are particularly susceptible to oxidation by ROS and are converted to methionine sulfoxide (MetO) with the formation of two stereoisomers, referred to as MetO-S and MetO-R. The S and R forms of MetO can be reduced back to methionine by methionine sulfoxide reductase A (Msr-A) and B (Msr-B), respectively. Each cycle of methionine oxidation and reduction will destroy one equivalent of ROS, which might represent a major natural scavenging system for ROS. Evidence of this antioxidant role suggested by Levine et al. [88] has been further provided by studies using knockout or overexpression of MsrA gene in various cells and organisms (see Refs. [82,87] for reviews): for instance, mutant strains of yeast, bacteria and mice that lack the MsrA gene were found to be more sensitive to ROS, whereas transgenic flies overexpressing Msr-A had increased resistance to ROS. Methionine sulfoxide reductases have been associated with increased longevity, and Msr activity is, for instance, markedly reduced during aging in the rat [89]. These enzymes are also involved in the repair of oxidized proteins [83,86]. In fact, damaged proteins can be eliminated from cells by two processes, that is, protein degradation and protein repair. The only oxidative damage that can be repaired is that involving sulfur-containing amino acids, through the "Msr" system with regard to methionine. Msr can therefore prevent cellular accumulation of damaged proteins.

Interestingly, certain nutritional conditions have been reported to cause up-regulation of Msr-A and Msr-B including, in relation to the present review, amino acid starvation in *Escherichia coli* [90] and the source of dietary protein in growing pigs [91]; one possible explanation for the up-regulation of Msr observed in pigs fed restricted protein diets based on soy protein isolate compared to casein might be the differences in protein amino acid patterns, particularly

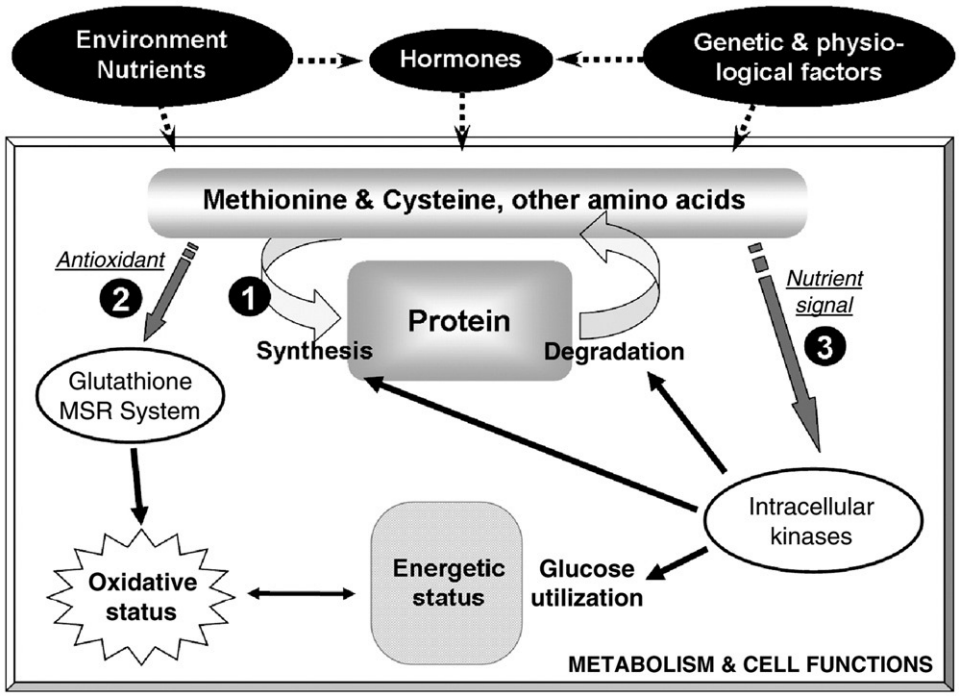

Fig. 4. Regulation of metabolism and cell functions; main roles of amino acids: (1) precursors of protein synthesis, (2) antioxidant function and (3) nutrient signal. The figure represents a composition of findings discussed in the present review.

sulfur amino acids. Pamplona and Barja [92] and Sanz et al. [93] recently reported an effect of dietary methionine levels on oxidative stress and longevity: lowering of methionine levels apparently decreases the sensitivity of proteins to oxidative damage and ROS production.

6. Conclusion

Although protein metabolism is regulated by numerous factors such as physiological, genetic and environmental factors and hormones, amino acids are recognized to be essential in such regulation. Protein synthesis is affected when an insufficient level of a specific amino acid (limiting amino acid) is provided. This defect may originate from dietary amino acid deficiency and/or excessive amino acid utilization for other purposes such as the synthesis of acute-phase proteins during catabolic stress. Recent studies have indicated that amino acids, particularly sulfur amino acids, also participate in the control of oxidative status and may act as a nutrient signal, which is illustrated in Fig. 4. It is noteworthy that the role of amino acids as a nutrient signal appears to be important for cell functions and metabolic pathways other than those directly concerning protein turnover. For example, overstimulation of mTOR/p70S6K by amino acids mediates a feedback loop, promoting insulin resistance (i.e., inhibition of insulin-stimulated glucose transport; see Refs. [31,94]). Nevertheless, despite this increased knowledge on the role of amino acids, many questions still remain unanswered and the underlying mechanisms need to be elucidated.

An understanding of the mechanisms of amino acid action is essential to optimize dietary amino acid requirements whatever the conditions, including physiopathologic conditions such as disease, heat stress and aging. The signal function of sulfur amino acids remains to be clarified. Studies should be performed to explore the cascade involved in the action of sulfur amino acids (i.e., methionine and its analogues and/or metabolites) and the consequences on the regulation of protein turnover and to examine any possible synergy between sulfur compounds. Moreover, the effects of sulfur amino acids should be analyzed under circumstances affecting the oxidative status of animals and humans since, for example, sulfur amino acids have an antioxidant function via glutathione synthesis. Further studies more specifically focused on sulfur amino acids are likely to have new nutritional applications in the future.

The concept of nutrient signal has been used to modulate metabolic pathways in some critical situations. For example, several studies have shown the value of dietary supplementation with leucine in aging, with effects on both postprandial stimulation of muscle protein synthesis (see Ref. [95] for a review) and postprandial inhibition of muscle proteolysis [96]. Recent findings have also suggested that leucine acts as a nutrient signal to stimulate protein synthesis in the cardiac and skeletal muscles of neonates [97]. However, more information is needed on the potential effects of chronic amino acid supplementation before use for nutritional purposes in aged humans or neonates. Moreover, although the role of amino acids as modulators of signal transduction pathways is now accepted, very little information is available on their possible effects on metabolic disorders. p70S6K may mediate deleterious effects such as obesity, insulin resistance and, potentially, type 2 diabetes when there is nutrient excess. Further studies are needed to elucidate the amino-acid-induced changes in signaling pathways that control energy metabolism.

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