REVIEW ARTICLE

Amino acid regulation of gene expression

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The impact of nutrients on gene expression in mammals has become an important area of research. Nevertheless, the current understanding of the amino acid-dependent control of gene expression is limited. Because amino acids have multiple and important functions, their homoeostasis has to be finely maintained. However, amino-acidaemia can be affected by certain nutritional conditions or various forms of stress. It follows that mammals have to adjust several of their physiological functions involved in the adaptation to amino acid availability by regulating the expression of numerous genes. The aim of the present review is to examine the role of amino acids in regulating mammalian gene expression and protein turnover. It has been reported that some genes involved in the control of growth or amino acid metabolism are regulated by amino acid availability. For instance, limitation of several amino acids greatly increases the expression of the genes encoding insulin-like growth factor binding protein-1, CHOP (C/EBP homologous protein, where C/EBP is CCAAT/enhancer binding protein) and asparagine synthetase. Elevated mRNA levels result from both an increase in the rate of transcription and an increase in mRNA stability. Several observations suggest that the amino acid regulation of gene expression observed in mammalian cells and the general control process described in yeast share common features. Moreover, amino acid response elements have been characterized in the promoters of the CHOP and asparagine synthetase genes. Taken together, the results discussed in the present review demonstrate that amino acids, by themselves, can, in concert with hormones, play an important role in the control of gene expression.

Key words: amino acid availability, amino acid response element, CHOP, IGFBP-1, protein turnover.

INTRODUCTION

The regulation of gene expression in response to changes in the nutritional environment is one of the most well documented events in prokaryotes and lower eukaryotes. These single-cell organisms are able to adjust their metabolic capacity in response to variations in the nutrient supply in the culture medium. For example, the nutrient-dependent regulation of the lactose, histidine and tryptophan operons by their respective substrates has been well characterized in bacteria [1]. A limitation in the supply of these substrates will activate genes coding for enzymes involved in their biosynthesis. These mechanisms involve the conditional regulation of specific genes in the presence (or absence) of appropriate nutrients.

In multicellular organisms, the control of gene expression differs in many aspects from that operating in single-cell organisms, and involves complex interactions of hormonal, neuronal and nutritional factors. Although not as widely appreciated, nutritional signals also play an important role in controlling gene expression in mammals. It has been shown that major (carbohydrates, fatty acids, sterols) and minor (minerals, vitamins) dietary constituents participate in the regulation of gene expression [2–5]. However, much less is known about the role of amino acids [6]. The present review summarizes recent work on the effects of amino acid availability on the regulation of gene expression. On the basis of the physiological concepts of amino acid homoeostasis, which are first reviewed, we will discuss a specific example of the role of amino acids: the regulation of expression of insulin-like growth factor binding protein-1 (IGFBP-1). We will finally focus on the regulation of gene expression and protein turnover by amino acids.

PHYSIOLOGICAL CONCEPTS IN AMINO ACID HOMOEOSTASIS

Among the amino acids present in Nature, only 20 are involved in the synthesis of proteins. These 20 amino acids, together with a few others, such as ornithine, citrulline and taurine, are of physiological importance in the nutrition of the mammalian organisms. The supply of free amino acids to the tissues plays an important role in the maintenance of organ and body protein homoeostasis. However, besides their role as substrates for protein synthesis, amino acids have multiple and important functions. They can act as glucogenic substrates, nitrogen carriers, neurotransmitters, regulators of protein turnover, enzyme activity and ions fluxes. Amino acids can also be the precursors of signal transducers, nucleotides and neurotransmitters (for review, see Young et al. [10]).

The requirements for amino acids in humans have been very well studied [7–9]. In healthy adult humans, nine amino acids (valine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and histidine) have been shown to be indispensable. Therefore these amino acids must be supplied in the diet, and a deficiency of any one of them can lead to a

Abbreviations used: AARE, amino acid response element; AS, asparagine synthetase; C/EBP, CCAAT/enhancer binding protein; CHOP, C/EBP homologous protein; eEF-2, eukaryotic elongation factor-2; eIF2, eukaryotic initiation factor 2; 4E-BP1, eIF4E binding protein-1; IGF, insulin-like growth factor; IGFBP, IGF binding protein; mTOR, mammalian target of rapamycin; ODC, ornithine decarboxylase; UPR, unfolded protein response.

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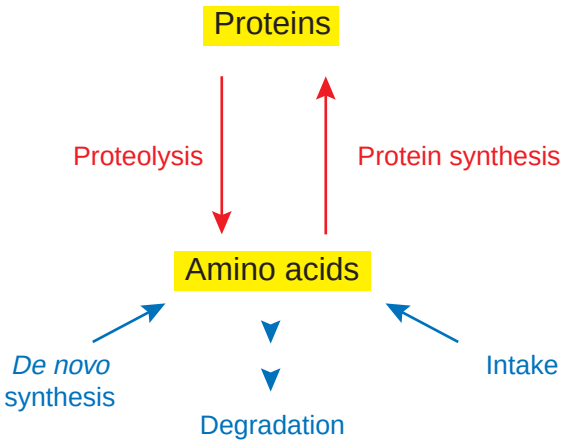

Figure 1 Biochemical systems involved in the homoeostasis of protein and amino acids

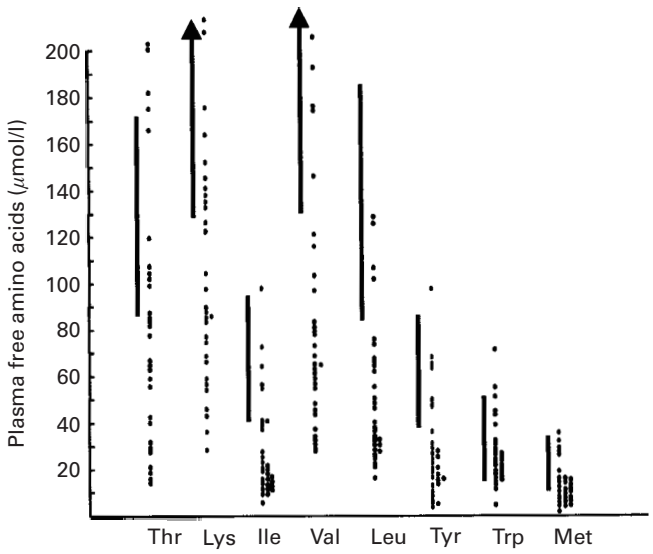

Figure 2 Concentrations of plasma free amino acids in severely malnourished young children at the time of admission to hospital

The solid lines represent the range of concentrations found for well-nourished children. The highest control concentrations for lysine and valine (arrows) are approx. 295 and 255 μM respectively. Data from Baertl et al. [20].

negative nitrogen balance and clinical symptoms. However, under a particular set of conditions, dispensable amino acids may become indispensable. These amino acids are called 'conditionally indispensable'. For example, enough arginine is synthesized by the urea cycle to meet the needs of an adult, but not those of a growing child.

Amino acid and protein homoeostasis have to be finely maintained by the integrated action of all the tissues and organs. The main metabolic systems responsible for the maintenance of whole-body protein and amino acid homoeostasis are: protein synthesis and degradation, amino acid oxidation, amino acid intake and *de novo* synthesis (Figure 1) [10,11]. However, in certain situations nitrogen metabolism can become deranged, and because (in contrast with lipids or glucose) there is no large store of dispensable amino acids, a loss of body protein and/or amino acids is at the expense of essential elements. As a consequence, the plasma amino acid profile is changed. The main factors affecting nitrogen metabolism and amino-acidaemia are the nutritional status and various forms of stress.

Nutritional status

The amino acid profile has been shown to be altered in humans and animals when there is a deficient intake of protein, a dietary imbalance of amino acids or a deficiency in any one of the essential amino acids. Early studies on mice, rats, chicks and pigs demonstrated that a diet low in one essential amino acid resulted in a decrease in the concentration of that amino acid in the plasma [12–15]. It has also been shown that prolonged feeding of a low-protein diet causes a fall in the plasma levels of most essential amino acids [16,17]. Moreover, extreme malnutrition is often associated with high levels of infection and infestation (kwashiorkor). Therefore the profile of amino acids in the plasma of malnourished subjects is also influenced by the effects of infection and loss of appetite [18]. In such a situation, the plasma concentrations of certain essential amino acids can be dramatically lowered. For example, leucine and methionine concentrations can be reduced from approx. 100–150 μM and 18–30 μM to 20 μM and 5 μM respectively in the plasma of children affected by kwashiorkor [19,20] (Figure 2).

In contrast with the situation of protein undernutrition, the level of amino acids in the plasma has been reported to rise after a protein-containing meal is given to an animal or a human subject. The concentrations of leucine and certain other amino acids approximately double in the peripheral blood after a protein-rich meal [21], and reach much higher levels within the portal system [22]. As a consequence, hepatocytes and intestinal cells are in contact with highly variable concentrations of nutriments. Although most of the essential amino acids are degraded in the liver, the branched-chain amino acids and methionine are poorly used by hepatocytes. Consequently, after a protein-rich meal, these amino acids pass through the liver into the general circulation and cause a much greater increment in systemic blood levels than is the case for other essential amino acids, which are removed efficiently by the liver. Data suggesting that some responses to a protein-rich meal might be due to postprandial increases in the concentrations of circulating amino acids [23,24] will be discussed below.

Stress

Various forms of stress (trauma, thermal burn, sepsis, fever, etc.) can lead to a state of negative nitrogen balance and significant loss of lean body mass. Cachexia and wasting syndromes are also observed during several chronic illnesses (chronic renal, cardiac, hepatic and pulmonary diseases), AIDS and cancer (for references, see [25–30]). In such situations, changes in nitrogen metabolism can be ascribed to several hormonal, metabolic and behavioural alterations. The hypercatabolic state of severe injury is characterized by a marked increase in nitrogen loss [31]. Briefly, amino acids are released by muscle proteolysis and provide substrates for the synthesis of proteins associated with inflammation. In such a situation, changes are observed in the profiles of free amino acids in plasma and urine.

Taken together, these numerous examples show that amino-acidemia can be affected by various nutritional or pathological

situations, with one major consequence being a large variation in the concentration of amino acids in the blood. It follows that mammals have to adjust several of their physiological functions involved in defence/adaptation to amino acid limitation by regulating the expression of numerous genes. An example is given in the next section of the role of amino acids in the regulation of gene expression following an extreme nutritional situation.

A SPECIFIC EXAMPLE OF THE ROLE OF AMINO ACIDS: GROWTH INHIBITION FOLLOWING PROTEIN UNDERNUTRITION IS MEDIATED BY THE REGULATION OF IGFBP-1

Animals used for meat production can be limited in essential amino acids because dietary proteins are not sufficiently diverse. Studies on pigs or chicks show a close correlation between the ability to grow and the level of limiting essential amino acids in the diet [32,33]. In humans, a limitation in amino acid intake can occur in cases of malnutrition.

Growth is controlled by a complex interaction of genetic, hormonal and nutritional factors. A large part of this control is due to growth hormone and to the IGFs (insulin-like growth factors) [34–36]. The biological activities of the IGFs are modulated by the IGFBPs, which bind IGF-I and IGF-II specifically [37,38]. Of the six IGFBPs, IGFBP-1 is the only one that displays rapid dynamic regulation *in vivo*, with serum levels varying by 10-fold or more depending on the nutritional state. The plasma IGFBP-1 level is increased by fasting [39], malnutrition [40,41] and diabetes [42]. IGFBP-1 is synthesized mainly by the liver and, *in vivo*, its expression is controlled by insulin, growth hormone and glucose. Using transgenic mice that overexpress hepatic IGFBP-1 [43–46], it has been shown that permanent hepatic expression of IGFBP-1 leads to growth retardation during both the ante- and post-natal periods, and to impaired development of organs such as the brain.

The growth defects associated with protein malnutrition occur in the presence of normal serum levels of growth hormone [47], low levels of IGFs and very high expression of hepatic IGFBP-1 [36]. Furthermore, Straus et al. [48] have reported a higher level of IGFBP-1 expression in protein-restricted animals than in animals starved for 24 h. This difference cannot be explained by variations in the plasma levels of glucose, insulin or growth hormone, which are considered to be the regulators of IGFBP-1 expression *in vivo*. These authors suggested that other additional metabolic factors may be involved in IGFBP-1 gene regulation. It can be hypothesized that a fall in the amino acid concentration may be responsible for IGFBP-1 induction, since: (i) the blood amino acid concentration was decreased by protein undernutrition, whereas it was almost unaffected in response to 24 h of starvation [49]; and (ii) in rat hepatoma cells, amino acid starvation increased the abundance of IGFBP-1 mRNA [48,50].

Indeed, it has been established that amino acid limitation, as it occurs during dietary protein deficiency, induces IGFBP-1 expression in hepatic cells [51]. Depletion of any one of all the essential amino acids plus arginine and cysteine significantly affects the IGFBP-1 mRNA level in freshly isolated rat hepatocytes as well as in the human hepatoma cell line HepG2. Moreover, IGFBP-1 expression is significantly induced by amino acid concentrations in the range of those observed in the blood of rats that have been fed a low protein diet or in humans affected by kwashiorkor. Therefore amino acid limitation, as occurs during dietary protein deficiency, induces IGFBP-1 expression in hepatic cells and thus participates in the down-regulation of growth.

Table 1 Genes regulated *in vitro* by amino acids

Abbreviations: AP-1, activator protein-1; ApoB100, apolipoprotein B100.

Gene	Regulation
Genes repressed by amino acid starvation	
Fatty acid synthase [85]	
Prepro-glucagon [86]	
Genes induced by amino acid supplementation	
Argininosuccinate synthase [81]	Cell swelling
ODC [82–84]	Stabilization of the mRNA; turnover of the protein
Collagenase [75]	AP-1-dependent
Tissue inhibitor of metalloproteinases [75]	
Genes induced *in vitro* by amino acid starvation	
Amino acid transporters (see the text)	
IGFBP-1 [48]	
CHOP [65]	Transcription, stabilization of the mRNA
C/EBPβ [65]	
ApoB100 [66]	
AS [62]	Transcription; post-transcriptional regulation
Argininosuccinate synthase [11]	Transcription
Ribosomal proteins [64]	

GENES REGULATED BY AMINO ACIDS

Genes or enzymic activities that are up-regulated by amino acid starvation

In mammalian cells, the first enzymic activity that was described to be regulated in response to amino acid starvation was the ubiquitous transporter for neutral amino acids, system A. Although the gene(s) encoding system A have not been identified to date, the regulation of its activity by amino acids has been intensively studied. Gazzola et al. [52,53] were the first to demonstrate that incubation of cells in amino acid-free medium results in up-regulation of system A-mediated transport. Since the original description, the regulation of system A by amino acids has been studied extensively in a wide variety of cell types. Several models for the amino acid regulation of system A activity have been proposed (for reviews, see [54–56]). Although there are some discrepancies between the different models, all postulate that amino acids enhance the transcription of a gene coding for a regulatory protein that can repress system A activity. Similar regulation has been described for other amino acid transport systems, such as system L and system X_{AG}^- [54,57].

More recently, specific examples of mRNAs for which synthesis is enhanced in response to amino acid deprivation have been described (Table 1). They include the mRNAs for IGFBP-1 [48], argininosuccinate synthase [58–60], asparagine synthetase (AS) [61,62], ribosomal proteins L17 and L25 [63,64], CHOP (C/EBP homologous protein, where C/EBP is CCAAT/enhancer binding protein), C/EBPα, C/EBPβ, β-actin, ubiquitin c [65], apolipoprotein B100 [66,67], calreticulin [68], glutamine synthetase [69], ornithine decarboxylase (ODC) [70], Jun, Myc [70], the growth-arrest genes (*gas* and *gadd*) [71] and genes encoding unidentified proteins [72–74]. The mechanisms by which levels of these mRNAs are elevated in response to a limitation in amino acid supply are discussed later in this review.

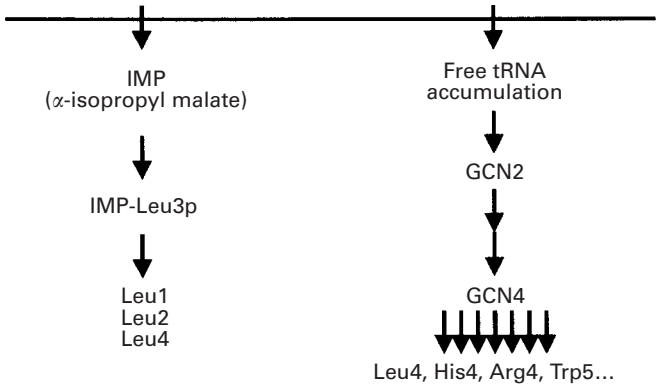

Figure 3 Amino acid control of gene expression in yeast

Two types of gene regulation in response to leucine limitation have been characterized. The general control process can be activated in response to a deficiency of any single amino acid. See the text for details.

Genes specifically up-regulated by amino acid supplementation

Genes that are specifically up-regulated in response to a supra-physiological concentration of amino acids have been described. For example, a high concentration of L-tryptophan enhances the expression of collagenase and of tissue inhibitor of metallo-proteinases [75]. Although the molecular mechanisms involved are not known, it has been shown that activation of collagenase gene expression by tryptophan is mediated through AP-1 (activator protein-1) binding elements in the promoter [76]. The physiological significance of this observation is uncertain, since the tryptophan concentration required to stimulate collagenase gene expression is higher than that found in the blood. However, the authors hypothesized that intracellular tryptophan depletion that occurs in response to interferon-γ treatment could play an important role in the regulation of collagenase expression [77,78]. Indeed, in human fibroblasts, collagenase is strongly induced in response to interleukin-1β treatment, and interferon-γ abrogates the effects of interleukin-1β. Moreover, interferon-γ treatment leads to a marked depletion of intracellular tryptophan levels, due to a substantial increase in the activity of indoleamine 2,3-dioxygenase, which catabolizes tryptophan. Supplementation of the cells with tryptophan completely overcomes the inhibitory effects of interferon-γ on collagenase mRNA expression. The authors concluded that overexpression of indoleamine 2,3-dioxygenase and subsequent tryptophan depletion resulting from interferon-γ treatment may account, at least in part, for the inhibitory effect of interferon-γ on collagenase expression.

It is notable that argininosuccinate synthase and ODC, which are induced by amino acid starvation, are also up-regulated in response to high amino acid concentrations. It was demonstrated that the cell swelling that results from the addition of amino acids could be involved in the regulation of gene expression [79,80]. A high glutamine concentration was found to induce the accumulation of argininosuccinate synthase mRNA in isolated hepatocytes [81]. Similarly, L-asparagine supplementation induces ODC expression, primarily by post-transcriptional stabilization of the ODC mRNA [82–84]. In addition, asparagine specifically stimulates the synthesis and suppresses the degradation of the ODC protein [82].

In addition, genes that are specifically down-regulated by amino acid limitation have been described. In HepG2 cells, starvation of any essential amino acid, or of arginine, specifically represses fatty acid synthase expression [85]. Similar experiments have shown that histidine removal inhibits the accumulation of the prepro-glucagon mRNA in α-TC6 cells [86].

Taken together, these examples show that amino acids can activate several control processes that can specifically regulate the expression of target genes. However, for most of the examples described in this section the molecular mechanisms involved in the regulation of gene expression by amino acids are poorly understood.

MOLECULAR MECHANISMS INVOLVED IN THE REGULATION OF GENE EXPRESSION BY AMINO ACID LIMITATION

The molecular mechanisms involved in the control of gene expression by amino acid deprivation have been extensively studied in yeast. After a brief summary of these processes, we will focus on the regulation of gene expression by amino acids in mammalian cells.

Amino acid control of gene expression in yeast

In yeast, two types of gene regulation in response to amino acid availability have been characterized: a specific and a general control process (Figure 3).

Specific control

It is well documented that numerous operons are regulated by the specific end-products of the corresponding enzymes [87]. A small effector molecule can induce the transition of transcriptional activators from their inactive to an active form. For example, leucine biosynthesis is controlled by the transcriptional activator Leu3p in response to leucine availability. Leu3p is activated by the levels of the metabolic intermediate α-isopropyl malate, which serves as a sensor of leucine availability ([88]; see also [88a]). This type of regulation has also been described for the control of amino acid catabolism. The transcriptional activator PUT3p senses the presence or absence of intracellular proline, and then regulates proline degradation as a function of the availability of the amino acid in the medium [89].

General control process

In addition to this type of specific control, yeast use a general control process whereby a subset of genes is co-ordinately induced by starvation of the cell of any single amino acid. Under starvation conditions, the activity of the transcriptional machinery is affected by amino acid deprivation. Uncharged tRNAs accumulate and thus stimulate the activity of the protein kinase GCN2, which phosphorylates the α-subunit of eukaryotic initiation factor 2 (eIF2) which, in turn, impairs the synthesis of the 43 S pre-initiation complex (Met-tRNA, GTP and eIF2). The major consequence is the translational up-regulation of the transcription factor GCN4. This control is due to the particular structure of the 5′-untranslated region of the GCN4 mRNA. As a result, GCN4 induces more than 30 different genes in nine different biosynthetic pathways [90–92].

Certain genes involved in amino acid metabolism can be regulated by both the specific and the general control processes. For example, genes encoding enzymes involved in the leucine biosynthesis pathway are subject to regulation by the general control system in response to starvation of any amino acid. In addition, these genes are regulated independently by the Leu3p transcriptional activator in response to leucine availability [93].

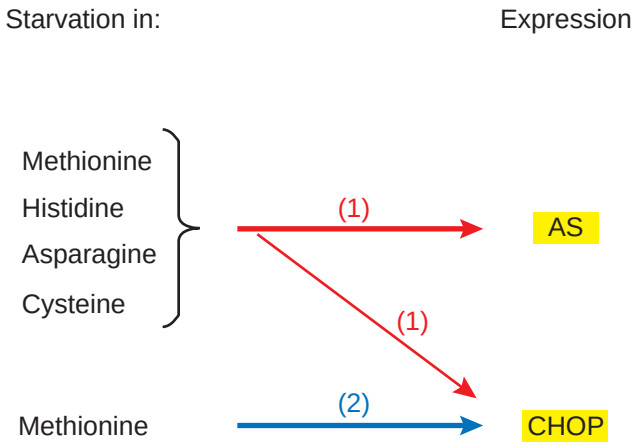

Figure 4 Regulation of *CHOP* and *AS* gene expression by amino acid starvation

Taking the example of the regulation of CHOP and AS expression in response to starvation of methionine, histidine, asparagine and cysteine, we can propose the following model. Starvation of any one of these amino acids can activate a signalling pathway (1) leading to overexpression of AS or CHOP, with the *AS* gene being more inducible than *CHOP*. In addition, methionine starvation can also turn on a specific control process (2) that activates CHOP expression.

Amino acid regulation of gene expression in mammalian cells

Considerably less information is available concerning the amino acid control of gene expression in mammalian cells compared with yeast. Since no general accumulation of mRNAs in amino acid-starved cells has been observed [65], cells must possess specific mechanism(s) that enable(s) them to alter one specific pattern of gene expression in response to amino acid deprivation. At the molecular level, most results have been obtained by studying the up-regulation of CHOP and AS gene expression in response to amino acid limitation. AS is expressed in most mammalian cells as a 'housekeeping' enzyme responsible for the biosynthesis of asparagine from aspartate and glutamine [94]. The level of AS mRNA increases in response not only to asparagine starvation but also to deprivation of leucine, isoleucine and glutamine [61,95]. *CHOP* encodes a ubiquitous transcription factor that heterodimerizes with the other members of the C/EBP family [96–98]. *CHOP* induction is linked to the activation of an endoplasmic-reticulum stress, itself presumably mediated by the accumulation of misfolded proteins [99].

Signalling pathways involved in the response to amino acid limitation

The studies presented in this section aim at a better understanding of the mechanisms responsible for the amino acid regulation of gene expression. In prticular we discuss the role of a stress of the endoplasmic reticulum [the unfolded protein response (UPR)] and the possibility that several mechanisms are involved in this process.

Since the expression of CHOP and AS can be induced both by a stress of the endoplasmic reticulum and by a limitation in amino acid availability [99,100], two hypotheses were considered: (i) amino acid limitation indirectly affects protein folding in the endoplasmic reticulum, thus turning on the UPR pathway, and in this way affecting the expression of target genes [99]; and (ii) amino acid limitation modulates gene expression through a specific mechanism independent of the UPR pathway. Using the induction of the *CHOP* gene by amino acid starvation as a model, it was shown that amino acid limitation regulates gene expression through a specific pathway that is distinct from the UPR signalling cascade [101]. This conclusion was drawn from two lines of evidence. First, the induction of gene expression in response to amino acid depletion is not correlated with an overexpression of the endoplasmic-reticulum chaperone BiP (immunoglobulin heavy-chain binding protein)/GRP78 (glucose-regulated protein 78), a marker of the UPR. Secondly, amino acid starvation and endoplasmic reticulum stress regulate *CHOP* promoter activity using distinct *cis* DNA elements.

The pathway linking amino acid limitation to gene regulation remains unknown. However, from studies of the regulation of AS and CHOP expression in response to depletion of one individual amino acid, it has been demonstrated that, as in yeast, several distinct mechanisms may be involved in the amino acid regulation of gene expression. CHOP expression is strongly induced in response to methionine starvation, but is only slightly affected by histidine, asparagine or cysteine starvation. Under the same experimental conditions, AS expression is induced equally in response to a limitation in any one of these amino acids [101a]. The discrepancy between the regulation of AS and CHOP expression by the limitation of individual amino acids can be explained by the existence of at least two regulatory mechanisms (Figure 4). Depletion of any one of these amino acids could activate a signalling pathway that controls the expression of a large number of genes, including those encoding AS and CHOP. In addition, methionine starvation could also turn on a more specific control process that induces CHOP expression specifically.

Several observations suggest that one of these pathways could be related to the general control process that exists in yeast: (i) the expression of several genes (e.g. those encoding CHOP, IGFBP-1 and AS) is regulated by the levels of many different amino acids [50,95,102]; (ii) Andrulis et al. [103] have shown a correlation between asparagine starvation, amino-acylation of $tRNA^{Asn}$ and AS activity; (iii) inhibition of leucyl-tRNA synthetase induces CHOP and AS expression [101]; and (iv) a homologue of yeast GCN2 has been identified in *Drosophila* [104,105] and in mammalian cells [106]. Nevertheless, regulation of the kinase activity of this protein by amino acids has not yet been described.

However, other observations suggest that, in mammalian cells, mechanisms involved in the amino acid control of gene expression may be different from that described in yeast. (i) eIF-2α kinase activity is similar in extracts prepared from fed and starved cells [107,108]. Nevertheless, for studies involving cell extracts, the possibility still remains that the results are affected by technical problems associated with preparation of the extracts [107,108]. (ii) In the perfused liver, inhibition of histidyl-tRNA synthetase by histidinol treatment increases eIF2-α phosphorylation, as in yeast [108,109]. However, in contrast with the situation in yeast, the main factor controlling eIF-2α phosphorylation is the regulation of phosphatase rather than kinase activity [108,109].

In summary, from the above discussion it is clear that the general control process described in yeast and the amino acid regulation of gene expression observed in mammalian cells share common features. However, some important distinctions are also apparent. Our knowledge of the signalling pathways that are activated in response to amino acid availability is limited. Further work will be necessary in order to determine precisely the cascade of molecular events involved in this control process.

An amino acid response element (AARE) on a mammalian gene promoter

The transcriptional regulation of argininosuccinate synthase expression in response to arginine starvation has been well

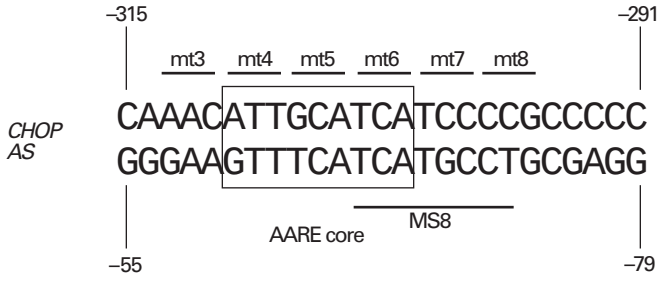

Figure 5 The *CHOP* AARE shares high similarity with the *AS* AARE

Shown is a sequence comparison of the *CHOP* AARE (−315 to −291) with the *AS* AARE (−55 to −79). Identical nucleotides are boxed in pink. The minimum AARE core sequence is boxed. The positions of mutations in the *CHOP* AARE (mt3, mt4, mt5, mt6, mt7, mt8) and in the *AS* AARE (MS8) sequences are represented.

documented [110,111]. However, the *cis* elements of the gene involved in this regulation have not been identified to date. The molecular mechanisms involved in the amino acid control of gene transcription have been studied, using the regulation of CHOP and AS expression by leucine availability as a model. It has been shown that (i) a limitation in leucine availability induces CHOP and AS expression in several cell lines; and (ii) this regulation involves both transcriptional and post-transcriptional components [62,102].

Guerrini et al. [62] have studied the regulation of the *AS* promoter by leucine availability and characterized a DNA fragment spanning nucleotides −164 to +44 that is sufficient to abolish the regulation by leucine. Mutation analysis of this DNA region identified the nucleotides between positions −70 and −66 (part of the MS8 mutation) as being essential for amino acid regulation (see Figure 5).

In the case of the *CHOP* promoter, we have recently shown that a DNA sequence located between positions −313 and −295 is essential for activation by amino acids [112]. Mutations (mt4, mt5 and mt6) affecting a stretch of nine nucleotides (5′-ATTG-CATCA-3′) between positions −310 and −302 result in a complete loss of amino acid responsiveness (Figure 5). This CHOP DNA sequence is the first element to be characterized that can regulate, in all human cell lines tested so far (HeLa, CaCo-2 and HepG2), a basal heterologous promoter in response to starvation of several individual amino acids [152]. Transcriptional activity of this element was induced rapidly and for leucine concentrations ranging from 70 to 0 μM, as described previously for the endogenous CHOP mRNA. From their functional properties, these *AS* and *CHOP cis* DNA sequences were called AAREs. When *CHOP* and *AS* AARE sequences were compared, high identity was found in a stretch of nine nucleotides of both AAREs (called the 'AARE core'), with only two variant nucleotides (Figure 5). Thus we propose the sequence 5′-(A/G)-TT(G/T)CATCA-3′ as the first AARE consensus sequence to be described that is essential for transcriptional regulation of eukaryotic genes by amino acids.

It has been shown that the core sequence of the *AS* and *CHOP* AAREs binds a multiprotein complex in a gel mobility-shift assay. Mutations in the core AARE, which impair the amino acid regulation of the promoter, also abolish the binding of the multiprotein complex [152]. Moreover, no differences in the DNA binding activity of this protein complex were observed for nuclear extracts from starved and non-starved cells. These data suggest that the mechanism of activation of the *AS* and *CHOP* genes does not occur at the DNA binding level, and may involve a post-translational modification of the DNA binding protein(s). The identity and role of such a modification remain to be determined. It is notable that sequences of the *AS* and *CHOP* AARE regions show some identity with the specific binding sites of the C/EBP and ATF (activating transcription factor)/CREB (cAMP-response element binding protein) leucine-zipper tran-

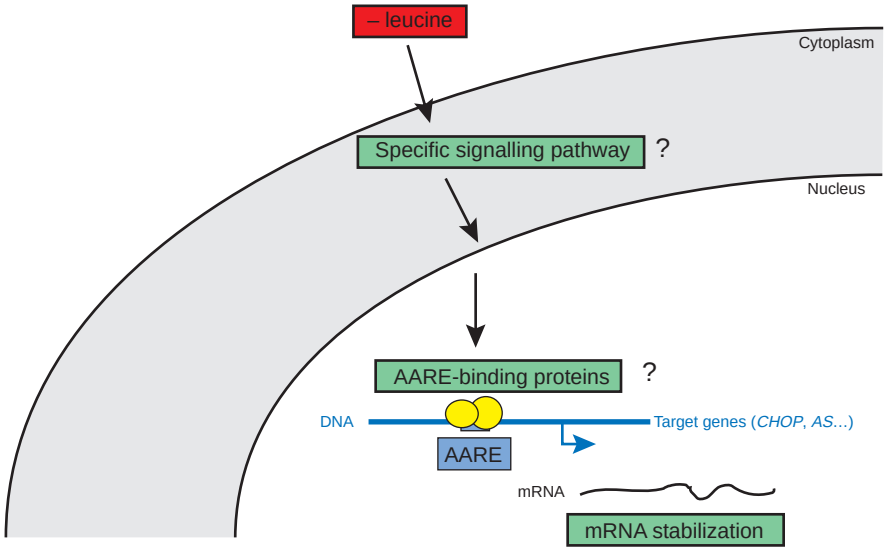

Figure 6 Scheme for the transcriptional activation of specific mammalian genes by leucine starvation

Leucine starvation can activate a specific signalling pathway that is distinct from the endoplasmic-reticulum stress-signalling cascade. Unknown nuclear proteins bind specifically to the AARE sequence in the mammalian gene promoters. No difference in the DNA-binding activity of the proteins is observed, suggesting that amino acid activation of transcription involves a post-translational modification of these AARE-binding proteins. The regulation of mammalian gene mRNA levels by leucine also has a post-transcriptional component that affects RNA stability.

scription factors. However, the role of members of the C/EBP and ATF transcription factor family in the activation of the *AS* and *CHOP* promoters by amino acids remains to be demonstrated (Figure 6).

Post-transcriptional component of amino acid regulation

The data presented for the genes encoding AS, CHOP, c-Jun, c-Myc and ODC clearly establish that the regulation of mammalian gene mRNA levels by amino acids also has a post-transcriptional component affecting RNA stability [62,70,102]. However, the molecular mechanisms that affect mammalian gene stability in amino acid-starved cells remain to be characterized.

REGULATION OF PROTEIN TURNOVER BY AMINO ACID AVAILABILITY

In mammals, amino acids have been shown to stimulate protein synthesis and inhibit proteolysis in several tissues, such as pancreatic β-cells, liver, heart and skeletal muscle. Although this regulation affects overall protein turnover, certain families of transcripts or proteins are affected differently by amino acid availability.

Regulation of mRNA translation by amino acid availability

Beside their role as substrates for proteins synthesis, amino acids also have important regulatory roles in the control of the initiation phase of mRNA translation. In this section we summarize the different steps of translation and the key players in this process, and then we review current knowledge on the regulation of those steps by amino acids.

The initiation of mRNA translation is a complicated process, involving several multiprotein complexes (for reviews, see [113–118]). The first step of protein translation (Figure 7a) is the formation of the 43 S pre-initiation complex containing methionyl-tRNA, eIF2, GTP and the 40 S ribosomal subunit. The preliminary phase is the activation of eIF2. To be active, eIF2 has to bind GTP. This is followed by the association of methionyl-tRNA and eIF2–GTP, which bind to the 40 S ribosomal subunit. The GTP is hydrolysed late in the initiation process, and eIF2 is released from the ribosome as an inactive eIF2–GDP complex. The recycling of eIF2 in an active form, eIF2–GTP, is mediated by the guanine-nucleotide exchange factor eIF2B, which is the first regulatory step of translation initiation. Two different mechanisms regulate eIF2B activity: phosphorylation of the α-subunit of eIF2 and phosphorylation of the ϵ-subunit of eIF2B. The rate of translation is decreased when these factors are phosphorylated.

The second regulated step in translation initiation is the binding of mRNA to the 43 S pre-initiation complex (Figure 7b). Proteins collectively referred to as eIF4 mediate this step. eIF4E binds to the cap structure of the mRNA and, through its association with the scaffolding protein eIF4G, also binds the helicase eIF4A and the 43 S ribosomal pre-initiation complex. This step is regulated through changes in: (i) the phosphorylation

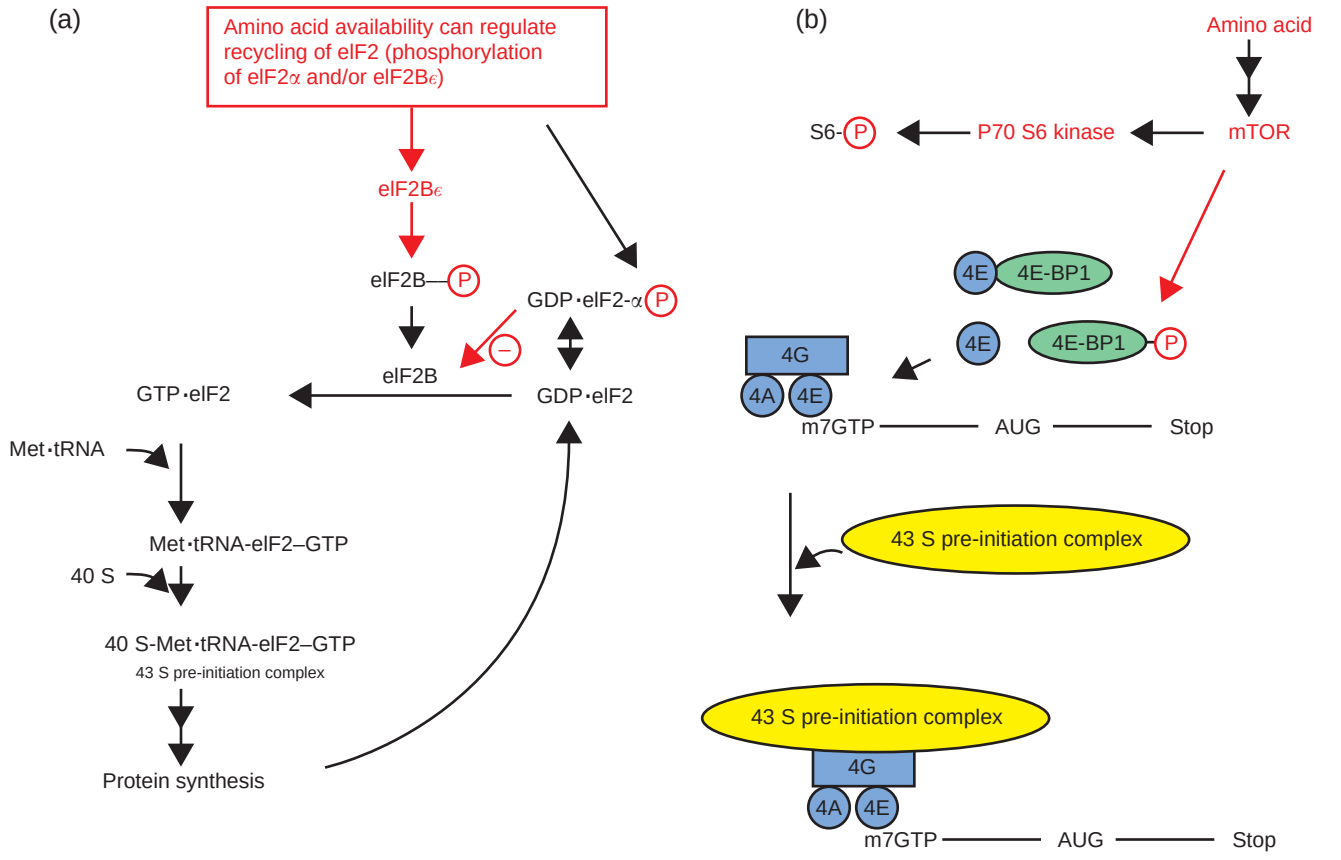

Figure 7 mRNA translation and its regulation by amino acid availability

Shown are the principal stages in peptide-chain initiation. The amino acid-regulated steps are emphasized (in red). (**a**) Formation of the 43 S pre-initiation complex; (**b**) binding of the mRNA to the 43 S pre-initiation complex. 4A (etc.) represents eIF4A (etc.).

of eIF4E: the affinity of eIF4E for the cap structure is increased when eIF4E is phosphorylated; and (ii) the availability of eIF4E: changes in eIF4E availability occur though its association with 4E-BP1 (eIF4E binding protein 1; also known as PHAS protein). When bound to 4E-BP1, the eIF4E protein is not able to bind eIF4G and the cap structure of the mRNA. The binding of eIF4E and 4E-BP1 is regulated by the phosphorylation of 4E-BP1 in response to a variety of stimuli. The complex dissociates when 4E-BP1 is phosphorylated.

The third regulated step in protein synthesis may be at the level of the ribosomal protein S6 and eukaryotic elongation factor-2 (eEF-2), which are phosphorylated in response to many agents, including growth factors and hormones. Although the relevance of eEF-2 phosphorylation in the physiological control of translation still remains unclear [116], S6 protein phosphorylation could be involved in the regulation of translation of proteins that are encoded by mRNAs containing oligopyrimidine tracts at the 5′ end of the message ('TOPS' mRNAs) [119,120].

Amino acid-regulated steps in protein translation

Amino acids regulate protein translation through modulation of eIF2B activity, 4E-BP1 phosphorylation and protein S6 phosphorylation.

(i) eIF2B activity. Amino acid deprivation has been shown to cause a significant decline in eIF2B activity. The change in eIF2B activity can be explained by increased phosphorylation of eIF2ϵ [121], and possibly by phosphorylation of eIF2Bϵ. Although the kinases involved in eIF2ϵ phosphorylation in response to amino acid availability are well known in yeast [91], they have not yet been identified in mammalian cells. Concerning the phosphorylation of eIF2Bϵ, Kimball et al. [121] have shown that, in L6 myoblast cell extracts, amino acid deprivation causes a decrease in eIF2Bϵ kinase activity, suggesting a modulation of the phosphorylation state of eIF2Bϵ.

(ii) 4E-BP1 phosphorylation. In addition to the modulation of eIF2B activity, amino acids cause a redistribution of eIF4E from the inactive eIF4E–4E-BP1 complex. This redistribution results from a change in the phosphorylation of 4E-BP1. Amino acid deprivation induces a marked dephosphorylation of 4E-BP1, resulting in the sequestration of eIF4E. Resupplying amino acids rapidly reverses these effects. This regulation has been observed in a large variety of cell lines, such as CHO cells ([96,122]; see also [122a]), myoblasts [121,123], pancreatic β-cells [124], adipocytes [125,126] and hepatoma cells [127,128]. Branched-chain amino acids, and particularly leucine ([125–127,129]; see also [129a]), play an important role in mediating this regulatory effect.

(iii) Protein S6 phosphorylation. It has been well demonstrated that withdrawal of amino acids from the nutrient medium results in a rapid deactivation of the p70 protein S6 kinase ([121–123,129]; see also [129a]). Re-addition of amino acids quickly reverses this effect, resulting in increased phosphorylation of ribosomal protein S6.

Several groups have demonstrated that the amino acid concentration in the culture medium regulates 4E-BP1 phosphorylation and p70 S6 kinase activity in a parallel manner. Moreover, p70 S6 kinase activation and 4E-BP1 phosphorylation induced by amino acid addition are inhibited by rapamycin, a specific inhibitor of the protein kinase mTOR (mammalian target of rapamycin). Data from several groups indicate that mTOR is (i) the upstream regulator of both p70 S6 kinase and 4E-BP1 [130,130a,131], and (ii) required for the response to amino acids [132–136]. In addition, it has been established that p70 S6 kinase and 4E-BP1 are regulated by separate mTOR-controlled pathways that bifurcate at or downstream of mTOR [134,137,138]. The mechanisms by which amino acids regulate the mTOR pathway are not known. However, recent results suggest that tRNA amino-acylation could be involved in this regulation [139].

Regulation of protein breakdown by amino acid availability

The breakdown of intracellular protein is carried out by both extra- and intra-lysosomal pathways. Extralysosomal proteolytic activities are due mainly to the 26 S proteasome in association with ATP and ubiquitin for protein targeting, and to the Ca^{2+}-dependent proteases or calpains (for reviews, see [140–142]). Intracellular proteins can also be targeted for their degradation to the lysosome, which is enriched in numerous peptidases [143]. Targeting to the lysosome can be achieved by macroautophagy, defined as the sequestration of cytosolic components in structures generated from the endoplasmic reticulum free of ribosomes [141,142]. The contribution of the different proteolytic systems to general protein breakdown differs according to the tissue. In the liver, the lysosomal pathway is responsible for the degradation of most proteins, whereas the extralysosomal pathways are involved mainly in the degradation of a small pool of proteins with a high turnover rate. Presumably for this reason, most studies on lysosomal proteolysis have been carried out in the liver. In muscle, the relative contribution of extralysosomal proteolytic systems is larger, particularly the breakdown of myofibrillar protein, which proceeds mainly via the ubiquitin/proteasome system.

The lysosomal pathway of protein breakdown is finely controlled by hormones and amino acids: glucagon stimulates, while insulin and amino acids inhibit, macroautophagy. Under *in vitro* experimental conditions, such as in the perfused rat liver, amino acids are the major regulators of lysosomal proteolysis. The hormonal regulation is not efficient in the absence of amino acids (when autophagic flux is maximal), nor in the presence of high amino acid concentrations (when macroautophagy is maximally inhibited). It has also been demonstrated that not all amino acids are equally effective as inhibitors of macroautophagy. Investigations with the perfused liver and isolated hepatocytes have shown that eight amino acids (Leu, Tyr, Phe, Gln, Pro, Met, His and Trp) plus alanine, which has a co-regulatory role, contribute to the control of hepatic proteolysis. The amino acid regulation of lysosomal proteolysis has also been described in other tissues, such as kidney [144], heart [145] and skeletal muscle [153].

In mammals, the molecular mechanisms involved in the regulation of lysosomal proteolysis by amino acids are poorly understood. It has been demonstrated that amino acids do not affect the level of expression of the enzymes involved in the proteolytic system, but regulate the sequestration step at the level of autophagy. Recently, a new concept has emerged that both autophagy and protein synthesis are under the control of mTOR [146]. Recent studies in yeast on the mechanisms of autophagy should allow a better understanding of the role of amino acids in the regulation of this process [147–149].

Physiological consequences of the regulation of protein turnover by amino acids

In summary, amino acids can regulate protein synthesis through changes in eIF2B activity, and through phosphorylation of 4E-BP1 and S6 proteins. They can also be involved in the control of protein degradation through effects on macroautophagy. These roles of amino acids can be associated with the changes in peripheral metabolism that occur after a meal [23,24,150,151].

Feeding a complete diet rapidly reverses the inhibition of protein synthesis that occurs after a short fast. In the past, these effects have been attributed to postprandial changes in circulating insulin concentrations. However, more recent evidence suggests that responses to a protein-rich meal might be due to postprandial increases in the blood amino acid content. Thus refeeding with a diet lacking protein or amino acids has no significant effect on protein synthesis, whereas the plasma insulin concentration is increased [23,24,151]. In addition, using mice with diabetes (type I and type II), Svanberg et al. [24] demonstrated that an increase in the plasma insulin concentration is not required for the restoration of protein synthesis in skeletal muscle in response to refeeding, but may play a permissive role.

CONCLUSIONS

In mammals, the plasma amino acid concentration shows striking alterations as a function of nutritional or pathological conditions. The amino-acidaemia can rise after a protein-rich meal, whereas, under poor nutritional conditions, the organism can experience limitations in the supply of essential amino acids. The organisms then have to adjust several of their physiological functions involved in the adaptation to amino acid supply by regulating numerous genes.

The idea that amino acids can regulate gene expression has emerged only recently. It is now clear that amino acids by themselves can play, in concert with hormones, an important role in the control of gene expression; however, the underlying processes have only just begun to be discovered. It has been shown that amino acid availability can modify the expression of target genes at the levels of transcription and mRNA stability. Moreover, amino acids can also regulate protein turnover. Further investigations will be necessary in order to characterize the molecular steps by which the cellular concentration of amino acids can regulate gene expression, particularly to determine: (1) the AAREs in the regulated genes; (2) the nature of the protein complexes bound to these elements; (3) the identity of the intracellular metabolites that mediate transcriptional activation by amino acid limitation; and (4) the signalling pathways involved in the control of translation by amino acids. Such studies will increase our understanding of the role of amino acids in the control of cellular functions such as cell division, protein synthesis, proteolysis, etc. The molecular basis for gene regulation by dietary protein intake is important with respect to the regulation of physiological functions of individuals living under conditions of restricted or excessive food intake.

Note added in proof (received 5 August 2000)

Since this review was typeset, two important articles have been accepted for publication. M. S. Kilberg's group [154] have shown that the induction of the AS gene by amino acid deprivation and by endoplasmic-reticulum stress response occurs via the same set of genomic elements. Compared with our data, these results demonstrate that the mechanisms involved in AS regulation by amino acid and/or endoplasmic-reticulum stress response are different from those involved in CHOP regulation. Our group [152] has precisely characterized the CHOP AARE and demonstrated that expression of the transcription factor ATF2 (activating transcription factor 2) is essential for the transcriptional activation of CHOP by amino acid starvation.

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