CELLULAR ADAPTATION TO AMINO ACID AVAILABILITY:
MECHANISMS INVOLVED IN THE REGULATION OF GENE EXPRESSION

ADAPTATION CELLULAIRE À LA DISPONIBILITÉ DES ACIDES AMINÉS :
MÉCANISMES IMPLIQUÉS DANS LA RÉGULATION DE L’EXPRESSION DES GÈNES

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In mammals, the impact of nutrients on gene expression has become an important area of research. Nevertheless, the current understanding of amino acid-dependent control of gene expression is limited. Amino acids have multiple and important functions, so their homeostasis has to be finely maintained. However, the amino acidemia can be affected by certain nutritional conditions or various forms of aggression. It follows that mammals have to adjust several of their physiological functions involved in the adaptation to amino acid availability by regulating expression of numerous genes. The aim of this review is to examine the role of amino acids in regulating mammalian gene expression and physiological functions.

A limitation for several amino acids strongly increases the expression of target genes such as IGFBP-1, CHOP and asparagine synthetase (AS) genes. The molecular mechanisms involved in the regulation of CHOP and AS gene transcription in response to amino acid starvation have been partly identified. Particularly, a signalling pathway requiring the protein kinase GCN2 and the transcription factor ATF4 has been described to sense the amino acid limitation. In case of an amino acid imbalanced food source, this pathway has been shown to decrease food intake by activating a neuronal circuit. Taken together, the results discussed in this review demonstrate that amino acids by themselves can act as “signal” molecules with important roles in the control of gene expression and physiological functions.

Keys words: amino acid, gene expression, signalling pathway, GCN2, ATF4, nutrition, protein.

SUMMARY

In mammals, the impact of nutrients on gene expression has become an important area of research. Nevertheless, the current understanding of amino acid-dependent control of gene expression is limited. Amino acids have multiple and important functions, so their homeostasis has to be finely maintained. However, the amino acidemia can be affected by certain nutritional conditions or various forms of aggression. It follows that mammals have to adjust several of their physiological functions involved in the adaptation to amino acid availability by regulating expression of numerous genes. The aim of this review is to examine the role of amino acids in regulating mammalian gene expression and physiological functions.

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Keys words: amino acid, gene expression, signalling pathway, GCN2, ATF4, nutrition, protein.
Regulation of amino acid metabolism and homeostasis in the whole animal

Amino acids exhibit two important characteristics compared with other macronutrients (lipids or sugars). First, in healthy adult humans, 9 amino acids (valine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, histidine, and tryptophan) are indispensable or essential. However, the mechanisms involved in the amino acid control of gene expression have just begun to be understood in mammalian cells (Kilberg et al. 1994; Fafournoux et al. 2000; Bruhat & Fafournoux, 2001). This review summarizes recent work on the effect of amino acid availability in the regulation of physiological functions, particularly focusing on the mechanisms involved in the amino acid regulation of gene expression.

For several amino acids, a decrease in their concentration stimulates the expression of specific genes such as IGFBP-1, CHOP, and asparagine synthetase (AS). The molecular mechanisms implicated in the activation transcriptional level of CHOP and AS in response to an amino acid deficiency have been partially identified. In particular, a signalization pathway involving the GCN2 kinase and the transcription factor ATF4 plays the role of sensor of amino acid deficits. At the physiological level, recent studies show that the activation of this pathway permits, through a neuronal circuit, to inhibit food intake when the nutritional amino acid supply is imbalanced.

The data presented in this review demonstrate that amino acids can act as signal molecules controlling gene expression in mammals. It has been shown that major (carbohydrates, fatty acids, steroids) and minor (minerals, vitamins) dietary constituents participate in the regulation of gene expression (Towle 1995; Foufelle et al. 1998; Pegorier 1998; Duplus et al. 2000; Vaulont et al. 2000; Grimaldi 2001). However, the mechanisms involved in the amino acid control of gene expression have just begun to be understood in mammalian cells (Kilberg et al. 1994; Fafournoux et al. 2000; Bruhat & Fafournoux, 2001). This review summarizes recent work on the effect of amino acid availability in the regulation of biological functions. On the basis of the physiological concepts of amino acid homeostasis, we will discuss specific examples of the role of amino acids in the regulation of physiological functions, particularly focusing on the mechanisms involved in the amino acid regulation of gene expression.

Mots-clés : acide aminé, expression des gènes, voie de signalisation, GCN2, ATF4, nutrition, protéine.
Amino acids are synthesized by the urea cycle to meet the needs of an adult but not those of a growing child. Secondly, there are no large stores of amino acids. Consequently, when necessary, an organism has to hydrolyze muscle proteins to produce free amino acids. This loss of protein will be at the expense of essential elements. Therefore, complex mechanisms that take into account these amino acid characteristics are needed for maintaining the free amino acid pools.

**Free amino acids pool**

The size of the pool of each amino acid is the result of a balance between input and removal (figure 1). The metabolic outlets for amino acids are protein synthesis and amino acid degradation whereas the inputs are de novo synthesis (for non-essential amino acids), protein breakdown and dietary supply. Changes in the rates of these systems lead to an adjustment in nitrogen balance. For example, a protein-containing meal given to an animal or a human subject has been reported to increase both the nitrogen balance and the level of amino acids in the plasma. Particularly, the concentration of leucine and certain other amino acids approximately doubles in peripheric blood plasma. Particularly, the concentration of leucine and certain other amino acids approximately doubles in peripheric blood following a protein-rich meal (Aoki et al. 1976) and reaches much higher concentrations within the portal vein (Fafournoux et al. 1990). It is now well established that the effect of a protein-rich meal on protein turnover is due to postprandial increases in the concentrations of circulating amino acids (Yoshizawa et al. 1995; Svanberg et al. 1997).

A nother example of the nitrogen balance adjustment is the adaptation to an amino acid deficient diet. A dramatic decrease in the plasma concentrations of certain essential amino acids has been shown to occur following a dietary imbalance, a deficiency of any one of the essential amino acids or a deficient intake of protein (Grimble & Whitehead, 1970; Peng & Harper, 1970; Ozalp et al. 1972; Baertl et al. 1974). Long-term feeding with such diets can lead to a negative nitrogen balance and clinical symptoms.

The examples cited above show that amino acid metabolism can be affected by various nutritional and/or pathological situations, with two major consequences: a large variation in blood amino acid concentrations and a negative nitrogen balance. In these situations, individuals have to adjust several of their physiological functions involved in the defence/adaptation to amino acid limitation by regulating numerous genes.

In the next section the specific role of amino acids in the adaptation to amino acids deficient diets will be considered.

**Specific examples of the role of amino acids in the adaptation to protein deficiency**

*Protein undernutrition*

Protein undernutrition has its most devastating consequences during growth. Prolonged feeding on a low protein diet causes a fall in the plasma level of most essential amino acids. For example, leucine and methionine concentrations can be reduced from about 100-150 µM and 18-30 µM to 20 µM and 5 µM, respectively, in plasma of children affected by kwashiorkor (Grimble & Whitehead, 1970; Baertl et al. 1974). It follows that individuals have to adjust several physiological functions in order to adapt to this amino acid deficiency. One of the main consequences of feeding a low protein diet is the dramatic inhibition of growth. Growth is controlled by a complex interaction of genetic, hormonal and nutritional factors. A large part of this control is due to growth hormone (GH) and insulin-like growth factors (IGFs). The biological activities of the IGFs are modulated by the IGF-binding proteins (IGFBPs) that specifically bind IGF-I and IGF-II (Lee et al., 1993; Straus, 1994). Straus et al. (1993) demonstrated that a dramatic overexpression of IGFBP-1 was responsible for growth inhibition in response to prolonged feeding on a low protein diet. Known regulators of IGFBP-1 expression are GH, insulin or glucose. However, the high IGFBP-1 levels found in response to a protein-deficient diet cannot be explained by these factors. It has been demonstrated that a fall in the amino acid concentration was directly responsible for IGFBP-1 induction (Straus et al. 1993; Jousse et al. 1998). Therefore, amino acid limitation, as occurring during dietary protein deficiency, participates in the down-regulation of growth through the induction of IGFBP-1.

*Imbalanced diet*

The ability to synthesize protein is essential for survival, and protein synthesis is dependent on the simultaneous supply of the 20 precursor amino acids. Because mammals cannot synthesize all of the amino acids, the diet must provide the remaining ones. Thus, in the event of a deficiency of one of the indispensable amino acids body proteins are broken down to provide the limiting amino acid and the remaining amino acids are catabolised and lost (M unro 1976). It follows that mammals (with the exception of the ruminants) need mechanisms that provide for selection of a balanced diet. The capacity to distinguish balance from imbalance among the amino acids in the diet and to select for the growth-limiting essential amino acid provides an adaptive advantage to animals.

After eating an amino acid imbalanced diet, animals first recognize the amino acid deficiency and then respond by reducing their food intake. Recognition and anorexia resulting from an...
AMINO ACID CONTROL OF GENE EXPRESSION IN MAMMALIAN CELLS

Amino acids as “signal” molecules

• Genes up-regulated by amino acids

Genes that are specifically up-regulated in response to supraphysiological concentrations of amino acids have been described. For example, a high concentration of L-tryptophan enhances the expression of collagenase and of tissue inhibitors of metalloproteinase. In rat hepatocytes, Na+-cotransported amino acids like glutamine, alanine or proline stimulate acetyl-coA carboxylase, glycogen synthetase and arginino succinate synthetase activity. It has been demonstrated that the swelling resulting from the addition of amino acid could be involved in the regulation of gene expression (Watford 1990; Hausinger 1996), although the molecular mechanisms involved in these processes are poorly understood.

• Genes up-regulated by amino acid starvation

Specific mRNA s that are induced following amino acid deprivation have been reported (Marten et al. 1994) in mammalian cells. Most molecular mechanisms involved in the amino acid regulation of gene expression have been obtained by studying the up-regulation of C/EBP homologous protein (CHOP), A speragine synthetase (ASNS) and the cationic amino acid transporter (Cat-I) genes.

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

Transcriptional activation of mammalian genes by amino acid starvation

It has been established that the increase in CHOP or ASNS mRNA following amino acid starvation is mainly due to an increased transcription (Hutson & Kilberg, 1994; Bruhat et al. 1997). By first identifying the genomic cis-elements and then the corresponding transcription factors responsible for regulating these specific target genes, it is anticipated that one can progress backwards up the signal transduction pathway to gain an understanding of the individual steps required.

• Regulation of the human CHOP gene by amino acid starvation

CHOP encodes a ubiquitous transcription factor that heterodimerises avidly with the other members of the C/EBP (Fawcett et al. 1996) and Jun/Fos (Ubeda et al. 1999) family. The CHOP gene is tightly regulated by a wide variety of stresses in mammalian cells (Luethy & Holbrook, 1992; Sylvester et al. 1994; Wang et al. 1996). Leucine limitation in human cell lines leads to induction of CHOP mRNA and protein in a dose dependent manner (Bruhat et al., 1997).

We identified a cis-positive element in the CHOP promoter located between –313 and –295 that is essential for amino acid regulation of the gene transcription (Bruhat et al. 2000) (figure 2). This short sequence can regulate a basal promoter in response to starvation of several individual amino acids, which is then termed an amino acid regulatory element (AARE). The sequence of the CHOP AARE region exhibits some homology with the specific binding sites of the C/EBP and ATF/CREB transcription factor families. Using gel shift experiments and chromatin immunoprecipitation, we have shown that ATF2, ATF3, ATF4 and CCAAT enhancer-binding protein (C/EBP) that belong to the ATF or C/EBP family have the ability to bind to the CHOP AARE. Among these factors ATF2 and ATF4 are involved in the amino acid control of CHOP expression: when knockout cell line for these two proteins were tested, amino acid regulation of CHOP expression was abolished (Bruhat et al. 2000; Averous et al. 2004). This work was broadened to the regulation of other amino acid regulated genes and confirmed that ATF4 and ATF2 are key components in the amino acid control of gene expression (Averous et al. 2004).

Amino acid signalling pathway

It appears that more than one amino acid signalling pathway exists in mammalian cells (Joussen et al. 2000; Bain et al. 2002). However, the individual steps required for these pathways are not well understood.
• AT F4 and the amino acid signalling pathways

D. Ron’s group has identified a signalling pathway for regulating gene expression in mammals that is homologous to the well-characterised yeast general control response to amino acid deprivation (Harding et al. 2000). Its components include (figure 3) the mammalian homologue of the GCN2 kinase, the initiation factor eIF2α and AT F4. Like GCN4, the ACC4 mRNA contains U0RF in its 5’UTR that allows translation when the cap-dependent translation is inhibited. The authors showed that GCN2 activation, phosphorylation of eIF2α and translational activation of AT F4 are necessary but not sufficient for the induction of CHOP expression in response to leucine starvation. These data are in good agreement with the analysis of the CHOP and A SN5 promoters showing that AT F4 can bind to the promoter sequences involved in the response to amino acid starvation.

• AT F2 and the amino acid signalling pathways

Transactivating capacity of AT F2 is activated via phosphorylation of N-terminal residues Thr-69, Thr-71 and Ser-90 (Gupta et al. 1995; Livingstone et al. 1995). There are two lines of evidence suggesting that AT F2 phosphorylation belongs to the amino acid response pathway leading to transcriptional activation of CHOP by amino acids: (i) Leucine starvation induces AT F2 phosphorylation in human cell lines (Averous et al. 2004) and (ii) an AT F2 dominant negative mutant (Sano et al. 1999) in which the three residues cannot be phosphorylated inhibits AT F2 promoter activity enhanced by leucine starvation (Bruhat et al. 2000). These data suggest that a specific amino acid-regulated pathway that leads to the transcriptional activation of CHOP may involve a phosphorylation of prebound AT F2 rather than an increase in AT F2 binding. However, the identity of any kinases involved in AT F2 phosphorylation by amino acid starvation remains to be discovered (see figure 2).

It appears that at least two different pathways that lead to AT F2 phosphorylation and to AT F4 expression are necessary to induce CHOP expression in response to one stimulus (amino acid starvation). In addition, AT F4 and AT F2 belong to the bZIP transcription factor family. These proteins have the ability to interact with several transcription factors to bind the target DNA sequence. In the case of amino acid regulation of CHOP expression, we have no evidence that AT F2 and AT F4 form a dimer that bind the AARE sequence but they could be included in a larger regulatory protein complex. However, it has been shown that AT F2 is able to interact with the coactivator of transcription p300 which has histone acetyltransferase activity (Kawasaki et al. 1998) and also with JDP2, a repressor that recruits a histone deacetylase complex (Jin et al. 2002), suggesting that this transcription factor could modulate transcription by interacting directly or indirectly with the chromatin structure.

ROLE OF THE GCN2/ATF4 PATHWAY IN NUTRITION: AMINO ACID DEFICIENCY SENSING BY GCN2 TRIGGERS FOOD AVERSION

Food intake results from a complex behavioural pattern in which innate factors play an important role, particularly in the case of omnivores. A remarkable example of an innate mechanism governing food choice is presented by the fact that omnivorous animals will consume substantially less of an otherwise identical experimental meal lacking a single essential amino acid (Harper et al. 1970; Gietzen et al. 1993). Although it seems likely that the signalling pathway leading to this response comprises the sensing of amino acid variations, the basis for this innate aversive response is poorly understood. As described above, previous studies have implicated the anterior piriform cortex (APC) in controlling food intake according to amino acid levels (Leung & Rogers, 1971; Gietzen 1993). Furthermore, it has been recently reported that consumption of an amino acid imbalanced diet rapidly elevates levels of phosphorylated eIF2α in A PC neurons (Gietzen et al. 2004).

Blood concentration of an essential amino acid decreases rapidly when this amino acid is missing in the diet. As a consequence, the protein kinase GCN2, which is ubiquitously expressed, could be activated in most tissues. Its only known substrate is the serine 51 of the eIF2α subunit. Therefore GCN2 could be an important sensor of amino acid homeostasis.

Figure 2: cis-acting elements required for induction of CHOP, AT A2 and ASNS genes following amino acid starvation

Sequence comparison of the CHOP AARE with the ASNS SRE-1 and the AT A2 AARE. The positions of sequences from the transcription start site are shown in brackets. The minimum sequence (core sequence) required for the response to amino acid starvation is in boxes. Identical nucleotides to the CHOP sequence are in red.

Figure 3: Comparison between the general control process of gene expression by amino acid availability in yeast and the amino acid regulation of CHOP or ASNS expression in mammals

The mammalian pathway appears to be more complex than the yeast pathway; for example, AT F2 needs to be phosphorylated to allow CHOP transcriptional induction in response to leucine starvation. In addition, eIF2α can be phosphorylated by 4 different kinases (GCN2, PERK, PKR, and HRI).
inside cells and could activate downstream rectifying responses mediated by phosphorylated eIF2α. The latter affect gene expression programs at the level of mRNA translation and transcription (Hinnebusch 1994; Harding et al. 2000; this review).

Recent results (Maurin et al. 2005; Hao et al. 2005) establish that the aversive response of wild-type mice to a diet deficient in one essential amino acid is likewise blunted in GCN2-/- mice whereas serum amino acid levels are decreased to similar levels by the imbalanced diet in both genotypes (figure 4). These results indicate an altered response to amino acid deficiency in mice lacking GCN2 activity. Moreover, we confirmed the previously described increase in phosphorylated eIF2α levels in the A PC of wild-type animals after consumption of an imbalanced meal, and further showed that no such signal occurs in the GCN2-/- . This observation indicates a role for GCN2 in mediating eIF2α phosphorylation in the A PC of mice fed an imbalanced diet. Using conditional GCN2 knockout mice, we further demonstrated that GCN2 ablation specifically in the brain also impairs the aversive response to an imbalanced diet. Thus, even if the consumption of an imbalanced meal also activates GCN2 and promotes eIF2α in peripheral tissues, particularly in the liver, our observation implicates brain GCN2 signalling in initiating the aversive response. This example highlights the need to rely on physiological observations of molecular events described in vitro to improve our knowledge on physiological consequences of nutritional status.

**CONCLUSION**

The idea that amino acids can regulate gene expression is now well established. Amino acids by themselves can play an important role in the control of gene expression, in concert with hormones, but the underlying processes are only now being discovered. Amino acid availability can modify the expression of target genes at the level of transcription, mRNA stability and translation.

Defining the precise cascade of molecular events by which the cellular concentration of an individual amino acid regulates gene expression will be an important contribution to our understanding of metabolite control in mammalian cells. These studies will provide insight into the role of amino acids in the regulation of cellular functions such as cell division, protein synthesis or proteolysis.

![Figure 4](image-url)
BIBLIOGRAPHY


