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Joris G. Winderickx • Peter M. Taylor (Eds.)

Nutrient-Induced Responses in Eukaryotic Cells

With 48 Figures, 2 in Color; and 1 Table

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The cover illustration depicts pseudohyphal filaments of the ascomycete *Saccharomyces cerevisiae* that enable this organism to forage for nutrients. Pseudohyphal filaments were induced here in a wild-type haploid MATa Σ1278b strain by an unknown readily diffusible factor provided by growth in confrontation with an isogenic petite yeast strain in a sealed petri dish for two weeks and photographed at 100X magnification (provided by Xuewen Pan and Joseph Heitman).

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Introduction

Joris Winderickx and Peter M. Taylor

Cells of all living organisms are able to sense environmental stimuli and respond appropriately. Especially for unicellular organisms, the environment largely controls growth, metabolism, and differentiation. In higher multicellular organisms, most cells experience relative environmental homeostasis. However, growth and metabolism of cells within multicellular organisms require coordination between the cells in a tissue, an organ, and the whole organism. These cells communicate by cell-to-cell contact, gap-junctions, and integrins, or by using molecules such as hormones and growth factors, which allow cell-to-cell signalling.

For unicellular and multicellular organisms alike, nutrients provide the essential building blocks and energy supply to make the necessary cellular components and drive metabolism. Therefore, the availability of nutrients is essential to survive, proliferate, and be productive. Cells have developed mechanisms to sense nutrient availability and produce appropriate responses whereby nutrients are able to influence gene transcription and mRNA processing as well as translation and post-translational modifications. Such mechanisms may, in certain cases, involve direct or near-direct interactions between a nutrient and the regulatory sequences of specific genes involved in its metabolism. There are also reports of metabolite-binding domains in particular mRNA species (so-called "riboswitches"), which serve as metabolite-responsive genetic control elements. Nevertheless, many nutrients appear to affect cell and organismal function largely through intermediate nutrient-responsive signalling pathways. Such nutrient-dependent signalling allows for optimal nutrient consumption in a dynamic integrated manner and particularly in unicellular organisms it enables coordinated induction of a resting phase where the cells cease proliferation upon nutrient limitation, but rapidly resume the process once the conditions are more suitable.

In recent years, our understanding of nutrient sensing and the responses triggered by altered nutrient availability have greatly advanced. The emerging picture is that nutrient signalling mechanisms have evolved early in evolution and that the so-called nutrient-responsive signalling cascades used by microorganisms provide core elements of the more sophisticated regulatory pathways found in multicellular organisms, where hormonal controls have assumed increasingly greater importance. For example, many of the genes regulated by glucose alone in lower eukaryotes are additionally dependent upon the presence of insulin (and to some extent thyroid hormones) in higher eukaryotes. However, perhaps surprisingly, insulin was also found to trigger regulatory effects in microorganisms (Muller et al. 1998). The availability of genomic and proteomic data for an increasing number of eukaryotic species has highlighted the conservation of these basic pathways and, in many cases, conservation has been confirmed by functional complementation of several key proteins. The success of genome and proteome research has led to other exciting and new approaches dealing not only with top-down elucidation

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of a single nutrient sensing pathway but with the more global investigation of nutrient signalling networks and the identification of converging effector branches that explain the dynamical but very coordinated nutritional response. Indeed, every step in a particular nutrient pathway represents a potential convergence point for yet another cascade, which may modulate or otherwise alter the final overall response. A nice example of this is the pseudohyphal growth pathway in yeast, which combines modules of the pheromone pathway and the Ras-cAMP cascade (Gancedo 2001).

Several nutritional factors have now been implicated as specific regulators of signal transduction and these include organic nutrients such as glucose, amino acids and fatty acids as well as inorganic compounds like carbon dioxide, ammonia, nitrates, and key micronutrients such as zinc, calcium, and phosphate ions. It has not always been possible to differentiate whether a nutritional stimulus acts really as a signal initiator or whether it merely triggers a local metabolic response. However, in a growing number of cases, specific receptors or transporters of a particular nutrient have been identified which appear to function as sensors. One of the best examples of this has been the discovery of two glucose transporters, i.e. Snf3 and Rgt2, that have been implicated in glucose sensing in yeast (Oscan et al. 1998) and, most recently, a similar function has been recognised for the human sodium/glucose cotransporter SGLT3 (Diez-Sampedro et al. 2003). Other nutrients may bind to GPCR-receptors such as Gpr1, a glucose receptor in yeast (Rolland et al. 2000) and GPR105/P2YX, the UDP-glucose receptor in mammals (Chambers et al. 2000).

Nevertheless, as might be expected there are also some important and fundamental differences in nutrient-induced responses between lower eukaryotes such as yeast and more complex organisms such as mammals. For example, glycogen is stored during glucose abundance in mammals, but in yeast it is only stored at the end of fermentation, before glucose becomes limiting. This is at least in part due to opposing end-point effects of the orthologous signalling pathways in the different organisms. Thus, the nutrient signal itself (in this case glucose availability) does not always induce the same (or even the equivalent) response, depending on the species studied, although the overall response in all cases is regarded as adaptive to the prevailing conditions and the specific biology of the species concerned. Another phylogenetic difference is the apparently unusual importance of free fatty acids as sensed molecules for metabolic regulation in animals. Lipids (fats and oils) are major sources and stores of fuel in higher animals but much less so in most plants and lower eukaryotes. A notable exception occurs during post-germinative growth of oilseeds, which is initially dependent on the breakdown of stored lipid reserves, which can be converted to sugar and other metabolites via the glyoxylate cycle (this is important for seedlings which have large lipid reserves but cannot yet photosynthesize). Recent studies have demonstrated inhibitory effects of sucrose on glyoxylate cycle activity (Borek et al. 2003). This represents an intriguing and important example of a nutrient-induced regulatory response in plants, although the mechanism is not yet known.

A common theme throughout the eukaryotic systems considered here is the link between nutrient availability and cellular energy status (notably as judged by the

absolute or relative concentrations of ATP and ADP/AMP). Reduction in cellular ATP levels is also an index of cellular stress and indeed nutritional deprivation *per se* may be considered as a stress. Such stresses are typically associated with activation of one or more "stress-related" signalling or endocrine pathways, which have substantial downstream effects on organismal function, typically involving shut-down of nonessential processes and the induction of the genetic and metabolic program directed towards the protection of the cell.

In this volume on nutritional responses, we brought together experts on nutrient signalling in yeast and animals. The different chapters give an overview of recent advances in the field to guide the reader in the complex but dynamic system of nutrient sensing. We hope that reader will appreciate the dedication of all the scientists whose research has been cited in the reviews presented. Without such expertise, this book would not have been possible.

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1 Transcriptional regulatory mechanisms for the response to amino acid deprivation of mammalian cells

Michael S. Kilberg, Can Zhong, Randall McClellan, and YuanXiang Pan

Abstract

Dietary protein is critical to mammalian nutrition and on a cellular level this translates into amino acid availability. Cells monitor amino acids and respond with changes in cellular processes, including gene transcription. Thus, amino acids serve as signal molecules to transmit the nutritional status of the organism to individual cells. Using two target genes, CHOP and asparagine synthetase, this chapter will review the transcriptional control mechanisms triggered by amino acid limitation, a pathway named the amino acid response. The transcription factors, identified thus far, belong to two subfamilies, C/EBP and ATF, of the bZIP superfamily. There is much yet to learn about the signal pathways and the molecular mechanisms responsible for transcriptional regulation by nutrients. Beyond gaining a basic understanding of these biological control mechanisms, characterizing how these processes contribute to the pathology of various disease states represents an exciting aspect of molecular nutrition.

1.1 Introduction

Dietary protein is an important factor in the general nutrition of an entire organism, and on a cellular level this translates into amino acid availability. Although the amino acid content in the bloodstream and protein turnover both act to buffer variation in dietary protein/amino acid intake, fluctuations in the intracellular levels of individual amino acids do occur in response to diet, disease, and metabolic status. Obviously, the cellular metabolic stance must be altered in an attempt to adapt to these changes, and yet, how mammalian cells monitor amino acid levels and respond with changes in fundamental cellular processes is not completely understood. In this context, amino acids are serving as signal transduction messengers to transmit the nutritional status of the organism to individual cells. One of the target mechanisms of amino acid-dependent signaling is altered transcription for specific genes. This chapter will focus on the mechanisms associated with modulation of transcription triggered by amino acid limitation, a signaling pathway that will be referred to as the amino acid response (AAR). Detection of a limiting amount of any single amino acid has been linked to a ribosome-associated

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Table 1. Example genes that exhibit increased mRNA content following amino acid limitation

Protein	References
Asparagine synthetase	(Gong et al. 1991; Kilberg and Barbosa-Tessmann 2002; Siu et al. 2002)
Amino Acid Transporters	
CAT1	(Hyatt et al. 1997)
SNAT2	(Bain et al. 2002; Gazzola et al. 2001)
CHOP	(Bruhat et al. 1997; Jousse et al. 1999; Marten et al. 1994)
C/EBPα	(Marten et al. 1994)
C/EBPβ	(Marten et al. 1994)
IGFBP-1	(Jousse et al. 1998; Straus et al. 1993)
Ribosomal proteins	
L17	(Laine et al. 1991)
S25	(Laine et al. 1994)
L35	(Hitomi et al. 1993)
S13	(Hitomi et al. 1993)

kinase, GCN2, that binds and therefore, monitors the level of uncharged tRNAs (Hinnebusch 1997). Starvation-activated GCN2 kinase phosphorylates eIF2α and then the ensuing changes in eIF2α-mediated translation initiation favor increased synthesis of a specific transcription factor. In yeast, this transcription factor is GCN4 (Hinnebusch 1997), which has been reported to alter the transcription rate of up to 1000 genes (Natarajan et al. 2001). This translational detection mechanism has not been as extensively studied in mammalian cells, but a mammalian counterpart to yeast GCN2 has been identified (Berlanga et al. 1999; Sood et al. 2000). It appears from several studies that ATF4 (discussed in more detail below) may represent the mammalian counterpart to GCN4. The translation of pre-existing ATF4 mRNA is rapidly increased following amino acid deprivation (Harding et al. 2000), and ATF4 protein has been shown to mediate the increased transcription of AAR pathway target genes (Siu et al. 2002).

1.2 Examples of mammalian activities altered by amino acid availability

A wide range of enzymatic and transport activities, protein content, mRNA content, and transcription of specific genes have been reported to be regulated by amino acid availability both *in vivo* and *in vitro*. Given current screening technologies using gene arrays, it is anticipated that many more mammalian genes will be identified for which transcription is regulated by amino acid availability. Table 1 presents a partial and ever-increasing representative list of genes for which the corresponding mRNA content is increased following amino acid deprivation of

mammalian cells. In some instances, the elevation in mRNA content is known to result from a change in transcription, but for several others, the mechanism remains to be established, and mRNA stabilization may contribute (Abcouwer et al. 1999; Gong et al. 1991). Marten et al. (1994) showed that C/EBPα and C/EBPβ mRNA content was increased by amino acid deprivation of rat hepatoma cells. As discussed in more detail below, C/EBP family members are of particular interest given the participation of C/EBPβ as one of the transcription factors that mediates induction of the human asparagine synthetase gene in response to activation of both the amino acid response (AAR) and the ER stress response (ERSR) nutrient sensing pathways (Siu et al. 2001). Also of note is the amino acid-dependent transcriptional regulation of the C/EBP homologous protein, CHOP (Bruhat et al. 1997; Fafournoux et al. 2000). Future characterization of amino acid-dependent changes in transcription factor expression, especially those (e.g. C/EBPβ, ATF4, and ATF3) that appear to be an integral part of the AAR pathway signaling process, will provide valuable insight into the mechanisms of gene expression following amino acid limitation. Amino acid-dependent regulation of transcription factor synthesis and action is discussed more extensively below.

Although increased transcription of ribosomal protein genes in the face of amino acid limitation appears to be counter-intuitive, the mRNA content for several ribosomal proteins has been shown to be increased (Laine et al. 1994) and for ribosomal proteins L17 and S25 this increase has been demonstrated to be transcriptional in nature (Laine et al. 1994). Interestingly, the newly synthesized mRNA molecules for L17 and S25 are retained within the nucleus for the duration of the amino acid deprivation period, and only released into the cytoplasm for translation following amino acid re-feeding (Laine et al. 1994). Adilakshmi and Laine (2002) have demonstrated that p53 binds to the S25 mRNA in the nucleus and may be associated with this nuclear retention process. Further investigation into how this nuclear retention is controlled should provide mechanistic insight into an interesting and novel cellular process regulated by amino acids.

Amino acid-dependent regulation of insulin-like growth factor binding protein-1 (IGFBP-1) has been reviewed by Bruhat et al. (1999). Among the amino acid-regulated genes identified to date, IGFBP-1 may be of particular importance because nutrient-dependent control of its expression is likely to have significant metabolic effects on a number of tissues and organs. IGFBP-1 may also serve as a prototype for nutrient feedback on metabolism-regulating hormones. A comprehensive examination of how amino acid availability may influence hormone and cytokine expression and/or action has not been undertaken, but such studies will greatly contribute to our understanding of the inter-organ effects that protein nutrition has on cell growth and metabolism. Beyond IGFBP-1, only a limited number of examples are known thus far. It has been demonstrated that histidine deprivation of murine pancreatic cells suppresses the synthesis of glucagon (Paul et al. 1998).

The substrate-dependent regulation of the sodium-dependent zwitterionic amino acid transporter System A activity has been investigated for three decades (Gazzola et al. 1972), and the subject has been reviewed periodically during this period of time (Kilberg et al. 1993; Palacín et al. 1998). The more recent identifi-