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Review article

Homeostatic responses to amino acid insufficiency

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ABSTRACT

This article provides a brief overview describing how two key signaling pathways, namely the integrated stress response and the mammalian target of rapamycin complex 1, work together to facilitate cellular adaptation to dietary amino acid insufficiency. A deeper understanding of these mechanisms is leading to identification of novel targets which aid in disease treatments, improve stress recovery and increase health span through slowed aging and enhanced metabolic fitness.

1. Introduction

All living organisms possess cellular strategies to ensure survival during times of nutrient scarcity. These survival responses alter organismal metabolism and cell mass to adapt to availability of nutrients and maintain essential functions for life. Reductions in amino acid supply are sensed within the cell by a number of overlapping signal transduction mechanisms which differentiate amino acid sufficiency from amino acid deficiency. This article will provide a brief overview of these mechanisms and provide examples how these responses are important for prevention and treatment of disease.

2. Amino acid sensing networks

Sensing of amino acid availability occurs in multiple locations in and around the cell. Amino acid transporters expressed at the cell surface play an important role in sensing, carrying, and thus regulating supply and demand of substrate (Taylor, 2014). These transporters can also function as transceptors, serving as or interacting with intracellular sensors which deliver information to signaling networks. The generation of amino acids from endogenous proteolysis via autophagy and/or the ubiquitin-proteasome system identifies these subcellular organelles and membranes as additional key sites of amino acid sensing. Further examination into the anatomical organization and the molecular identity of the sensing molecules in each space remain to be discovered.

Two major signal transduction networks responsive to changes in amino acid supply are the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) pathway and the integrated stress response (ISR). The activities of these two signal transduction cascades in relation to amino acid supply are coordinated together in order to provide a spectrum of cellular responses, ranging from the induction of growth to activation of cell death. Exactly how these pathways are coordinated is for the most part a mystery, requiring additional research.

3. Mammalian/mechanistic target of rapamycin senses amino acid sufficiency

The mTORC1 pathway serves as a hub for cellular decision-making on matters that relate to homeostatic control of the proteome (Laplante and Sabatini, 2012). Located within the endosomal-lysosomal compartment, assembly of this complex at the surface of late endosomes and lysosomes promotes mRNA translation and dampens autophagy. Assembly of mTORC1 is regulated by many environmental stimuli including insulin/insulin-like growth factors, energy state, redox status and amino acids. Amino acids stimulate the assembly and translocation of mTORC1 at the lysosomal surface in a manner that requires the Rag, Ragulator and vacuolar adenosine triphosphatase protein complexes. Reductions in amino acid availability diminish mTORC1

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assembly and signaling to ribosomal proteins and mRNA translation factors, reducing protein synthesis. At the same time, disassembled mTORC1 permits elevations in autophagy, increasing proteolysis. The sensing mechanism activating assembly of mTORC1 upon increased amino acid supply is yet to be clearly defined. Recent reports by three separate labs have identified SLC38A9 as a component of the lysosomal amino acid sensing machinery that controls activation of mTORC1 upon arginine sufficiency (Jung et al., 2015; Rebsamen et al., 2015; Wang et al., 2015). This protein transports arginine and interacts with the Rag guanosine triphosphatases, suggesting it functions as a transceptor. How other amino acids are sensed intracellularly remain to be discovered. Furthermore, the role of SLC38A9 and other putative transceptors in mammalian tissues and organ systems remain to be explored.

Studies in animals show that dietary restriction of essential amino acids reduces mTORC1 signaling in liver (Anthony et al., 2001, 2004). Pharmaceutical depletion of asparagine by L-asparaginase, a drug used to treat childhood leukemia and canine lymphoma, also reduces mTORC1 signaling in liver and pancreas but not spleen, suggesting tissue-specific effects (Reinert et al., 2006). Reductions in mTORC1 signaling correlate with reductions in general protein synthesis rates and tissue growth. Temporary reductions in growth help the organism adapt to acute nutrient stress whereas longer term reductions correspond with life extension. More specifically, recent studies show that chronic reductions in mTORC1 activity by diet, drug or genetic knock down promote longevity (Drake et al., 2013, 2014). Thus the role of mTOR in controlling the homeostatic control of the proteome under dietary restriction paradigms is important to further understand.

4. General control nonderepressible 2 (GCN2) senses amino acid insufficiency

While mTORC1 mediates cellular growth responses to AA sufficiency, the cellular reaction to amino acid insufficiency is primarily driven by the ISR. Originally defined in yeast as the general amino acid control pathway, the ISR in mammals comprises 2 signal transduction networks operating in parallel with some cross-talk (Kilberg et al., 2012; Balasubramanian et al., 2013). The first signal network is initiated by the protein kinase called GCN2. In the cytosol, GCN2 acts as an amino acid sensor, autoactivating itself upon binding deacylated tRNA present in the vicinity of the ribosome. Activated GCN2 then phosphorylates eukaryotic initiation factor 2 (eIF2), causing the translation initiation machinery to slow down and conserve energy while at the same time favoring mRNA translation of specific genes. The best characterized gene in this regard is activating transcription factor 4 (ATF4), a transcription factor which functions to promote cellular survival and adaptation during AA insufficiency (Kilberg et al., 2009). It accomplishes this by altering the transcriptome to prevent cellular oxidative damage and promote macronutrient metabolism. It also promotes the transcription of genes encoding translation initiation factors and autophagy-related proteins regulated by mTORC1. The second signal network activated is the G-protein coupled receptor (GPCR)-mitogen activated protein kinase (MAPK) pathway. Activation of this pathway results in increased synthesis of transcription factors such as cJUN and ATF2 which, similar to ATF4, bind amino acid response elements (AARE) in DNA promoter region to modulate gene expression. Less is understood about this pathway and the identity of the proximal sensor is unknown.

Work from our lab and others show GCN2 is essential for adapting to amino acid deprivation induced by diet (Anthony et al., 2004; Guo and Cavener, 2007), drug (Wilson et al., 2013, 2014) or genetic condition (She et al., 2013) by regulating both protein synthesis and expression of ISR-regulated genes. Mice deleted for GCN2 (GCN2KO) demonstrate hepatic steatosis and reduced muscle mass when maintained on a leucine-devoid diet (Anthony et al., 2004; Guo and Cavener, 2007). Treatment with the chemotherapy agent asparaginase induces immunosuppression, hepatic failure and pancreatitis in GCN2KO mice (Bunpo et al., 2010; Wilson et al., 2013). Furthermore, genetic deletion of both GCN2 and the branched chain keto acid dehydrogenase kinase (BDK) in mice results in a novel leukodystrophy that limits lifespan to less than 2 weeks postnatal (She et al., 2013). All of these dysfunctional and maladaptive outcomes are not present in GCN2KO mice maintained in an environment that provides a sufficient supply of amino acids. Indeed, when provided standard nourishment for rodents, GCN2KO mice reproduce normally and live normal, healthy lives. These data are interpreted to mean that other mammalian species including humans could harbor a deficient or defective GCN2 gene yet possess a silent phenotype, revealed only when challenged with amino acid insufficiency. Identifying these genotypes may help with precision medicine or individualized nutrition.

5. Amino acid sensing to improve health span

While the critical importance of GCN2 and the ISR is starting to become recognized, large gaps in knowledge about how GCN2 regulates cytoprotection during amino acid limitation in the whole animal remain unfilled. Current studies in the lab are focused on the molecular basis for health benefits of dietary essential amino acid restriction (Anthony et al., 2013) and the role of ATF4 in mediating the actions of GCN2 and mTORC1 during amino acid stress. This information is needed to further delineate the relative importance of global protein synthesis versus gene-specific translation in determining overall health outcomes to nutritional stress. Furthermore, rising rates of obesity and metabolic dysfunction combined with an aging population have prompted exploration into how dietary restriction of essential amino acids can improve metabolic health and promote lifespan. In this regard, dietary restriction of methionine promotes a metabolically younger phenotype by increasing metabolic flexibility and reducing body fat (Lees et al., 2014). A better understanding of how dietary methionine restriction regulates mTORC1 in concert with the ISR may lead to new and improved strategies to improve health span and treat chronic diseases of aging.

Conflict of interest

There are no conflicts of interest to report.

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