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Minireview

Nutritional stress in eukaryotic cells: Oxidative species and regulation of survival in time of scarceness

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ABSTRACT

The survival response to glucose limitation in eukaryotic cells involves different signaling pathways highly conserved from yeasts to mammals. Upon nutritional restriction, a network driven by kinases such as the AMP dependent protein kinase (AMPK/Snf1), the Target of Rapamycin kinase (TOR), the Protein kinases A (PKA) or B (PKB/Akt) control stress defenses, cell cycle regulators, pro and anti apoptotic proteins, respiratory complexes, etc. In this work we review the state of the art in this scenario of kinase pathways, i.e. their principal effectors and links, both in yeasts and mammals. We also focus in downstream actors such as sirtuins and the *Forkhead* box class O transcription factors. Besides, we particularly analyze the participation of these kinases on the balance of Reactive Oxygen Species and their role in the regulation of survival during glucose deprivation. Key results on yeast stationary phase survival and the contribution of such genetics studies are discussed.

Contents

Introduction

Low-glucose stress denotes a situation common to physiological and pathological scenarios in mammalian cells. The cellular response to such condition entails diverse signal transduction pathways implicated in survival regulation, most of them ancestrally present for more than one thousand million years, as long ago as the mitochondrion and eukaryotes' history begins. This turns signaling during glucose deprivation in a matter where comparative studies are especially useful.

During the last years numerous works have been performed analyzing different aspects of the effects of glucose lack on metabolism, bioenergetics, stress response, regulation of cell death, etc. in a great diversity of organism models and cell types, including tumor cells. Nevertheless, such an extensive scope makes it difficult to integrate all the related results and information and to develop a complete picture of this network which regulates eukaryotic cells viability.

In the following sections we intend to carry out a resumed survey on many outstanding topics and novel findings both in yeast and mammalian cells subjected to depletion of the principal carbon source. In addition, we discuss the importance of genetics approaches and comment some examples that show the current advances of knowledge in the mechanisms of regulation of cell death during glucose deprivation.

1. Glucose metabolism and cell death

Glucose is the foremost source of energy in the majority of eukaryotic cells. Glycolysis and mitochondrial respiration represent

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the sequential possibilities in the route of oxidation of glucose, even though their metabolic fluxes and accumulated byproducts change depending on the cell type and its quiescence/proliferation state [1]. It is worthy of note that, in the two extremes of the evolutionary range, many yeasts and cancer cells share the particularity of adopting the fermentation of glucose with high metabolic flux independently of the presence of oxygen, which is known as the Warburg's effect [2].

Both glycolysis and respiration are linked to oxidative stress, thus conditioning cell survival, the former in a protective way bypassing glucose-6P to oxidative Pentose Phosphate Pathway and providing NADPH reducing capacity, and the latter in the opposite fashion, constituting the mitochondrion an important source of superoxide [3]. Furthermore, other connections exist linking glucose metabolism to regulation of cell death in eukaryotes, the most important associated to signal transduction pathways that include metabolic enzymes such as hexokinase/s [4,5]. Mitochondrial localization of mammalian hexokinases (HK) is in fact an integrative bridge in the pro survival signaling of Akt, since such HKs (the mitochondrial isoforms, HK1 and HK2) interact with the Voltage-dependent anion channel (VDAC), thus releasing it from its pro-apoptotic phosphorylation by GSK3β, which is concomitantly inhibited by Akt. The connection between HK1/2 and the unphosphorylated form of VDAC in the outer membrane prevents further interactions of this channel with pro-apoptotic proteins, which would lead to cytochrome c release and apoptosis [6]. Even though this link between mitochondrial hexokinases and the yeast orthologue of Akt, Sch9, is absent in *Saccharomyces cerevisiae*, the yeast hexokinase isoform 2 (Hxk2) have regulatory roles in this organism which associate this isozyme with survival signaling already in the simplest eukaryotes. In fact, Hxk2 participates in the inactivation of the kinase Snf1, the orthologue of the mammalian AMP activated kinase, AMPK, by favoring its desphorylation by the phospatase Reg1/Glc7 complex, and it acts as nuclear repressor in association with Mig1, localizing at the nucleus in the presence of glucose [5,7]. Among other effects on the metabolism of alternative carbon sources during carbon stress, Snf1 converges with Protein kinase A (PKA) and the Target of Rapamycin (TOR) kinase in regulating the localization of the transcriptional factor Msn2, a transcriptional activator of stress responsive genes [8]. Activation of Snf1 by disrupting their negative regulators Reg1 or Hxk2 leads to inhibition of Msn2 nuclear import during glucose starvation [9]. Besides this role of Hxk2 in this signal transduction pathway that can modulate stress response during glucose deprivation, the deletion of HXK2 in *S. cerevisiae* provokes different changes in yeast replicative and chronological lifespans, which are not completely understood: *hxk2*Δ shows a 30% increase in the replicative lifespan in comparison with the wild type, mimicking the effect of caloric restriction [10], whilst the double mutant *hxk2*Δ *mig1*Δ, but not the single mutant, shows a decreased chronological lifespan that can be explained by synergic effect on Snf1 [7].

The aim of the present work is to review some aspects on the survival response to glucose restriction present in eukaryotic organisms from yeast to mammals. In this connection, it is remarkable that Reactive Oxygen Species (ROS) are involved as part of initial steps of this regulation in all eukaryotes. In fact, it is well known that high glucose levels promote oxidative damage and can induce apoptosis in diverse mammalian cells and tissues [11–13], or in stationary phase yeasts in the absence of complementing nutrients [14]; while the opposite condition, i.e. low glucose levels, also entails induction of different mechanisms of cell death depending on the cell type or its transformation state [15]. Glucose deprived cells can die either by mitochondrial apoptosis [16], caspase 8 driven apoptosis [17], or necrosis [18]. Therefore, mitochondrion emerges as a central scenario, if not the main, of survival regulation in mammalian cells undergoing glucose restriction. Furthermore, it also intervenes in similar situations in a basic eukaryotic organism such as the budding yeast. In this connection, mitochondrial apoptosis can be induced in yeast cells shifted from logarithmic growth in glucose to a respiratory medium such as glycerol [19].

Mitochondrial respiration is one of the major cellular sources of superoxide anion, which is mainly generated from complex I through reverse and from complex III through forward electron transfer [3]. Given that this superoxide production is tightly dependent on the proton motive force through the mitochondrial membrane, the relative contribution of respiration to any cellular ROS imbalance can be determined by mildly uncoupling mitochondria with the ionophores FCCP or CCCP [3,20]. Following this rationale, experiments with *S. cerevisiae* strains with disruption or overexpression of the gene coding one of the yeast catalytic subunits of PKA, Tpk3, show that cAMP/Tpk3 signaling during glucose exhaustion leads to mitochondrial dysfunction, which is mainly associated to the downregulation of cytochrome c oxidase (COX) subunits 2 and 4 that results in respiratory collapse (loss of mitochondrial membrane potential), ROS generation and apoptotic death [21]. This is likely conserved in mammals. In fact, we have recently found that glucose restriction in normal rat hepatocytes may induce bioenergetics adjustments in mitochondria entailing a concomitant increase in mitochondrial ROS production that leads to apoptotic activation, a mechanism also signalized by PKA [22]. Our results demonstrate that this effect is at least partly due to intramitochondrial PKA signaling, since significant increases in the levels of the endogenous PKA activator cAMP are detected in mitochondria of glucose deprived hepatocytes [22]. Interestingly, even when the study in yeast mentioned above reports that none of the three yeast catalytic subunits of PKA localizes at mitochondria, compartmentalization of Tpk3 signaling in these organelles cannot be discarded [21]. Another similarity between these results is that in both cases PKA activation also limits the expression of stress responsive genes such as superoxide dismutase and catalase, which contribute to ROS imbalance, thus indicating extramitochondrial PKA signaling at the transcriptional level [21,22]. A previous work shows conclusive data about the expression of adenylate cyclase (AC) at the mammalian mitochondria matrix and the constitution of a local PKA signaling pathway which modulates mitochondrial bioenergetics by phosphorylation of diverse targets of the respiratory chain [23]. Altogether these results imply novel unexpected roles of PKA signaling and, at the same time, an emergent field for further research. In this connection, it is possible that mitochondrial AC isozyme is sensitive to glucose availability; however, the putative actors upstream AC and PKA either in mammals or in yeasts are still uncertain, and it is unknown whether cytosolic glucose levels can be sensed by any metabolic step that connects with mitochondria and further internal signaling pathways involving luminal AC and PKA (Fig. 1). Interestingly, this PKA mediated signal transduction pathway that links the lack of glucose with respiratory adjustments which impact on ROS generation and induction of cell death seems not to be unique in the yeast. It was recently described that TOR kinase signaling is also involved in a similar mechanism of modulation of mitochondrial bioenergetics in *S. cerevisiae*: A reduction of TOR signaling upregulates respiratory complexes codified by mitochondrial and nuclear genes, thus extending survival in stationary phase [24]. TOR inhibition also extends lifespan in worms and flies [25,26]. It is appealing to study whether this TOR mediated regulation of oxidative phosphorylation and survival is conserved from yeast to mammals. It is feasible that both PKA and TOR signal transduction pathways share different effectors in such a mitochondria axis that can tune viability in response to glucose scarceness in all eukaryotic cells. Another question is to identify not only other targets in these signaling pathways but also the precise way of glucose sensing and the relations among respiratory activity, ROS production and cell survival regulation.

2. Glucose responsive signaling pathways and regulation of cell survival: role of oxidative stress

Different signaling pathways are involved in transducing responses to glucose availability in eukaryotic cells [15]. We will first