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COMMENTARY

A new adaptation strategy to glucose starvation: modulation of the gluconate shunt and pentose phosphate pathway by the transcriptional repressor Rsv1

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Survival upon glucose starvation requires a delicate balance between different metabolic pathways. A recent work by the Roe laboratory provides a mechanistic link between glucose deprivation and the regulation of the pentose phosphate pathway, with the transcriptional repressor Rsv1 playing a key role in the process. Rsv1 regulates the flow of glucose into its possible metabolic fates and promotes long-term survival under low glucose.

Living cells have to survive in diverse environments, and several mechanisms have been developed through evolution in order to adapt to differences in nutrient availability. Glucose is the preferred carbon source for a vast majority of living organisms. Internalized cellular glucose is phosphorylated to glucose 6-phosphate (G6P) in the first step of glycolysis. G6P can then be further broken down in the process of glycolysis to provide energy (ATP) or shunted into the pentose phosphate pathway (PPP) to provide reducing equivalents [nicotinamide adenine dinucleotide phosphate (NADPH)] and pentose sugars, as precursors of nucleotide and serine/glycine/threonine biosynthesis [1]. Balance between these two fates of glucose, glycolysis or PPP, is tightly controlled depending on the environmental situation, modulating them differentially depending on the nutrient concentration, or the presence or absence of stressors [2]. It is worth mentioning that glucose can enter the PPP through the so-called gluconate shunt, in which glucose is oxidized to gluconate. While the importance of the gluconate shunt in plants, algae, and bacteria is well defined [3], little has been described regarding its relevance in yeast or mammals, being only known that it is regulated via zinc proteins and that it may functionally overlap with the classical entrance to PPP [3]. Furthermore, extracellular gluconate uptake can be achieved via specific transporters (Ght3 and Ght4

Abbreviations
cAMP, cyclic adenosine monophosphate; G6P, glucose 6-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate; PPP, pentose phosphate pathway.

in fission yeast [4]), providing an alternative source for the PPP.

When glucose is depleted, cells trigger different responses to allow adaptation. Growing in low glucose concentration induces different genetic changes such as a shift from fermentation to respiration, activation of gluconeogenesis, expression of high-affinity glucose uptake systems, induction of stress response pathways, and a temporary stall of the cell cycle (see Fig. 1) [5]. These gene expression programs triggered by low glucose conditions lead to cell adaptation, and they also participate in the calorie restriction-dependent long-term survival [6]. Thus, mutants of the low glucose-sensing signaling cascades often display longer or shorter life span.

In fission yeast, several pathways have been described to trigger the biological effects described above. Thus, the mTORC2 cascade (and its main kinase, Tor1) participates in the transient cell cycle arrest of glucose-starved cells [7], as well as in the up-regulation of glucose uptake by controlling the synthesis and localization of Ght5, a high-affinity glucose transporter [8]. However, the two main cascades involved in the regulation of the gene expression programs described in Fig. 1 are the Sty1 and the Pka1 pathways. Sty1 is a mitogen-activated protein kinase which becomes activated in response to different stresses, such as osmotic, oxidative, heat shock, or nutrient deprivation [9], to induce a wide antistress gene expression program through the transcription factor Atf1 [10]. Regarding the Pka1 pathway, in the presence of high glucose, this kinase phosphorylates the transcription activator Rst2, keeping it away from its target genes. When glucose is depleted, the secondary metabolite cyclic adenosine monophosphate (cAMP) also decreases and the regulatory subunit Cgs1, which is normally bound to cAMP, is now free to bind and inactivate Pka1; nonphosphorylated Rst2 accumulates in the nucleus and promotes transcription of its target genes (Fig. 2) [11]. Some genes, such as *fbp1* (coding for the gluconeogenic enzyme fructose-1,6-bisphosphatase), are target of both Atf1 and Rst2 during glucose deprivation and are kept repressed in the presence of glucose by the Scr1 repressor cascade; the Ssp2 kinase inactivates Scr1 during low glucose conditions [12] (Fig. 2).

In a very elegant study led by Jung-Hye Roe published in The FEBS Journal [13], the role of the transcription factor Rsv1 in the regulation of glucose metabolism is analyzed, with a special focus on the regulation of the *rsv1* gene and the consequences of its up-regulation during glucose starvation. Rsv1 is homologous to *Aspergillus nidulans* CreA, *Saccharomyces cerevisiae* Mig1, and mammalian EGR-1/NGFI-A [13], and is synthesized only in low glucose environments, based on the work by the Roe laboratory. They describe the transcriptional up-regulation of *rsv1* during glucose starvation, demonstrating that it depends on the Sty1-Atf1 cascade and also on the Scr1 and Rst2 transcription factors, resembling the well-known regulation of *fbp1* (Fig. 2). Interestingly, they show by ChIP that Atf1, Rst2, and Scr1 can be detected not only in the promoter of *rsv1* but also in the 3' region of the gene [13]. This downstream position of the transcription factors could be related to the loop-formation hypothesis previously reported for the *fbp1* gene [14], in which loops would be formed under glucose starvation conditions in order to induce nucleosome eviction and achieve full transcription. It is also interesting the fact that the transcription factor Pcr1 is not detected at the *rsv1* promoter. This transcription factor is a classical partner for Atf1 not only in the

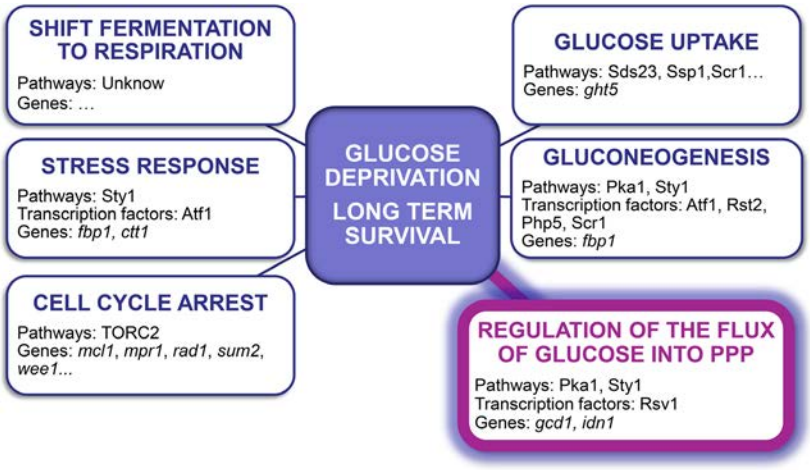

Fig. 1. Growth in low glucose conditions induces a number of metabolic adaptations such as a shift from fermentation to respiratory metabolism, or the activation of glucose uptake to promote long-term survival. Among them, the regulation of the flux from glucose toward the PPP is tightly regulated, according to the work by Roe and colleagues [13]. The molecular pathways and transcription factors involved in the regulation of each of these adaptation strategies, and the genes downstream of the pathways, are indicated (based on fission yeast).

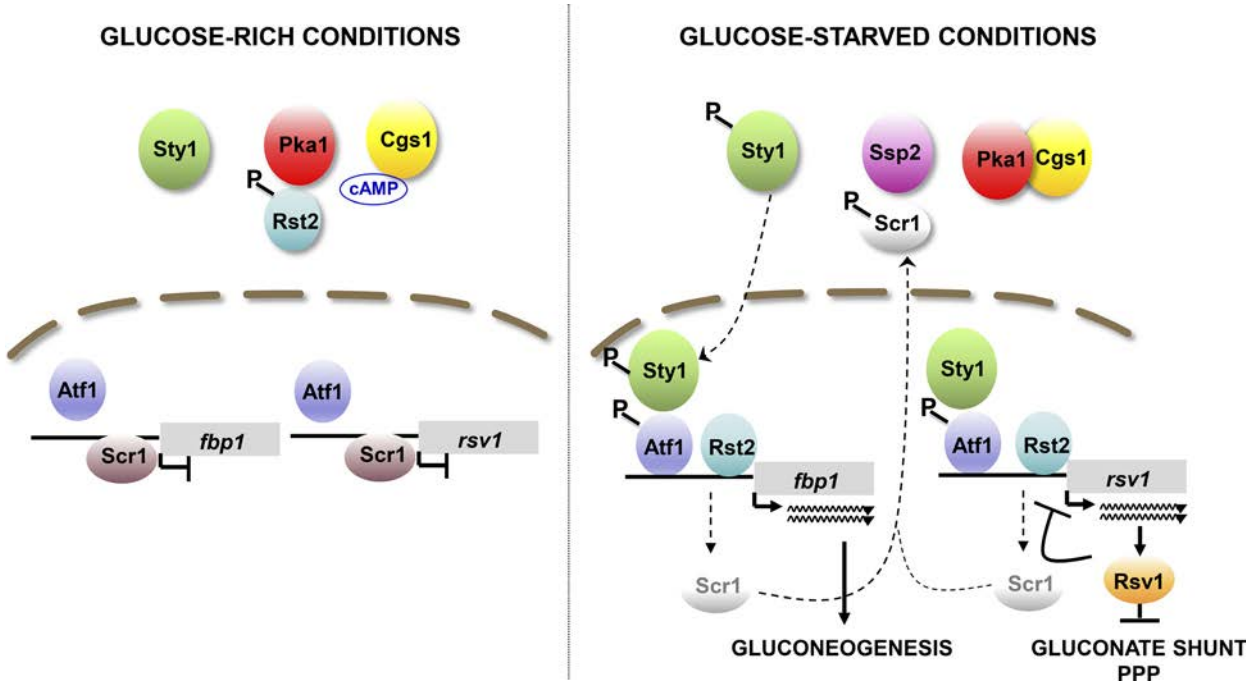

Fig. 2. Regulation of glucose response genes *fbp1* and *rsv1* by different signaling cascades. When glucose is present in the media, transcription of the *Schizosaccharomyces pombe* genes *fbp1* and *rsv1* is repressed by Scr1. After glucose starvation, activation of the Sty1 pathway and inactivation of the cAMP-dependent Pka1 kinase by Cgs1 promote the binding of both transcription factors to gene promoters, in parallel to the release from promoters of the Scr1 repressor. Then, up-regulated Fbp1 and Rsv1 participate in the induction of gluconeogenesis and in the regulation of the glycolysis-to-PPP flux, respectively.

activation of oxidative stress genes but also in *fbp1* activation [15], and heterodimer Atf1/Pcr1 is supposed to share a target sequence (TGACGT) [15]. So, the absence of Pcr1 in *rsv1* suggests an Atf1-mediated regulation different than usual. In conclusion, the low glucose-dependent transcriptional activation of *rsv1* and *fbp1* genes shares many regulatory features (involvement of Sty1/Atf1 and PKA pathway, activated by Atf1 and Rst2, repressed by Scr1 and Tup family corepressors...).

What is the role of Rsv1 during glucose starvation? Once Rsv1 protein levels are up-regulated at the level of transcription in a Sty1- and Pka1 pathway-dependent manner, the group of Roe reveals by ChIP-sequencing and RNA-sequencing that it acts as a repressor of some genes and an activator of other genes. Thus, Rsv1 directly represses the expression of 13 genes, among them *rsv1, gcd1,* encoding an enzyme essential to enter the PPP through the gluconate shunt, and *gnd1*, encoding 6-phosphogluconate dehydrogenase in the PPP. It is worthy to remark that Rsv1 binds its own promoter, creating a feedback loop. Rsv1 also activates directly 8 genes such as *gut2*, encoding the mitochondrial glycerol-3-phosphate dehydrogenase which indicates activation of the glycerol-3-phosphate shuttle pathway.

The report by Kim and colleagues [13] provides a mechanistic link between glucose deprivation and the regulation of the PPP. Thus, they show that the shortened life span of strain *Δrsv1* is partially suppressed when enzymes from the gluconate shunt such as Gcd1 are removed. Furthermore, a double delete mutant *Δrsv1 ΔSPCC16c4.10* (encoding a protein involved in the classical entrance of G6P into the PPP) is able to suppress the short life span phenotype of Δ*rsv1* to a larger extent than deletion of *gcd1*. This links the role of Rsv1 with the modulation of glucose entry into the PPP, not only via the gluconate shunt, but also through the classical G6P entry site. Interestingly, most PPP genes repressed by Rsv1 upon glucose deprivation are transcriptionally up-regulated upon stress in a Sty1-Atf1-dependent manner [10], probably as a general mechanism to enhance NADPH production for antioxidant enzymes. The experiments described by the group of Roe suggest that this up-regulation may not be beneficial for glucose-deprived conditions, and the synthesis of the Rsv1 repressor would, by dampening the Sty1-Atf1 up-regulation of the PPP genes, optimize the glucose flux between glycolysis and PPP. To the

best of our knowledge, this is the first report demonstrating the relevance of modulating the PPP to promote adaptation to glucose starvation and long-term survival. In summary, the report by Kim and colleagues [13] describes a new mechanism to regulate the flow of glucose into its possible fates allowing the metabolic reorganization to overcome the nutrient changes in the environment.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

RF, LS-M, and EH wrote the manuscript.

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