PERSPECTIVES: PROTEIN SYNTHESIS

The Perks of Balancing Glucose

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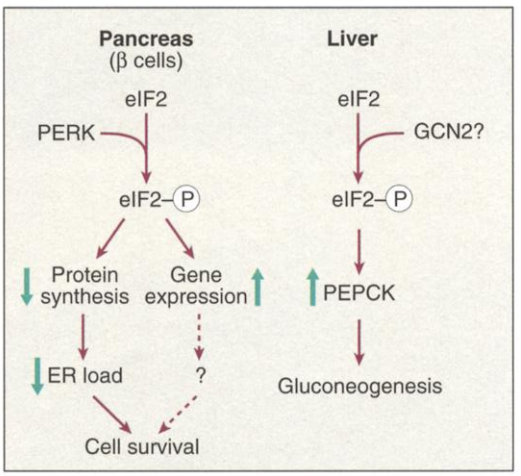
Development, differentiation, and growth of eukaryotic cells can be regulated by modulating the translation of mRNAs into proteins. Many signaling pathways regulate mRNA translation and protein synthesis, and perturbations in these pathways can result in metabolic dysregulation and disease. Two recent reports in *Molecular Cell* by Harding *et al.* (*1*) and Scheuner *et al.* (*2*) now tie together translational regulation and glucose metabolism. Both groups show in genetically engineered mice that a protein kinase that phosphorylates a master regulator of translation—eukaryotic translation initiation factor–2 (eIF2)—promotes the survival of insulin-secreting pancreatic β cells, thus contributing to glucose homeostasis.

Genetic and biochemical analyses of the yeast *Saccharomyces cerevisiae* (*3*) has shown that a single known kinase, Gcn2p, that phosphorylates the α subunit of eIF2, leading to inhibition of protein synthesis. Gcn2p itself is activated by uncharged transfer RNAs (tRNAs without attached amino acids) under starvation conditions when the amino acid pool is depleted. Paradoxically, synthesis of the transcription factor Gcn4p is enhanced in response to activation of Gcn2p and phosphorylation of eIF2α (*3*). Gcn4p switches on expression of genes encoding enzymes that make amino acids. Thus, in yeast, phosphorylation of eIF2α serves predominantly to regulate gene expression at the transcriptional level in response to nutritional deprivation.

In eukaryotes, translational control operates primarily during the first steps of translation when the small (40*S*) ribosomal subunit, charged with an initiator tRNA, is recruited to the 5′ end of mRNA. Initiation of translation is modulated in part by the activity of the eIF4F complex that recognizes the modified 5′ end of mRNA, and in part by eIF2, which recruits the charged initiator tRNA to the 40*S* ribosomal subunit. Phosphorylation of a serine at position 51 (Ser^{51}) in the α subunit of eIF2 is crucial for preventing this step and for halting protein synthesis. When phosphorylated, eIF2 inhibits the guanine nucleotide exchange factor eIF2B, becoming trapped in its inactive (guanosine diphosphate–bound) form and unable to initiate translation (*3*).

Four distinct kinases are known to phosphorylate eIF2α on Ser^{51} in mammals. Each is activated by specific signals that elicit translational control in response to distinct needs. RNA-activated protein kinase is activated by double-stranded RNA produced during viral infection and halts protein synthesis, thus preventing production of viral proteins. Heme-regulated inhibitor kinase is activated when heme concentrations in maturing red blood cells become too low, switching off synthesis of globin. Perturbed protein folding in the endoplasmic reticulum (ER) induces eIF2α phosphorylation and the attenuation of protein synthesis (see the figure) (*4*). The kinase responsible for this unfolded protein response is PERK (*5*). Identified as the eIF2 kinase enriched in pancreatic cells (*6*), PERK is a transmembrane protein resident in the ER membrane whose activity is repressed by the ER chaperone BiP. When too many unfolded proteins accumulate in the ER, BiP dissociates from PERK, resulting in the activation of this kinase, which then phosphorylates eIF2α (*7, 8*). Through halting translation initiation and protein synthesis, PERK may relieve ER stress by reducing the number of unfolded proteins in the ER (*9, 10*).



Perking up protein synthesis. (Left) Phosphorylation of eIF2α by PERK in response to unfolded proteins in the ER inhibits translation initiation and protein synthesis in pancreatic β cells. This results in a decrease in the number of unfolded proteins in the ER and promotion of β cell survival. eIF2α phosphorylation also results in the transcriptional activation of genes that are important for cell survival. **(Right)** Control of glucose production (gluconeogenesis) in the liver by eIF2α phosphorylation may be mediated by GCN2 because deletion of the other eIF2α kinases (including PERK) does not result in hypoglycemia. eIF2α phosphorylation may increase the synthesis of gluconeogenic enzymes (such as PEPCK) or of transcription factors that activate the expression of their genes.

By engineering PERK-deficient mice (*1*) or mice with a mutation in the eIF2α phosphorylation site ($Ser^{51} \rightarrow$ Ala) (*2*), Harding, Scheuner, and their colleagues reveal that eIF2α phosphorylation is connected to glucose metabolism. The PERK-deficient and Ser^{51} mutant mice exhibited severe but opposing defects in glucose homeostasis. PERK-deficient animals developed marked hyperglycemia (elevated blood glucose) at 4 weeks of age, whereas the Ser^{51} mutant mice were normal at birth but died of severe hypoglycemia 18 hours later. Both mutant strains had defects in pancreatic β cells; these defects were apparent in Ser^{51} mutant embryos, but only became apparent in PERK-deficient animals several weeks after birth.

The difference between the two animal models suggests that more than one type of eIF2 kinase may be operating in the insulin-producing β cells of the pancreas. A feature common to both mouse models is the decrease (but not complete absence) of β cells, large numbers of which undergo apoptosis in the PERK-deficient mice (*11*). That the loss of β cells appears in the setting of hyperglycemia in one model and hypoglycemia in the other implies that this β cell insufficiency is directly caused by loss of normal eIF2α-mediated translational control and is not a secondary effect in response to disruption of glucose homeostasis.

The fatal hypoglycemia in the Ser^{51} mutant mice may be caused by defects in glucose production in the liver (gluconeogenesis). In utero, the fetus is supplied with ample amounts of glucose through the placental circulation. At birth, this source of glucose is extinguished and the newborn mammal must activate enzymes that promote the conversion of gluconeogenesis precursors to glucose. The induction of this gluconeogenesis program is defective in the eIF2α mutant mice, as reflected by their failure to increase the amount of the gluconeogenic enzyme phosphoenol-pyruvate carboxykinase (PEPCK). Intriguingly, synthesis of one of the transcription factors that induces expression of the *PEPCK* gene is regulated by eIF2α phosphorylation. It will thus be important to determine whether loss of eIF2α phosphorylation decreases synthesis of this transcription factor in the liver cells of mutant mice. Phosphorylation of eIF2α is clearly

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important for regulation of PEPCK expression. Any broad conclusions, however, must await further studies on other key gluconeogenic enzyme genes and their expression in response to perturbations other than birth, such as fasting and refeeding.

Why is it that mice with defective eIF2α phosphorylation exhibit both β cell insufficiency and defective liver gluconeogenesis, whereas PERK-deficient animals only exhibit β cell insufficiency? The Harding *et al.* work (*1*) provides a possible answer: PERK-deficient mice have a reduced ratio of phosphorylated to total eIF2α in pancreas, lung, and thymus, but a normal ratio in liver and spleen. This finding suggests that an eIF2α kinase other than PERK may be the key modulator of translational control of gluconeogenic enzyme expression in the liver. If eIF2α phosphorylation does prove to be important in this pathway, the mammalian homolog of yeast *GCN2* (which is activated by amino acid deprivation) may be involved (see the figure). Defective gene expression downstream of GCN2, however, is unlikely to account for all of the characteristics of the eIF2 mutant mice, given that *GCN2*-deficient animals do not manifest any impairment in neonatal survival (*12*).

The importance of the new mouse models is underscored by the discovery of mutations in the *PERK* gene in an inherited autosomal recessive disease in humans, called the Wolcott-Rallison syndrome (*13*). This disease is classified as a form of type 1 diabetes because it develops in early infancy and is characterized by the destruction of pancreatic β cells. A key question posed by the Harding and Scheuner studies is why β cells are selectively destroyed in Wolcott-Rallison patients and in the mutant mice. It is very likely that β cells die because they need both PERK (which is extraordinarily abundant in the pancreas) and eIF2α phosphorylation to survive.

In yeast, eIF2α is phosphorylated in response to nutritional cues and directs adaptations in intermediary metabolism. The new work suggests that, despite considerable diversification in upstream signals, metazoans have retained the kinases that phosphorylate eIF2α and control translation, adapting them for the regulation of glucose homeostasis.

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PERSPECTIVES: EARTH HISTORY

The Rise of Atmospheric Oxygen

James F. Kasting

Geologists and evolutionary biologists have long speculated about when atmospheric oxygen, O_2, first became abundant and how rapidly it reached its present concentration. Cloud's groundbreaking studies of reduced and oxidized iron and uranium minerals (*1*), coupled with later studies of microfossils and ancient soils (*2*), convinced most observers that atmospheric O_2 underwent a dramatic increase in concentration between 2200 and 2400 million years ago. But some geologists remained skeptical (*3*) because the geochemical and biological data allowed room for alternative interpretations.

Recently, an entirely new line of geochemical evidence removed much of the uncertainty regarding the timing of the initial rise in atmospheric O_2. Farquhar *et al.* (*4*) showed that sulfur isotope ratios in rocks older than about 2300 million years are unusual. In younger rocks, the difference in abundance between ^{33}S and ^{32}S is about half that between ^{34}S and ^{32}S. Such "mass-dependent" isotope fractionation results from a variety of aqueous chemical and biochemical reactions. But the sulfur isotopes in rocks older than 2300 million years consistently deviate from the mass-dependent fractionation line. The "mass-independent" fractionation seen in these rocks is thought to result solely from gas-phase photochemical reactions such as photolysis of SO_2 (*4*).

In a high-O_2 atmosphere like the present one, the isotopic signature of such reactions is unlikely to be seen in sediments because almost all sulfur gases emitted into the atmosphere are oxidized to sulfuric acid and accumulate in the ocean as dissolved sulfate, SO_4^{-}. In a low-O_2 atmosphere, however, sulfur can exit the atmosphere in a variety of oxidation states from −2 (H_2S) to +6 (H_2SO_4) (see the figure) (*5*). The likelihood of transferring a mass-independent fractionation pattern to sediments is thus much higher in a low-O_2 atmosphere. Two independent sets of sedimentary S isotope data (*6*, *7*) agree with those of Farquhar *et al.* (*4*), strongly supporting the hypothesis that the transition from an anoxic to an oxic atmosphere occurred near 2300 million years ago.

The timing of the initial O_2 rise is thus relatively well established, but the question of what triggered it remains hotly debated. Researchers agree that O_2 was produced initially by cyanobacteria, the only prokaryotic organisms (Bacteria and Archea) capable of oxygenic photosynthesis. But cyanobacteria are thought to have emerged by 2700 million years ago, on the basis of evidence from organic biomarkers in well-preserved sedimentary rocks (*8*). [Earlier claims for the existence of cyanobacteria 3500 million years ago (*9*) have recently been called into question (*10*).] Why was there a gap of at least 400 million years between the emergence of cyanobacteria and the rise in atmospheric O_2?

A clue may come from the carbon isotope composition of sedimentary carbonates, which has remained essentially constant through most of the geologic record. This suggests that organic carbon was buried at a more or less constant rate since at least 3200 million years ago (*11*). If this organic carbon was produced by photosynthesis, then net O_2 production would have been constant as well (or, more precisely, it would have remained proportional to the rate of volcanic CO_2 emission). If so, the sink for oxygen must have changed rather than its source. This in turn suggests a geological rather than a biological explanation for the timing of the rise in O_2.

On page 839 of this issue, Catling *et al.* (*12*) suggest that the transition from low to high O_2 was caused by enhanced hydrogen escape into space as a result of high methane concentrations in the Late Archean/Early Proterozoic atmosphere (3000 to 2300 million years ago). As was realized a long time ago (*13*), hydrogen should escape rapidly from low-O_2 atmospheres rich in reduced gases such as H_2 and CH_4. The vast majority of Earth's hydrogen was originally incorporated into the planet as water. The escape of hydrogen to space thus results in a net accumulation of oxygen somewhere on the planet. Kasting *et al.* have argued (*14*) that most of the escaping hydrogen originated from

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