New Phytologist

Review

Tansley insight

Energy and sugar signaling during hypoxia

Author for correspondence:
Pierdomenico Perata
Tel: +39 050 881901
Email: p.perata@santannapisa.it

Received: *26 September 2019*
Accepted: *1 November 2019*

Hsing-Yi Cho[1]*, Elena Loreti[2]*, Ming-Che Shih[1] and Pierdomenico Perata[3]

[1]Agricultural Biotechnology Research Center, Academia Sinica, Taipei 115, Taiwan; [2]Institute of Agricultural Biology and Biotechnology, CNR, National Research Council, Via Moruzzi 1, 56124 Pisa, Italy; [3]PlantLab, Institute of Life Sciences, Scuola Superiore Sant'Anna, Via Giudiccioni 10, 56010 San Giuliano Terme, Pisa, Italy

Contents

New Phytologist (2021) **229:** 57–63
doi: 10.1111/nph.16326

Key words: energy sensing, ERF-VI, hypoxia, oxygen sensing, SnRK1, sugar sensing.

Summary

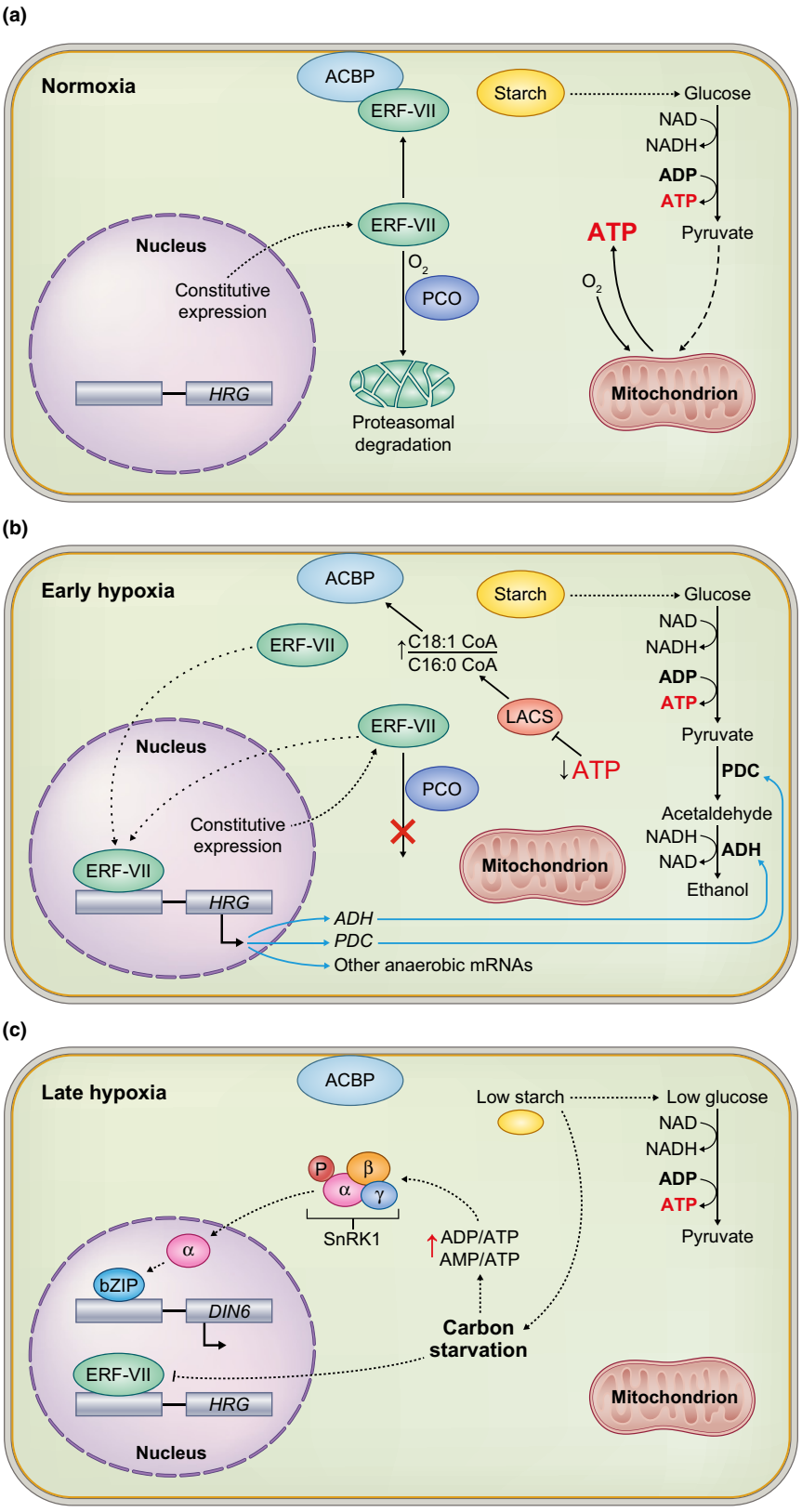
The major consequence of hypoxia is a dramatic reduction in energy production. At the onset of hypoxia, both oxygen and ATP availability decrease. Oxygen and energy sensing therefore converge to induce an adaptive response at both the transcriptional and translational levels. Oxygen sensing results in stabilization of the transcription factors that activate hypoxia-response genes, including enzymes required for efficient sugar metabolism, allowing plants to produce enough energy to ensure survival. The translation of the resulting mRNAs is mediated by SnRK1, acting as an energy sensor. However, as soon as the sugar availability decreases, a homeostatic mechanism, detecting sugar starvation, dampens the hypoxia-dependent transcription to reduce energy consumption and preserves carbon reserves for regrowth when oxygen availability is restored.

I. Introduction

In the absence of oxygen (hypoxia), the production of ATP in mitochondria is hindered because the oxidation of NADH to NAD^+ cannot occur. This leads to an accumulation of NADH and a shortage of NAD^+, which can, eventually, lead to inhibition of glycolysis. During hypoxia, production of ATP through glycolytic flux is indeed of the utmost importance, and oxidation of NADH to NAD^+ occurs as a result of the hypoxia-dependent induction of genes encoding fermentative enzymes (Perata & Alpi, 1993). Initially, pyruvate, the end-product of glycolysis, is converted to lactate by the action of lactate dehydrogenase (LDH), with concomitant re-oxidation of NADH to NAD^+. However, the lowering of cytosolic pH due to lactic acid accumulation redirects the fermentative pathway towards ethanol production, allowing continued recycling of NAD^+ through the combined action of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) (Perata & Alpi 1993). The onset of hypoxic conditions in plant tissues is therefore characterized by a drop in ATP availability due to the absence of mitochondrial ATP production, followed by a readjustment of glucose metabolism (Fig. 1). The availability of sugars to fuel glycolysis is also of primary importance for plant survival during submergence (Loreti *et al.*, 2018). However, the

*These authors contributed equally to this work.

New Phytologist



induction of sugar-utilizing enzymes under hypoxia is an energy consuming process that needs to be fine-tuned to avoid an imbalance between energy consumption and production.

In this review, we will describe the most recent discoveries that demonstrate the interaction of energy and sugar signaling with oxygen sensing.

Fig. 1 Interplay between the oxygen sensing machinery and sugar signaling pathways. (a) Under normoxia, sugar metabolism occurs with starch degradation producing sugars that are initially channeled to glycolysis, followed by mitochondrial respiration. This latter pathway requires molecular oxygen and produces most of the ATP. In the presence of oxygen the expression of hypoxia responsive genes (*HRG*s) is absent, since Plant Cysteine Oxidases (PCOs) enzymatically oxidize the N-terminal cysteine of ERF-VII transcription factors, leading to their proteasomal degradation. Some ERF-VII protein is stable even in normoxia, thanks to its binding to ACYL-COA BINDING PROTEIN (ACBP), which is located at the plasma membrane. (b) At the onset of hypoxia (early hypoxia), the reduced availability of oxygen leads to impairment of the mitochondrial activity producing ATP. Some ATP is still produced though glycolysis, but a drop in the ATP content soon occurs. This leads to reduced LACS activity, altered Acyl-CoA composition (i.e. increased C18:1 CoA/C16:0 CoA ratio) and release of the ERF-VII proteins from ACBP. *De novo* synthesis of ERF-VIIs also occurs, which are stable under hypoxia, since in the absence of oxygen PCOs cannot oxidize the ERF-VII N-terminus. The stable ERF-VII proteins migrate to the nucleus, where they activate *HRG* transcription. Among *HRG*-encoded proteins, PDC and ADH are required for the fermentative steps required to allow continued glycolytic activity. (c) Approximately 12 h after the onset of dark hypoxia, most of the starch available in the cell is consumed (late hypoxia). Glycolysis slows down and the expression of *HRG*s involved in glycolysis and fermentation are no longer required. Carbon starvation occurs and represses the action of the ERF-VII, dampening the transcription of *HRG*s. Translocation of the α-subunit of SnRK1 activates starvation-dependent genes such as the SnRK1 target *DARK INDUCED6* (*DIN6*), possibly by activating the bZIP transcription factors, such as bZIP11, involved in regulation of this gene. ADH, ALCOHOL DEHYDROGENASE; *HRG*, hypoxia responsive genes; PDC, PYRUVATE DECARBOXYLASE.

II. The onset of hypoxia: energy crisis and oxygen sensing

Plants respond rapidly to a lack of oxygen through transcription of hypoxia regulated genes (*HRG*s); this process is clearly evident as early as 40 min after the onset of anoxia (Loreti *et al.*, 2005; van Dongen *et al.*, 2009). *HRG*s are transcriptionally activated (Gasch *et al.*, 2016) by proteins belonging to the group VII Ethylene Responsive Factors (ERF-VII; see Fig. 1) that are unstable under aerobic conditions because of the action of PLANT CYSTEINE OXIDASEs (PCOs; Weits *et al.*, 2014) but are stabilized under hypoxia, since PCOs require molecular oxygen for their activity (Gibbs *et al.*, 2011; Licausi *et al.*, 2011; Weits *et al.*, 2014). Among ERF-VII proteins, RAP2.12 and RAP2.2 play a redundant role (Kosmacz *et al.*, 2015). ERF-VII proteins are protected from degradation in aerobic conditions by interaction at the plasma membrane with ACYL-COA BINDING PROTEIN (ACBP). It is believed that induction of *HRG*s is triggered by the combined action of RAP2.12 that is released from ACBP and *de novo* synthesis of RAP2.12 (Licausi *et al.*, 2011; Kosmacz *et al.*, 2015). Although the induction of *HRG*s occurs within 1 h of hypoxia, RAP2.12 localization in the nucleus is obvious only after 3 h (Kosmacz *et al.*, 2015). This could be explained by a faster nuclear re-localization of RAP2.2/RAP2.3, or by the fact that only a small amount of RAP2.12 is required for the transcription of *HRG*s (Kosmacz *et al.*, 2015). Although the contribution of *de novo* synthesized ERF-VII compared to the release of ERF-VII from ACBP is still unresolved, it was recently demonstrated that the drop in ATP content in plants exposed to hypoxia represents a trigger for RAP2.12 release from ACBP. In Arabidopsis, it has been observed that within 2 h of the onset of hypoxia, the ATP content starts to decrease (Schmidt *et al.*, 2018). Lower ATP levels reduce the activity of LONG-CHAIN ACYL-COA SYNTHETASE (LACS), leading to a shift in the composition of the acyl-CoA pool (Schmidt *et al.*, 2018). Interestingly, the increased hypoxia-dependent level of oleoyl-CoA triggers the release of RAP2.12 from ACBP and consequently activates *HRG* transcription (Schmidt *et al.*, 2018). This evidence provided a remarkable first link between energy sensing (ATP level) and the activation of the oxygen signaling cascade. The release of ERF-VII from ACBP probably requires up to 4 h of hypoxia, since this is the time required to observe a 50% reduction in ATP content in the cell, a decrease that is probably required to significantly inhibit LACS activity (Schmidt *et al.*, 2018). Reduction by 50% of the ATP level by chemical inhibition of mitochondrial activity could only moderately induce some *HRG*s, therefore, together with the oleoyl-CoA-dependent release of RAP2.12 from ACBP, *de novo* ERF-VII synthesis is therefore likely required for a full anaerobic response at the transcriptional level.

III. During hypoxia: enhanced sugar utilization followed by sugar starvation

The hypoxia-dependent RAP2.12 stability and translocation to the nucleus leads to the transcription of *HRG*s, several of which are involved in carbohydrate utilization (Fig. 1). The ERF-VII proteins thus regulate central metabolic processes to support growth, development, and anoxic resistance of plants (Paul *et al.*, 2016). Remarkably, it is not only important that hypoxia stabilizes the ERF-VII proteins, but also that under aerobic conditions these transcription factors are actively degraded. The expression of *HRG*s under aerobic conditions is indeed detrimental to plant performance. Under normoxia, overexpression of a version of RAP2.12 (named Δ-RAP2.12) that is constitutively expressed and stable even under aerobic conditions resulted in enhanced expression of several *HRG*s and led to increased activity of fermentative enzymes. This led to the accumulation of fermentation products even in air, which were accompanied by decreased adenylate energy states and, most importantly, decreased starch levels. This indicates that the *HRG*s induced by ERF-VIIs are deeply involved in carbohydrate consumption. Plants overexpressing Δ-RAP2.12 exhibit decreased carbohydrate reserves and also decreased resistance to anoxia, while the latter can be prevented by using an external sucrose supply (Paul *et al.*, 2016). Interestingly, very young seedlings overexpressing Δ-RAP2.12 were shown to be more tolerant to anoxia, possibly because they still depend on the sugar supply of the seed's cotyledons (Hartman *et al.*, 2019).

As already described above, an adequate sugar supply is of great importance for plants' tolerance to hypoxia. An external supply of sugars, especially sucrose, improves anoxia tolerance (Loreti *et al.*, 2005), and plants deprived of starch reserves are extremely intolerant to submergence (Loreti *et al.*, 2018).

Sugar availability needs to be coupled to the induction of specific *HRG*s that are involved in sugar and fermentative metabolism to enhance tolerance to hypoxia. Given that the expression of *HRG*s is

a costly process, plants also need to ensure that the synthesis of sugar catabolic enzymes occurs only when the carbohydrate reserves are sufficient to guarantee enough energy production to repay the debt and provide extra ATP for driving survival-related processes. Loreti *et al.* (2018) showed that the expression of *HRG*s increases sharply at the onset of hypoxia, peaks after 12 h, and then declines. The reduction in *HRG* expression during late hypoxia (Fig. 1) coincides with the consumption of most of the sugar reserves in the plant, and it was indeed found that expression of *HRG*s is much reduced in a starchless mutant or after an extended-night treatment (Loreti *et al.*, 2018). Sugar starvation thus appears to trigger a marked dampening of the efficacy by which ERF-VII proteins activate the expression of *HRG*s. This happens downstream of RAP2.12 stabilization and independently of the energy sensor SnRK1 (which is described in the forthcoming paragraph). The fact that carbon starvation after extended-night treatment has negligible effects on the ATP level supports the idea that the starvation effects are independent of SnRK1 (Wagner *et al.*, 2019). Dampening the anaerobic response by carbon starvation ensures that the *HRG* expression matches the availability of metabolic carbohydrates, without unnecessary costs for the transcriptional and translational machinery.

IV. Energy sensing under hypoxia

The highly-conserved animal AMP-activated protein kinase (AMPK), yeast sucrose non-fermenting 1 (SNF1) and the plant SNF1 related protein kinase 1 (SnRK1) function as cellular energy sensors that modulate the metabolic processes and repression of energy consumption under energy crisis (Baena-Gonzalez *et al.*, 2007; Polge & Thomas, 2007). AMPK, SNF1 and SnRK1 are heterotrimeric enzymes comprising a catalytic α subunit and regulatory β and γ subunits. The reversible phosphorylation of α subunit T-loop threonine (Thr175 in the Arabidopsis SnRK1 α_1 isoform, and Thr176 in α_2) is critical for SnRK1 activity (Crozet *et al.*, 2014). During hypoxia, the suppression of aerobic respiration leads to energy starvation, resulting in the activation of SnRK1 (Branco-Price *et al.*, 2008; Cho *et al.*, 2012, 2016). However, SnRK1 is an atypical AMPK, and it could not be directly activated by ADP or AMP, as in the cases of AMPK and SNF1 (Sugden *et al.*, 1999; Emanuelle *et al.*, 2015). SnRK1 appears to sense energy charge primarily through high energy sugar phosphates, especially trehalose-6 phosphate (T6P), whose concentration is high under high sugar, high energy conditions. T6P directly binds to the SnRK1 α subunit and interferes with its T-loop phosphorylation by upstream kinases (Geminivirus Rep interacting kinase 2 and 1) to inhibit the activity of SnRK1 (Zhang *et al.*, 2009; Zhai *et al.*, 2018). Notably, the level of T6P is reduced under submergence in Arabidopsis (Cho *et al.*, 2016), suggesting that SnRK1 could be de-repressed by the reduction of T6P under hypoxia (Fig. 2). However, under submergence SnRK1 was activated earlier than the decline in T6P (Cho *et al.*, 2016), indicating that there could be other factors coordinately activating SnRK1 under hypoxia.

More recently, the catalytic α subunit of SnRK1 was shown to have independent activity and to translocate into the nucleus by releasing from the restriction of the β subunit in the cytosol under low energy conditions (Cho *et al.*, 2012; Ramon *et al.*, 2019), suggesting that the translocation of the α subunit may be involved in energy sensing under hypoxia. The role of the β subunit under hypoxia remains unclear. There are four β subunit genes in Arabidopsis. β1 and β2 were N-myristoylated and were detected in the plasma membrane, and the βγ and β3 subunits were not N-myristoylated and were detected in the nucleus and cytosol (Pierre *et al.*, 2007; Ramon *et al.*, 2019). N-myristoylation is known to mediate membrane association, and the knock-out mutant of main N-myristoyl transferase was shown to have increased SnRK1 activity in the soluble fraction (including the nucleus and cytosol) (Pierre *et al.*, 2007), which also raises the possibility that the α subunit of SnRK1 dissociates from N-myristoylated β subunits under energy starvation. Collectively, these findings suggest an energy sensing mechanism under hypoxia whereby SnRK1 is activated via the turnover of metabolites caused by low energy, including the reduction of T6P, and translocated into the nucleus to activate the transcription of genes involved in the energy-stress responses under hypoxia, such as *DARK INDUCED 6* (*DIN6*) (Ramon *et al.*, 2019; Fig. 2).

V. Control of translation under hypoxia by SnRK1

Protein synthesis in cells requires a significant amount of energy. Under hypoxia, general translation is repressed, especially cap-mediated translation (Branco-Price *et al.*, 2008; Juntawong *et al.*, 2014). Based on the functional conservation of SnRK1 and AMPK, it has been proposed that the SnRK1-Target of Rapamycin (TOR) relay mediates the repression of mRNA 5′ capping dependent translation. When AMPK represses the activity of TOR, eIF4E binding protein (4E-BP), which was not phosphorylated by TOR, can compete with eIF4G for eIF4E binding to repress the cap-mediated translation (Hardie, 2011). Due to their small size and low abundance, 4E-BP-like identification in plants might prove difficult (Schepetilnikov & Ryabova, 2017).

However, a set of mRNAs of genes functioning in energy generation and stress adaption can bypass the translational repression via eIFiso4G1 phosphorylated by SnRK1 under hypoxia (Cho *et al.*, 2019). The eIFiso4Gs belonging to the eIF4G family are plant-specific eIF4Gs. In Arabidopsis, there are two *eIFiso4G* genes, *eIFiso4G1* and *eIFiso4G2,* and one *eIF4G* gene (Lellis *et al.*, 2010; Browning & Bailey-Serres, 2015). Compared to eIF4G, eIFiso4G lacks an N-terminal domain, but it has two HEAT domains, which allows it to bind to eIFiso4E and eIF4A to form the eIFiso4F (Cheng & Gallie, 2007). Under hypoxia, eIFiso4G1 is the dominant regulator in translational regulation, although both eIFiso4Gs are SnRK1 targets (Cho *et al.*, 2019). Phosphorylation of eIFiso4G1 by SnRK1 has been shown to result in the enrichment of specific mRNAs – including mRNAs of genes involved in fermentation, sucrose degradation, and fatty acid biosynthesis – being translated under hypoxia (Cho *et al.*, 2019). Interestingly, genes involved in fermentation and sucrose degradation were also shown to have higher chromatin accessibility and ribosome association under hypoxia (Lee & Bailey-Serres, 2019; Reynoso *et al.*, 2019), indicating that specific genes related to energy production were ensured to be transcribed and translated under hypoxia.

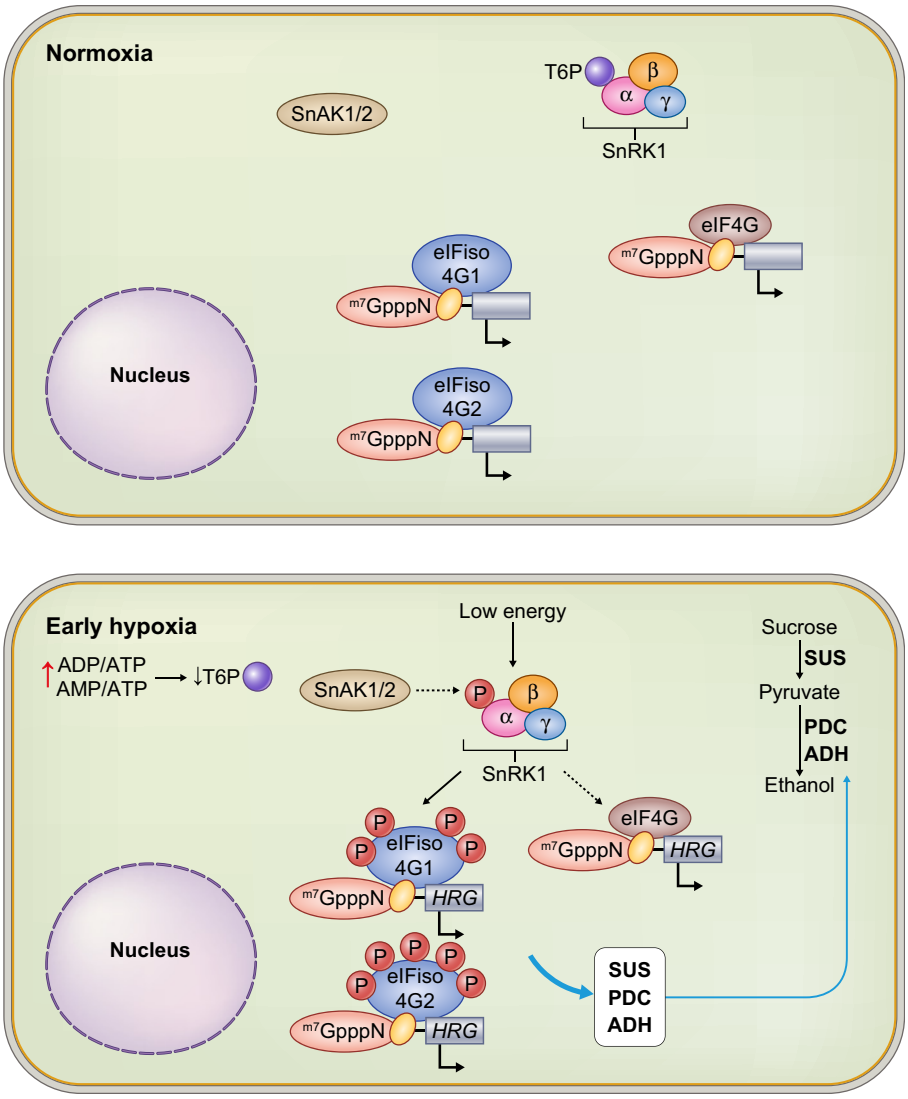


Fig. 2 Proposed signaling model for energy sensor SnRK1. Under normoxia, trehalose-6 phosphate (T6P) binds to SnRK1, preventing it from being phosphorylated by SnAK1/2. Under early hypoxia, low energy triggers the activation of SnRK1 to activate the stress responses. In addition, the turnover of T6P caused by hypoxia might also result in the activation of SnRK1. The activated SnRK1 in the cytosol phosphorylates eIFiso4Gs or activates eIF4G to form a complex that enhances the translation of specific mRNAs, such as the key enzymes of sucrose degradation and fermentation under hypoxia. The dashed arrow indicates predicted, hypothetical regulation. ADH, ALCOHOL DEHYDROGENASE; *HRG*, hypoxia responsive genes; PDC, PYRUVATE DECARBOXYLASE; SUS, SUCROSE SYNTHASE; m7GpppN, 7-methygluanosine cap structure.

To enhance the translation of specific mRNAs, eIFiso4G1, which is phosphorylated by SnRK1, is able to recognize the 5′ untranslated region (5′UTR) of *ADH1* (Cho *et al.*, 2019), a region providing effective translation of mRNA in maize, rice, and Arabidopsis (Bailey-Serres & Dawe,1996; Mardanova *et al.*, 2008). In addition, eIF4G can complement eIFiso4G function *in vitro* (Gallie & Browning, 2001) and contains SnRK1 recognized phosphorylation sites, suggesting that the eIF4G family is involved in SnRK1-mediated translational regulation under hypoxia (Fig. 2).

VI. Conclusions

Energy sensing was considered for decades to be a possible mechanism by which plants detect hypoxia. With the discovery of the oxygen sensing mechanism based on the oxygen-dependent protein destabilization of the ERF-VII proteins, the role of energy as a proxy for hypoxic conditions had to be reconsidered. However, energy sensing is still of great importance for plants' adaptation to hypoxia. Energy and sugar sensing converge with oxygen sensing to provide the plant with a homeostatic mechanism which ensures that the energy status of the plant contributes to the definition of the intensity of the transcriptional and translational responses. Oxygen sensing mediated by the ERF-VII proteins mostly contributes to the control of transcriptional activation of *HRG*s (Giuntoli & Perata, 2018), while energy sensing through SnRK1 makes efficient translation of *HRG*s' mRNAs possible. Translocation of the α subunit of SnRK1 to the nucleus controls activation of another set of genes that are required under hypoxia, namely sugar-starvation genes (Ramon *et al.*, 2019). Furthermore, another energy sensing mechanism, based on the requirement of LACS activity for an

adequate ATP level, controls the release of ERF-VII bound to ACBP, contributing to the induction of *HRG*s (Schmidt *et al.*, 2018). Finally, should hypoxia last for over 12 h in the dark, sugar starvation represses the action of ERF-VIIs and reduces the expression of *HRG*s, possibly to preserve carbon resources for regrowth during the post-hypoxic phase (Loreti *et al.*, 2018). Under sugar starvation, autophagy may contribute to tolerance in submerged plants. In Arabidopsis, submergence induces the transcription of autophagy-related genes and the formation of autophagosomes (Chen *et al.*, 2015). Furthermore, the autophagy-defective mutants are hypersensitive to submergence (Chen *et al.*, 2015). Remarkably, a link between selective autophagy and NO-levels under hypoxia was described (Zhan *et al.*, 2018). Given the involvement of NO in the destabilization of ERF-VII (Gibbs *et al.*, 2014), this is an area of research that deserves additional investigation.

The timing of the different converging pathways is, however, still unclear. Induction of *HRG*s' mRNAs takes less than an hour to be observed, while the drop in ATP takes much longer. This suggests that the ATP content dynamics in the hypoxic cell may not be accurately defined by the biochemical analysis of plant tissues, or the early hypoxic induction of *HRG*s by ERF-VII is temporarily uncoupled from ATP-based signaling. Under dark hypoxia, induction of sugar starvation genes occurs after > 12 h (Loreti *et al.*, 2018), suggesting that translocation of SnRK1 to the nucleus occurs quite late during hypoxia. Nuclear localization of the α subunit of SnRK1 occurs only after an extended night (Ramon *et al.*, 2019), in line with this timing. The contribution of SnRK1 to efficient *HRG* mRNA translation likely occurs much earlier, probably in concomitance with activation of the *HRG* transcription, suggesting that distinct energy-dependent signaling pathways may operate during hypoxia.

Acknowledgements

This work was supported by the Italian Ministry of Education, Research and Universities, Special Program PRIN 2015, the Ministry of Science and Technology (MOST 108-2311-B-001-037) and Academia Sinica, Taiwan.

ORCID

Hsing-Yi Cho https://orcid.org/0000-0002-2631-4476
Elena Loreti https://orcid.org/0000-0002-5255-4983
Pierdomenico Perata https://orcid.org/0000-0001-9444-0610
Ming-Che Shih https://orcid.org/0000-0002-7590-793X

References

Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J. 2007. A central integrator of transcription networks in plant stress and energy signalling. *Nature* **448**: 938–942.

Bailey-Serres J, Dawe RK. 1996. Both 5′ and 3′ sequences of Maize *adh1* mRNA are required for enhanced translation under low-oxygen conditions. *Plant Physiology* **112**: 685–695.

Branco-Price C, Kaiser KA, Jang CJ, Larive CK, Bailey-Serres J. 2008. Selective mRNA translation coordinates energetic and metabolic adjustments to cellular oxygen deprivation and reoxygenation in *Arabidopsis thaliana*. *The Plant Journal* **56**: 743–755.

Browning KS, Bailey-Serres J. 2015. Mechanism of cytoplasmic mRNA translation. *Arabidopsis Book* **13**: e0176.

Chen L, Liao B, Qi H, Xie LJ, Huang L, Tan WJ, Zhai N, Yuan LB, Zhou Y, Yu LJ *et al.* 2015. Autophagy contributes to regulation of the hypoxia response during submergence in *Arabidopsis thaliana*. *Autophagy* **11**: 2233–2246.

Cheng S, Gallie DR. 2007. eIF4G, eIFiso4G, and eIF4B bind the poly(A)-binding protein through overlapping sites within the RNA recognition motif domains. *Journal of Biological Chemistry* **282**: 25247–25258.

Cho HY, Lu MJ, Shih MC. 2019. The SnRK1-eIFiso4G1 signaling relay regulates the translation of specific mRNAs in *Arabidopsis* under submergence. *New Phytologist* **222**: 366–381.

Cho HY, Wen TN, Wang YT, Shih MC. 2016. Quantitative phosphoproteomics of protein kinase SnRK1 regulated protein phosphorylation in *Arabidopsis* under submergence. *Journal of Experimental Botany* **67**: 2745–2760.

Cho YH, Hong JW, Kim EC, Yoo SD. 2012. Regulatory functions of SnRK1 in stress-responsive gene expression and in plant growth and development. *Plant Physiology* **158**: 1955–1964.

Crozet P, Margalha L, Confraria A, Rodrigues A, Martinho C, Adamo M, Elias CA, Baena-Gonzalez E. 2014. Mechanisms of regulation of NF1/AMPK/SnRK1 protein kinases. *Frontiers in Plant Science* **5**: 190.

van Dongen JT, Fröhlich A, Ramírez-Aguilar SJ, Schauer N, Fernie AR, Erban A, Kopka J, Clark J, Langer A, Geigenberger P. 2009. Transcript and metabolite profiling of the adaptive response to mild decreases in oxygen concentration in the roots of Arabidopsis plants. *Annals of Botany* **103**: 269–280.

Emanuelle S, Hossain MI, Moller IE, Pedersen HL, van de Meene AM, Doblin MS, Koay A, Oakhill JS, Scott JW, Willats WG *et al.* 2015. SnRK1 from *Arabidopsis thaliana* is an atypical AMPK. *The Plant Journal* **82**: 183–192.

Gallie DR, Browning KS. 2001. eIF4G functionally differs from eIFiso4G in promoting internal initiation, cap-independent translation, and translation of structured mRNAs. *Journal of Biological Chemistry* **276**: 36951–36960.

Gasch P, Fundinger M, Müller JT, Lee T, Bailey-Serres J, Mustroph A. 2016. Redundant ERF-VII transcription factors bind to an evolutionarily conserved cis-motif to regulate hypoxia-responsive gene expression in Arabidopsis. *The Plant Cell* **28**: 160–180.

Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J *et al.* 2011. Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* **479**: 415–418.

Gibbs DJ, Isa NM, Movahedi M, Lozano-Juste J, Mendiondo GM, Berckhan S, Marín-de la Rosa N, Vicente Conde J, Sousa Correia C, Pearce SP. 2014. Nitric oxide sensing in plants is mediated by proteolytic control of group VII ERF transcription factors. *Molecular Cell* **53**: 369–379.

Giuntoli B, Perata P. 2018. Group VII ethylene response factors in Arabidopsis: regulation and physiological roles. *Plant Physiology* **176**: 1143–1155.

Hardie DG. 2011. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. *Genes & Development* **25**: 1895–1908.

Hartman S, Liu Z, Van Veen H, Vicente J, Reinen E, Martopawiro S, Zhang H, van Dongen N, Bosman F, Bassel GW *et al.* 2019. Ethylene-mediated nitric oxide depletion pre-adapts plants to hypoxia stress. *Nature Communications* **10**: 1–9.

Juntawong P, Girke T, Bazina J, Bailey-Serresa J. 2014. Translational dynamics revealed by genome-wide profiling of ribosome footprints in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **111**: E203–E212.

Kosmacz M, Parlanti S, Schwarzländer M, Kragler F, Licausi F, Van Dongen JT. 2015. The stability and nuclear localization of the transcription factor RAP 2.12 are dynamically regulated by oxygen concentration. *Plant, Cell & Environment* **38**: 1094–1103.

Lee TA, Bailey-Serres J. 2019. Integrative analysis from the epigenome to translatome uncovers patterns of dominant nuclear regulation during transient stress. *Plant Cell* **31**: 2573–2595.

Lellis AD, Allen ML, Aertker AW, Tran JK, Hillis DM, Harbin CR, Caldwell C, Gallie DR, Browning KS. 2010. Deletion of the eIFiso4G subunit of the *Arabidopsis* eIFiso4F translation initiation complex impairs health and viability. *Plant Molecular Biology* **74**: 249–263.

Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LACJ, Perata P, van Dongen JT. 2011. Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature* **479**: 419–422.

Loreti E, Poggi A, Novi G, Alpi A, Perata P. 2005. A genome-wide analysis of the effects of sucrose on gene expression in Arabidopsis seedlings under anoxia. *Plant Physiology* **137**: 1130–1138.

Loreti E, Valeri MC, Novi G, Perata P. 2018. Gene regulation and survival under hypoxia requires starch availability and metabolism. *Plant Physiology* **176**: 1286–1298.

Mardanova ES, Zamchuk LA, Skulachev MV, Ravin NV. 2008. The 5' untranslated region of the maize alcohol dehydrogenase gene contains an internal ribosome entry site. *Gene* **420**: 11–16.

Paul MV, Iyer S, Amerhauser C, Lehmann M, van Dongen JT, Geigenberger P. 2016. Oxygen sensing via the ethylene response transcription factor RAP2. 12 affects plant metabolism and performance under both normoxia and hypoxia. *Plant Physiology* **172**: 141–153.

Perata P, Alpi A. 1993. Plant responses to anaerobiosis. *Plant Science* **93**: 1–17.

Pierre M, Traverso JA, Boisson B, Domenichini S, Bouchez D, Giglione C, Meinnel T. 2007. N-Myristoylation regulates the SnRK1 pathway in *Arabidopsis*. *Plant Cell* **19**: 2804–2821.

Polge C, Thomas M. 2007. SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control? *Trends in Plant Science* **12**: 20–28.

Ramon M, Dang TVT, Broeckx T, Hulsmans S, Crepin N, Sheen J, Rolland F. 2019. Default activation and nuclear translocation of the plant cellular energy sensor SnRK1 regulate metabolic stress responses and development. *Plant Cell* **31**: 1614–1632.

Reynoso MA, Kajala K, Bajic M, West DA, Pauluzzi G, Yao AI, Hatch K, Zumstein K, Woodhouse M, Rodriguez-Medina J. 2019. Evolutionary flexibility in flooding response circuitry in angiosperms. *Science* **365**: 1291–1295.

Schepetilnikov M, Ryabova LA. 2017. Recent discoveries on the role of TOR (Target of Rapamycin) signaling in translation in plants. *Plant Physiology* **176**: 1095–1105.

Schmidt RR, Fulda M, Paul MV, Anders M, Plum F, Weits DA, Kosmacz M, Larson TR, Graham IA, Gerrit T *et al.* 2018. Low-oxygen response is triggered by an ATP-dependent shift in oleoyl-CoA in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **115**: E12101–E12110.

Sugden C, Crawford RM, Halford NG, Hardie DG. 1999. Regulation of spinach SNF1-related (SnRK1) kinases by protein kinases and phosphatases is associated with phosphorylation of the T loop and is regulated by 5′-AMP. *The Plant Journal* **19**: 433–439.

Wagner S, Steinbeck J, Fuchs P, Lichtenauer S, Elsässer M, Schippers JH, Nietzel T, Ruberti C, Van Aken O, Meyer AJ *et al.* 2019. Multiparametric real-time sensing of cytosolic physiology links hypoxia responses to mitochondrial electron transport. *New Phytologist* **224**: 1668–1684.

Weits DA, Giuntoli B, Kosmacz M, Parlanti S, Hubberten HM, Riegler H, Hoefgen R, Perata P, van Dongen JT, Licausi F. 2014. Plant cysteine oxidases control the oxygen-dependent branch of the N-end-rule pathway. *Nature Communications* **5**: 3425.

Zhai Z, Keereetaweep J, Liu H, Feil R, Lunn JE, Shanklin J. 2018. Trehalose 6-phosphate positively regulates fatty acid synthesis by stabilizing WRINKLED1. *Plant Cell* **30**: 2616–2627.

Zhan N, Wang C, Chen L, Yang H, Feng J, Gong X, Ren B, Wu R, Mu J, Li Y *et al.* 2018. S-Nitrosylation targets GSNO reductase for selective autophagy during hypoxia responses in plants. *Molecular Cell* **71**: 142–154.

Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RA, Powers SJ, Schluepmann H, Delatte T, Wingler A, Paul MJ. 2009. Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiology* **149**: 1860–1871.

See also the Editorial on this article by Sasidharan *et al.*, **229**: 5–7.