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SHORT COMMUNICATION

The ACBP1-RAP2.12 signalling hub: A new perspective on integrative signalling during hypoxia in plants

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ABSTRACT

During their lifetime, plants are frequently exposed to a variety of stresses which negatively impact on growth and vitality. In order to respond specifically to a given stress situation, integration of multiple signal inputs is of utmost importance. Recently, we demonstrated that recognition and adaptation to low-oxygen stress requires integration of signals from energy metabolism, lipid metabolism and oxygen availability. Low oxygen which results in an energy crisis causes a shift in lipid intermediate ratios. Binding of C18:1-CoA by ACYL-COA BINDING PROTEIN 1 (ACBP1) at the plasma membrane concomitantly leads to release and nuclear accumulation of the ERFVII transcription factor RELATED TO APETALA 2.12 (RAP2.12) which is central to the activation of anaerobic metabolism during stress. Moreover, RAP2.12 protein stability is oxygen-dependently regulated and its oxidation results in degradation by the N-end rule pathway. Here, we illuminate the concept of multiple-signal integration under hypoxia and discuss signal inputs merging at the ACBP1-ERFVII signaling hub.

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The mitochondrion as important source of energy equivalents is disturbed in its function once molecular oxygen as final acceptor of the electron transport chain becomes restricted. Considering the dramatic consequences for diverse cellular processes, giving the mitochondrion a "voice", i.e., the opportunity to initiate adaptation programs upon its dysfunction appears natural. Impaired mitochondrial energy production needs to be sensed to initiate a coordinated response, including a matched decrease in energy consumption and the activation of fermentative pathways in order to prevent a cellular collapse.[1] Mitochondria produce next to ATP a diverse array of bioactive molecules of which adenylate nucleotides, tricarboxylic acid (TCA) cycle intermediates, acetyl-CoA and reactive oxygen species (ROS) have been identified as signaling molecules.[1,2] In addition, calcium and several mitochondrial peptides and proteins appear to participate in mitochondrial signaling pathways.[3,4] Though hypoxia impacts immediately on plant mitochondrial functioning, the role and identity of mitochondrion-derived signals during oxygen limitations and their integration into existing transcriptional response pathways is poorly explored.[5,6]

Group VII ETHYLENE-RESPONSE FACTOR (ERFVII) transcription factors are well known for their crucial role in providing low-oxygen tolerance in diverse plant species. Their protein stability has in many cases been found to be regulated in an oxygen-dependent fashion.[7–9] However, a mechanistic link between ERFVII action and mitochondrial dysfunction under natural oxygen constraints such as flooding remained missing. Recently, we discovered that the molecular response to hypoxia not only requires low levels of oxygen but also an energy crisis in order to induce nuclear accumulation of the ERFVII factor RELATED TO APETALA 2.12 (RAP2.12).[10] Importantly, RAP2.12 is under non-stress conditions located at the plasma membrane through association with ACYL-COA BINDING PROTEIN 1 (ACBP1) (Figure 1(a)). Lipid intermediate signals under hypoxia promote release of RAP2.12 from ACBP1 and its subsequent accumulation and activation in the nucleus.[10] ERFVII and ACBP family members are found together in higher plants.[7,11] Moreover, the ankyrin repeat domain of ACBP1 and the DNA-binding domain of RAP2.12 enable interaction between both proteins and are highly conserved within plants.[10] Therefore, we consider the ACBP-ERFVII complex as an important signaling hub in plants with potential to integrate signals from different intracellular origins in order to modulate hypoxic responsestpe.

The N-end rule pathway, specifically the oxygen-dependent branch, promotes proteasome-assisted dismantling of soluble RAP2.12 protein under aerobic conditions. However, RAP2.12 protein that is attached to ACBP1 is protected from breakdown by a so far unknown mechanism.[7] Premature release of RAP2.12 from ACBP1 during normoxia will readily result in transcription factor degradation, while upon oxygen shortage, N-end rule dependent degradation is prevented leading to RAP2.12 stabilization[7] [Figure 1(b)]. Despite its unquestionable importance in regulating RAP2.12 abundance, a limitation of considering the N-end rule pathway the only cellular entity modulating RAP2.12 function is that it takes into account the cellular oxygen concentration but does not integrate information concerning the cellular energy status. As a consequence, low cellular oxygen concentrations due to fully active mitochondrial respiration or high cellular metabolic activity in general cannot be distinguished

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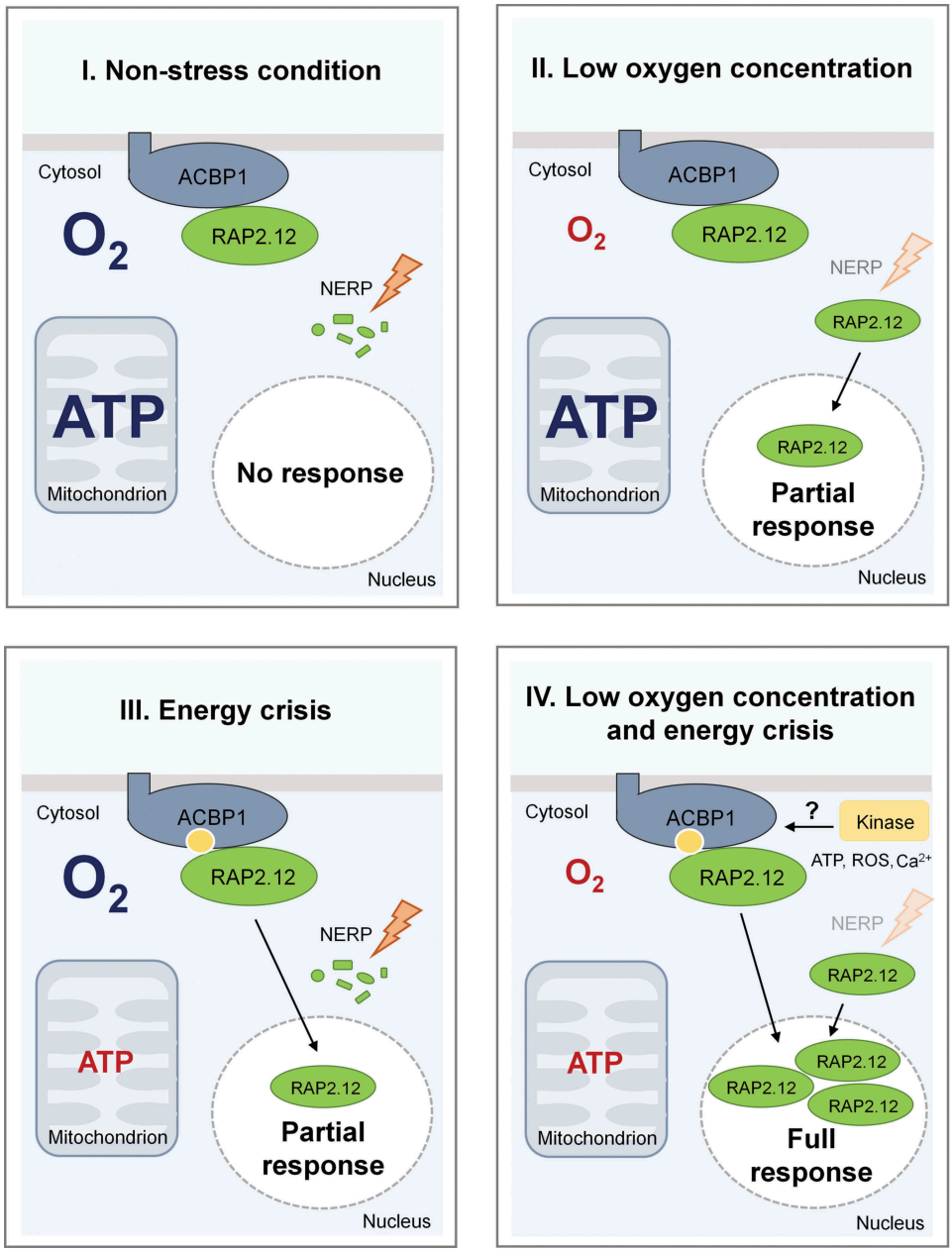

Figure 1. Model of integrative signaling under hypoxia involving the ACBP1-RAP2.12 hub. (a) Under non-stress conditions, cytosolic RAP2.12 protein is constantly destabilized by the oxygen-dependent branch of the N-end rule pathway (NERP). RAP2.12 protein which is sequestered at the plasma membrane by binding to ACBP1 can escape degradation. (b) A low oxygen concentration not affecting the cellular energy status inhibits NERP activity and results in stabilization of cytoplasmic RAP2.12 protein. However, dissociation of ACBP1-bound RAP2.12 protein is prevented. Therefore, only partial activation of hypoxic transcripts is achieved. Of note, a low oxygen concentration does not necessarily lead to a low energy status as long as the flux of oxygen into the cell meets up with mitochondrial demands. In contrast, oxygen shortage, i.e., insufficient oxygen supply, will lead in either case to low energy. (c) A low energy status, e.g., caused by reduced mitochondrial ATP synthesis or high cellular metabolic activity, modifies cellular acyl-CoA levels leading to a shift in C18:1-CoA/C16:0-CoA ratios. C18:1-CoA (shown as yellow circle) binds as ligand to ACBP1 promoting the release of RAP2.12. Due to the still active NERP, only a fraction of RAP2.12 protein reaches the nucleus to activate hypoxic genes. (d) Under a low oxygen concentration that results in an energy crisis, RAP2.12 is released from ACBP1 and gets stabilized upon NERP inhibition, leading to full activation of hypoxic gene expression. Kinases activated upon (mitochondrial) ROS, calcium or ATP signals potentially phosphorylate ACBP1 thereby further affecting its affinity towards RAP2.12.

from an actual stress situation in which oxygen is limited for oxidative phosphorylation leading to an energy crisis.[6]

We recently showed that a signal derived from an energy crisis is mandatory for inducing dissociation of the ACBP1-RAP2.12 complex.[10] A hypoxia-induced shift in cellular C18:1-CoA/C16:0-CoA ratios acts as trigger for movement of RAP2.12 from the plasma membrane to the nucleus in order to activate hypoxic gene expression. Within this mechanism, C18:1-CoA, which is a long-chain acyl-CoA, is bound as ligand by ACBP1 via its acyl-CoA binding domain. Most likely due to conformational changes within ACBP1, RAP2.12 is subsequently set free,[10] [Figure 1(c)]. With our work, we demonstrate for the first time that acyl-CoAs have signaling function in plants. In non-plant organisms, acyl-CoAs are well-recognized intracellular signals which regulate diverse functions, including the activity of enzymes, ion

channels and transcription factors. As ACBPs sequester specific acyl-CoA molecules, they actively participate in acyl-CoA -facilitated signaling processes.[12] The master regulator of transcriptional reprogramming under hypoxia in animals and humans, HYPOXIA-INDUCIBLE FACTOR 1 (HIF), is in *C.elegans* also linked to ACBP. Remarkably, though not related to a hypoxic environment, it is suggested that an unknown bioactive lipid compound derived from the acyl-CoA pool affects HIF-1 nuclear localization and transcriptional activity.[13] Several other studies have shown that acyl-CoAs can furthermore serve as direct ligands of transcription factors. In most cases, the transcription factor ability to activate target genes is altered upon ligand binding, as shown for FadR in bacteria and HEPATIC NUCLEAR FACTOR-4 ALPHA (HNF4α) in hepatocytes.[14–16] The presence of certain acyl-CoAs in hepatocytes is an indicator of higher fatty acid availability due to food supply. Interestingly, acyl-CoA-dependent regulation of HNF4α activity impacts genes involved in fatty acid oxidation. In this way, acyl-CoA levels can pass on information on lipid availability and thereby fine-tune energy metabolism directly through adaptive transcript responses. In our study, the drop in ATP levels under hypoxia is sensed and translated into acyl-CoA signals, as the enzymes responsible for acyl-CoA synthesis (see below) act in an ATP-dependent manner.[10] The subsequent signal promotes activation of fermentative metabolism through the ACBP1-RAP2.12 complex. Strikingly, next to C18:1-CoA being involved in hypoxic gene regulation, we found two other acyl-CoAs, C16:0-CoA and C18:0-CoA, to provoke distinct transcript responses related to specific biological processes.[10] It points to additional roles of acyl-CoAs in plant signaling pathways beyond hypoxia which deserves future research effort.

Once fatty acids are synthesized in the chloroplast, they are exported into the cytosol in the form of acyl-CoA thioesters.[17] The activation of long-chain fatty acids to long-chain acyl-CoAs is catalyzed by the LONG-CHAIN ACYL-COA SYNTHETASE (LACS) family.[18] The LACS reaction requires next to CoA also ATP, and not surprisingly, LACS activity is decreased upon a drop in ATP levels as found under hypoxic stress.[10] LACS enzymes therefore establish a direct link between ATP deficiency upon mitochondrial dysfunction and acyl-CoA pool composition. Consistently, disabled mitochondrial ATP production provoked by antimycin-A application results in induction of RAP2.12-dependent genes even under aerobic conditions.[10] Thus, activation of a part of the RAP2.12-dependent hypoxia pathway takes place through energy deficiency signals. The combination of (i) energy crisis sensing via acyl-CoA signals acting on ACBP1-RAP2.12 affinity and (ii) oxygen sensing via RAP2.12 protein stabilization upon N-end rule impairment may help to generate a stress signal which is specific to hypoxic stress (Figure 1(d)).

ACBP1 – a phosphorylation target?

The discovery of C18:1-CoA as trigger to release RAP2.12 from ACBP1 under energy constraints is an important step forward in understanding hypoxia signaling in plants. However, it is very likely that additional, unknown signals are detected by the ACBP1-RAP2.12 hub. In our recent study, C18:1-CoA application under aerobic conditions could not fully promote dissociation of the RAP2.12 protein from ACBP1, both *in vitro* and *in vivo*.[10] Pharmacologically inhibited mitochondrial function indeed induces RAP2.12 target genes even in air, but at levels lower than observed during hypoxia, which among others may be partially traced back to still active RAP2.12 destabilization.[7,10] These observations leave room for further regulatory mechanisms and signals participating in ACBP1-RAP2.12 complex dissociation. Central to eukaryotic energy signaling during stress and growth are TARGET OF RAPAMYCIN (TOR) and SUCROSE NON-FERMENTING 1 (SNF1)-RELATED PROTEIN KINASE 1 (SnRK1) signaling cascades.[19,20] In both pathways, reversible phosphorylation of downstream components results in altered activity, localization or ability to interact.[21] Potentially, also the ACBP1-RAP2.12 hub is a phosphorylation target during energy and oxygen limitation in plants. In line with this idea, it was shown that ACBP in rat liver cells is phosphorylated upon stress, leading to an altered localization.[22] Another study on Mueller retinoma cells suggested that phosphorylation of ACBP may influence its interaction with other proteins, since binding to GABA receptors is reduced upon ACBP dephosphorylation.[23] We speculate that phosphorylation of ACBP1 may modulate its binding affinity towards RAP2.12 or C18:1-CoA. This exciting possibility of ACBP1 modifications under low-oxygen stress and the identification of candidate kinases are part of our current research focus. As signals produced by the mitochondrion encompass among others ATP, calcium and ROS, individual kinases from the CALCINEURIN-B-LIKE INTERACTING PROTEIN KINASE (CIPK), CALCIUM-DEPENDENT PROTEIN KINASE (CDPK) and MITOGEN-ACTIVATED PROTEIN KINASE (MPK) family may facilitate the integration of mitochondrial signals at the ACBP1-RAP2.12 complex (Figure 1(d)). Studying such integration events represents a unique opportunity to uncover the identity and origin of early low oxygen-relevant signals in plants.

Disclosure statement

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