

Plant Cell Monographs

Joost T. van Dongen
Francesco Licausi *Editors*

Low-Oxygen Stress in Plants

Oxygen Sensing and
Adaptive Responses to Hypoxia

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Editors

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Oxygen Sensing and Adaptive Responses
to Hypoxia

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Cover picture: Image of an *Arabidopsis thaliana* leaf epidermis after modified pseudo-Schiff propidiumiodide (mPS-PI) staining showing basal cells of a removed trichome. Courtesy of Dr. Ruth Eichmann.

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Preface

Throughout the history of Earth, a tight relationship was established between the high availability of oxygen in the atmosphere and the biological population. Oxygen enrichment of the Archean Earth's atmosphere was initiated by cyanobacteria and further boosted as a consequence of the colonization of the planet's surface by photosynthetic and multicellular eukaryotes which developed into land plants (Bendall et al. 2008). Nowadays, oxygen accounts for about one-fifth of our atmosphere and represents an essential element, which sustains the life of most multicellular organisms, including fungi, animals, and plants. Not only oxygen is required as terminal electron acceptor to ensure respiratory energy production via oxidative phosphorylation, but it also acts as primary substrate in a majority of metabolic reactions that produce structural and signaling components throughout all kingdoms of life. Consequently, when oxygen availability is reduced below the levels required to sustain these biological processes, a situation of crisis is generated. This is especially serious for sessile organisms, such as plants, which are limited in their possibility to move towards area where oxygen availability is sufficient.

For plants, the most common case of restriction in oxygen availability is caused by submergence, due to the slower diffusion of gases in water than in air and exacerbated by the competition for oxygen consumption by soil microorganisms, whose anaerobic metabolism in turn leads to the accumulation of phytotoxic metabolites (Bailey-Serres and Voesenek 2008). Plants do not need to be completely submerged to suffer from oxygen deficiency: reduced oxygen levels (hypoxia) or complete absence of oxygen (anoxia) in waterlogged soil is sufficient to put plant's survival at stakes and has dramatic effect on crop yield (Ahmed et al. 2013). The consequent reduction in size and functionality of the root apparatus of a flooded or waterlogged plant reduce water and nutrient transport towards the shoot. The high probability that every plant will experience restriction in oxygen availability at sometimes throughout its lifespan suggested the concept that these organisms must have evolved efficient strategies to cope with this situation orchestrated by perception and signaling mechanism that integrated them into developmental and growth programs (Bailey-Serres et al. 2012). Additionally, the notion

that oxygen availability is not equal to all plant cell types and certain tissues or organs actually develop at oxygen levels lower than those available in the atmosphere put this element in the perspective of a developmental signal.

Initially, research on plant anaerobiosis developed in the fields of biochemistry, due to its direct effect on primary metabolism, and ecology, as broad differences in tolerance to flooding were traditionally known in wild and cultivated species. Biochemical studies first focused on the metabolic switch from the aerobic respiration to fermentative pathways (reviewed in Davies 1980), but later explored the global adjustment and re-routing of primary metabolic reactions opening the debate over an adaptation of respiratory rates to hypoxia. On the other hand, ecophysiological approaches aimed at the identification and characterization of the strategies adopted by different plant species to restricted oxygen availability throughout evolution (Jackson and Colmer 2005). The characterization of the molecular elements, involved in low oxygen sensing and signaling, initiated at the end of the 1990s and beginning of the current century (Hoeren et al. 1998; Klok et al. 2002) but, in comparison with other abiotic stresses such as heat, cold, and high salinity, knowledge in this field lagged behind. Nevertheless at the descriptive level, very detailed overviews of the transcriptomic adjustment to oxygen deprivation were produced, including time-, oxygen-concentration-, and cell type-specific-resolved analyses (Mustroph et al. 2009; Mustroph et al. 2010). This generated a deep knowledge related to the dynamics of the anaerobic response and greatly contributed to the identification of general and tissue-specific responses. In the last 10 years, two main breakthrough set milestones in the applied and theoretic knowledge with respect to the plant adaptation to low oxygen availability, respectively. First, a joint team of agronomists, physiologists, and molecular biologists revealed the genetic basis for submergence tolerance in wild rice varieties and described for the first time a quiescent metabolic adaptation aimed at saving energy and resources for short-lasting floods (Xu et al. 2006). An opposite strategy was shown a couple of years later to occur in deep-water rice varieties (Hattori et al. 2009). More recently, studies conducted in *Arabidopsis* converged to the identification of an oxygen-dependent pathway for the degradation of transcription factors that orchestrate the core of the anaerobic response in plants (Gibbs et al. 2011; Licausi et al. 2011). Not surprisingly, these three studies hit on the same class of transcription factors, suggesting that species-specific modes of action to respond to low-oxygen stress evolved from the same basic genetic elements. Concomitantly, the gaseous phytohormone ethylene emerged as a key-regulator of the response to flooding and its interaction with other growth regulators such as gibberellins, auxin, and abscisic acid was shown to shape plant growth (Bailey-Serres and Voesenek 2008).

With this book, we bring together the different fields of research which deal with low oxygen conditions in plants and algae to provide an overview of the deep interconnection between their achievements. The monograph consists of seven sections, starting from the mechanisms adopted by plant cells to *perceive oxygen availability and initiate the signaling cascade* that leads to the activation of conserved and species-specific adaptive responses. In this section, both direct

oxygen sensing (Kosmacz and Weits, Chap. 1) and biochemical parameters that are affected as consequence of decreased oxygen availability are discussed, including the level of reactive oxygen species (Blokhina et al., Chap. 2), nitric oxide (Igamberdiev et al., Chap. 3), and pH (Ishizawa, Chap. 4). The *molecular response of plants to hypoxia* is presented in the following section, with a focus at the transcriptional (Giuntoli and Perata, Chap. 5) and the posttranscriptional (Sorenson and Bailey-Serres, Chap. 6) level with an additional chapter dedicated to the hormonal interplay that integrate the adaption to oxygen deficiency into growth and developmental programs (Steffens and Sauter, Chap. 7). The third section of this book is dedicated to the *metabolic adaptations* that take place as consequence of a decrease in the oxygen—and thus energy—availability. This section is not limited to higher plants but takes into consideration also green algae whose anaerobic metabolism is of potential economic interest, such as *Chlamydomonas reinhardtii* (Yang et al., Chap. 8). The role of alternative energy storage units, such as PPI, is discussed by Mustroph et al. (Chap. 9) while the effect of changing oxygen availability on respiratory energy production is described by Paepke et al. (Chap. 10). Oxygen-dependent effect on nitrogen and amino acid metabolism is reviewed by Limami (Chap. 11) and Geigenberger (Chap. 12) describes storage metabolism under oxygen limitations. Most of the molecular and metabolic changes described in the previous sections are ultimately aimed at sustaining prolonged conditions of hypoxia, which is also achieved via *morphological adaptations* that ameliorate oxygen supply and transport within the plant tissues (Armstrong and Armstrong, Chap. 14), namely the formation of aerenchyma (Takahashi et al., Chap. 13) and the production of adventitious roots (Sauter and Steffens, Chap. 15). Species-specific strategies which have been developed by plants to maintain photosynthetic activity under water (Pedersen and Colmer, Chap. 16) and cope with flooding conditions (van Veen et al., Chap. 17) are discussed in a specific section dedicated to the *ecophysiological aspects* of the response to low oxygen. Furthermore, the occurrence and impact of low oxygen responses in agricultural practice are discussed taking into consideration the difficulty of oxygen diffusion into bulky fruits (Nicolai et al., Chap. 18), the oxygen supply in artificial substrates used in horticulture (Wessel et al., Chap. 19), and presenting the effect of herbicides that mimic the hypoxic response in plants (Zabalza and Royuela, Chap. 20). Our book concludes with a review about the state-of-the-art techniques used in the past *to measure oxygen concentrations in vivo* and the novel molecular strategies that are being developed to do so in the least intrusive way (Ast and Draaijer, Chap. 21).

We expect that the detailed survey about the various aspects of low-oxygen stress in plants as it is discussed in this monograph will not just contribute to our understanding of the adaptation of plant to low oxygen stress but also extend its potential to the improvement of crops against the damage caused by flooding. Even more so, we hope it will pave the way towards new discoveries that are expected to further boost our knowledge in this field in the next years.

We would like to express our gratitude to all authors and reviewers that contributed to this book. Furthermore, we acknowledge Christiane Welsch for her excellent help in preparing the final manuscript.

Aachen and Pisa
June 2013

Joost van Dongen
Francesco Licausi

References

- Ahmed F, Rafii MY, Ismail MR, Juraimi AS, Rahim HA, Asfaliza R, Latif MA (2013) Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects. *BioMed Res Int* 2013:10
- Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voisenek LACJ, van Dongen JT (2012) Making sense of low oxygen sensing. *Trends Plant Sci* 17:129–138
- Bailey-Serres J, Voisenek LACJ (2008) Flooding stress: acclimations and genetic diversity. *Annu Rev Plant Biol* 59:313–339
- Bendall DS, Howe CJ, Nisbet EG, Nisbet RER (2008) Introduction. Photosynthetic and atmospheric evolution. *Phil Trans Roy Soc Lond B Biol Sci* 363(1504):2625–2628
- Davies DD (1980) Anaerobic metabolism and the production of organic acids. In: Davies DD (ed) *The biochemistry of plants*, Vol 2. Academic, New York, p 581–611
- Gibbs DJ, Lee SC, Md Isa N, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J, Holdsworth MJ (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* 479:415–418
- Hattori Y, Nagai K, Furukawa S, Song X-J, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature* 460:1026–1030
- Hoeren FU, Dolferus R, Wu Y, Peacock WJ, Dennis ES (1998) Evidence for a role for AtMYB2 in the induction of the Arabidopsis alcohol dehydrogenase gene (ADH1) by low oxygen. *Genetics* 149:479–490
- Jackson MB, Colmer TD (2005) Response and adaptation by plants to flooding stress. *Ann Bot* 96:501–505
- Klok EJ, Wilson IW, Wilson D, Chapman SC, Ewing RM, Somerville SC, Peacock WJ, Dolferus R, Dennis ES (2002) Expression profile analysis of the low-oxygen response in Arabidopsis root cultures. *Plant Cell* 14:2481–2494
- Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voisenek 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
- Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Masihi A, Fukao T, Bailey-Serres J (2010) Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. *Plant Physiol* 152:1484–1500
- Mustroph A, Zanetti ME, Jang CJH, Holtan HE, Repetti PP, Galbraith DW, Girke T, Bailey-Serres J (2009) Profiling transcriptomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. *Proc Natl Acad Sci* 106:18843–18848
- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* 442:705–708

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Part I
Sensing and Signalling Hypoxic Stress

Oxygen Perception in Plants

Monika Kosmacz and Daan A. Weits

Abstract In aerobic organisms oxygen is a rate-limiting substrate for the efficient production of energy, and therefore they need to adjust their metabolism to the availability of oxygen. For this reason, eukaryotes and prokaryotes independently developed mechanisms to perceive oxygen availability and integrate this into developmental and growth programs. Despite their ability to produce oxygen in the presence of light, plants can experience low oxygen conditions when the oxygen diffusion from the environment cannot satisfy the demand set by metabolic rates. The oxygen-sensing mechanism recently identified in plants shares striking similarities with those previously described in animal cells. While in bacteria the different oxygen-sensing pathways reported involve protein dimerization and phosphorylation cascades, in plants and animals this function is mediated by oxygen-dependent proteolysis. The plant oxygen-sensing pathway is regulated via the oxygen-dependent branch of the N-end rule, which regulates the stability of the group VII of the Ethylene Response Factors, key activators of the anaerobic response. Additionally, constitutively expressed ERF-VII proteins, such as RAP2.12, are bound to the acyl-CoA-binding proteins (ACBPs) at the plasma membrane and protected from aerobic degradation. In hypoxia, RAP2.12 is released from the membrane and relocalizes into the nucleus, where it activates the molecular response to oxygen deficiency. Additional factors, indirectly affected by oxygen availability, have also been suggested to play roles in the fine tuning of oxygen sensing in plants.

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1 Introduction

Over 2,500 million years ago, the first cyanobacteria, which were able to produce oxygen via photosynthesis, appeared on Earth. The increase in oxygen concentrations into the atmosphere dramatically changed the composition of life forms and led to the emergence of aerobic organisms. These organisms exploit oxygen as final electron acceptor in the oxidative phosphorylation to produce energy in a process called “respiration.” Being aerobic organisms, plants also consume oxygen to produce energy and therefore can experience oxygen deficiency when the availability of this element in the environment becomes limiting. This occurs, for example, as a consequence of complete submergence or soil waterlogging, since oxygen diffusion is 10,000 times slower in water than in air. Plant organs also experience low oxygen when oxygen diffusion cannot keep up with its consumption by cells, such as in highly metabolically active tissues. Since, in contrast to animals, plant species lack an efficient oxygen transport system (van Dongen et al. 2009), oxygen-deprived cells have no other option than to adapt energy metabolism to the lower availability of oxygen in order to avoid energy shortage.

The first metabolic response that can be observed upon low oxygen conditions is a reduction of non-essential, energy-consuming processes such as the metabolism of storage products like starch, protein, and lipids (Geigenberger 2003; Bailey-Serres and Voesenek 2008). Moreover, P_{Pi}-dependent reactions are favored above those which use ATP as substrate (Greenway and Gibbs 2009). Oxygen consumption via respiration is also reduced, supposedly to avoid the plant to incur anoxic conditions (Zabalza et al. 2009). Indeed, when oxygen is completely depleted, ATP production via oxidative phosphorylation in the mitochondria is impossible and plant cells can only rely on the little ATP that is produced via glycolysis. In this situation, the activity of this pathway is strongly upregulated to maximize energy production, a phenomenon known as the Pasteur Effect (Summers et al. 2000). Since glycolysis consumes NAD⁺, continuous NADH oxidation in the absence of oxidative phosphorylation is ensured by the activation of fermentative pathways (Tadege et al. 1999). This regulation of the energy metabolism in plants demands an efficient and tunable sensing mechanism for the oxygen availability, similarly to what has been described for other organism such as bacteria, fungi, and animals.

For long, scientists have searched in plants for homologous systems to the oxygen-sensing pathways described for other organisms. However, no ortholog for sensor proteins was discovered. Oxygen signaling in plants has been suggested to be mediated by metabolic alterations caused by oxygen depletion such as cytosolic pH acidification, production of reactive oxygen species (ROS), NO or Ca²⁺ fluxes (Licausi and Perata 2009). In 2011, a plant-specific asset of proteins was identified as involved in the regulation of gene expression in response to oxygen deficiency (Gibbs et al. 2011; Licausi et al. 2011b). This plant oxygen-sensing pathway shares striking similarities to a branch of the mechanisms that regulates NO-dependent responses in animals (Hu et al. 2005). In this review, we describe the

recent discoveries about the molecular mechanisms by which the hypoxic responses in plants are regulated and provide a critical comparison with oxygen-sensing systems in other organisms.

2 Oxygen Sensing in Bacteria Relies on Three Independent Mechanisms

The facultative anaerobes within the Bacteria kingdom have the ability to thrive in both presence and absence of oxygen. In order to do so, these microorganisms need to switch their metabolism between aerobic respiration and fermentation depending on the oxygen availability in the environment.

Three different mechanisms that regulate separate sets of anaerobiosis-related genes were described. The first relies on the Fumarate Nitrate Reductase (FNR) transcriptional regulator, a close homolog of Cyclic AMP Receptor Proteins (CRP) (Guest et al. 1996; Körner et al. 2003). This protein can exist in a monomeric or homodimeric form depending on the oxygen availability (Kiley and Beinert 2003). In its dimeric form, FNR regulates the metabolic switch from aerobic to anaerobic metabolism, inducing Nitrate Reductase and Nitrite Reductase, which are required to utilize nitrate as electron acceptor instead of oxygen. The formation of dimers relies on a cluster of sulfur-containing cysteine residues coordinated to iron atoms ([4Fe-4S]²⁺ cluster), which is oxidized in presence of oxygen (Khoroshilova et al. 1995).

A second, well-studied, mechanism in *E. coli* involved in the control of gene expression under condition of reduced oxygen availability is the two component system consisting of the membrane-bound ArcB kinase and its cognate transcriptional activator ArcA (Iuchi et al. 1988, 1989). When oxygen availability decreases, the increase of reduced ubiquinol promotes ArcB autophosphorylation which, in turn, phosphorylates and activates ArcA (Georgellis et al. 1999; Rodriguez et al. 2004). This results in the up-regulation of operons involved in carbon catabolism and cellular redox status (Unden and Bongaerts 1997; Alexeeva et al. 2003).

A third, wide, class of oxygen sensors in bacteria consists of heme-binding domains (Taylor and Zhulin 1999). The PAS (Per-ARNT-Sim) domain which is contained in the FixL and EcDos (Delgado-Nixon et al. 2000) is an example of heme-dependent perception of oxygen. Usually, these sensory domains are associated with transmitter domains such as histidine kinase, phosphodiesterase, or diguanylate cyclase to direct the signal to transcriptional regulators (Gilles-Gonzalez et al. 2008). A paradigm of this mechanism is provided by FixL, the master regulator of nitrogen fixation in diazotrophic bacteria. In fact, when oxygen concentrations drop below the levels that would inactivate the nitrogen fixing nitrogenases, the deoxygenation of the heme group associated to FixL triggers its autophosphorylation (Da Re et al. 1994). Once active, FixL transfers the phosphate to the transcription factor FixJ causing a change in its conformation, thereby

inducing the genes involved in nitrogen fixation (Fischer 1994; Gong et al. 2000). Orthologs of FixL have been identified in plants and were found to contain a functional PAS domain. However, in plants they were shown not to be involved in low oxygen stress, but in transduction of light stress signals (Taylor and Zhulin 1999).

3 The Oxygen-Sensing Mechanism in Animal Cells Relies on the Oxygen-Dependent Stability and Activity of Hypoxia Inducible Factor 1

Opposite to plants and fungi, maintenance of oxygen homeostasis in animals relies on the efficient transport of oxygen through blood vessels. Therefore, the importance of oxygen gradients as cues for specific developmental programs has been widely studied. The key regulator of oxygen homeostasis in animal cells is the Hypoxia Inducible Factor (HIF)1, which is a transcriptional activator, required for the development of new blood vessels (angiogenesis) in response to hypoxia. HIF-1 is a heterodimer of two basic helix loop-helix/PAS proteins containing HIF-1 α and the aryl hydrocarbon nuclear translocator (ARNT or HIF1 β) (Wang et al. 1995). The molecular regulation of HIF-1 by oxygen is controlled by the alpha subunit. In fact, the HIF1 β protein is constitutively expressed and its level is not significantly affected by oxygen (Kallio et al. 1997). In contrast, the HIF-1 α protein is stabilized within minutes of exposure to hypoxia. In presence of oxygen, prolines in HIF-1 α are hydroxylated and this promotes the polyubiquitination and proteasomal degradation by an E3 ubiquitin ligase complex that contains the von Hippel-Lindau tumor suppressor protein (pVHL), elongin B, elongin C, Cul2, and Rbx (Maxwell et al. 1999 and Ohh et al. 2000). A second oxygen-dependent regulation of HIF-1 α relies in hydroxylation of an asparagine residue by the Factor Inhibiting HIF-1 (FIH-1) (Lando et al. 2002). The hydroxylated asparagine residue hinders the interaction of HIF1 with transcriptional co-activators such as CBP and p300 and thereby represses its transcriptional activity (Lando et al. 2002). Upon hypoxia, reduced oxygen availability leads to accumulation of HIF1, and restored interaction with CBP and p300, allowing it to activate its target genes (Lando et al. 2002).

4 Perception of Oxygen Availability in Plants Is Regulated via the Oxygen-Dependent Branch of the N-End Rule Pathway (NERP)

To understand which transcriptional regulators are involved in the anaerobic response to oxygen deficiency in plants, the expression of TF-encoding genes was analyzed using microarrays and qPCR platforms in different plant species, such as

Arabidopsis thaliana (Licausi et al. 2011b; Branco-Price et al. 2005), rice *Oryza sativa* (Lasanthi-Kudahettige et al. 2007), poplar *Populus x canescens* (Kreuzwieser et al. 2009), and cotton *Gossypium hirsutum* L. (Christianson et al. 2010). Among the TF gene families conservatively induced in response to hypoxia, the Ethylene Response Factor (ERF) family attracted the attention of several research groups (Licausi et al. 2010; Hinz et al. 2010). Group VII in particular has been strongly associated with submergence in rice (Xu et al. 2006; Hattori et al. 2009). Indeed, other studies showed that members of this group can activate the expression of the hypoxic genes upon low oxygen (Licausi et al. 2010; Hinz et al. 2010). The unique feature of this group is the highly conserved N-terminal MCGGAI(I/L) motif, which was shown to dictate the stability of proteins belonging to the ERF group VII via the N-end rule in an oxygen-dependent manner (Gibbs et al. 2011; Licausi et al. 2011b). This provided the first demonstration of a mechanism by which plants sense oxygen and regulate the anaerobic response. Among the ERF-VII transcription factors, the constitutively transcribed RAP2.2 and RAP2.12 have been suggested to trigger the initial induction of the anaerobic response (Bailey-Serres et al. 2012).

Upon reoxygenation, ERF VII proteins are quickly degraded via the NERP to repress the anaerobic response. In more detail (Fig. 1), the conserved N-terminal motive MCGGAI/L allows the methionine amino peptidase (MAP) to cleave of the methionine and leave the cysteine exposed (Bradshaw et al. 1998). The sulfur atom on the Cys residue is subsequently oxidized in presence of oxygen yielding cysteine sulfinic or sulfonic acid (Hess et al. 2005). The oxidized cysteine, in turn, is recognized by the arginine-transferases ATE1 and ATE2, which add an arginine to the N-terminus via a peptide bond. Finally, this is recognized by the E3 ligase PRT6 that polyubiquitinates the protein and causes its degradation via the proteasome (Garzón et al. 2007). Indeed, mutants of the enzymes involved in the N-end rule such as *ate1* and *prt6* showed an up-regulation of core hypoxic genes in normoxia and a stronger up-regulation upon hypoxia (Gibbs et al. 2011; Licausi et al. 2011b), providing the first evidence of the involvement of the N-end rule in hypoxia signaling. In addition, Licausi et al. (2011b) showed that transgenic plants expressing a truncated version of RAP2.12 that lacked the conserved N-terminus upregulate the hypoxic genes even in aerobic conditions. In summary, the penultimate cysteine oxidation requires oxygen and this makes the stability of the ERF VII proteins oxygen-dependent: when the oxygen availability decreases, cysteine oxidation is reduced and the ERF VII proteins are stabilized and activate the hypoxic response. To test if ERF group VII members in *Arabidopsis* are substrates of the NERP, Gibbs et al (2011) tested their stability by performing a heterologous rabbit reticulocyte lysate assay. This in vitro assay showed that all five members are degraded by the proteasome (Gibbs et al. 2011). However, when the cysteine was substituted for an alanine their protein degradation via the NERP was abolished. This experiment strongly indicates that the oxidation of the penultimate cysteine is the key determinant of ERF VII stability (Gibbs et al. 2011; Licausi et al. 2011b).

Interestingly, a member of the ERF VII family in rice, SUB1A, was found to be uncoupled from the N-end rule (Gibbs et al. 2011). This may be explained due to

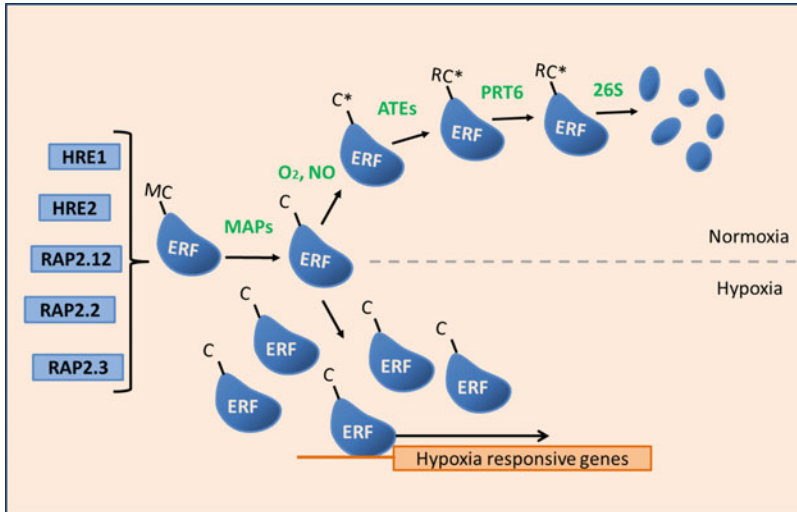


Fig. 1 The induction of the hypoxic response is regulated via the N-end rule pathway for protein degradation. During normoxic conditions ERF group VII TFs are degraded via the N-end rule pathway. The first step is carried out by the methionine aminopeptidase (MAP) which removes the methionine residue, to expose free cysteine for further oxidation. The oxidized form of the cysteine is recognized by the Arginine transferase (ATE) which conjugates an arginine residues to the protein. This in turn triggers the Proteolysis 6 (PRT6) to polyubiquitinate the protein and causes the protein to be degraded by the 26S proteasome. When oxygen becomes limited during hypoxia, cysteine oxidation is reduced and the oxygen-dependent branch of the N-end rule pathway is inhibited. Therefore, ERFs can induce hypoxia response in plants to shift the metabolism from aerobic to fermentation, which results in increased tolerance and survival of plants

the absence of a lysine in the protein sequence downstream of the N-terminus which is, required for its ubiquitination. Another explanation is based on the speculation that the tertiary structure of the SUB1A protein protects it from the N-end rule. Consequently, the higher stability of SUB1A has been associated with submergence tolerance in rice.

5 Role of Acyl-CoA-Binding Proteins (ACBP) in Oxygen Sensing

When Licausi et al. (2011b) studied the subcellular localization of RAP2.12, they found that, in normoxic condition, this protein is localized at the membrane (Fig. 2). This was surprising, since RAP2.12 does not contain any hydrophobic domains. However, former reports in the literature indicated that members of the ERF-VII family can interact with membrane-bound acyl-CoA-binding proteins (ACBPs) (Li and Chye 2004). Using yeast-two-hybrid and biomolecular fluorescence complementation, Licausi et al. (2011b) demonstrated that indeed RAP2.12

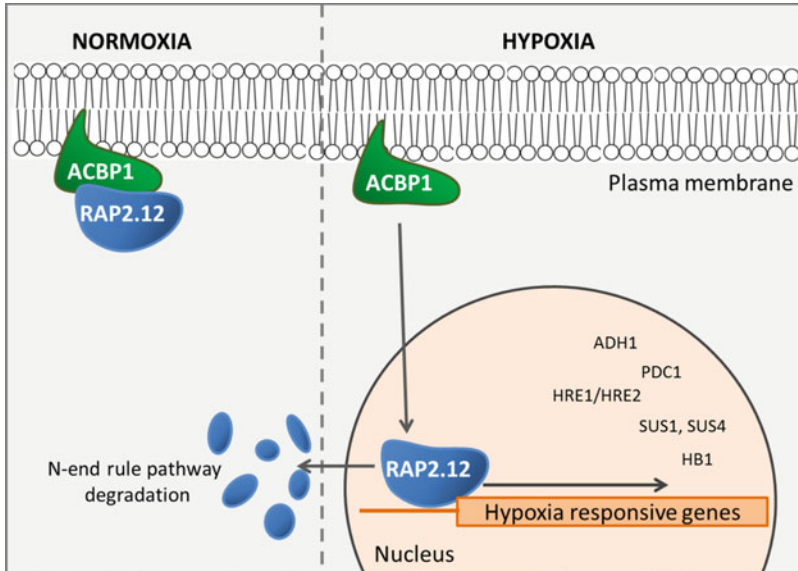


Fig. 2 During hypoxia, RAP2.12 is moving from membrane to the nucleus to trigger the hypoxia response. In normoxic condition RAP2.12 interacts with Acyl-CoA-Binding Proteins (ACBP) and therefore localizes to the membrane. As soon as the oxygen availability in the cell decreases, RAP2.12 moves to the nucleus to induce hypoxia responsive genes such as Hemoglobin1 (HB1), Alcohol Dehydrogenase (ADH1), Pyruvate Decarboxylase (PDC1), Sucrose Synthase 1 and 4 (SUS1 and SUS4), and Hypoxia Responsive Factors 1 and 2 (HRE1 and HRE2). When oxygen level again increases to normoxic condition (reoxygenation), RAP2.12 is degraded via N-end rule pathway

can interact with ACBP1 and ACBP2. Upon hypoxia, RAP2.12 was found to localize to the nucleus suggesting that this translocation from the membrane into the nucleus depends on oxygen as well (Licausi et al. 2011b). It still remains unclear, whether ACBP moves to the nucleus together with RAP2.12, or if, upon low oxygen, RAP2.12 dissociates from the ACBPs and moves to the nucleus alone. The latter hypothesis is supported by the fact that ACBPs contain a membrane-binding domain and therefore is not expected to enter to the nucleus (Li et al. 2003).

Until now, it is still not known whether the interaction of RAP2.12 with the ACBPs is required to prevent its degradation in normoxic conditions or if additional factors are involved. The presence of RAP2.12:GFP at the membrane even under normoxic conditions indicated that a reservoir of RAP2.12 is accumulated in the cell. This reservoir of RAP2.12 units might represent a mechanism which allows plant to respond quickly to a decline in oxygen levels via the relocalization of RAP2.12 from the membrane into the nucleus, instead of relying on *de novo* translation. Indeed the observation that the induction of the hypoxic genes occurs already after 30 min of hypoxia supports this hypothesis (Licausi et al. 2011b).

6 Role of Nitric Oxide in the N-End Rule Pathway

In animals, nitric oxide (NO) is produced by NO synthases and plays a role in a vast range of processes including glycolysis, apoptosis, and cardiovascular homeostasis (Ignarro 2002; Packer et al. 2003; Barouch et al. 2002). Part of NO's effect in animals is mediated by its ability to modify amino acids within proteins (Hess et al. 2005). In particular the role of NO on Cys residues has been extensively studied. It has been shown that NO can convert Cys residues to S-nitrosothiols, via a process called S-nitrosylation (Hu et al. 2005). This reaction alone can directly affect protein function, but in other cases, such as within the N-end rule pathway, additional oxygen-dependent reactions must proceed to yield oxidized Cys (cysteine-sulphinic or sulphonic acid) (Hess et al. 2005)

The Cys-dependent branch of the N-end rule in animals has been characterized as a NO-dependent developmental program. Similarly to the ERF-VII in Arabidopsis, the *Mus musculus* RGS4, RGS5, and RGS16, which are involved in angiogenesis and other tubulogenesis pathways (Kwon et al. 2002) are directed towards proteolysis when their penultimate cysteine residue is oxidized in the concomitant presence of NO and oxygen (Lee et al. 2005; Hu et al. 2005). In fact, in vivo reduction of NO levels through inhibition of NO synthases or direct addition of NO scavengers was shown to increase RGS4 protein levels (Hu et al. 2005), indicating that both NO and O₂ are required for RGS4 degradation via the N-end rule pathway.

In plants, NO has also been implicated to play a role in a wide range of processes, which include pathogen defense, root nodule symbiosis, growth, and development (Wendehenne et al. 2001). Whether NO also affects the arginylation pathway of the N-end rule in plants is not known. Interestingly, low oxygen leads to a strong increase in NO levels (Dordas et al. 2003). As NO was shown to promote arginylation of MC proteins in animals, this could theoretically lead to their destabilization upon low oxygen. At least for the ERF-VII proteins involved in oxygen sensing, this appears to be not the case since they, instead, were shown to be stabilized upon hypoxia (1 % O₂) (Licausi et al. 2011b). Perhaps at these strong hypoxic conditions, it is not NO, but oxygen which becomes the limiting factor. In plants, class 1 hemoglobins were shown to modulate NO levels (Perazzolli et al. 2004) and it may be speculated that through their role in NO homeostasis (Dordas et al. 2003), they may also play a role in Cys oxidation. Interestingly, atHb1 was shown to be a direct target of RAP2.12 and belongs to the core genes which are strongly upregulated in response to hypoxia (Licausi et al. 2011b). Since hypoxia is associated with a strong increase in NO that could potentially promote RAP2.12 degradation, it may be speculated that class 1 hemoglobins boost and support the molecular response to hypoxia by protecting RAP2.12 from degradation (see also Chap. 3, Igamberdiev et al. 2014). According to this hypothesis, it would be expected that AtHb1 overexpressors have a stronger induction of the anaerobic genes in response to hypoxia. This appeared to be not the case: while wild-type Arabidopsis seeds exposed to mild hypoxia (10 % O₂) strongly induce the hypoxic genes, AtHb1 overexpressors show a more moderate induction of these genes (Thiel et al. 2011).

Interestingly though, AtHb1 overexpression increased survival of Arabidopsis seedlings following hypoxia (Hunt et al. 2002). Taken together, these experiments do not support a role for class 1 hemoglobin in control of RAP2.12 stability.

7 Fine Tuning of Oxygen Sensing in Plants is Mediated by Additional Factors

In addition to hemoglobin and NO signaling, other factors can be indirectly involved in the regulation of oxygen homeostasis and signaling in the cell. A very popular example is the phytohormone ethylene. Since gas diffusion is 10,000 times slower in water than in air, under submergence conditions ethylene is trapped and accumulates in the intracellular spaces of plant tissues (Voesenek et al. 1993). This accumulation of ethylene has been shown to promote submergence escape strategies and plays a crucial role in flooding tolerance in rice (Jackson et al. 1985; Voesenek et al. 2004).

Other molecules that change consequentially with a decline in oxygen are also thought to play a role in oxygen sensing. Examples of such molecules include: ROS, pH, and energy status. A paradigmatic example of such an indirect effect is provided by pH on the regulation of fermentation. In fact, initial regeneration of NAD⁺ to sustain glycolysis is provided by lactic fermentation (Drew 1997). The accumulation of lactic acid, together with the inhibition of proton extrusion pumps as a consequence of energy crisis, lowers the cytosolic pH to an optimal level for PDC activity, therefore channeling pyruvate towards ethanolic fermentation (Davies 1980). Ethanol production is less harmful in comparison to lactate accumulation as it does not affect pH levels. Pyruvate consumption is also beneficial because this metabolite has been shown to stimulate oxygen consumption via respiration. A decrease in pyruvate levels may therefore aid to reduce respiration and protect the plant tissue from reaching anoxic conditions (Zabalza et al. 2009).

Upon hypoxia, local changes in the Ca²⁺ level occur. In fact, low oxygen levels stimulate the accumulation of cytosolic calcium which is required for induction of ADH1 (Subbaiah et al. 1994), one of the core- hypoxia responsive genes. Interestingly, Ca²⁺ is also involved in the signal transduction that leads to ethylene biosynthesis (He et al. 1996). In addition, changes in calcium level inside the cell can alter gene response, which imply that hypoxia response can be indirectly calcium-dependent.

Microarray studies indicated that also oxidative stress-related genes are induced by oxygen deficiency (Klok et al. 2002; Loreti et al. 2005; Branco- Price et al. 2008; Blokhina et al. 2010). Additionally, the transient accumulation of ROS was shown to be a common response to both anoxia and heat stress (Banti et al. 2008, 2010). Accordingly, Arabidopsis plants overexpressing Heat Shock Factor A2 (HSFA2), a sensor for ROS (Miller et al. 2006), showed increased tolerance to anoxic conditions (Banti et al. 2010). Anoxia induces ROS production via plasma membrane localized NADPH oxidases as well as in mitochondria by affecting the electron

transport chain. Baxter-Burrell et al. (2002) proved that the accumulation of hydrogen peroxide (H₂O₂) under low O₂ is required for the expression and activity of alcohol dehydrogenase (ADH) and thus tolerance.

8 Concluding Remarks

Looking at the recently identified plant oxygen-sensing pathway, one cannot avoid noticing its striking functional similarity with the animal mechanism. In fact, both systems rely on the oxygen-dependent regulation of the stability of a master regulator of the anaerobic response. Instead, the pathways downstream activated are extremely divergent between plants and animals, as is expected by the intrinsic properties of each kingdom (autotrophy versus heterotrophy, stillness against motility). Since the MC-branch of the N-end rule pathway exists in both animals and plants, it is tempting to speculate that this mechanism may represent an ancient oxygen perception mechanism, while an additional one, based on HIF-1, was evolved later in animals. However, the functional homology shared by the two mechanisms suggests that a strategy based on oxygen-controlled proteolysis is optimal in both kingdoms.

No orthologs of pVHL, HIF-1, or PHD has been identified in green organisms. Plants do possess a plethora of prolyl hydroxylase enzymes, some of them also low oxygen-inducible (Mustroph et al. 2010). However, the plant PHDs have been described as involved in cell wall or peptide modifications (Velasquez et al. 2011), rather than in the regulation of the hypoxic response. The fact that molecular oxygen is anyway required by PHD to catalyze the hydroxylation of proline residues has led to the speculation that they may still be involved in oxygen-dependent signaling (Vlad et al. 2007).

Both HIF-1 and RAP2.12 are destabilized in the presence of oxygen. Interestingly, in animals the rate of HIF-1 mRNA translation into protein depends on Target of Rapamycin (mTOR), a protein known to sense cellular energy status through the AMP-activated protein kinase (AMPK) (Wullschleger et al. 2006). An ortholog of the yeast/animal mTOR pathway exists in plants, and has been shown to be directly involved in the control of diverse cellular processes such as autophagy, protein translation, ribosome biogenesis, and actin dynamics (Wullschleger et al. 2006). Until now however, the relation between the mTOR signalling pathway and low oxygen in plants has not been studied and represents an interesting research perspective. Concerning RAP2.12 degradation, one of the most crucial questions deals with the oxidation of its penultimate cysteine: does it occur spontaneously or is it catalyzed enzymatically? Enzymes with cysteine oxidase activity exist in animals and are known to catalyze the oxidation of the free amino acid cysteine to cysteine sulfinic acid. These cysteine dioxygenases are highly induced upon dietary consumption of cysteine and function in maintaining cysteine homeostasis (Stipanuk et al. 2008). *Arabidopsis thaliana* possesses five genes that contain motifs associated with thiol oxidation. The function of these genes has not been studied, but it is known that two of these are strongly induced upon hypoxia (Mustroph

et al. 2010). This makes these genes an interesting starting point to look for enzymes with the capacity to oxidize penultimate cysteine. On the other hand, spontaneous oxidation of cysteine *in vitro* has been reported (Stipanuk et al. 2009). However, the presence of several anti-oxidants and reductants within the cell keep its redox status highly reduced (Kamata and Hirata 1999). Therefore, spontaneous oxidation of cysteine is not likely to occur at high rates within the cell.

Following the finding of oxygen-sensing mechanisms in bacteria, fungi, and animals, also in plants an oxygen-sensing mechanism has now been identified. This mechanism, like the one described in animals, relies on the oxygen-dependent regulation of the protein stability of a master regulator of the hypoxic response. Nevertheless, there are still lots of open questions which provide exciting new research perspectives for the future.

References

- Alexeeva S, Hellingwerf KJ, Teixeira de Mattos MJ (2003) Requirement of ArcA for redox regulation in *Escherichia coli* under microaerobic but not anaerobic or aerobic conditions. *J Bacteriol* 185(1):204–209
- Bailey-Serres J, Voesenek LACJ (2008) Flooding stress: acclimations and genetic diversity. *Annu Rev Plant Biol* 59:313–339
- Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voesenek LA, van Dongen JT (2012) Making sense of low oxygen sensing. *Trends Plant Sci* 17(3):129–138
- Banti V, Loreti E, Novi G, Santaniello A, Alpi A, Perata P (2008) Heat acclimation and cross-tolerance against anoxia in *Arabidopsis*. *Plant Cell Environ* 31:1029–1037
- Banti V, Mafessoni F, Loreti E, Alpi A, Perata P (2010) The heat-inducible transcription factor HsfA2 enhances anoxia tolerance in *Arabidopsis*. *Plant Physiol* 152:1471–1483
- Barouch LA, Harrison RW, Skaf MW, Rosas GO, Cappola TP, Kobeissi ZA, Hobai IA, Lemmon CA, Burnett AL, O'Rourke B, Rodriguez ER, Huang PL, Lima JA, Berkowitz DE, Hare JM (2002) Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. *Nature* 416(6878):337–339
- Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J (2002) RopGAP4-dependent Rop GTPase rheostat control of *Arabidopsis* oxygen deprivation tolerance. *Science* 296(5575):2026–2028
- Blokhina O, Fagerstedt KV (2010) Oxidative metabolism, ROS and NO under oxygen deprivation. *Plant Physiol Biochem* 48:359–373
- Bradshaw RA, Brickey WW, Walker KW (1998) N-terminal processing: the methionine aminopeptidase and N α -acetyl transferase families. *Trends Biochem Sci* 23:263–267
- Branco-Price C, Kawaguchi R, Ferreira RB, Bailey-Serres J (2005) Genome-wide analysis of transcript abundance and translation in *Arabidopsis* seedlings subjected to oxygen deprivation. *Ann Bot* 96(4):647–660
- 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*. *Plant J* 56:743–755
- Christianson JA, Llewellyn DJ, Dennis ES, Wilson IW (2010) Global gene expression responses to waterlogging in roots and leaves of cotton (*Gossypium hirsutum* L.). *Plant Cell Physiol* 51(1):21–37
- Da Re S, Bertagnoli S, Fournent J, Reyrat JM, Kahn D (1994) Intramolecular signal transduction within the FixJ transcriptional activator: *in vitro* evidence for the inhibitory effect of the phosphorylatable regulatory domain. *Nucleic Acids Res* 22:1555–1561

- Davies DDD (1980) Anaerobic metabolism and the production of organic acids. In: Davies DDD (ed) *The biochemistry of plants*, vol 2. Academic, New York, pp 581–611
- Delgado-Nixon VM, Gonzalez G, Gilles-Gonzalez MA (2000) Dos, a heme-binding PAS protein from *Escherichia coli* is a direct oxygen sensor. *Biochemistry* 39:2685–2691
- Dordas C, Hasinoff BB, Igamberdiev AU, Manac'h N, Rivoal J, Hill RD (2003) Expression of a stress-induced hemoglobin affects NO levels produced by alfalfa root cultures under hypoxic stress. *Plant J* 35(6):763–770
- Drew MC (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annu Rev Plant Physiol Plant Mol Biol* 48:223–250
- Fischer HM (1994) Genetic regulation of nitrogen fixation in rhizobia. *Microbiol Rev* 58(3):352–386
- Garzón M, Eifler K, Faust A, Scheel H, Hofmann K, Koncz C, Yephremov A, Bachmair A (2007) PRT6/At5g02310 encodes an Arabidopsis ubiquitin ligase of the N-end rule pathway with arginine specificity and is not the CER3 locus. *FEBS Lett* 581(17):3189–3196
- Geigenberger P (2003) Response of plant metabolism to too little oxygen. *Curr Opin Plant Biol* 6:247–256
- Georgellis D, Kwon O, Lin EC (1999) Amplification of signaling activity of the Arc two-component system of *Escherichia coli* by anaerobic metabolites. An in vitro study with different protein modules. *J Biol Chem* 274:35950–35954
- Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW, Correia CS, Corbineau F, Theodoulou FL, Bailey-Serres J, Holdsworth MJ (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. *Nature* 479(7373):415–418
- Gilles-Gonzalez MA, Gonzalez G, Sousa EHS, Tuckerman J (2008) Oxygen-sensing histidine-protein kinases: assays of ligand binding and turnover of response-regulator substrates. *Methods Enzymol* 437:173–189
- Gong W, Hao B, Chan MK (2000) New mechanistic insights from structural studies of the oxygen-sensing domain of Bradyrhizobium japonicum FixL. *Biochemistry* 39(14):3955–3962
- Greenway H, Gibbs J (2009) Review: mechanisms of anoxia tolerance in plants. II. Energy requirements for maintenance and energy distribution to essential processes. *Funct Plant Biol* 30(10):999–1036
- Guest JR, Green J, Irvine AS, Spiro S (1996) The FNR modulon and FNR-regulated gene expression. In: Lin ECC, Lynch AS (eds) *Regulation of gene expression in Escherichia coli*. RG Landes and Co, Austin, TX, pp 317–342
- Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H, Ashikari M (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature* 460(7258):1026–1030
- He CJ, Morgan PW, Drew MC (1996) Transduction of an ethylene signal is required for cell death and lysis in the root cortex of maize during aerenchyma formation induced by hypoxia. *Plant Physiol* 112(2):463–472
- Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS (2005) Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* 6(2):150–166
- Hinz M, Wilson IW, Yang J, Buerstenbinder K, Llewellyn D, Dennis ES, Sauter M, Dolferus R (2010) Arabidopsis RAP2.2: an ethylene response transcription factor that is important for hypoxia survival. *Plant Physiol* 153(2):757–772
- Hu RG, Sheng J, Qi X, Xu Z, Takahashi TT, Varshavsky A (2005) The N-end rule pathway as a nitric oxide sensor controlling the levels of multiple regulators. *Nature* 437(7061):981–986
- Hunt PW, Klok EJ, Trevaskis B, Watts RA, Ellis MH, Peacock WJ, Dennis ES (2002) Increased level of hemoglobin 1 enhances survival of hypoxic stress and promotes early growth in Arabidopsis thaliana. *Proc Natl Acad Sci U S A* 99(26):17197–17202
- Igamberdiev AU, Stasolla C, Hill RD (2014) Low oxygen stress, non-symbiotic hemoglobins, NO and programmed cell death. In: van Dongen JT, Licausi F (eds) *Low oxygen stress in plants*. Springer, Heidelberg

- Ignarro LJ (2002) Nitric oxide as a unique signalling molecule in the vascular system: a historical overview. *J Physiol Pharmacol* 53:503–514
- Iuchi S, Lin EC (1988) *arcA* (dye), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. *Proc Natl Acad Sci U S A* 85:1888–1892
- Iuchi S, Cameron DC, Lin EC (1989) A second global regulator gene (*arcB*) mediating repression of enzymes in aerobic pathways of *Escherichia coli*. *J Bacteriol* 171:868–873
- Jackson MB (1985) Ethylene and responses of plants to soil waterlogging and submergence. *Annu Rev Plant Physiol* 36:145–174
- Kallio PJ, Pongratz I, Gradin K, McGuire J, Poellinger L (1997) Activation of hypoxia-inducible factor 1 α : posttranscriptional regulation and conformational change by recruitment of the Arnt transcription factor. *Proc Natl Acad Sci U S A* 94:5667–5672
- Kamata H, Hirata H (1999) Redox regulation of cellular signalling. *Cell Signal* 11(1):1–14
- Khoroshilova N, Beinert H, Kiley PJ (1995) Association of a polynuclear iron-sulfur center with a mutant FNR protein enhances DNA binding. *Proc Natl Acad Sci USA* 92:2499–2503
- Kiley PJ, Beinert H (2003) The role of Fe-S proteins in sensing and regulation in bacteria. *Curr Opin Microbiol* 6(2):181–185
- Klok EJ, Wilson IW, Wilson D, Chapman SC, Ewing RM, Somerville SC, Peacock WJ, Dolferus R, Dennis ES (2002) Expression profile analysis of the low oxygen response in *Arabidopsis* root cultures. *Plant Cell* 14(10):2481–2494
- Körner H, Sofia HJ, Zumft WG (2003) Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. *FEMS Microbiol Rev* 27:559–592
- Kreuzwieser J, Hauberg J, Howell KA, Carroll A, Rennenberg H, Millar AH, Whelan J (2009) Differential response of gray poplar leaves and roots underpins stress adaptation during hypoxia. *Plant Physiol* 149(1):461–473. doi:10.1104/pp.108.125989
- Kwon YT, Kashina AS, Davydov IV, Hu RG, An JY, Seo JW, Du F, Varshavsky A (2002) An essential role of N-terminal arginylation in cardiovascular development. *Science* 297(5578):96–99
- Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK (2002) FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev* 16:1466–1471
- Lasanthi-Kudahettige R, Magneschi L, Loreti E, Gonzali S, Licausi F, Novi G, Beretta O, Vitulli F, Alpi A, Perata P (2007) Transcript profiling of the anoxic rice coleoptile. *Plant Physiol* 144(1):218–231
- Lee MJ, Tasaki T, Moroi K, An JY, Kimura S, Davydov IV, Kwon YT (2005) RGS4 and RGS5 are *in vivo* substrates of the N-end rule pathway. *Proc Natl Acad Sci U S A* 102(42):15030–15035
- Li HY, Chye ML (2003) Membrane localization of *Arabidopsis* acyl-CoA binding protein ACBP2. *Plant Mol Biol* 51(4):483–492
- Li HY, Chye ML (2004) *Arabidopsis* Acyl-CoA-binding protein ACBP2 interacts with an ethylene-responsive element-binding protein, AtEBP, via its ankyrin repeats. *Plant Mol Biol* 54(2):233–243
- Licausi F, Perata P (2009) Low oxygen signaling and tolerance in plants. *Adv Bot Res* 50:189–198
- Licausi F, van Dongen JT, Giuntoli B, Novi G, Santaniello A, Geigenberger P, Perata P (2010) HRE1 and HRE2, two hypoxia-inducible ethylene response factors, affect anaerobic responses in *Arabidopsis thaliana*. *Plant J* 62(2):302–315
- Licausi F, Weits DA, Pant BD, Scheible WR, Geigenberger P, van Dongen JT (2011a) Hypoxia responsive gene expression is mediated by various subsets of transcription factors and miRNAs that are determined by the actual oxygen availability. *New Phytol* 190(2):442–456
- Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LA, Perata P, van Dongen JT (2011b) Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. *Nature* 479(7373):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 Physiol* 137:1130–1138
- Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399(6733):271–275
- Miller G, Mittler R (2006) Could heat shock transcription factors function as hydrogen peroxide sensors in plants? *Ann Bot* 98(2):279–288
- Mustroph A, Lee SC, Oosumi T, Zanetti ME, Yang H, Ma K, Yaghoubi-Masihi A, Fukao T, Bailey-Serres J (2010) Cross-kingdom comparison of transcriptomic adjustments to low-oxygen stress highlights conserved and plant-specific responses. *Plant Physiol* 152(3):1484–1500
- Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, Pavletich N, Chau V, Kaelin WG (2000) Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. *Nat Cell Biol* 2(7):423–427
- Packer MA, Stasiv Y, Benraiss A, Chmielnicki E, Grinberg A, Westphal H, Goldman SA, Enikolopov G (2003) Nitric oxide negatively regulates mammalian adult neurogenesis. *Proc Natl Acad Sci U S A* 100(16):9566–9571
- Perazzolli M, Dominici P, Romero-Puertas MC, Zago E, Zeier J, Sonoda M, Lamb C, Delledonne M (2004) *Arabidopsis* nonsymbiotic hemoglobin AHb1 modulates nitric oxide bioactivity. *Plant Cell* 16(10):2785–2794
- Rodriguez C, Kwon O, Georgellis D (2004) Effect of D-lactate on the physiological activity of the ArcB sensor kinase in *Escherichia coli*. *J Bacteriol* 186:2085–2090
- Stipanuk MH, Dominy JE Jr, Ueki I, Hirschberger LL (2008) Measurement of cysteine dioxygenase activity and protein abundance. *Curr Protoc Toxicol* 38:6.15.1–6.15.25
- Stipanuk MH, Ueki I, Dominy JE Jr, Simmons CR, Hirschberger LL (2009) Cysteine dioxygenase: a robust system for regulation of cellular cysteine levels. *Amino Acids* 37(1):55–63
- Subbiah CC, Bush DS, Sachs MM (1994) Elevation of cytosolic calcium precedes anoxic gene expression in maize suspension-cultured cells. *Plant Cell* 6:1747–1762
- Summers JE, Ratcliffe RG, Jackson MB (2000) Anoxia tolerance in the aquatic monocot *Potamogeton pectinatus*: absence of oxygen stimulates elongation in association with an unusually large pasture effect. *J Exp Bot* 51:1413–1422
- Tadege M, Dupuis II, Kuhlemeier C (1999) Ethanolic fermentation: new functions for an old pathway. *Trends Plant Sci* 4:320–325
- Taylor BL, Zhulin IB (1999) PAS domains: internal sensors of oxygen, redox potential, and light. *Microbiol Mol Biol Rev* 63(2):479–506
- Thiel J, Rolletschek H, Friedel S, Lunn JE, Nguyen TH, Feil R, Tschiersch H, Müller M, Borisjuk L (2011) Seed-specific elevation of non-symbiotic hemoglobin AtHb1: beneficial effects and underlying molecular networks in *Arabidopsis thaliana*. *BMC Plant Biol* 11:48
- Uden G, Bongaerts J (1997) Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors. *Biochim Biophys Acta* 1320(3):217–234
- 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. *Ann Bot* 103(2):269–280
- Velasquez SM, Ricardi MM, Dorosz JG, Fernandez PV, Nadra AD, Pol-Fachin L, Egelund J, Gille S, Harholt J, Ciancia M, Verli H, Pauly M, Bacic A, Olsen CE, Ulvskov P, Petersen BL, Somerville C, Iusem ND, Estevez JM (2011) O-glycosylated cell wall proteins are essential in root hair growth. *Science* 332(6036):1401–1403
- Vlad F, Spano T, Vlad D, Daher FB, Ouelhadj A, Fragkostefanakis S, Kalaitzis P (2007) Involvement of *Arabidopsis* prolyl 4 hydroxylases in hypoxia, anoxia and mechanical wounding. *Plant Signal Behav* 2(5):368–369

- Voesenek L, Banga M, Thier RH, Mudde CM, Harren F, Barendse G, Blom C (1993) Submergence-induced ethylene synthesis, entrapment and growth in two plant species with contrasting flooding resistances. *Plant Physiol* 103:783–791
- Voesenek LACJ, Rijnders JHGM, Peeters AJM, van de Steeg HM, de Kroon H (2004) Plant hormones regulate fast shoot elongation underwater: from genes to communities. *Ecology* 85:16–27
- Wang GL, Jiang B-H, Rue EA, Semenza GL (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A* 92:5510–5514
- Wendehenne D, Pugin A, Klessig DF, Durner J (2001) Nitric oxide: comparative synthesis and signaling in animal and plant cells. *Trends Plant Sci* 6(4):177–183
- Wullschlegel S, Loewith R, Hall MN (2006) TOR signaling in growth and metabolism. *Cell* 124(3):471–484
- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* 442(7103):705–708
- Zabalza A, van Dongen JT, Froehlich A, Oliver SN, Faix B, Gupta KJ, Schmäzlin E, Igal M, Orcaray L, Royuela M, Geigenberger P (2009) Regulation of respiration and fermentation to control the plant internal oxygen concentration. *Plant Physiol* 149(2):1087–1098

Oxidative Stress Components Explored in Anoxic and Hypoxic Global Gene Expression Data

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Abstract Global gene expression data were analyzed to search for the genes related to oxidative stress response, to examine the differences between hypoxia and anoxia, and to reveal new components of oxygen deprivation response escaped from the previous analyses. Gene Set Z-score (GSZ) was used to report gene ontology (GO) classes that showed significant regulation and also partial up- and downregulation in Arabidopsis anoxic and hypoxic microarray data sets. Under both anoxia and hypoxia significant upregulation was reported for anaerobic respiration, response to low oxygen levels, and response to hypoxia. Comparable high GSZ scores were shown for several oxidative stress-related GO classes and for functional groups of biological processes known to involve oxygen radical formation such as: cellular respiration, wounding, and response to high light and UV-B. Availability of oxygen in hypoxic experimental sets was marked by upregulation of several oxygenases, including ACC-oxidase responsible for ethylene synthesis. Consistent strong induction of several Fe-dependent ketoglutarate oxygenases (FeKGO) in the majority of hypoxic conditions analyzed suggests an important and yet unidentified function for these enzymes. Based on metabolic and gene expression studies we suggest that FeKGO may function in a bypass route for part of the TCA cycle (citrate-isocitrate) inhibited under hypoxia. This would incorporate 2-ketoglutarate supplied by activated GABA shunt and form succinate, a TCA cycle and mitochondrial electron transport chain substrate. FeKGO turnover is sustained by the putative route coupled to ascorbate–monodehydroascorbate cycling and hemoglobin-dependent NO elimination. The analysis strongly supports earlier findings that formation of activated oxygen and oxidative stress is an integral part of the response to oxygen deprivation. Several novel functional gene groups

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were highlighted by the analysis: upregulation of cysteamine dioxygenase activity and FeKGO and downregulation of circadian rhythm-related genes.

1 Introduction

During the past 2 decades reactive oxygen species (ROS) have developed in our minds from damaging stress molecules to stress-signaling agents important for the development of stress responses in practically all stresses that plants have to endure (Bailey-Serres and Chang 2005; Van Breusegem et al. 2008; Jaspers and Kangasjärvi 2010; Petrov and Van Breusegem 2012). The presence of ROS under oxygen deprivation stress is somewhat a paradox and indeed some oxygen is needed for ROS production under oxygen deprivation stress, and hence it is preferable to talk about severe hypoxia in most cases, and of a reoxygenation period when atmospheric oxygen conditions are again introduced (Branco-Price et al. 2008). The biochemistry behind ROS production and antioxidative defense as well as the damage produced under oxygen deprivation is well documented (Yan et al. 1996; Biemelt et al. 1998, 2000; Blokhina et al. 2000, 2001, 2003; Fukao and Bailey-Serres 2004; Santosa et al. 2007). The evidence for the regulatory role of ROS under oxygen deprivation in the control of gene expression (Pucciariello et al. 2012), the negative feedback regulation of H₂O₂ levels by Rop–RopGAP4 interaction (Baxter-Burrell et al. 2002), and the activation of MAPK kinases in response to mitochondrial ROS resulting in better survival under hypoxia (Chang et al. 2012), all point to a complex relationship between hypoxic metabolic rearrangements, ROS levels, and their cellular localization and affect the physiological outcome of oxidative stress. Naturally, the most important processes from the plant's point of view are the adaptations preserving the adenylate energy charge (Greenway and Gibbs 2003; Bailey-Serres and Voeselek 2008; Lee et al. 2011). During the past few years multiple routes have been elucidated for the production and consumption of ATP such as the pyrophosphate-dependent glycolysis (Huang et al. 2008), nitrate-dependent ATP synthesis under oxygen deprivation (Stoimenova et al. 2007), and ATP hydrolysis in animal mitochondria under anaerobic conditions (St-Pierre et al. 2000). On the other hand, bulky plant organs seem to avoid total anoxia in the tissues by metabolic control of respiration (Borisjuk et al. 2007; Zabalza et al. 2009).

It has been reassuring to note, as we show in this chapter, that bioinformatics analysis of oxygen deprivation arrays picked up multiple classes related to oxidative stress and ROS. We have also noticed that many different stresses such as high light and wounding are leading to the upregulation of oxidative stress-related genes also shared by oxygen deprivation array data.

In addition to ROS, during the recent years a vast amount of data has accumulated in favor of reactive nitrogen species (RNS) in plant tissues and their regulative role in adjusting metabolic events (Qiao and Fan 2008; Igamberdiev et al. 2010; Gupta et al. 2011a, b; Hebelstrup et al. 2012). Plant non-symbiotic hemoglobins are