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Invited Expert Review

New Role for an Old Rule: N-end Rule-Mediated Degradation of Ethylene Responsive Factor Proteins Governs Low Oxygen Response in Plants[F]

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Abstract

The N-end rule pathway regulates protein degradation, which depends on exposed N-terminal sequences in prokaryotes and eukaryotes. In plants, conserved and specific enzymes stimulate selective proteolysis. Although a number of developmental and growth phenotypes have been reported for mutants in the N-end rule, its function has remained unrelated to specific physiological pathways. The first report of the direct involvement of the N-end rule in stress responses focused on hypoxic signaling and how the oxygen-dependent oxidation of cystein promotes the N-end rule-mediated degradation of ethylene responsive factor (ERF)-VII proteins, the master regulators of anaerobic responses. It has been suggested that plants have evolved specific mechanisms to tune ERF-VII availability in the nucleus. In this review, we speculate that ERF-VII proteins are reversibly protected from degradation via membrane sequestration. The oxidative response in plants subjected to anoxic conditions suggests that reactive oxygen and nitrogen species (reactive oxygen species and reactive nitrogen species) may interact or interfere with the N-end rule pathway-mediated response to hypoxia.

Keywords: Ethylene response factor; flooding; hypoxia; N-end rule pathway; ubiquitin.

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Introduction

N-end Rule Pathway for Proteolysis

Protein abundance in a cell is regulated by the rate of protein synthesis and degradation (Li et al. 2012). Although certain cell types possess specific organelles dedicated to protein degradation (Swansona et al. 1998; Samaj et al. 2005), proteolysis is universally carried out via the proteasome, an enzymatic complex involved in breaking peptidic bonds into partially-unfolded protein substrates (Hough et al. 1987). Its association with substrate proteins is mediated by the addition of ubiquitin units to K residues in the target, in a process called polyubiquitination (Goldknopf and Busch 1977).

Ubiquitin is a small protein (~8.5 kDa) which is extremely conserved among eukaryotes, with a plethora of regulatory functions including mediating recognition by the 19S-subunits of the proteasome (Sharon et al. 2006). The addition of ubiquitin units is mediated by a precisely-defined sequence of events which are themselves mediated by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3) (Kimura and Tanaka 2010). Ubiquitin ligases

make up one of the largest protein families encoded by plant genomes, and function as adaptors between E2 and the protein to be degraded. The recognition motif exposed on the proteasome targets (degron) is either generated via covalent modification within the amino acidic sequence, or, more commonly, is represented by specific N-terminal residues followed by a flexible region, and is in spatial proximity to a Lys residue (N-degron) (Tasaki et al. 2012).

N-degrons can be generated via proteolytic cleavage or by the removal of the initial methionine present in each protein. The RING E3 ligases involved in binding N-degrons are called N-recognins (Tasaki et al. 2012). The existence of N-degrons was first described by Bachmair et al. (1986), and they were thought to be limited to a small number of proteins. However, over the following years, it became clear that they represent a more general mechanism that orchestrates the degradation of most proteins in a cell. The process involving covalent modification on N-terminal amino acids, ubiquitin ligation, and proteasomal degradation was defined as the N-end rule pathway.

N-terminal amino acids are classified as stabilizing and destabilizing, with the latter further divided into primary, secondary, and tertiary destabilizing residues (Varshavsky 1997). Stabilizing amino acids hinder ubiquitin ligation and confer a long half-life to the corresponding proteins, while primary destabilizing residues correspond to N-degrons. In eukaryotes, primary amino acids are further subdivided into type I (positively charged amino acids Arg, His, and Lys) and type II (bulky hydrophobic amino acids Phe, Tyr, Trp, Ile, and Leu) (Tasaki et al. 2012). Type I and type II N-degrons are recognized by distinct domains of E3 Ub-ligases (Xia et al. 2008). The UBR-box binds positively charged residues, whereas recognition of bulky hydrophobic amino acids also requires the N domain, which is similar in structure to the prokaryotic Clp-like proteases (Tasaki et al. 2009). N-degrons can also be generated via the acetylation of N-terminal residues by N-terminal acetylases (Nats) in a separate branch of the N-end rule. This modification occurs frequently in animal and yeast cells (Polevoda et al. 2009) and is mediated by different Nat isoforms depending on the exposed N-terminal residue (Poledoda and Sherman 2003). In yeast, the N-recognin Doa10 is involved in the degradation of N-acetylated proteins (Hwang et al. 2010). Secondary destabilizing residues are substrates for the addition of an Arg destabilizing residue by arginyl-tRNA transferases (ATEs). Finally, tertiary destabilizing amino acids are subjected to covalent modification, such as the deamidation of Asn and Gln to Asp and Glu, and the oxidation of Cys, *de facto* turning them into secondary destabilizing amino acids.

The identity of stabilizing and destabilizing residues is not conserved among organisms (Tasaki et al. 2012). In bacteria, which lack ubiquitin-coding genes, the proteolytic role in the N-end rule pathway is assumed by the Clp protease family (Tobias et al. 1991). Primary destabilizing residues are the same as the eukaryotic type II with the exclusion of Ile, while secondary destabilizing amino acids (Met, Lys, Arg, Asp, and Glu) are leucylated or phenilalanylated (Shrader et al. 1993). In eukaryotes, the production of N-degrons from tertiary or secondary amino acids is catalyzed enzymatically. Removal of the initial methionine is carried out by methionine amino-peptidases (Tasaki et al. 2012). Methionine amino-peptidases preferentially remove methionine residues followed by amino acids with short side-chains (Frottin et al. 2006). N-terminal N and Q can be deamidated via Nt^N- and Nt^Q-amidase, respectively, to produce the corresponding destabilizing amino acid (Grigoryev et al. 1996; Wang et al. 2009). Oxidation of cystein in animals and plants is believed to occur spontaneously in the presence of oxygen (O_2) and nitric oxide (NO), although the need for enzymatic catalysis has not been ruled out (Hu et al. 2005).

The N-end Rule Pathway in Plants

The enzymes involved in the N-end rule pathway were initially identified in plants by forward genetics and subsequently by the sequence similarity with animal sequences (Graciet and Wellmer 2010). For instance, the terminal Nt^N- and Nt^Q-amidases are conserved between plants and animals, but are structurally different from the general $Nt^{N,Q}$-amidase in yeast (Baker and Varshavsky 1995; Graciet et al. 2010). The oxidation of N-terminal cystein that leads to the formation of secondary destabilizing amino acids has not yet been analyzed in detail. Most of the information related to it is derived from observations in animal models where both oxygen (O_2) and nitric oxide (NO) seem to be involved (Hu et al. 2005). It is still unclear whether both O_2 and NO are required and if enzymatic activity is also necessary. Three levels of oxidation are possible for cystein: sulfenic (SOH), sulfinic (SO_2H), or sulfonic (SO_3H) acid. Kwon et al. (2002) showed that Regulator of G-protein Signaling 4 (RGS4) is arginylated *in vitro* to cystein sulfonic acid, and Hu et al. (2005) confirmed that this type of oxidation is necessary for arginylation by ATE *in vivo*.

Arabidopsis possess two Arginyl-tRNA transferase genes which are functionally redundant: *ATE1* and *ATE2* (Yoshida et al. 2002). To now, two N-recognins have also been described in *Arabidopsis*: PRT1 (PROTEOLYSIS 1) and PRT6 (PROTEOLYSIS 6). PRT1 was discovered in a screening for mutants impaired in N-end rule degradation by Bachmair et al. (1993) by selecting mutagenized plants unable to degrade an N-end rule reporter represented by dihydrofolatereductase (DHFR) with an exposed N-terminal Phe residue. Stable DHFR enabled the mutant plant to grow on methotrexate, which wild-type plants were unable to do. On the other hand, PRT6 was identified by sequence similarity to the yeast N-recognin

UBR1 (Garzón et al. 2007). PRT1 and PRT6 share little similarity: while PRT6 contains a UBR-box, which is typical of N-recognins, plus a C3HC4 zinc finger at the C-terminus, PRT1 possesses two C3HC4 zinc finger motifs followed by a ZZ motif. In line with this structural difference, the two PRTs also differ in terms of substrates. PRT1 promotes the degradation of proteins with N-terminal aromatic hydrophobic residues, while PRT6 recognizes basic N-degrons (**Figure 1**). Neither PRT6 nor PRT1 possess an N domain, which is typical of animal UBRs able to degrade type II degrons. No gene coding for protein possessing an N domain has yet been identified in plant genomes.

Additional candidate proteins able to act as N-recognins in plants have been proposed by Graciet and Wellmer (2010), and include proteins with a sequence and size that are similar to the mouse UBR4: the proteins TIR3 (TRANSPORT INHIBITOR RESPONSE3), DOC1 (DARK OVEREXPRESSION OF CAB1) and BIG (named due to its enormous size).

To date, the acetylation-dependent branch of the N-end rule has not been investigated in plants. Proteins with homology to the yeast N-recognin Doa10 are present in the *Arabidopsis* genome, one of which was characterized as CER9, a factor involved in cuticle biosynthesis (Lue et al. 2012). N-acetyl transferase complexes exist in plants, although their activity has only been associated with protein targeting and not with proteolysis (Pesaresi et al. 2003). N-degrons in plants were characterized by Graciet et al. (2009) using the N-end rule reporter consisting of Ub fusions to firefly luciferase (LUC), where the authors varied the exposed amino acid resulting from deubiquitination. Using this strategy, N-terminal Met, Gly, Val, Thr, Ser, and Ala were shown to be stabilizing residues in plants, whereas Gln, Asn, Cys, Glu, Asp, Arg, Lys, His, Leu, Ile, Phe, Trp, and Tyr were shown to be destabilizing residues (**Figure 1**). The existence of such a large group of destabilizing amino acids and the specificity of PRT6 and PRT1 suggested the presence of further, yet undisclosed, N-recognins (Graciet and Wellmer 2010).

Graciet and Wellmer (2010) also speculated on the possible function of ClpT and Clp-like proteins in the N-end rule pathway in chloroplasts. In fact, Apel et al. (2010) reported that major stability determinants are located in the N-terminus of chloroplast proteins, and they also identified chloroplast N-degrons (**Figure 1**). Given their symbiotic origin, chloroplasts are expected to have a prokaryote-type N-end rule. This was not entirely confirmed because N-terminal Leu increased protein stability, while Cys and His reduced it (Apel et al. 2010). No

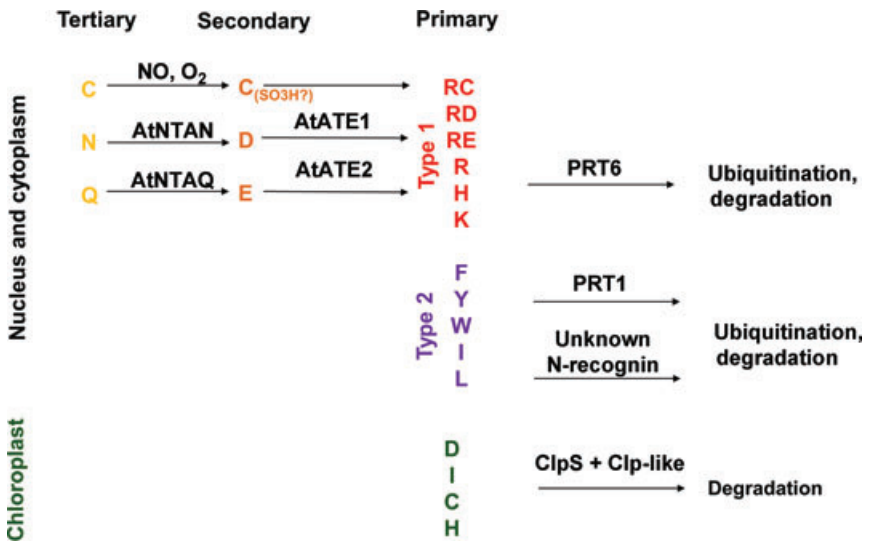

Figure 1. Scheme summarizing the N-end rule pathway in plants.

The nuclear and cytosolic pathways are mediated by the 26S proteasome, which degrades proteins polyubiquitinated by N-recognins PROTEOLYSIS 1 (PRT1) and PRT6. Two types of N-terminal tags (N-degrons) are recognized. Basic N-terminal amino acids characterize type 1 N-degrons, while apolar aromatic residues are typical of type 2 N-degrons. Together, they act as primary destabilizing residues. Secondary destabilizing residues are substrates for Arginyl-transferases (*ATE1* and *ATE2*) that add an Arg to their N-termini, thus forming new primary destabilizing residues. In turn, secondary destabilizing amino acids are produced via covalent modification to tertiary destabilizing residues. In detail, Asn and Gln are deamidated by specific N-terminal amidases (NTAN and NTAQ). Cys is oxidized, likely to Cys-sulfonic acid (SO_3H) as in animals, via oxidizing agents such as oxygen or nitric oxide. In chloroplasts, only one type of destabilizing residue, which is likely to be recognized and degraded by bacterial-type caseinolytic proteases (ClpT and Clp-like), is present.

protein with sufficient homology to the Phe/Leu-transferase was identified in the *Arabidopsis* genome. The instability conferred by His and Cys, which do not occur at the N-terminus of any plastome protein in *Arabidopsis*, could account for the short half-life of nuclear encoded proteins that are targeted at the chloroplast.

Nothing is known about the identity of N-degron in plant mitochondria. Clp-like enzymes also exist in these organelles, which are therefore presumed to have inherited the same pattern as the Gram-negative bacterium from which they are derived (Varshavsky 2011). Studies in yeast mitochondria revealed that most nuclear-encoded proteins imported in the inner matrix are cleaved by mitochondrial processing proteases (Voegtle et al. 2009). This cleavage removes exposed destabilizing residues, thus protecting the imported protein from degradation by the prokaryotic Leu/Phe N-end rule pathway. The existence of homologous protein in plant genomes suggests that the same process takes place in plant mitochondria; however, this hypothesis still needs to be validated experimentally.

Involvement of the N-end Rule Pathway in Plant Growth and Development

After the discovery of the N-end rule pathway in yeast and animal systems, major players in the N-end rule in plants were quickly identified. However, the physiological processes in which this pathway is involved required a longer time to be identified. A major role for the N-end rule in the perception of oxygen availability has only recently been reported (Gibbs et al. 2011, Licausi et al. 2011a), and will be discussed in the following section. The involvement of the ATE1 in the promotion of senescence was discovered in a genetic screening for mutants with a delayed senescent phenotype (Yoshida et al. 2002). A second genetic screening for mutants with impaired seed-to-seedling transition revealed the involvement of the N-end rule pathway in abscisic acid (ABA) signaling and germination (Holman et al. 2009). *prt6* mutant seeds appeared to require a longer after-ripening time to germinate than those of the wild-type, probably due to increased ABA sensitivity. PRT6 was also shown to be necessary for oil body degradation during germination. Interestingly, double *ate1ate2* mutants and single *prt6* knockout plants exhibited high mRNA levels of a gene coding for a hypoxia-inducible, stearoyl-coenzyme A (CoA) desaturase-like protein (Gibbs et al. 2011; Licausi et al. 2011a) which may counteract lipid degradation and mobilization.

The same mutants were also shown to diverge from the wild-type in terms of rosette morphology. *ate1ate2* have wavy serrated leaves, a phenotype that becomes more evident as the plant grows older (Graciet et al. 2009). It was found that the cause for this phenotype could not be explained solely by the misexpression of BREVIPEDICELLUS, a KNOX transcription factor involved in leaf shape determination. It therefore seems that the N-end rule might regulate the expression of other KNOX genes. Other developmental defects of *ate1ate2* plants included loss of apical dominance and phyllotaxis and internode elongation, a process associated with the gibberellin-mediated elongation of cells (Graciet et al. 2009).

To date, no evident phenotype has been reported for *prt1* mutant plants. However, it is possible that further analyses that take more aspects of plant growth and development into account will provide clues regarding the involvement of the N-end rule in more physiological processes. Already, the plethora of phenotypes observed in *Arabidopsis prt6* and *ate1ate2* indicate cross-talk with stress- and hormone-related signaling pathways. The identification of direct targets of the N-end rule in *Arabidopsis* as well as in other plant species will help in identifying how each component specifically contributes to the physiology of plants.

Involvement of the N-end Rule Pathway in Oxygen Sensing in Plants

Apart from its implication in growth and developmental programs, the identification of N-end rule substrates in plants has remained elusive. Recently, two independent reports described the major role of N-degrons characterized by oxidized Cys in a specific group of proteins. In *Arabidopsis,* more than 200 proteins have N-terminal residues corresponding to Met-Cys; however, the stability of these proteins *in vivo* has not yet been explored on a global scale. The recent identification of a group of ethylene responsive factors (ERF) associated with hypoxia as N-end rule substrates (Gibbs et al. 2011; Licausi et al. 2011a) is a notable step forwards. In fact, it provides the first evidence of the involvement of the N-end rule pathway in stress-related processes, and sets a milestone in understanding the low O_2 sensing mechanism in plants.

Plants suffer from oxygen restrictions in many situations, the most common of which is caused by water submergence (for a review see Bailey-Serres and Voesenek 2010). When subjected to this stress, plants suffer from a deep energy crisis due to the switch from aerobic to anaerobic respiration, which is less favorable in terms of energy supply (Davies 1980). However, many plant species have developed adaptive mechanisms that rely on morphological, physiological, and molecular modification to survive the stress (Bailey-Serres et al. 2012). The activation of these various mechanisms depends primarily on a system of sensing variations in O_2 levels, which, unlike animals and microorganisms, has been elusive for years.

In plants, it was long hypothesized that low O_2 sensing relied on a transcriptional regulator modulated by O_2, thus acting as a molecular switch at a transcriptional level. In bacteria, a

similar mechanism of sensing exists with the fumarate nitrate reductase regulator (FNR) transcriptional regulator forming homodimers under low O_2, thus repressing aerobic metabolism genes and promoting anaerobic ones (Kiley and Beinert 2003). In addition, in animals, the master regulator of the hypoxic response, the hypoxia inducible factor (HIF)-1, is regulated post-transcriptionally in an O_2-dependent manner (Wenger, 2002). The existence of a similar transduction system in plants has been hypothesized (Licausi 2011); however, as of yet no functional ortholog of HIF-1 has been found (Licausi and Perata 2009). The two recent independent studies mentioned above have, on the other hand, suggested that the N-end rule is the key regulator of low O_2 responses in plants (Gibbs et al. 2011; Licausi et al. 2011a). Both studies identified ERFs belonging to group VII as being subjected to N-end rule degradation when their N-terminal cystein isoxidized in the presence of O_2. The effect of NO on the oxidation state of the terminal Cys residue in the ERF-VII group has not yet been reported (**Figure 2**).

This transcription factor (TF) family has been extensively studied in relation to its prominent role in acclimative responses

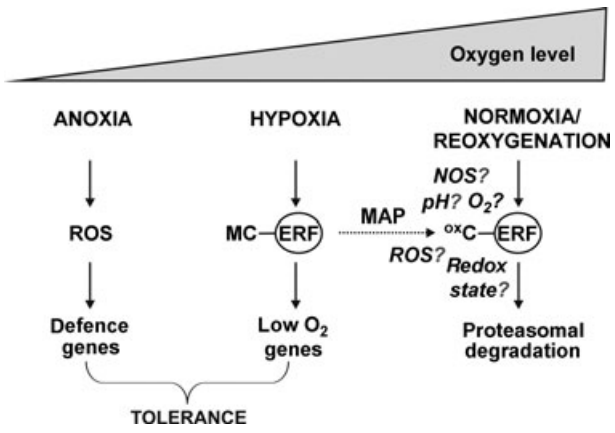

Figure 2. Model of signal transduction under low O_2.

Under normoxia and re-oxygenation, ethylene responsive factor (ERF)-VII transcription factors are targets of proteasomal degradation via the N-end rule pathway. The formation of an $_{ox}$C-degron is mediated by Met cleavage by methionine amino-peptidase and Cys oxidation by a mechanism that depends on O_2 availability and may involve cytosolic acidification, alteration of the redox state, reactive oxygen species (ROS) or reactive nitrogen species (NOS) action and enzymatic activities. Under hypoxia, the N-degron is stabilized and ERF-VII transcription factors migrate to the nucleus to activate anaerobic gene expression. Under anoxia, a mitochondrial imbalance or an nicotinamide adenine dinucleotide phosphate oxidasemediated mechanism leads to the production of ROS, promoting the activation of genes involved in oxidative responses. Both these processes contribute to plant tolerance to low O_2 stress. GFP, green fluorescent protein.

to low O_2 stress. In rice (*Oryza sativa*), two group VII ERFs have been shown to orchestrate opposite adaptive mechanisms to water submergence (Xu et al. 2006; Hattori et al. 2009). The SUBMERGENCE1A (*SUB1A*) gene, in its allelic form *SUB1A-1*, is the central component of the "quiescence strategy" that relies on growth repression under submergence in wild and cultivated rice (Xu et al. 2006; Niroula et al. 2012). This happens through the repression of GA-mediated elongation (Fukao and Bailey-Serres 2008), and fast regrowth when the water recedes (for a review see Perata and Voesenek 2007). The induction of *SUB1A-1* is a positive controller of the anaerobic metabolism, promoting the activity and gene expression of key enzymes for fermentation such as PYRUVATE DECARBOXYLASE (PDC) and ALCOHOL DEHYDROGENASE (ADH) (Fukao et al. 2006). On the other hand, the ERF SNORKEL1 (*SK1*) and *SK2* genes in deepwater rice varieties, again from group VII, promote the exceptionally fast growth of the plant internodes under submergence so that they reach the water surface and rapidly re-establish contact with the air, thus restoring the more efficient aerobic metabolism (Hattori et al. 2009).

In *Arabidopsis*, ortholog TFs of the same family, the hypoxia responsive factor (HRE)1 and HRE2 (Licausi et al. 2010), and RAP2.2 (Hinz et al. 2010), have been found to act in low O_2 signaling, thus enhancing anaerobic gene expression and improving plant tolerance to stress. Furthermore, *RAP2.12* has been shown to promote ADH transcription and enzyme activity (Papdi et al. 2008). All these TFs are characterized by a conserved N-terminal sequence MCGGAI, which constitutes a typical N-degron when the initial methionine is cleaved off and the cystein is oxidized.

One of the major confirmations that the N-end rule is involved in low O_2 sensing was the identification of fermentative genes (e.g. *ADH1* and *PDC1*) among those constitutively expressed by the *Arabidopsis* mutants *ate1ate2* and *prt6* (Gibbs et al. 2011). The same mutants not only displayed a constitutive response to hypoxia, but also germinated and survived better than wild-type plants under hypoxic conditions. *Arabidopsis* group VII ERFs were further investigated as N-end rule pathway substrates by analyzing their stability *in vitro*. HRE1, HRE2, RAP2.12, and RAP2.2 protein stability was enhanced by adding both the proteasome inhibitor MG132 and the N-end rule pathway competitive dipeptide Arg-β-Ala. The protein stability of HRE1 and HRE2 was also tested *in vivo* by mutating the Cys to Ala in the N-degron (C2A). This substitution was found to stabilize the protein compared to the wild form, concomitantly conferring higher tolerance to low O_2 treatments.

However, rice wild SUB1A-1 was not unstable *in vitro,* and the Cys2Ala substitution did not enhance its stability. Gibbs et al. (2011) hypothesized that its increased stability may depend on the presence of a Glu residue at position five, which is different from the MCGGAI consensus of the ERF-VII group. Surprisingly, the stability of a Glu5Ala mutated version

did not vary, suggesting a certain independence of SUB1A-1 regulation from the N-end rule. This independence may be the determinant of the high submergence tolerance of rice varieties harboring the *SUB1A-1*. Indeed, while RAP2.12 is widely conserved in plants (Licausi et al. 2011a), *SUB1A-1* is not, and is only present in a subgroup of rice varieties belonging to the AA genomic group (Xu et al. 2006; Niroula et al. 2012). An additional difference between RAP2.12 and SUB1A is in their transcription regulation. RAP2.12 is induced by hypoxia but not by the ethylene precursor ACC, while *SUB1A-1* is strictly linked to ethylene regulation, because it is positively regulated by this hormone and downregulates ethylene synthesis in a feedback mechanism (Fukao et al. 2006). This suggests that submergence-tolerant plants could use ethylene as an indirect mechanism to sense low O_2, because its entrapment often occurs under water submergence.

Because in *Arabidopsis* *RAP2.12* and *RAP2.2* are constitutively and ubiquitously expressed, they are likely to act as primary O_2 sensors, while HRE1 and HRE2 become involved after their transcription has been activated (**Figure 2**). Indeed, silencing *RAP2.12* and its homologous *RAP2.2* reduced the induction of anaerobic genes under low O_2. The overexpression of RAP2.12 was shown to enhance tolerance of *Arabidopsis* to flooding stress, whereas the deletion of its N-degron increased plant sensitivity. In addition, the fusion of RAP2.12 to a green fluorescent protein enabled the drafting of an exciting model of its subcellular localization and movement under low O_2 sensing. Under normoxia, RAP2.12 is located in the plasma membrane, where it interacts with the membrane-localized Acyl-CoA-binding proteins ACBP1 and ACBP2. ACBP-unbound RAP2.12 would, in this hypothesis, therefore be targeted at the proteasome via the N-end rule, whereas membrane binding by ACBPs protects RAP2.12 from Cys oxidation and consequent degradation. Under hypoxia, RAP2.12 is dissociated from the membrane and is rapidly translocated to the nucleus to regulate the transcription of anaerobic genes. Upon re-oxygenation, nuclear RAP2.12 is exposed to oxidation at the second cystein and is thereby degraded by the N-end rule, thus switching off the transcription of anaerobic genes (**Figure 2**). It is still not known whether this mechanism is active for other proteins; however, ACBP1 and ACBP2 have been shown to be partners of other genes belonging to the ERF group VII (Li and Chye 2004; Li et al. 2008).

Together with the N-end rule-related induction of the genes involved in anaerobic metabolism, tolerance to low O_2 includes at least one other mechanism that enhances plant survival under oxygen deprivation. A group of genes correlated with oxidative stress protection has been shown to be activated by very low O_2 levels, but not directly regulated by the N-end rule (**Figure 2**) (Pucciariello et al. 2012a). In fact, these genes are not constitutively expressed in the *Arabidopsis* mutants *ate1 ate2* and *prt6*. A transient and rapid peak of H_2O_2 is observed soon after the beginning of very low O_2 stress, indicating a possible specific mechanism to modulate reactive oxygen species (ROS) production rather than a general stress condition (Pucciariello and Perata 2012). The production of ROS may be an important link between this protection pathway and the Cys-related branch of the N-end rule. Because it is still unclear whether the ERF-VII instability conferred by Cys oxidation depends directly on O_2 or on cellular changes associated with its reactivity, a possible candidate for the role of regulator is the ROS/reactive nitrogen species balance (**Figure 2**). Thus, the oxidation state of the terminal Cys of ERF-VII proteins could be the result of different oxidative processes that are integrated at the N-degron site. Interestingly, the exogenous or endogenous induction of ROS is known to play a role in the stabilization of the mammalian hypoxia-inducible TF HIF-1 under normoxia (Chandel et al. 2000; Hamanaka and Chandel 2009).

In this context, the activation of a protection mechanism that is disjointed by the metabolic rearrangement could be part of the complex system that finally leads to low O_2 stress tolerance (Licausi et al. 2011b; Pucciariello et al. 2012b). Indeed, the activation of anaerobic genes is conserved across species regardless of their tolerance to low O_2 (Mustroph et al. 2010). The near isogenic rice plant M202(*SUB1A*), which contains the *SUB1A-1* allele from the submergence-tolerant FR13A rice landrace, seems to restrict the production of ROS, or is at least better able to remove ROS during submergence and after de-submergence (Jung et al. 2010).

Concluding Remarks

The identification of the mechanisms that through or together with N-end rule are responsible for variations in low O_2 tolerance in plants is definitely one of the most challenging current aspects in this field. As protein degradation and inhibition represent a rapid switch for signaling cascades, it seems reasonable to speculate that other yet-undiscovered N-degrons may play a role in hormone or stress pathways. Cellular proteolysis is just one of the aspects related to the involvement of the N-end rule in the regulation of protein activity. Selective proteolysis may be involved in the activation or repression of enzymes, DNA binding properties, or protein–protein interactions. The identification of novel N-recognins and enzymes involved in the production of specific N-degrons is likely to shed new light on the importance of this pathway in plant physiology. On the other hand, traditional breeding or the biotechnology-assisted modification of N-end rule pathway components could be a useful means for improving plant crops in terms of stress tolerance, or could reveal new properties that may be of industrial interest.

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