Update on group VII ethylene response factors:

Group VII Ethylene Response Factors co-ordinate oxygen and nitric oxide signal transduction and stress responses in plants

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This update discusses advances in understanding of ERFVII regulation and function, highlighting their role as regulators of signal transduction at the interface of ethylene, oxygen and NO signalling.

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The group VII ethylene response factors (ERFVIIs) are plant-specific transcription factors that have emerged as important regulators of abiotic and biotic stress responses, in particular low oxygen stress. A defining feature of ERFVIIs is their conserved N-terminal domain, which renders them oxygen- and nitric oxide-(NO-) dependent substrates of the N-end rule pathway of targeted proteolysis. In the presence of these gases, ERFVIIs are destabilised, whilst an absence of either permits their accumulation; ERFVIIs therefore coordinate plant homeostatic responses to oxygen availability and control a wide range of NO-mediated processes. ERFVIIs have a variety of context-specific protein- and gene-interaction partners, and also modulate GA and ABA signalling to regulate diverse developmental processes and stress responses. This update discusses recent advances in our understanding of ERFVII regulation and function, highlighting their role as central regulators of gaseous signal transduction at the interface of ethylene, oxygen and NO signalling.

ERFVII transcription factors

The ERF transcription factors are plant-specific proteins characterized by a single DNA-binding APETALA 2(AP2)/EREBP domain (Nakano et al., 2006; Licausi et al., 2013). This domain was first discovered in ethylene-responsive element (ERE) binding proteins (EREBPs) in tobacco and shown to bind to the GCC-element of EREs in promoters (Ohme-Takagi and Shinshi, 1995). ERFs constitute one of the largest transcription factor families in plants: a genome wide analysis found that rice has 139 and Arabidopsis 122, clustered into 15 and 12 subgroups respectively, based on the presence of conserved features (Nakano et al., 2006). One of these subgroups, the group VII ERFs (ERFVIIs), has been heavily studied in recent years due to the roles its members play in orchestrating a wide range of plant growth, development and stress responses. In addition to the AP2 domain, ERFVIIs are characterised by several other motifs including, most importantly, N-terminal (Nt-) MCGGAI2/L (Nakano et al., 2006; van Veen et al., 2014), which is a conserved feature of ERFVIIs in all flowering plant species (Fig.1 a,b). A recent study investigated the phylogenetic origin of ERFVIIs using an analysis of synteny in 16 angiosperm species, revealing that ERFVIIs originated from two ancestral genes, and that subsequent gene duplication resulted in the appearance of five members before mono- and
dicotyledonous plants divided (Fig. 1a). Of the five Arabidopsis ERFVIIIs, HYPOXIA RESPONSIVE1 (HRE1), RELATED TO AP2.2 (RAP2.2) and RAP2.12 were shown to share syntenic origins, while HRE2 and RAP2.3 evolved from a separate ancestral protein (van Veen et al., 2014).
ERFVIIs control flooding responses

A major role for ERFVIIs in controlling flooding- and low oxygen- (hypoxia-) tolerance in plants has been discovered in recent years. For example, in Arabidopsis *hre1hre2* mutant seedlings showed drastically reduced survival in anoxia (0% oxygen), while ectopic overexpression of *HRE1* enhanced survival by increasing the expression of core hypoxia-responsive genes, including *ALCOHOL DEHYDROGENASE 1 (ADH1)* (Licausi et al., 2010; Hess et al., 2011). A similar function was observed for *RAP2.2*, where ectopic expression increased *ADH1* transcripts in hypoxia (5% oxygen), whilst *rap2.2* mutants had reduced survival under this stress (Hinz et al., 2010). Other studies in Arabidopsis have also pointed to key roles for these proteins in controlling hypoxia and ethylene-regulated submergence responses (Papdi et al., 2008; Yang et al., 2011).

Flood tolerant plants typically cope with submergence through two opposite survival strategies: quiescence, during flash floods, and escape, during deep-water floods (Voosenek and Bailey-Serres, 2015). The quiescence strategy is characterised by reduced growth and respiration, metabolic changes that enhance use of carbohydrate reserves, induced responses against oxidative damage, and inhibition of floral initiation (Pena-Castro et al., 2011; Bailey-Serres et al., 2012). In contrast, the deep water escape strategy requires rapid growth of petioles and stems, and vascular changes to facilitate gas diffusion (Bailey-Serres et al., 2012; Voosenek and Bailey-Serres, 2015). In rice, three different members of the ERFVII family control these antithetical survival strategies: SUBMERGENCE (SUB)1A, one of three ERFVIIs located at the *Sub1* locus, promotes quiescence, whilst SNORKEL1 and 2 regulate escape (Xu et al., 2006; Hattori et al., 2009). These ERFVIIs are transcriptionally induced by ethylene, which rapidly accumulates in flooded tissues, but regulate their respective growth strategies by modifying GA signalling in opposite directions. Ethylene induction of *SUB1A* increases the accumulation of SLENDER RICE 1 (SLR1) and SLR1 LIKE-1 (SLRL1), two GA-labile signalling repressors that inhibit the transcription of GA-inducible genes, arresting elongation and promoting catabolism of carbohydrates (Fukao et al., 2006; Fukao and Bailey-Serres, 2008; Hirano et al., 2012). Furthermore, there is a strong up regulation of GA-deactivating GA2 oxidase in *SUB1A* containing lines, which would reduce the levels of active GA and enhance SLR1 stability (Jung et al., 2010). In contrast, during flooding in deep water-rice varieties, the induction of *SNORKEL1* and 2 by ethylene triggers, together
with the near isogenic lines NIL1 and NIL3, internode elongation by up-regulating
GA20 oxidase, which increases the accumulation of active GA (Raskin and Kende,
1984; Hattori et al., 2009; Ayano et al., 2014). Similar quiescent and escape-like
flooding responses have also recently been identified in two related *Rumex* species
from contrasting hydrological niches, which may also be regulated by ERFVIIs (van
Veen et al., 2013).

Together with ethylene and GA, ABA is also an important hormone involved
in the signalling network regulated by *SUB1A* in rice (Hoffmannbenning and Kende,
1992; Fukao and Bailey-Serres, 2008; Chen et al., 2010; Fukao et al., 2011). When
flood waters subside, de-submergence can lead to rapid dehydration of the leaves
(Setter et al., 2010; Fukao et al., 2011). ABA levels decline upon submergence
(Fukao and Bailey-Serres, 2008), and *SUB1A* prevents leaf desiccation by increasing
ABA responsiveness, which promotes the expression of several drought-associated
*DEHYDRATION RESPONSE ELEMENT-BINDING PROTEIN 1* (*DREB1*) and *LATE
EMBRYOGENESIS ABUNDANT* (*LEA*) genes, limits the spread of reactive oxygen
species (ROS) and induces enzymes (e.g. superoxide dismutases and catalases) that
neutralise the oxidative damage associated with dehydration (Fukao et al., 2011). The
role of *SUB1A* in fine tuning ROS levels seems to be also essential for flooding
tolerance, specifically regulating the role of hydrogen peroxide in aerenchyma
formation, but at the same time limiting its toxicity during the stress (Jung et al.,
2010; Parlanti et al., 2011). Furthermore, there is evidence supporting a role for
*SUB1A* in maintaining the levels of chlorophyll and carbohydrates during leaf
senescence promoted by ethylene, a process associated with several stresses including
drought (Fukao et al., 2012). It has also been reported that GA2 oxidase may inhibit
leaf growth and promote root growth during drought (Wang et al., 2011). Intriguingly,
recent data show a positive role in drought of *GA-INSENSITIVE DWARF* (*GID1*),
the GA receptor, that counteracts *SUB1A* action by degrading SLR1 (Du et al., 2014);
future studies will be required to unravel the interaction between these factors during
dehydration.

**ERFVIIs are substrates of the N-end rule pathway of proteolysis**

As well as ethylene accumulation, another key signal associated with flooding
is reduced oxygen availability (van Dongen and Licausi, 2015). It has been shown
that ERFVIIs function as homeostatic sensors of hypoxia via the N-end rule pathway
of targeted proteolysis (Gibbs et al., 2011; Licausi et al., 2011), an ancient and conserved branch of the ubiquitin proteasome system that relates the stability of a protein to the nature of its N-terminus (Fig. 2) (Bachmair et al., 1986; Varshavsky, 2011; Gibbs et al., 2014). Moreover, this ERFVII-based system has also emerged as a critical mechanism for nitric oxide (NO) sensing (Gibbs et al., 2014), highlighting a...
central role for ERFVIIIs and their proteolytic control by the N-end rule pathway in
gaseous signal transduction.

**ERFVIIIs and oxygen sensing**

A defining feature of the ERFVIIIs is the presence of an N-terminal motif that
initiates with the residues Met-Cys (**Fig. 1a,b**) (Gibbs et al., 2011; Licausi et al.,
2011). This motif is highly conserved across ERFVIIIs in flowering plants, and
functions as an N-degron, rendering these proteins substrates of the N-end rule
pathway. The Nt-Met of ERFVIIIs is co-translationally cleaved by cytosolic
METHIONINE AMINOPEPTIDASEs (MAPs) to reveal a tertiary destabilising Nt-
Cys residue, that is susceptible to oxidative modifications to produce an oxidised Cys
(Cys sulfenic or sulfonic acid; (Hu et al., 2005), which is then arginylated by
ARGINYL tRNA TRANSFERASES (ATEs). Nt-Arg-Cys- is then recognised by the
N-end rule pathway E3 ligase (N-recognin) PROTEOLYSIS6 (PRT6), which targets
the ERFVII for destruction via polyubiquitination (**Fig 2**). In the presence of oxygen
(and NO; see next section), oxidation of Nt-Cys therefore catalyses protein
degradation, whilst under hypoxia ERFVIIIs accumulate to coordinate the
transcriptional response to oxygen limitation (Gibbs et al., 2011; Licausi et al.,
2011).

In Arabidopsis, **RAP2.12, RAP2.2 and RAP2.3** are all constitutively expressed,
whereas **HRE1** and **HRE2** are hypoxia-inducible, suggesting that there is a cascade of
transcription and stabilisation in response to declining oxygen levels, and that
individual ERFVIIIs have different contributions to the response (Licausi et al., 2010;
Bui et al., 2012).

It was hypothesised that reliance upon spontaneous Nt-Cys oxidation alone
would not allow plants to fine tune their response to hypoxia, and would instead
expose ERFVIIIs to unregulated fluctuations in cell redox status. The stability of the
mammalian hypoxia sensor protein HYPOXIA INDUCIBLE FACTOR 1-ALPHA
(HIF1α) is regulated in an oxygen dependent manner by prolyl hydroxylases (Kaelin
and Ratcliffe, 2008). A survey of hypoxia-responsive genes in Arabidopsis identified
several plant cysteine oxidase (PCO) enzymes, which were shown to oxidise Nt-Cys
using oxygen as a co-substrate (Weits et al., 2014). These enzymes promote
degradation of RAP2.12 in the presence of oxygen, and therefore play a similar
regulatory role to mammalian prolyl hydroxylases (Weits et al., 2014). PCOs were
shown to counteract RAP2.12-mediated induction of hypoxia-responsive reporter
genes, and hypoxic induction of PCO1 and 2 indicates that they are important for
dampening anaerobic gene transcription through negative regulation of RAP2.12, and
likely the other ERFVIIIs (Weits et al., 2014).

Interestingly, analysis of rice SUB1A-1 demonstrated that it is not an N-end
rule substrate in vitro, in contrast to all five of the Arabidopsis ERFVIIIs and at least
one barley ERFVII (Gibbs et al., 2011; Mendiondo et al., 2015). Enhanced tolerance
of rice varieties carrying the SUB1A-1 gene might therefore be due to the increased
stability of the protein, although more research is needed to fully support this
hypothesis. Perhaps the semi-aquatic nature of rice has placed evolutionary pressure
on ERFVII dynamics to enhance survival in fluctuating water schemes; it will be
interesting to see whether ERFVIIIs from other wetland species, for example those
from Rumex (van Veen et al., 2014), are also uncoupled from N-end rule regulation.

Arabidopsis N-end rule pathway mutants have altered responses to hypoxia or
flooding, either enhancing or negatively impacting survival rates depending upon the
context of the stress and recovery conditions (Gibbs et al., 2011; Licausi et al., 2011;
Riber et al., 2015). This indicates that the N-end rule pathway is a promising target for
manipulating flooding tolerance in crops. In barley (Hordeum vulgare) the ERFVII
BERF1, was shown to be a putative N-end rule substrate in vitro, whilst
posttranscriptional accumulation of an artificial N-end rule reporter protein consisting
of the ERFVII Nt-domain fused to beta-glucuronidase (MCGGAIL-GUS) was
observed under waterlogged conditions, indicating that ERFVIIIs are also stabilised by
low-oxygen in monocots (Mendiondo et al., 2015). Barley PRT6 (HvPRT6) RNAi
lines with reduced HvPRT6 expression had increased levels of anaerobic response
gene transcripts (including ADHs), similarly to what is observed in Arabidopsis N-end
rule mutants (Gibbs et al., 2011; Licausi et al., 2011). Furthermore, RNAi lines
performed better than null controls under waterlogging stress, as evidenced by
retention of chlorophyll, increased biomass and sustained yield post-stress
(Mendiondo et al., 2015). This translational study highlights the value of targeting
ERFVIIIs and the N-end rule pathway for engineering flooding tolerance in
agronomically important species.

A recent study has shown that hypoxia can also act as an important
environmental positional cue (Abbas et al., 2015). It was found that low oxygen
levels repress photomorphogenesis in dicot species, promoting the maintenance of a
skotomorphogenic developmental program. This response was linked to stabilised
ERFVIIs, which actively maintained a closed apical hook, and repressed chlorophyll biosynthesis and cotyledon greening. Counterintuitively, hypoxic conditions were beneficial to seedlings, helping to protect them from photooxidative damage following extended darkness and dramatically enhancing survival rates once light was perceived (Abbas et al., 2015). Remarkably, hypocotyl elongation still occurred under hypoxia, demonstrating an active role for oxygen sensing by the ERFVIIs in protecting the stem cell niche, as opposed to inducing a quiescent-like state as can occur during flooding stress. This study indicates that oxygen availability may have a wider role in regulating general plant growth and development than has been previously considered.

Nitric oxide signal transduction via proteolytic control of ERFVIIs

An important signal associated with flooding-induced hypoxia is the accumulation of nitric oxide (NO), which alongside the activity of class-1 nonsymbiotic hemoglobins plays a role in balancing the antioxidant status of the cell (van Dongen and Licausi, 2015). NO is a highly reactive gaseous signalling molecule that is known to regulate a diverse range of processes in plants (Yu et al., 2014). In contrast to animals, which produce NO through the action of NO-synthases, the origins of NO in plants are less well defined, and production can occur through several different reductive and oxidative pathways (Yu et al., 2014). NO typically induces effects through covalent modification of proteins thereby altering function, such as via S-nitrosylation, Y-nitrilation and metal nitrosylation (Besson-Bard et al., 2008). Cysteine S-nitrosylation has been shown to control a number of key regulatory proteins during plant stress responses. For example, in Arabidopsis, S-nitrosylation of the NADPH oxidase AtRBOHD regulates the Salicylic Acid (SA) induced hypersensitive response (Yun et al., 2011), whilst S-nitrosylation of (SNF1)-related protein kinase 2.6/OST1 negatively regulates ABA-signalling in guard cells (Wang et al., 2015). However, in these instances, the downstream effect of this modification is process- or stress-specific, and no unifying sensing mechanism for coordinating multiple transcriptional, developmental and physiological responses to NO had been identified until recently.

Destabilisation of Nt-Cys-initiating REGULATOR OF G PROTEIN SIGNALLING (RGS) substrates during cardiovasculature development in mammals requires NO in addition to oxygen (Hu et al., 2005; Jaba et al., 2013). It was
hypothesised that Nt-Cys of RGS proteins is first S-nitrosylated, and then subsequently further oxidised to permit arginylation and degradation (Hu et al., 2005). An analysis of ERFVII stability revealed that ERFVII degradation is also dependent on NO, indicating that a similar mechanism occurs in plants (Fig.2) (Gibbs et al., 2014). Under NO-limited conditions - such as in the nitrate reductase nia1nia2 double mutant or using pharmacological NO-scavengers - ERFVII proteins are stabilised. Remarkably, prt6 and ate1ate2 N-end rule mutants, in which ERFVIIs constitutively accumulate, were completely insensitive to exogenous NO for a wide range of responses, including induction of germination, inhibition of hypocotyl elongation in the dark, and stomatal closure, suggesting that N-end rule-mediated proteolysis is essential for NO signal transduction. Furthermore, the NO-insensitivity of N-end rule mutants was genetically linked to ERFVIIs for each of the processes investigated, revealing that ERFVIIs play a key role in regulating plant NO responses (Gibbs et al., 2014). It will be important to elucidate the exact mechanism by which NO controls the stability of these transcription factors and the relationship between NO and oxygen during this process; for example, it is not yet known if NO spontaneously modifies ERFVIIs, or whether the effect of NO on their stability is dependent on enzymatic activity or occurs indirectly.

Regulation of plant responses to other environmental stresses by ERFVIIs

In addition to hypoxia, ERFVIIs from a range of flowering plant species enhance tolerance to other abiotic and biotic stresses. For example, a recent study of Arabidopsis ERFVIIs found that the constitutively expressed group members also regulate responses to oxidative and osmotic stresses, which are both also associated with submergence (Papdi et al., 2015). ERFVII genes are up regulated in response to phytohormones and stresses, including ethylene, ABA, NaCl, SA, cold and heat, drought and osmotic stress (Yi et al., 2004; Jung et al., 2007; Xu et al., 2007; Zhang et al., 2009; Zhang et al., 2010; Park et al., 2011; Chen et al., 2012; Zhu et al., 2013; Yang et al., 2014). Pathogen infection was shown to increase expression of RAP2.2 in Arabidopsis in response to Botrytis cinerea (Zhao et al., 2012), OsBIERF1 and 4 in rice in response to Magnaporthe grisea (Cao et al., 2006), CaPFI in Capsicum annum in response to Xanthomonas axonopodis (Yi et al., 2004), GmERF3 in soybean in response to soybean mosaic virus (Zhang et al., 2009) and wheat TaERF1 in response to infection with Blumeria graminis (Xu et al., 2007). Ectopic expression
of ERFVIIs in transgenic plants increased cross-tolerance to multiple stresses. Arabidopsis HRE2 over-expression increased tolerance to salt and mannitol, whereas the hre2 mutant showed higher sensitivity to these stresses (Park et al., 2011). CaPF1 overexpressed in arabidopsis and tobacco increased tolerance to freezing and Pseudomonas syringae (Yi et al., 2004), and in Pinus virginiana (Virginia pine) to the heavy metals Cadmium, Copper and Zinc, to heat, and to the pathogens Bacillus thuringiensis and Staphylococcus epidermidis (Tang et al., 2005). Ectopic expression of GmERF3 in transgenic tobacco enhanced resistance toRalstonia solanacearum, Alternaria alternata, and tobacco mosaic virus, and improved tolerance to salt and dehydration (Zhang et al., 2009). Expression of tomato JERF1 in tobacco and rice led to increased tolerance to salt and drought (Zhang et al., 2004; Zhang et al., 2010) and in rice to resistance to Rhizoctonia solani (Pan et al., 2014). Ectopic expression of TaERF1 and barley HvRAF1 in Arabidopsis led to increased tolerance respectively to salt, drought, cold, B. cinerea and salt and R. solanacearum (Jung et al., 2007; Xu et al., 2007). Transgenic tobacco expressing the Jatropha curcas ERFVII JcERF1 showed increased salt tolerance (Yang et al., 2014).

In many cases similar alterations associated with ectopic expression hint at the downstream molecular and biochemical mechanisms that are enhanced by overexpressing ERFVIIs. There is certainly evidence of repression of reactive oxygen species (ROS) production by ERFVIIs. Tobacco BY-2 cells expressing arabidopsis RAP2.3 were more resistant to H$_2$O$_2$, and showed increased expression of the H$_2$O$_2$ induced GLUTATHIONE S-TRANSFERASE (GST)6 gene (Ogawa et al., 2005), whilst transgenic Arabidopsis expressing HRE2 showed increased tolerance to methyl viologen (MV)-induced oxidative stress, and lower levels of ROS in response to high salt (Park et al., 2011). Levels of several antioxidant enzymes were increased in Virginia pine overexpressing CaPF1 (Tang et al., 2005). It was also previously shown that the Subl locus increases seedling tolerance to MV and H$_2$O$_2$ due to enhanced transcript levels of genes encoding ascorbate peroxidase, superoxide dismutase and catalase (Fukao et al., 2011). In fact, transcripts for SUB1A and several other ERFVIIs increase in response to MV treatment (Jung et al., 2007; Fukao et al., 2011; Park et al., 2011). In addition, in several cases overexpression of ERFVIIs from a variety of species resulted in increased expression of Pathogenesis Related (PR) genes, many of which contain GCC-boxes in their promoters, suggesting the
mechanism through which ERFVIIs may increase tolerance to pathogens (Yi et al., 2004; Ogawa et al., 2005; Jung et al., 2007; Zhang et al., 2009) (Xu et al., 2007).

To understand how ectopic expression of ERFVIIs regulates plant responses to diverse stresses it will be essential to determine the mechanism controlling N-end rule (and potentially other) mediated protein stabilization and destabilization. None of the published studies of ectopically expressed ERFVIIs from non-Arabidopsis species have analyzed levels of transgenic ERFVII protein, but as similar phenotypes are invariably observed, it is very likely that their over-expression overrides the destabilizing function of the N-end rule pathway. As it has been shown (at present only in vitro) that SUB1A is not a substrate of the N-end rule pathway (Gibbs et al., 2011), it is likely that this protein is constitutively stable, thus mimicking the phenotypes of ectopically expressed ERFVIIs. It is unlikely that oxygen levels vary under the non-hypoxic abiotic and biotic stresses analysed, suggesting either that NO (Gibbs et al., 2014), or protection of the N-terminus (Shemorry et al., 2013) may be involved in modulating stability.

**Key interactions mediating ERFVII function**

How do the ERFVII transcription factors control such a diverse range of plant developmental and stress responses, and how do they distinguish between oxygen and NO signals to appropriately regulate gene expression? It is probable that several factors are involved, including diversity in gene targets, differences in temporal and tissue-specific expression or subcellular localisation and context-specific protein-protein interactions. Variation in gene targets and interaction partners is conceivable, since comparative analysis of ERFVIIs reveals that they are highly variable in sequence length and identity outside of the N-terminal and AP2 domains (Fig.1a) (Nakano et al., 2006). For example, Arabidopsis RAP2.3 was shown to associate with OCS ELEMENT BINDING FACTOR 4 (OBF4) (Buttner and Singh, 1997), and RAP2.2 with SEVEN IN ABSENTIA OF ARABIDOPSIS2 (SINAT2) (Welsch et al., 2007). SINAT2 is a RING E3 ligase, and a recent study showed that GFP-RAP2.12 - in which the N-degron is removed - was stabilised in SINAT1/2 silenced Arabidopsis lines (Papdi et al., 2015). Furthermore, an N-terminal YFP-RAP2.3 fusion was also shown to be degraded in response light independently of the Cys-2 N-degron (Abbas et al., 2015). Both of these findings indicate that ERFVIIs stability is regulated by more than one proteolytic mechanism, suggesting that complex post-translational
control of ERFVIIs occurs in plants. ERFVIIs have previously been shown to associate with a range of promoter DNA-motifs, including GCC boxes (Buttner and Singh, 1997; Zhang et al., 2004; Gibbs et al., 2014) and the ATCTA sequence (Welsch et al., 2007), suggesting they have diverse gene targets. Furthermore, there is evidence that post-translational phosphorylation by kinases might affect ERFVII activity (Cheong et al., 2003; Xu et al., 2007). Combined with these previous reports, recent studies indicate that ERFVIIs function as promiscuous transcription factors, providing mechanistic insight into how they regulate diverse signal- and context-specific responses.

**ERFVII-protein interactions during the low-oxygen response**

Licausi et al. (2011) discovered that under normoxic conditions, RAP2.12 is localised to the plasma membrane (PM) via interacting with Acyl-CoA binding protein (ACBP)1 and ACBP2, two PM-associated members of the six-member ACBP family (Fig.3a) (Xiao and Chye, 2009; Licausi et al., 2011). RAP2.3 had also previously been identified as a direct interaction partner of ACBP2 (Li and Chye, 2004). In normoxia, this protein association seems to protect a pool of ERFVIIs from degradation, whilst hypoxia induces a localisation shift from the PM to the nucleus (Licausi et al., 2011). This relocalisation is triggered once oxygen levels decrease to approximately half that of normal air, and accumulation in the nucleus at this oxygen tension coincides with the first induction of hypoxia-responsive gene expression (Kosmacz et al., 2014). In addition, reduction in oxygen availability would also permit stabilisation of de novo synthesised ERFVIIs, therefore the pool of active ERFVIIs in the nucleus likely comprises factors of these two different origins. This is supported by the finding that RAP2.12 transcript levels in polysomal complexes are not dramatically altered by hypoxia (Mustroph et al., 2009). Once normal oxygen levels return, RAP2.12 degradation occurs within 3 hours, coinciding with down regulation of hypoxia-adaptive gene expression (Kosmacz et al., 2014). The PM-localisation of RAP2.12 is speculated to be a key component of the hypoxia sensing mechanism, and it will be interesting to see whether other members of the ERFVII family behave similarly, whether interactions with soluble ACBP proteins also occur, and whether this mechanism is conserved across species. A key focus should be placed on understanding how RAP2.12 relocalisation occurs in response to low oxygen, and whether changes in NO availability play a role. Furthermore, exactly
how a small pool of RAP2.12 evades degradation to make it to the PM remains unanswered.

Once ERFVIIs accumulate in the nucleus a number of essential anaerobic response genes are switched on, including many of the ‘core 49’ hypoxia response mRNAs previously identified (Mustroph et al., 2009). Sustained expression of many of these genes can be detrimental, and so counterbalancing mechanisms must be in place. In addition to the PCO enzymes discussed earlier, the trihelix transcription factor HYPOXIA RESPONSE ATTENUATOR 1 (HRA1) plays such a role (Giuntoli et al., 2014) (Fig. 3b). HRA1 is both induced by RAP2.12, and counteracts anaerobic gene induction through physically interacting with RAP2.12. HRA1 was shown to be particularly important for attenuating hypoxia responses in young tissues and meristematic regions, and may play a role in dampening low-oxygen responses under aerobic conditions in regions of the plant that are experiencing physiological hypoxia. As well as antagonising RAP2.12-mediated gene expression, HRA1 also negatively regulates its own transcription, providing a second feedback loop. Interestingly, HRA1 did not interact with any of the other four Arabidopsis ERFVIIs, further indicating that each family member may have specific interaction partners (Giuntoli et al., 2014).

**ERFVII crosstalk with hormone signalling pathways**

As well as the discovery of functionally relevant protein-protein interactions, ERFVII proteins are known to interact with diverse hormone signalling pathways at the transcriptional and protein level. Gibbs et al (2014) discovered that ERFVIIs mediate crosstalk between NO-availability and ABA signalling during the regulation of seed dormancy, through their positive control of ABI5 expression, a major negative regulator of germination (Fig. 3c) (Holdsworth et al., 2008). Constitutively expressed RAP2.2, RAP2.12 and RAP2.3 all induced ABI5 expression in protoplasts, and a physical interaction between RAP2.3 and a GCC-box DNA motif in the ABI5 promoter was confirmed (Gibbs et al., 2014). This functional interaction retrospectively explained why both prt6 and atelate2 mutants, which constitutively accumulate ERFVIIs, have heightened levels of seed dormancy and ABA-hypersensitivity relative to wild type (Holman et al., 2009). It has long been known that NO acts as a potent disrupter of seed dormancy, able to trigger germination (Bethke et al., 2007), and that following seed imbibition, there is well-documented
burst of NO (Liu et al., 2009). Rapid increases in cellular NO as well as oxygenation of the seed during imbibition would stimulate ERFVII degradation, leading to a downregulation of ABI5 expression in the endosperm, reducing sensitivity to ABA and promoting germination (Gibbs et al., 2014). Future analyses should focus on whether other key ABA-regulated responses are also regulated by ERFVIIIs.
A recent large-scale yeast two-hybrid screen of Arabidopsis proteins identified RAP2.3 as an interaction partner of the DELLA protein GA-INSENSITIVE (GAI) (Marín-de la Rosa et al., 2014). Interactions between RAP2.3 and the DELLA protein REPRESSOR OF THE ga1-3 MUTANT (RGA), and between GAI and RAP2.12 were also identified. These DELLAs associate with the ERFVII DNA-binding domain, and disrupt their ability to interact with target genes (Fig. 3d) (Marín-de la Rosa et al., 2014). This suggests that in the absence of GA, accumulated DELLA proteins act as negative regulators of ERFVII-mediated gene expression, whilst GA-induced destruction of DELLA proteins would relieve this repression. This hypothesis was investigated within the context of seedling apical hook development. The apical hook is a hallmark feature of etiolated seedlings, its formation is promoted by both ethylene and GA, which have additive effects enhancing hook angle (Abbas et al., 2013). In the pentuple erfVII mutant, which lacks all 5 ERFVIIIs, hooks were able to respond similarly to wild type for ethylene, but the single and additive effects of exogenous GA were significantly reduced compared to wild type, indicating a role for ERFVIIIs in GA-regulation of early seedling development. As discussed earlier, there is a well-documented link between ERFVIIIs and GA signalling in rice: SNORKELs and SUB1A-1 promote and inhibit downstream GA responses respectively (Fukao and Bailey-Serres, 2008; Hattori et al., 2009). Furthermore, Arabidopsis ERFVIIIs regulate processes dependent on cell elongation (e.g. germination and hypocotyl elongation (Holman et al., 2009; Gibbs et al., 2014)) suggesting that ERFVII interactions with GA signalling pathways may be more significant than is currently understood.

Conclusions

Recent data indicate that the long-known distinctive N-terminal structure of the ERFVIIIs, conferring oxygen and NO-dependant stability, provides these proteins with a conditional activity that has diverse consequences during growth and development, and in response to environmental stress. Many aspects of ERFVII biology remain to be determined to enable an understanding of how changed stability is related to distinct responses to different conditions. Future studies aimed at defining the specificity of ERFVII protein-protein and protein-gene interactions, as well as the contribution of non-N-end rule-mediated ubiquitination to their stability, will shed
light onto the complex nature of ERFVII signal transduction and may help understand how this small family of transcriptional regulators controls such an array of plant responses. In addition, determining the overlapping and unique roles of individual members of the group will help in understanding adaptive molecular responses to evolutionary pressure related to specific stresses. Further study of these factors and mechanisms influencing the changes in their stability should also provide a deeper understanding of how plants integrate development and stress responses. The observation that ERFVIIIs from diverse species positively influence tolerance to multiple stresses in similar ways indicates that they are good targets for breeding and biotechnological approaches to increase stability of plant growth and yield in response to environmental stress.

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