Editorial: Plant proteostasis-a proven and promising target for crop improvement.

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Abstract

The Green Revolution of the 1960s accomplished dramatic increases in crop yields through genetic improvement, chemical fertilisers, irrigation, and mechanisation. However, the current trajectory of population growth, against a backdrop of climate change and geopolitical unrest, predicts that agricultural production will be insufficient to ensure global food security in the next three decades. Improvements to crops that go beyond incremental gains are urgently needed. Plant biology has also undergone a revolution in recent years, through the development and application of powerful technologies including genome sequencing, a pantheon of 'omics techniques, precise genome editing, and step changes in structural biology and microscopy. Proteostasis- the collective processes that control the protein complement of the cell, comprising synthesis, modification, localisation, and degradation- is a field that has benefitted from these advances. This special issue presents a selection of the latest research in this vibrant field, with a particular focus on protein degradation. In the current article, we highlight the diverse and widespread contributions of plant proteostasis to agronomic traits, suggest opportunities and strategies to manipulate different elements of proteostatic mechanisms for crop improvement, and discuss the challenges involved in bringing these ideas into practice.

Plant proteostasis- the basics

Proteostasis can be defined as the dynamic regulation of the functional proteome. This is achieved through the integration of biochemical pathways that comprise biogenesis, trafficking, modification, and degradation of proteins. Biogenesis begins with the interpretation of information encoded in DNA to guide the synthesis of proteins. Some aspects, such as transcription, are well studied, but it has been demonstrated repeatedly that the transcriptome can be a poor predictor of the proteome, highlighting the importance of post transcriptional processes, such as translational control and protein degradation [1]. Translational control is an understudied but emerging area in plants [2,3]. As the most energy demanding process in the cell, appropriate regulation is essential to adjust protein synthesis to demand, especially under stress conditions. Whilst the importance of translational regulation has been firmly established, for example in responses to light and abiotic stresses, in many cases, the molecular mechanisms remain to be determined [2].

In contrast, protein degradation has been the subject of intense study in plants [4,5]. Although- remarkably- proteins were initially believed to be stable, it is now known that eukaryotes possess a diverse and sophisticated collection of protein degradation mechanisms that play fundamental roles in cellular processes including protection of cells from mis-folded, aggregated, or damaged proteins, control of regulatory proteins, re-modelling of protein complexes, remodelling of the proteome, removal of damaged organelles, and background protein turnover [6]. These functions are carried out by several different degradation systems: the proteasome, endocytosis and vacuolar degradation, autophagy, and organellar proteolytic machines, in which ubiquitin and ubiquitin-like proteins play a key role. [7-14].

Ubiquitination is a versatile and tuneable post-translational modification (PTM) in which the 76 amino acid protein, ubiquitin is covalently attached via its C-terminus to a substrate protein, usually to a lysine (K) residue. This PTM can influence protein activity, protein-protein interactions, subcellular localisation, and protein stability, dependent on the precise nature of the modification [7]. Three enzymes control the ubiquitination reaction [8,9]. Ubiquitin residues are activated at their C-terminus by forming a thioester intermediate with a ubiquitin-activating enzyme (E1). Activated ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2). Ubiquitin residues are then attached to a target protein by the E2 enzyme with the assistance of a ubiquitin ligase (E3), either by direct transfer of ubiquitin from E2 to the substrate, in E3 ligases containing a really interesting new gene (RING) domain, or by formation of an intermediate ubiquitin-ubiquitin ligase complex, in ligases containing a homologous to the E6-AP carboxyl terminus (HECT) domain and in RING-in-Between-Ring (RBR) E3s. The multiplicity of E3 ligases in plants (~1400 in *Arabidopsis thaliana*) provides specificity and versatility [9,15]. Eukaryotes also contain ubiquitin-like proteins, such as small ubiquitin-like modifier (SUMO), which expand the PTM repertoire though an analogous enzymatic cascade [16]. Collectively, E1, E2 and E3 enzymes are considered the “writers” of the ubiquitin code, which is “read” by ubiquitin binding proteins and “erased” by the action of deubiquitinase enzymes (DUBs) [17]. DUBs display diverse substrate specificity, contributing to fine tuning of the ubiquitin-dependent proteostatic machinery [18].

Ubiquitin contains seven K residues (K6, K11, K27, K29, K33, K48, K63) that, together with the N-terminal methionine act as sites for conjugation of further ubiquitin moieties. Dictated by specific E2-E3 interactions, this enables the formation of polyubiquitin chains with different linkages and topologies [9]. Extension of polyubiquitin chains by attachment of a ubiquitin residue to K48 of the previously attached ubiquitin tags the substrate for degradation by the 26S proteasome, a multi-subunit, ATP-dependent protease belonging to the AAA+ (ATPases associated with diverse cellular activities) protein superfamily. Ubiquitinated proteins may then be relayed from pathway specific E3s to HECT ligases that mediate further chain modifications and promote proteasome processivity [19]. However, ubiquitin is also used in non-proteasomal degradation machineries, for example K63 linkages act as sorting signals in the endocytosis/vacuolar pathways that regulate abundance of plasma membrane receptors and transporters [10]. Ubiquitination also plays a role in autophagy, an evolutionarily conserved mechanism for the vacuolar degradation of large protein complexes, misfolded, insoluble protein aggregates, and damaged organelles [11,20]. A key feature is the formation of autophagosomes around proteins and organelles destined for degradation. Although these systems are often discussed as distinct entities, there is overlap and interplay between them and a given protein may be processed by more than one system. A good example of this is endoplasmic reticulum (ER)-associated degradation (ERAD), a mechanism for the removal and recycling of ER lumen or ER membrane anchored proteins that also participates in regulatory protein turnover. ERAD substrate proteins are handled by both the proteasomal and autophagic pathways [12].

Organelles house discrete proteostatic systems that work in concert with proteasomal and autophagic pathways. This ensures quality control of proteins that are post-translationally imported into organelles, regulation of the organellar proteome, and removal of organelles

during development and in response to damage [13,14]. Owing to their endosymbiotic origin, mitochondria and plastids do not have internal ubiquitin systems and rely on a network of chaperones and organelle proteases, including AAA+ caseinolytic protease (CLP), filamentation temperature sensitive (FTSH), long-filament phenotype (LON) and degradation of periplasmic proteins (DEG) proteases for intraorganellar proteostasis [13,21,22]. Similarly, peroxisomes employ luminal LON and DEG proteases and also interact intimately and extensively with the cytosolic proteasome system, as well as undergoing pexophagy [14].

Selection for dysregulated proteostatic mechanisms by crop breeding

A large body of literature bears witness to the extensive and important functions of proteostasis in plant biology, including responses to the environment, the circadian clock, and control of development. Importantly, ubiquitination plays key roles in all hormone signalling pathways, not only as an integral component of most perception mechanisms but also by fine-tuning levels of downstream signalling components and biosynthetic enzymes [4,5,15]. Through this and also via epigenetic processes, proteostatic mechanisms underlie key agronomic traits such as floral induction, determination of organ size, response to pathogens and responses to abiotic stress [5,7,8,11,16,18,23].

Given the importance of proteostasis in plants, it is perhaps not surprising that two of the most profound advances in modern crop improvement are underpinned by dysregulation of proteostatic mechanisms. In the Green Revolution of the 1960s, unprecedented increases in cereal production were achieved by the introduction of high-yielding wheat and rice varieties, coupled with the liberal use of fertilisers and pesticides [24]. Advances in yield could only be made by the introduction of dwarfing genes into cereal crops, to prevent stems collapsing under the weight of heavy ears (lodging) and permitting increased assimilate partitioning into grain. The best characterised of these are the *Reduced height* (*Rht*) genes of wheat. In hexaploid bread wheat, the *Rht-1* locus contains three homeologous *Rht-1* genes, designated as *Rht-A1a*, *Rht-B1a*, and *Rht-D1a*, which encode DELLA proteins, master regulators of gibberellin (GA) signalling that repress growth [25,26]. DELLA proteins belong to the GRAS family of transcriptional regulators and contain N-terminal motifs required for the interaction with the GA receptor, GIBBERELLIN INSENSITIVE DWARF1 (GID1), which promotes their rapid degradation in the presence of bioactive GAs [27,28] (Fig. 1). The semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* each contain a stop codon within their open reading frame that confers GA-insensitive semi-dwarfism [25]. Through translational re-initiation in the main open reading frame, these Green Revolution alleles produce N-terminally truncated proteins that lack the DELLA motif and therefore evade proteasomal degradation, leading to partial growth inhibition in the presence of GA, and consequently, reduced height [29,30] (Fig. 1).

The development of submergence tolerant rice arguably constitutes a second Green Revolution. Flooding is a significant cause of yield loss even in resilient crops: although rice is often grown in standing water (paddy conditions), elite high-yielding varieties typically have poor flood tolerance and over 18% of the global supply of rice is vulnerable to flash flooding [31]. It was long known that rice varies in flood tolerance, for example the Indian landrace, FR13A could recover and produce a viable harvest after at least a week of complete submergence (Fig. 2). However, attempts to transfer this trait to elite varieties were hampered by yield drag and it was not until the genes responsible were identified that precision breeding of submergence-tolerant mega-varieties became possible [31,32]. Fine mapping of the *SUBMERGENCE 1* (*SUB1*) quantitative trait locus (QTL) of FR13A revealed a cluster of genes encoding ethylene responsive factor (ERF) transcription factors. All rice accessions contain two genes, *SUB1B* and *SUB1C*, but an additional ERF, *SUB1A* is present in tolerant germplasm [32]. *SUB1A-1* is induced by ethylene under submergence and prevents the activation of genes that would otherwise promote elongation growth via GA

signalling and mobilisation of reserves; it also promotes the expression of genes required for survival of submergence and other stresses [33,34]. Thus, FR13A tolerates short-term flooding by using a quiescence strategy. A link to proteostasis emerged when the molecular basis of low oxygen signalling was discovered in Arabidopsis [35,36]. Arabidopsis has a large family of ERF transcription factors, five of which share a conserved N-terminal (Nt) domain [MCGGAI(I/L)] with a cysteine (C) at position two. Three of these ERFs, *RELATED TO APETALA* (*RAP*)*2.12*, *RAP2.2* and *RAP2.3* are constitutively expressed and undergo co-translational processing by methionine amino peptidases, to reveal an Nt C residue. In normoxia, this residue is oxidised by the action of dioxygenases and further modified by enzymatic addition of an Nt Arg residue which creates a degradation signal (degron), targeting the ERF proteins for proteasomal degradation via the Arg/N-degron pathway (Fig. 2). However, in hypoxic conditions typical of submergence, the degron is absent and the RAP proteins are stabilised, enabling activation of hypoxia responsive genes, such as fermentative enzymes alcohol dehydrogenase and pyruvate decarboxylase, and two *HYPOXIA RESPONSIVE ERF* (*HRE*) genes, which encode MC-initiating proteins. Upon reoxygenation, the HRE and RAP proteins are degraded via the Arg/N-degron pathway, effectively switching off the hypoxia response (Fig. 2). Intriguingly, SUB1A, despite bearing C at position 2, is not a substrate for the Arg/N-degron pathway [36], because it evades degradation via masking of the Nt degron by the C-terminus [34]. Two MC-ERFs, *ERF66/77* are direct transcriptional targets of SUB1A and promote survival of submergence. Unlike SUB1A, ERF66 and 67 are Arg/N-degron pathway substrates and consequently degraded in normoxia. Thus, whilst the specific hypoxia transcriptional response is silenced when not needed, SUB1A remains stable to coordinate the expression of a distinct group of genes required for survival of drought and reactive oxygen species upon desubmergence [34].

Proteostasis into practice- how can manipulating protein abundance serve agriculture?

Inspired by *Rht-1* semidwarf wheat and Sub1A rice, can we achieve step changes in crop performance by rational engineering or chemical manipulation of proteostatic mechanisms? Given the growing wealth of knowledge about plant proteostasis, genome editing has considerable potential to deliver desirable crop traits without the negative and unintended consequences of selection, as has already been demonstrated in *de novo* domestication projects [24,37]. Moreover, advances in structural biology and concepts borrowed from drug development offer promise for selective chemical intervention [38,39]. Below, we outline several different strategies to engineer or manipulate proteostasis for crop improvement, several of which have been demonstrated in model systems or crops.

1. Preventing degradation

Desirable phenotypes such as drought tolerance have been engineered by over expression of selected signalling components [24]. As the over expressed proteins can still be degraded, an attractive complementary strategy is to decouple selected proteins from endogenous post-translational regulation. Deleting or downregulating E3 ligases is an obvious approach, the impact of which will depend on the diversity and roles of the corresponding substrates. For example, knockdown of PUB 22/23/24, three U-Box E3 ligases that are negative regulators of immunity not only confers broad resistance against pathogens with distinct infection strategies but also drought tolerance [40]. Alternatively, removal of the degron, where known, provides a more targeted approach which has been used successfully to increase seed oil yield by site-directed mutagenesis of WRI1, a master transcription factor regulating lipid biosynthesis [41]. The concept was proven in transgenic plants but could in principle be achieved via CRISPR-Cas-induced deletions or base editing. An interesting case study is the engineering of flood tolerance by expressing stabilised ERF

transcription factors or by knocking down PRT6, the E3 ligase that controls their abundance in response to oxygen (Fig 1.). Whilst the latter strategy confers flood tolerance in barley without loss of seed yield [42], in other species, constitutive expression of the hypoxia response is deleterious, therefore more nuanced approaches are called for [39,43]. One possibility is rational engineering of plant cysteine oxidases, the dioxygenases involved in creating the degron, to tune the level of ERF proteins to specific O_2 levels [39]. Finally, an avenue not yet explored in plants is engineering of DUBs to control the ubiquitination level, and therefore abundance of specific proteins, for example using DUB-targeting chimera (DUBTAC) technology, in which a DUB is fused to a protein-targeting ligand [18,44].

2. Selective degradation

Many plant signalling pathways (most notably hormone signalling) rely on conditional degradation of repressors, providing an appealing target for engineering. Although there are several genetically encoded strategies for conditional degradation of proteins [45], in medicine, there is intense interest in the development of small molecule drugs that hijack proteostatic mechanisms, exemplified by proteolysis targeting chimaeras (PROTACs) and molecular glues [38]. PROTACs are heterobifunctional molecules that bind to a protein of interest and recruit an E3 ligase. The protein of interest is targeted for degradation by chemically induced proximity to the E3 ligase. Molecular glues are low molecular weight inducers of protein-protein interactions that upon binding one protein partner, create a new surface to enable binding of the second partner. The plant hormone, auxin is the original molecular glue, and has been used extensively to control rapid and specific protein degradation in mammalian systems, e.g. [46]. In plants, auxin mediates regulated degradation of Aux/IAA transcriptional regulators by TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFBs) co-receptor proteins [47]. An extensive literature encompassing genetics, biochemistry, biophysics, structural biology, synthetic biology, and mathematical modelling has produced a powerful toolkit with which to manipulate auxin signalling. Systematic characterisation of binding affinities and degradation dynamics for different IAA/receptor pairs has enabled development of a combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential auxin sensing [48,49], and chemical biology approaches have identified synthetic molecules that induce specific AUX/IAA protein degradation to modulate plant development [50]. Recently, an orthogonal auxin-TIR1 receptor pair that triggers auxin signalling without interfering with endogenous auxin or TIR1/AFBs has been engineered, enabling precise manipulation of auxin-mediated processes as a controllable switch [51]. In principle, these approaches to be applied to other plant hormones or a broader range of targets.

3. Selective translation

There are numerous examples of transgenic plants with increased resistance to pathogens, but trade-offs mean that superior immunity is often only achieved at the expense of growth [52]. An ingenious solution to this problem was the engineering in rice of a pathogen-responsive upstream open reading frame (uORF) cassette fused to NPR1, a central Arabidopsis disease resistance regulator, to produce a crop line that exhibits broad-spectrum disease resistance without a yield penalty [53]. This selective translation strategy was used subsequently to engineer pathogen resistance in Arabidopsis [54]. Salicylic acid-dependent immunity is compromised by transient heat, and transcription of CBP60g, a master immune regulator, was identified as a major thermosensitive step in the plant immune system. Driving CBP60g from the pathogen-responsive uORF ameliorated the impact of elevated temperature on plant immunity, without the growth penalty associated with constitutive over expression. These successes suggest that the large numbers of

uORFs encoded in plant genomes [55] provide an untapped resource that could be deployed more widely in crops.

4. Preventing hijack of proteostatic machinery by pathogens

Autophagy and proteasome-mediated protein degradation play multiple roles in defence against plant pathogens [11,23]. Conversely, pathogens such as bacteria, fungi, and oomycetes have evolved effector molecules that are secreted into the host to subvert plant cellular processes, and autophagy and the proteasome constitute a major hub where effectors converge [11,56]. For example, the *Xanthomonas campestris pv. vesicatoria* effector XopL suppresses host autophagy via its intrinsic E3 ligase activity, but XopL itself is targeted for degradation, revealing a complex antagonistic interplay between XopL and the host selective autophagy machinery [57]. In another example, SAP05, an effector from a plant pathogenic phytoplasma subverts plant morphogenesis by hijacking the proteasome subunit RPN10 to mediate degradation of transcription factors involved in developmental transitions [58]. This induces “witches’ broom”-like proliferations of leaves and facilitates parasitism by the phytoplasma. Intriguingly, substituting only two amino acids produced a functional RPN10 variant resistant to SAP05, paving the way to engineer resistance by genome editing. However, additional strategies would be needed to counter other effectors. Key considerations for more general adoption of this approach are whether it is constrained by conservation of essential proteostatic machinery and to what extent it delays the evolutionary “arms race” between host and pathogen.

5. Optimising stoichiometry of protein complexes and pathways

Manipulating proteostasis has utility in metabolic pathway engineering applications to produce novel high value products and increased yields of native products. For example, absolute protein quantification informed an over expression strategy to rebalance subunit stoichiometry of acyl-CoA carboxylase (the first committed step of de novo fatty acid biosynthesis) resulting in increased seed oil content in transgenic *Camelina sativa* [59]. Absolute protein quantification will also be important to facilitate tuning of protein expression levels when integrating heterologous enzymes into endogenous biochemical pathways.

6. Systems approaches

Although translatome data, protein quantitation and protein lifetime measurements are particularly relevant for understanding phenotypes associated with proteostasis, they are missing from the majority of gene expression studies [6]. Population-based proteomic studies in mammalian systems have provided new insights in the form of rich information on protein regulatory networks and protein complexes [60,61] and an extremely comprehensive catalogue of plant protein complexes has been published recently [62]. However, examples of protein centric genome-wide association studies (GWAS) and QTL analysis in plants are few and far between [63,64,65], and it remains to be determined whether and to what extent the genetic architecture of protein expression differs in plants. Given advances in quantitative proteomics and its application to plant systems [66,67,68,69] as well as the up scaling of translatome analysis [70], this is an area ripe for exploitation both in model plants and crops. In addition to identifying candidate genes, pathways, and regulatory networks for manipulation, use of recombinant inbred crop populations for protein QTL analysis potentially feeds directly into breeding pipelines. Proteomic analysis also has much to offer in terms of understanding impact of domestication and breeding on the proteome, including polyploidy, heterosis, and introgression [58,71,72,73,74,75,76], and will be important to understand and control how the proteome responds to transgenes.

Perspectives

In conclusion, there is enormous potential to manipulate proteostatic processes for agronomic improvement. Nevertheless, to design robust strategies for the development of resilient crops, it will be necessary to better understand plant proteostasis. Many gaps remain, such as knowledge of physiological E2-E3 pairs and their ubiquitin chain building properties, identification of substrates for the majority of E3 ligases, the elucidation of protein regulatory networks on a genome wide scale and understanding the interplay between different proteostatic systems. Here, technological advances will undoubtedly help. In addition to improvements to analytical techniques, such as protein lifetime measurements [77,78,79], increasingly sophisticated synthetic biology approaches will be possible, for example through new toolkits [80], design of Boolean logic gates [81], and rational design of components facilitated by advances in cryo-EM and protein structure prediction [82]. Finally, to bring these ideas into practice, it will be necessary to move beyond controlled environments and test crops bred or engineered for proteostatic traits in the field. In several countries there remain significant regulatory barriers to testing and commercialisation of genetically modified crops, but transgene-free solutions, including some forms of genome editing [83] and tuneable, transient reprogramming [84] offer exciting options to be explored.

Summary points

- Proteostatic mechanisms profoundly influence a wide range of important agronomic traits.
- There is enormous potential to improve crops by genetic or chemical intervention in proteostatic processes.
- A better understanding of plant proteostasis will underpin intelligent strategies for crop improvement in the face of climate change and population growth.
- Field testing will be an important element in delivering real world solutions, potentially aided by relaxation of the regulations that govern genome edited crops in an increasing number of countries.

Competing interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

AAA+, ATPases associated with diverse cellular activities; CLP, caseinolytic protease; DEG, degradation of periplasmic proteins; DUB, deubiquitinase; DUBTAC, DUB-targeting chimera; ERAD, Endoplasmic reticulum (ER)-associated degradation; ERF, ethylene response factor; FTSH, filamentation temperature sensitive; GA, gibberellin; GID1, GIBBERELLIN INSENSITIVE DWARF1; GWAS, genome-wide association study; HECT, homologous to the E6-AP carboxyl terminus; LON, long-filament phenotype; MC, methionine cysteine; Nt, amino-terminal; PROTAC, proteolysis targeting chimaera; PTM, post-translational modification; QTL, quantitative trait locus; RAP, RELATED TO APETALA; RBR, RING-in-Between-Ring; RING, really interesting new gene; SUB1, SUBMERGENCE 1; TIR/AFB, TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX; uORF, upstream open reading frame.

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Figure legends

Fig. 1 DELLA proteins encoded by Green Revolution *Reduced height* alleles evade GA-dependent degradation and truncated proteins are produced by translational re-initiation.

A. Nucleotide and predicted protein sequences of the N-terminal region of wheat (var Cadenza) *Rht-1* homeologues. Re-drawn after [29]. Positions of the *Rht-B1b* and *Rht-D1B* point mutations leading to stop codons are indicated by arrows; silent single nucleotide polymorphisms are indicated in lower case. B. Schematic of the wild type RHT-1 protein and protein products detected in *Rht-B1b* and *Rht-D1B*: a 62 or 63 amino acid N-terminal peptide and a truncated protein, ΔN-RHT produced by translational re-initiation at methionine residues (green) downstream of the DELLA motif (magenta). The GRAS subdomain is shaded grey. C. Model showing DELLA-regulated growth responses in hexaploid wheat carrying the wild type or *Rht-B1b* allele, adapted from [30]. In the absence of GA, DELLA proteins encoded by the three RHT homeologues repress stem elongation and other GA-responses. In the presence of GA, wild type RHT proteins bind the GID1 GA receptor and are degraded by the 26S proteasome, releasing repression of growth responses such as stem and rachis elongation. The low efficiency of translational re-initiation in *Rht-B1b* results in low amounts of N-terminally truncated RHT protein (ΔN-RHT-B1) which lacks the DELLA domain and consequently evades proteasomal degradation, resulting in weak repression of GA-responses and a semi-dwarf phenotype.

Figure 2. Group VII ERF transcription factors in hypoxia signalling: the rice SUB1A protein evades degradation by the Arg/N-degron pathway.

A. Submergence demonstration plot at the International Rice Research Institute, Philippines, showing the performance of pairs of Sub1 (+) and non-Sub1 (-) rice lines, in which the SUB1A locus from FR13A was introduced into several mega varieties: C, Ciherang (China), IR64 (Philippines), BR11 (Bangladesh), S, Swarna, and SM, Samba Mahsuri (India). B. Oxygen sensing and signalling mechanisms in Arabidopsis and submergence-tolerant rice. Arabidopsis (left) constitutively expresses three Group VII ERF transcription factors (ERVIIs; RAP2.12, RAP2.2 and RAP2.3), which possess an N-terminal methioine-cysteine motif (Nt MC-). Nt Met is cleaved co-translationally by methionine aminopeptidases (MetAP), revealing Nt C, which under normoxia, is oxidised (C^{OX}) by the action of plant cysteine oxidases (PCO), rendering it susceptible to arginylation by arginyl transferase enzymes (ATE). This creates a degradation signal (Nt Arg) recognised by the Arg/N-degron pathway E3 ligase PROTEOLYSIS6 (PRT6) and the RAP proteins are degraded by the 26S proteasome. Under hypoxia, the RAPs are stabilised and activate the transcription of various response genes, including *ALCOHOL DEHYDROGENASE* (*ADH*), *PYRUVATE DECARBOXYLASE* (*PDC*), *PHYTOGLOBIN1* (*PGB1*) and two additional ERFVII transcription factors, *HYPOXIA RESPONSIVE ERF* (*HRE*)*1* and *2*. As MC-initiating proteins, HRE1 and 2 are also degraded by the Arg/N-degron pathway when normoxia is restored. In rice (right), the SUB1A ERFVII transcription factor is transcriptionally induced in response to submergence and in turn induces expression of *ERF66* and *ERF67* in addition to a distinct set of target genes that confer stress tolerance. In hypoxic conditions, ERF66 and 67 trigger expression of further submergence responsive genes but are degraded by the Arg/N-degron pathway under normoxia. SUB1A is also an MC-initiating protein but escapes degradation via the Arg/N-degron pathway because the Nt MC motif is shielded by the C-terminal region of the protein. Thus, it remains active to coordinate the expression of other genes required for survival.