Review: Endoplasmic Reticulum-Associated Degradation (ERAD)-Dependent Control of (Tri)terpenoid Metabolism in Plants

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ABSTRACT

Plants are sessile organisms. Therefore, they developed the capacity to quickly respond to biotic and abiotic environmental stresses, for instance by producing a broad spectrum of bioactive specialized metabolites. In this defense response, the jasmonate phytohormones can instigate a signaling cascade that leads to the specific elicitation and reprograming of numerous metabolic pathways. Recent research progress has provided several insights into the regulatory networks of many specialized metabolic pathways, mainly at the transcriptional level. Nonetheless, our view on the regulation of defense metabolism remains far from comprehensive. Here, we describe the recent advances obtained with regard to one aspect of the regulation of plant specialized metabolism, namely the posttranslational regulation of enzyme stability. We focus on terpenoid biosynthesis and in particular on the rate-limiting and well-investigated enzyme of the terpenoid precursor pathway, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR). There are clear similarities, as well as important mechanistic differences, among the components involved in the posttranslational regulation of terpenoid biosynthesis via HMGR in plants, yeasts, and mammals. Furthermore, in plants, several of these components evolved to respond to specific signaling cues. Indeed, the elements of the plant endoplasmic reticulum-associated degradation (ERAD) and ER stress-associated processes can be induced upon environmental stresses and during specific developmental processes, thereby allowing a unique posttranslational regulation of terpenoid biosynthesis pathways.

Introduction

Plants are stationary by nature. Consequently, they are constantly challenged, often coping with biotic and abiotic stresses. Therefore, metabolic plasticity is crucial for their survival. A good example of this flexibility is the production of a myriad of bioactive specialized metabolites such as terpenoids, alkaloids, and phenolic compounds [1]. This review focuses on the terpenoids (also known as isoprenoids), which belong to a functionally and structurally diverse class of molecules with tens of thousands of different identified members in existing plant species and can be either widespread or restricted to a specific species or taxa. Numerous terpenoids can be considered essential, because they play a cru-

cial role in environmental adaptation, plant defense, and plant-environment interaction. For instance, they can serve as attractants for pollinators or seed-dispersing animals and repellents for herbivores or pathogens [2].

Terpenoids such as phytosterols, carotenoid pigments, electron transport chain components (quinones), and signaling molecules including gibberellins, ABA, BR, cytokinins, strigolactones, and phytoecdysteroids are associated with primary metabolism, regulating plant growth and development, photosynthesis, membrane permeability, and fluidity and are therefore occurring in all plant species [3–8].

Economically, many specialized terpenoids have beneficial properties for humans and are currently used for clinical purposes

ABA	abscisic acid
bHLH	basic helix-loop-helix
BiPs	binding proteins
BIS	basic helix-loop-helix (bHLH) iridoid synthesis
BR	brassinosteroids
bZIP	basic leucine-zipper
DMAPP	dimethylallyl pyrophosphate
Doa10	degradation of alpha 10
DRY2	drought hypersensitive 2
ER	endoplasmic reticulum
ERAD	endoplasmic reticulum-associated degradation
ERQC	ER protein quality control
FPP	farnesyl pyrophosphate
GGOH	geranylgeraniol
GGPP	geranylgeranyl pyrophosphate
gp78	glycoprotein 78
HMGR	3-hydroxy-3-methylglutaryl-coenzyme A reductase
HMG-CoA	HMG-coenzyme A
HR	hypersensitive response
HRD	HMGR degradation
IPP	isopentenyl pyrophosphate
IRE1	inositol-requiring protein 1
JA	jasmonic acid or jasmonate
lew1	leaf wilting 1
MEP	2-C-methyl-D-erythritol 4-phosphate
MKB1	makibishi 1
MVA	mevalonic acid or mevalonate
MYC	bHLH transcription factor
NAC	no apical meristem (NAM), *Arabidopsis thaliana* activating factor 1 and 2 (ATAF1 and -2), and cup-shaped cotyledon (CUC2)
OSER	organized smooth ER
P450	cytochrome P450s
RING	really interesting new gene
SA	salicylic acid
SAR	systemic acquired resistance
SSD	sterol-sensing domain
SQE	squalene monooxygenase
SUD1	suppressor of DRY2 effects 1
TSAR	triterpene saponin biosynthesis activating regulator
UbiA	polyubiquitin
UBIAD1	UbiA prenyltransferase domain-containing protein-1
UDP	uridine diphosphate
UPR	unfolded protein response

[9–14]. Among these terpenoids are the anticancer agent paclitaxel (taxol), which is a naturally occurring diterpenoid produced by *Taxus brevifolia* Nutt. (Coniferae) and *T. baccata* L. (Coniferae) and the antimalarial agent artemisinin, a sesquiterpenoid lactone from *Artemisia annua* L. (Asteraceae) [15–17]. The nonfood industry uses latex, extracted from *Hevea brasiliensis* Müll.Arg. (Euphorbiaceae) or the rubber tree, the main source of natural rubber for the production of tiers, tubing, elastics, and toys [18]. Another highly valued terpenoid in the flavor industry is the diterpenoid menthol produced by *Mentha x piperita* L. (Lamiaceae) [19, 20].

Despite their structural variability, all terpenoids are composed of a set amount of five-carbon (C_5) isoprene (2-methyl-1,3-butadiene) units, corresponding to their classes [21]. The biochemically active forms of isoprene are IPP and its allylic isomer DMAPP, both biosynthesized by two compartmentalized core pathways [22], the MVA pathway that operates in the cytosol, the ER and peroxisomes, and the MEP pathway that takes place in the plastids [23–29]. Plants uniquely harbor both pathways, although there is a set allocation of the two pathways among the different kingdoms of life [30]. In essence, in higher plants, the MVA pathway is responsible for the biosynthesis of the sesqui- and triterpenoids (C_{15}, C_{30}) and the MEP pathway for the formation of mono-, di-, some sesquiterpenoids (C_{10}, C_{20}, C_{15}), and plastoquinones [31].

To date, multiple studies have focused on the regulation of the core terpenoid biosynthesis reactions, in particular the rate-limiting ER-localized enzyme of the MVA pathway HMGR, mainly to decipher the control of the biosynthesis of specific end-products of the pathways [8]. This review will focus on posttranslational regulation of enzymes that are involved in terpenoid precursor biosynthesis as well as the pathway branches leading to the production of specialized metabolites and in particular the triterpenoids of which the biosynthetic enzymes are predominantly localized in the ER. Consequently, ER-centralized regulation of enzyme stability executed by ERAD machinery and its associated processes play an essential role in the regulation of these metabolic pathways.

Biosynthesis of Triterpenoids at the ER Membrane

The class of triterpenoids (C_{30}) in plants comprises primary metabolites such as phytosterols and BR and the specialized metabolites called saponins that serve as defense compounds against pathogens and herbivores [10].

Triterpenoid saponin biosynthesis starts in the cytosol with the "head-to-tail" condensation of two IPP units with one DMAPP unit, generating FPP. All consecutive biosynthesis steps towards triterpenoid saponins take place at the ER membrane. Two FPPs fuse "head-to-head" to form linear squalene, catalyzed by squalene synthase. Squalene is subsequently epoxidized by squalene monooxygenase (SQE) to 2,3-oxidosqualene, the last common intermediate in saponin and sterol biosynthesis. 2,3-oxidosqualene is further cyclized by specific oxidosqualene cyclases to either cycloartenol, the plant sterol precursor, or other cyclization products that are further oxidized by one or more P450s to form a wide variety of specific triterpenoid backbones or sapogenins. Finally, these sapogenins are further decorated by covalent attachment of sugar moieties by UDP-dependent glycosyltransferases, thereby further increasing the structural diversity of this metabolite class [32, 33]. For a complete overview of the triterpenoid biosynthesis in plants, we refer to Thimmappa et al. (2014) [34].

The amphipathic nature of triterpenoid saponins gives them economically valuable properties. For instance, saponin extracts from *Quillaja saponaria* Molina (Quillajaceae) have been used as emulsifiers and industrial soaps in the nonfood industry [35–39]. Furthermore, extracts of *Glycyrrhiza glabra* L. (Fabaceae) and *Panax ginseng* Baill. (Araliaceae), which contain glycyrrhizin and ginsenoside saponins, respectively, are commonly used as herbal medicines [40–42]. Glycyrrhizin is also used as natural sweetener in the food industry [43].

Transcriptional Regulation of Terpenoid Biosynthesis

Biologically, biosynthesis of plant triterpenoid saponins is controlled by a signaling cascade wherein the oxylipin-derived JA phytohormone is the protagonist [44–46]. In the last years, much effort has been made to identify transcription factors that control the terpenoid biosynthesis upon JA perception. First, a bHLH transcription factor MYC2 has been shown to control the sesquiterpenoid biosynthesis in *Arabidopsis thaliana* L.Heynh (Brassicaceae), *Solanum lycopersicum* L. (Solanaceae), and *A. annua* [47–50]. Shortly thereafter, two MYC2-unrelated bHLH transcription factors were identified in *Cucumis sativus* L. (Cucurbitaceae) that regulate the production of cucurbacitins [51]. Furthermore, in *Catharanthus roseus* (L.) G. Don (Apocynaceae), two bHLH transcription factors, bHLH iridoid synthesis 1 and 2, have been shown to induce the monoterpenoid branch of the monoterpenoid indole alkaloid pathway [52].

Recently, mechanisms that specifically control triterpenoid biosynthesis have also been identified. For example, in *Medicago truncatula* Gaertn. (Fabaceae), bHLH transcription factors TSAR1 and TSAR2, homologs of BIS1 in *C. roseus*, and therefore also unrelated to MYC2, were found to boost the nonhemolytic and hemolytic triterpenoid saponin biosynthesis, respectively [53, 54]. BIS and TSAR homologs also control the production of antinutritional triterpenoid saponins in quinoa seeds and soyasaponins in *Glycyrrhiza uralensis* Fisch. Ex DC. (Fabaceae) [55, 56].

Posttranslational Regulation of Enzyme Stability of the Terpenoid Biosynthetic Pathways in Plants

Substantial effort has been made to identify regulatory enzymes of the IPP precursor pathways and their mode of action [8, 57, 58]. One particular enzyme, HMGR, received much attention and is therefore the best-characterized enzyme of the MVA pathway in plants, yeasts, and mammals until now [59]. Because terpenoids are produced by all free-living organisms, it is not surprising that some regulatory and structural features of HMGR in the MVA pathway have been conserved and might have undergone parallel and functional evolution to cope with certain situations or suit specific requirements of organisms [60].

The mammalian genome and that of the fission yeast *Schizosaccharomyces pombe* contain only one gene encoding HMGR, whereas the genome of the budding yeast *Saccharomyces cerevisiae* harbors two isozymes, and in all plant species studied so far, HMGR is encoded by a multigene family [26, 61–66].

All eukaryotic HMGRs are targeted to the ER and consist of an N-terminal membrane domain with low sequence similarity, a C-terminal catalytic domain that is highly conserved and localized at the cytosolic site of the ER membrane, and a nonconserved flexible linker connecting the two [67]. Plant and mammalian HMGR is tetrameric, as a result of the oligomerization potential of the catalytic domain [68, 69]. Roitelman et al. (1992) predicted that the membrane domain of mammalian HMGR contains eight transmembrane sequences, contrasting with the model of Chin et al. (1982), wherein HMGR spans the membrane seven times. A similar eight membrane-span model was predicted for yeast HMGR [67, 70, 71]. In plants, HMGR only contains two transmembrane domains [26, 61, 72] (▶ **Fig. 1 A–C**). The mechanisms by which yeast and mammalian HMGR are regulated at multiple levels are profoundly described in a review by John S. Burg [73]. In this review, we will elaborate on the posttranslational control of HMGR activity by degradation.

The membrane domain of HMGR in mammals and HMG2 in *S. cerevisiae* is necessary for accelerated sterol or nonsterol (endproducts of the MVA pathway) feedback regulation by ERAD using the same machinery that is responsible for the elimination of improperly folded secretory proteins by proteasomal degradation [71, 74, 75]. Both HMGR in mammals and HMG2 in *S. cerevisiae* contain a SSD in the N-terminal membrane domain, consisting of five consecutive transmembrane spans [76, 77]. In mammals, it is this SSD that binds to Insig-1, an ER-retention protein, resulting in accelerated HRD by the gp78 machinery triggered by 24,25-dihydrolanosterol or oxysterol [78–81]. Furthermore, the nonsterol isoprenoid GGOH may act as an enhancer of degradation of mammalian HMGR by promoting the extraction of the ubiquitinylated HMGR from the ER, thereby facilitating degradation by the proteasome [82].

Likewise, two lipid signals control the rate of HMG2P turnover through ERAD, using the HRD machinery in *S. cerevisiae* [70, 83]. GGPP may act by altering the conformation of the membrane domain of HMG2P and thereby inducing Insig-independent HRD-mediated degradation of its incorrectly folded version and thus maintaining the lipid homeostasis in the cell [84]. An oxysterol-derived signal can further enhance the MVA-derived signal-induced degradation (▶ **Fig. 1 A, B**) [85]. A similar signal controlling the degradation of HMG1 in *S. pombe* remains to be discovered.

Furthermore, the ERAD E3 ubiquitin ligase Doa10 and TEB4 machinery in yeasts and mammals, respectively, are responsible for the sterol-dependent degradation of SQE to prevent accumulation of toxic sterol intermediates [86]. In plants, an analogous mechanism to target SQE has not been discovered yet.

Recently, it was discovered in mammals that sterols stimulate binding of UBIAD1, which uses GGPP to synthesize vitamin K_2, to a subset of HMGR, thereby inhibiting ERAD [87]. GGPP can trigger the release of UBIAD1 from HMGR, thereby enabling ERAD. This UBIAD1-dependent HMGR control system allows sterol-replete cells to synthetize MVA for the replenishment of GGPP [88].

That HMGR is also modulated at all levels in plants is reflected by the lack of correlation between enzyme activity, mRNA levels,
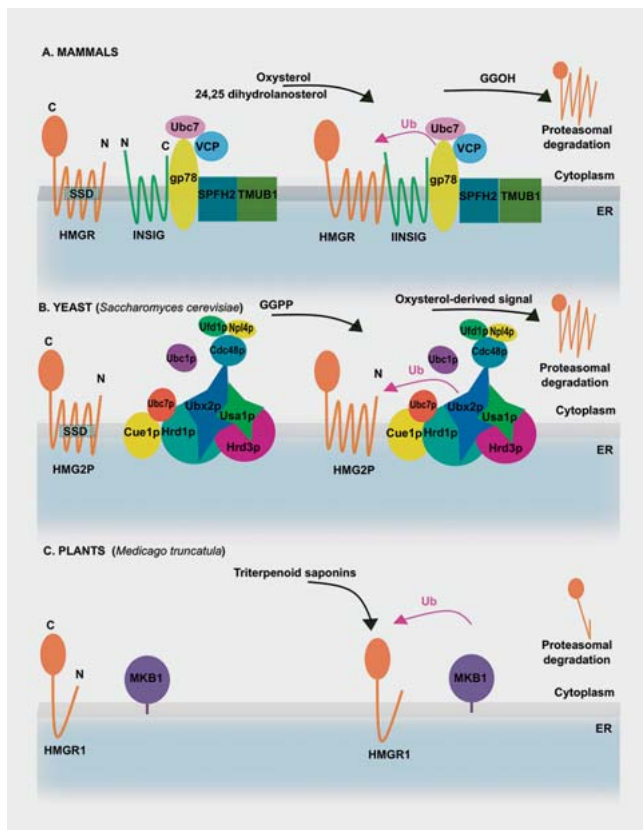
▶ **Fig. 1** Regulated degradation of HMGR in mammals (**A**), yeasts (*S. cerevisiae*) (**B**), and plants (*M. truncatula*) (**C**). **A** In the absence of sterols, HMGR does not bind Insig. In the presence of 24,25-dihydrolanosterol or oxysterols, Insig promotes ubiquitinylation (Ub) and proteasomal-dependent degradation of HMGR through sequential interactions with the E2 ubiquitin-conjugating enzyme Ubc7, the E3 ubiquitin ligase gp78, the ATPase VCP/p97, the SPFH domain-containing protein member 2 (SPFH2), and transmembrane and ubiquitin-like domain-containing protein 1 (TMUB1). GGOH possibly enhances the mammalian HRD by a mechanism downstream of ubiquitinylation. **B** GGPP induces a conformational change of HMG2P, thereby promoting recognition and ubiquitinylation by the HRD complex. HRDP3P is responsible for substrate recognition and delivery. HRD1P employs two E2 ubiquitin-conjugating enzymes: the primary soluble Ubc7p and Ubc1p. Ubc7p interacts with the membrane through the integral membrane protein Cue1p. Usa1p functions in the self-regulation of HRD1P. Ubx2p is responsible for the recruitment of the Cdc48p/Npl4p/Ufd1p ATPase complex, thereby catalyzing retrotranslocation of luminal and membrane-bound HRD substrates. An oxysterol-derived signal can also regulate HMG2P activity. **C** Coinciding with high triterpenoid saponin levels, HMGR is recruited to the MKB1 E3 ubiquitin ligase complex for ubiquitinylation and subsequent proteasomal-dependent degradation in *M. truncatula*.

and protein content [26, 89–92]. At the posttranslational level, plant HMGR activity can be controlled through noncovalent interactions with metabolic intermediates such as sterols, saponins, ABA, ubiquinone, and 4-hydroxybenzoic acid, but their mode of action awaits discovery [93]. Also, covalent modifications such as phosphorylation, glycosylation, and ubiquitinylation followed by proteasomal degradation can control HMGR activity [94–100].

Several observations support that an analogous type of proteasomal-dependent degradative control of HMGR in mammals and yeasts is also exerted in plants following diverse developmental and environmental cues, but the mode of action is still limitedly understood, even for a well-investigated enzyme such as HMGR. For example, in pea, HMGR activity declines rapidly when etiolated seedlings are irradiated with red light, which suggests that phytochrome-mediated signaling indirectly controls HMGR activity through a posttranslational mechanism [93]. Also, the linker region between the membrane domain and the catalytic domain of plant HMGR is rich in so-called PEST (Pro, Glu [and Asp], Ser, Thr) amino acids, which have been shown to be responsible for the turnover of ER-located proteins possibly by proteasomal degradation [101]. Contrarily, the membrane domain of plant HMGR is fairly small compared to HMGR in yeasts and mammals and does not contain a SSD, and plants do not express an ortholog of the ER-resident Insig-1 protein [61].

Nevertheless, in *M. truncatula*, a system that monitors the accumulation of bioactive triterpenoid saponins to secure plant development and integrity by turnover of HMGR was recently characterized. In particular, MKB1, a RING membrane-anchor E3 ubiquitin ligase without sequence homology to the yeast HRD and mammalian gp78 E3 ligases, was found to act as a posttranslational regulator of HMGR [102]. MKB1 recruits the ERAD system to regulate HMGR activity by controlling protein stability and hence the amount of saponin precursors generated via the MVA pathway (▶ **Fig. 1 C**). Therefore, silencing of MKB1 leads to an uncontrolled accumulation of monoglycosylated saponins and an aberrant root morphology.

Contrarily, SUD1, which is homologous in sequence and structure to the yeast Doa10 and human TEB4, was characterized as a positive posttranslational regulator of HMGR activity in *A. thaliana* [103]. In yeasts and mammals, the HDR and gp78 machinery, respectively, regulate HMGR activity by controlling protein stability, whereas SUD1 controls HMGR activity without changing the protein content but possibly by degrading a negative regulator of HMGR.

Besides clear similarities, there are also important mechanistic differences among the components involved in the terpenoid regulation through HMGR in plants, yeasts, and mammals. The nonconserved regions of plant HMGR may have steered the evolution of a specific control mechanism for HMGR activity, possibly regulated by specific saponins, their intermediates or plant-specific mediator proteins, since plants do not encode Insigs or Insig-like homologs. Identification of such mediators will be important for the understanding of this plant-specific degradative HMGR activity control mechanism. MKB1 possibly also targets other proteins than HMGR, such as P450s, SQE, chaperones, or regulators to protect itself against its own defense strategies.

In mammals for example, the ER-anchored hepatic P450s metabolize endo- and xenobiotics. Such agents can positively control liver P450 protein levels by increased synthesis or negatively via inactivation or degradation. Recently, an ERAD-dependent control mechanism using gp78 and C-terminus of Hsc70-interacting protein to target CYP3A4, the major human liver/intestinal P450, and the fast-turnover liver CYP2E1 for degradation, has been de-

scribed [104]. Likewise, it is possible that also in plants such regulators exist that target ER-located proteins such as P450s.

The membrane domain of plant, mammalian, and yeast HMGR is not only a determinant of its own turnover; it is also responsible for the morphology of its residency, the ER. The ER is a dynamic net of tubules, sheets and cisternae harboring terpenoid biosynthetic enzymes and the machinery controlling them. The constellation of the ER net and its tenants may vary, depending on the cellular demands. For example, gland cells in plants require an increased ER net to accommodate the production of terpenoids [105]. Likewise, the biosynthesis of steroid hormones requires an expanded ER in mammalian adrenocortical cells [106].

This ER expansion, also called ER hypertrophy, was first discovered in compactin (competitive inhibitor of reductase)-resistant Chinese hamster ovary UT-1 cells that were obtained by stepwise adaptation to grow at an increased concentration of compactin [107]. These cells generated crystalloid ER membranes consisting of hexagonal or cubical tubules/sheets [107–110].

Also, in *S. cerevisiae* a similar phenomenon was described. Overexpression of HMG1P resulted in the proliferation of karmellae, which are stacked cisternae on the outer nuclear envelope, whereas overexpression of *HMG2P* leads to the formation of peripheral ER membrane stacks and short karmellae [69, 111, 112]. All of these structures are categorized as OSER [113]. Although the mechanisms and signals that trigger this ER proliferation are to date still unknown, the membrane-spanning domains six and seven in yeast and mammal HMGR determine the ER morphogenesis potential, thereby regulating the catalytic activity of the C-terminal domain [112]. Nevertheless, a properly folded catalytic domain is indispensable to induce OSER biogenesis [69]. *A. thaliana* encodes three HMGR isoforms. HMG1 encodes HMGR1S and HMGR1L, the latter which holds a 50-amino acid residue extension at the N-terminus, whereas HMG2 encodes HMGR2. Expression of the membrane domain of HMGR1S and HMG2 leads to the reversible proliferation and morphogenesis of the ER in every cell type of diverse plant species [114]. Apparently, an N-terminal Arg-motif does not only serve as an ER-retention signal but it is also a requisite to trigger the biogenesis of OSER in plants [114].

The Regulatory Role of ERAD and Associated Processes and Signals that Activate or Modulate Them in Plants

Nearly one-third of the newly synthesized proteins allocated to the secretory pathway in the ER are misfolded. Therefore, a highly efficient ERQC mechanism is responsible to secure correct folding and removal by ERAD in case the proteins do not meet the conformational standard [115–119]. This process consists of four steps, namely recognition by E3 ubiquitin ligases embedded in the ER, ubiquitinylation, dislocation from the ER, and ultimately degradation of the ubiquitinylated folding-defective proteins by the 26S proteasome in the cytosol [116].

Adverse environmental conditions or certain developmental stages can trigger the accumulation of unfolded and misfolded proteins in the ER [120]. This imposed ER stress may elicit a conserved UPR, which consists of specific transcriptional and translational regulatory cascades that bring the ER folding capacity in line with demands by for example reducing the synthesis of secreted or membrane proteins or by increasing folding-assisting proteins such as chaperones and foldases or other components of the ERQC system.

ERQC, ERAD, or UPR deficiencies in plants can lead to an increased sensitivity to environmental stresses or an inadequate development. In the following sections, we will discuss the hitherto studied players in plant ERAD and ER stress-associated processes, their link to terpenoid biosynthesis regulation, and their involvement towards biotic and abiotic stresses and development.

ER Stress Response and Biotic Stress

Biotic stress triggers signaling molecules such as the plant hormones SA and JA to elicit a downstream defense response [121]. For instance, SA activates resistance against biotrophic pathogens by inducing a HR and SAR [122], whereas JAs activate a defense response against herbivorous insects and necrotrophic pathogens by the production of anti-insect proteins [123] and vegetative storage proteins [124] or specialized metabolites such as terpenoids, polyamines, quinones, alkaloids, phenylpropanoids, and glucosinolates [44, 45]. Depending on the situation, the interdependent SA- and JA-signaling pathways can work synergistically or antagonistically through modulation by other hormones [125–127].

Plants harbor several ER-localized stress sensors and transducers such as the bZIP transcription factors bZIP28, bZIP17, and bZIP60, the NAC transcription factors NAC062 and NAC089, and the RNA-splicing enzyme IRE1. Upon pathogen infection, IRE1 is activated by a still unknown mechanism, thereby enabling splicing of bZIP60 mRNA, resulting in the translation of an active form of bZIP60 that translocates to the nucleus, where it induces an ER stress response (▶ **Fig. 2**). The IRE1a mutant in *A. thaliana*, which is deficient in bZIP60 mRNA splicing, is more sensitive to pathogen infection and is defective in establishing SAR [128]. Likewise, in *Nicotiana benthamiana* Domin. (Solanaceae), silencing of bZIP60 resulted in plants that are more susceptible to *Pseudomonas cichorii* infection [129].

The link between the SAR and secretory pathway was further reinforced by the observation that an *A. thaliana* mutant in BiP2, encoding a highly abundant ER-luminal heat shock protein 70 chaperone, was impaired in the induction of the SA-responsive gene *PR1* [130]. It is possible that upon biotrophic pathogen-induced stress, the UPR is steering a SA response, thereby antagonizing a JA-response and thus also the production of specialized metabolites, including terpenoids.

ER Stress Response and Abiotic Stress

BiPs can also play a role in abiotic stress responses. Overexpressing BiPs in soybean or tobacco confers drought tolerance, possibly as the result of a dynamic interplay between drought stress responses and UPR [131, 132]. For example, a *lew1* mutant in *A. thaliana*, encoding a *cis*-prenyltransferase for the synthesis of dolichols, which are long-chain unsaturated polyterpenoids, showed a leaf-wilting phenotype under normal growth conditions [133].
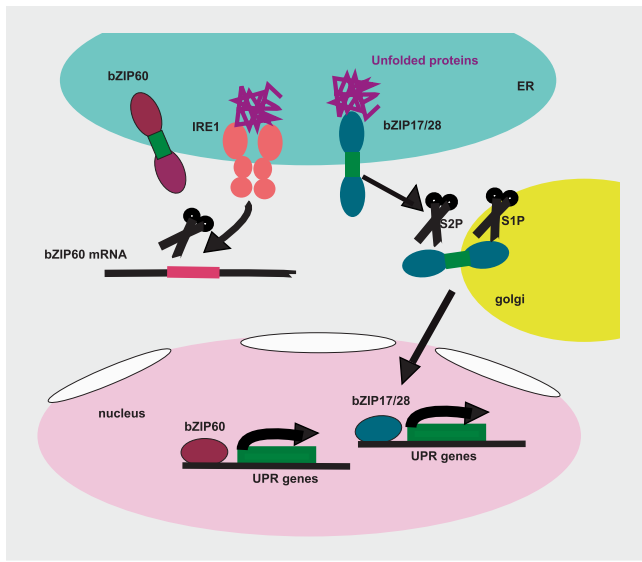
▶ **Fig. 2** A stylistic representation of the UPR in plants. When activated, by a still unknown mechanism, plant IRE1 splices out a 23-base intron of bZIP60 mRNA, inducing in a frame shift that results in the elimination of the transmembrane domain of the bZIP60p and thereby targeting active bZIP60 to the nucleus to induce expression of UPR genes. In a second mechanism, upon salt or heat stress, bZIP17/28 relocates to the nucleus through the Golgi where it is sequentially cleaved by the transcription factor peptidases, Site-2-proteases 1 and 2, to induce the transcription of several UPR genes.

Dolichols promote protein trafficking in the ER by carrying sugars for protein glycosylation. Consequently, a mutation in *lew1* can cause a loss of membrane integrity and reduced protein glycosylation. Drought stress resulted in higher expression of the UPR genes BiP and bZIP60 and earlier expression of the abiotic stress-responsive genes *Cold Regulated 47* and *Cold Regulated 29A* in the *lew1* mutant. This implicates that *lew1* links the MVA-dependent dolichol biosynthesis, the UPR pathway, and the abiotic stress response in *Arabidopsis*.

Furthermore, it has been shown in *A. thaliana* that the UPR can also be involved in the high salt or heat stress response. Upon heat stress, bZIP28 relocates from the ER to the nucleus through the Golgi, where it is cleaved and activated by the sequential action of the peptidases Site-2-protease 1 and 2 [134, 135]. A similar activation mechanism is triggered for bZIP17 upon salt stress (▶ **Fig. 2**) [136]. Mutating *A. thaliana* S2P impaired proteolytic activation and nuclear relocation of bZIP17 and bZIP28 [137].

Recently, a link was found between ER stress signaling, stress acclimatization, and BRs, which are terpenoid signaling molecules involved in growth and development. Abiotic stress can disable maturation and translocation of brassinosteroid insensitive 1, a leucine-rich repeat receptor kinase, from the endomembrane to the plasmamembrane, implicating that S2P-activated bZIPs can steer brassinosteroid insensitive 1 delivery to the plasma membrane and directly regulate BR signaling required for stress acclimatization and growth [137].

ER Stress Response and Plant Development

Deficiencies in the ERQC, ERAD, or UPR machinery can cause slower growth and sometimes even sterility. For example, *A. thaliana* S2P can control root development through BR signaling [137]. Furthermore, *A. thaliana* S2P monitors bZIP17 activation and therefore controls the expression of negative regulators of ABA signaling, enabling S2P to desensitize ABA signaling during seed germination [138]. Furthermore, a short root phenotype was observed in double *ire1a ire1b* mutants, suggesting that the UPR machinery can influence root development in normal conditions [139, 140].

Concluding Remarks and Perspectives

The past few years have yielded remarkable progress in our understanding of the molecular details underlying the posttranslational regulation of terpenoid biosynthesis through modulation of enzyme stability. However, the acquired insights are mostly limited to HMGR, the rate-limiting enzyme of the MVA-dependent IPP precursor pathway. Although there are important mechanistic differences across eukaryotic species in the regulatory control of this extremely conserved protein, there are also clear similarities. The signals that trigger each mode of regulation, although different in their exact identity, may be considered analogous in mammals, yeasts, and plants. Regulated degradation of HMGR seems indeed associated with a lipid signal: 24,25-dihydrolanosterol and an oxysterol in mammals, GGPP and oxysterols in yeasts and a yet unknown terpenoid intermediate in plants. The mechanistic differences may be caused by the divergent regions of HMGR in eukaryotes. As such, evolution may have allowed plants to develop multiple and plant-specific control mechanisms for HMGR activity and stability, possibly controlled by plant-specific triterpenoids, their intermediates, and/or by plant-specific protein mediators. For instance, orthologs of the HMGR-binding Insigs or Insig-like chaperones, conserved between yeasts and mammals, are not present in plants. Furthermore, it is possible that additional regulators, such as plant-specific E3 ubiquitin ligases, are able to target other ER-localized proteins than HMGR (e.g., triterpenoid biosynthesis enzymes such as SQE or P450s). This could be an ingenious system for plants to guarantee survival in a hostile environment while simultaneously ensuring protection against their own defense mechanism when being attacked. It will be of utmost importance to gain more knowledge about the posttranslational regulatory mechanism of the proteasome-dependent degradation of HMGR and the mechanisms that control the more downstream enzymes of the terpenoid biosynthesis pathway in plants. There are indications of involvement of ERAD and its associated processes such as the UPR in the regulation of terpenoid biosynthesis. Many members of the ERAD and associated mechanisms are responsive to environmental stresses and show engagement in certain developmental processes linked to terpenoid metabolism. Unraveling the links between the ERAD machinery, the ER stress response, and that of terpenoid metabolism can represent a major advance in the elucidation of plant hormonal and metabolic regulatory networks.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

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