Endomembrane System and Abiotic Stress in Plants

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The sustainable exploitation of agri-environmental systems focuses more and more on practices where crops and plant species are adapted to edaphoclimatic conditions. Recent studies have shown that increased stress tolerance is related to the reorganization of cell membranes that sometimes lead to major changes in the solutes' homeostasis and water transfer. When under stress, protein trafficking in plants is compromised, usually leading to changes in the endomembrane system that may include protein transport through unconventional routes and alteration of morphology, activity and content of key organelles, as the ER and the vacuole. Such events provide the tools for cells to adapt and overcome the challenges brought on by stress.

endomembrane system | vacuolar trafficking | stress | vacuole | endoplasmic reticulum | Golgi apparatus | unconventional routes

1. Introduction

Climate changes stand, nowadays, as the foremost threat to human and environmental health, causing crop failures worldwide and leading to food safety issues [1]. As sessile organisms, plants evolved the ability to adapt to, and take advantage of, changes in climate and environment [2]. The diverse environmental stresses often activate signals and pathways involved in similar cellular responses: overexpression of antioxidants, accumulation of solutes, changes in protein trafficking and endomembrane remodelling [3][4][5][6]. In recent years, by using high throughput screening techniques, such as microarrays and RNA sequencing, it was possible to identify many stress-related genes. These techniques provide us with important information and suggest genes among which it is possible to identify new markers for assisted selection of crop varieties resistant to stress. Nevertheless, changes in the transcriptome are still the result of a complex series of events, and the understanding of the mechanisms of stress response is only partial. One of the most relevant mechanisms occurs at the endomembrane level, in particular in what concerns inter-organellar communications [7][8]. The identification of the specific roles of each player in the game turns out to be an important factor for the genetic improvement of plants, because the positive adaptation probably depends on synergistic effects and balanced interactions among proteins that are normally not related [9]. Recent experimental evidence [10] suggests various classes of proteins (such as aquaporins, soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), ATPase pumps or channels) to control specific events of membrane transport, leading to important events of cell reorganization under adverse environmental conditions. Several research groups found interesting connections between stress tolerance and membrane rearrangements not observed before, as is the case for a potassium channel—AKT1/KC1 (a shaker-like

potassium channel)—selectively accumulated on small vacuoles [11] and sufficient to confer stress tolerance when overexpressed. Nonetheless, the connection between membranous structure architecture and stress tolerance is not sufficiently investigated.

2. The ER as a Cellular Stress Sensor

The Endoplasmic Reticulum (ER), being a network of tubules and cisternae that spreads along the entire cell and connects with several other organelles, plays a major role in maintaining cellular homeostasis and in perceiving and spreading external signals [7]. The ER is the organelle that mediates the stress response in animals and in plants [12][13][14]. Abiotic stress can be responsible for the misfolding of proteins and their accumulation, causing an ER stress situation [15][16][17]. In response, some mechanisms are activated by the cell in order to maintain the ER's homeostasis, for example, the expression of genes encoding chaperones and other proteins with protein folding capacity, degradation associated with the ER or the cutback of protein translation loaded to the ER [15][18]. One of these mechanisms is the binding of the unfolded proteins to BIP proteins (binding proteins), which will activate bZIP transcription factors, such as bZIP17/bZIP28, that are transported to the Golgi to be cleaved [14][15]. This transport will upregulate genes involved in the ER stress pathway in order to restore ER homeostasis [14]. Furthermore, this transport may mediate the overexpression of genes involved in stress response as the bZIP28 leading to the activation of heat stress response genes [14][19] and bZIP17 leading to the activation of salt stress responses [16][20]. In this way, it is possible to observe that, in heat and salt stress situations, ER stress responses are often activated [14][19][20]. However, there are other important proteins involved in the unfolded protein responses (UPR) that respond to adverse environmental conditions, such as inositol-requiring enzyme-1 (IRE1), an ER resident transmembrane protein [14][21]. This protein is described to be involved in the heat stress response. When it is activated by heat, IRE1 splices bZIP60 mRNA, which is required for the activation of genes involved in the ER's stress reaction [22]. This protein also regulates the stress transcriptome by degrading several mRNAs [23][24]. In addition to the mechanism previously described, other UPR are activated in this type of stress as the activation of BiP; however, its overexpression is not relevant, meaning that salt stress can simply promote the misfolding of a different group of proteins [14][20][25][26]. Recently, another transcriptional factor modulating plant UPR has been identified. The nonexpressor of PR1 genes 1 (NPR1), a crucial redox-regulated master regulator of salicylic acid (SA)-dependent responses to pathogens, has been demonstrated to suppress the transcriptional role of bZIP28 and bZIP60 in ER stress responses. Upon ER stress-induced reduction of the cytosolic redox potential, NPR1 is translocated to the nucleus and physically interacts with bZIP28 and bZIP60, acting as an antagonist of such UPR factors to optimize their cytoprotective role in the UPR. NPR1 functions in plant UPR-monitoring may promote a negative feedback loop that is important in balancing energy consumption and maintaining basal cellular homeostasis during ER stress signalling [27]. In addition to cell-intrinsic UPR signalling, a non-cell autonomous component has been demonstrated to be regulated by the intercellular movement of bZIP60 facilitating systemic UPR signalling. Evidences show that the sbZIP60 protein is able to traffic across cells and induces the activity of the promoter of a target gene, efficiently stimulating UPR gene expression in cells distally from the site where ER stress occurs. Such findings suggest that ER stress systemic signalling may constitute a mechanism of anticipation

to a potentially-upcoming ER stress, as the cells of yet-unchallenged tissues are prepared by inducing the accumulation of transcripts of ER stress attenuating proteins [28].

3. The Vacuole as a Key Organelle in Stress Response

Vacuoles can occupy up to 80% of the cell volume, serve physical and metabolic functions and are essential in cellular responses to general cell homeostasis, as well as abiotic and biotic factors [29][30]. These organelles usually store water, nutrients, ions and secondary metabolites, but can also be a deposit site for toxic cell residues, waste products and excess solutes [31][32][33][34] and are involved in programmed cell death [35]. In plant cells, there are two main types of vacuoles: the protein storage vacuole (PSV) and the lytic vacuole (LV). Typically, proteins accumulate in the PSVs due to their higher pH and lower hydrolytic activity, when compared to the LVs, and they predominate in storage tissues (as cotyledons, endosperm, tubers) and vegetative tissues of adult plants (bark, leaves, pods) [36][37]. In contrast, LVs are mostly found in vegetative tissues and are used for storage and deposit of unwanted products. Due to the LVs' acidic pH and high hydrolytic activity, this type of vacuole modulates the degradation of a panoply of macromolecules and other compounds [38][39]. Initially, it was not expected to find both types of vacuoles in the same cell, but studies performed in root tip cells of barley and pea seedlings showed otherwise [40][41]. Additionally, a study conducted using the model plant *Arabidopsis thaliana* reported that, during its germination, the LV is embedded in the PSV and then derives from it, instead of being generated de novo [42]. The existence of two different types of vacuoles implies that plants have distinct trafficking pathways and mechanisms for different proteins. Additionally, the presence of LVs and PSVs in the same cell might work as a plant flexibility mechanism that is hypothesized to relate with changing environmental conditions [43][44][45][46].

A recent publication by Neves and collaborators has highlighted that Arabidopsis plants under abiotic stress show a differential expression of genes related to vacuolar trafficking, with an enhancement of the route to the PSV [6]. In fact, plants under abiotic stress are able to modulate their development and growth by altering morphological and cellular mechanisms, and cells' responses/adaptations to stress might involve changes in the distribution and sorting of specific proteins and molecules. Several studies also show the important role of the vacuole as a defence mechanism against abiotic stress. Indeed, the vacuole seems to react to stress through multiple mechanisms, such as toxic product accumulation and cell-turgor pressure maintenance. A study using suspension-cultured cells of mangrove (*Bruguiera sexangula*) shows the rapid increase in vacuolar volume when cells are submitted to salt stress, at the expense of decreasing cytoplasm volume, to maintain turgor pressure, probably through the increase in Na^+ concentration in the vacuole [47]. Another study using *Arabidopsis thaliana* shows the importance of the vacuole when the plant is under oxidative stress. In fact, the vacuole accumulated high levels of GSSG (oxidized glutathione) as a mechanism to protect the cell from an excessively positive shift in cytosolic glutathione redox potential [48]. The vacuole is also involved in mechanisms to fight environmental stress, such as reducing high ion levels toxicity in the cytoplasm to avoid cell death. A study by Tang et al. [49] uncovered a novel function of the Calcineurin B-like (CLB) interacting protein kinases' (CIPK) (CBL–CIPK) signalling network in excessive $Mg2^+$ vacuolar sequestration to help plants thrive under $Mg2^+$ stress. The described $Mg2^+$ partitioning

process in the vacuole controlled by the CBL–CIPK pathway may represent a general mechanism underlying the detoxification of other ions, including Na^+.

4. Vacuolar Transport—A Cellular Response to Stress

The protein trafficking to the vacuole is a fine-tuned communication system mediated by vesicles and different types of receptors. This allows the existence of a differential sorting process of proteins, leading to a different destination, depending on the receptors and vesicles used [50][51]. The vacuolar sorting receptors (VSRs) are involved in the transport of soluble cargoes by the conventional pathway, being responsible for the binding and release of cargo and also the control of the trafficking from and to the prevacuolar compartment (PVC) [52][53]. Besides these receptors, receptor homology region-transmembrane domain-RING-H2 (RMR) proteins have been identified as part of the traffic to the PSV. However, these types of receptors cannot be recycled back [50][54][55]. Another distinctive factor for the final destination of the vacuolar proteins is the type of vesicles. Clathrin coated vesicles (CCVs) are involved in the post-Golgi transport, being localized at the *trans* Golgi Network (TGN), and are responsible for the trafficking of proteins to the LV [50][54][56]. In contrast to the CCVs, dense vesicles (DVs) are larger carriers that fuse with PVCs and travel to the PSV [52][57][58][59]. Taken together, given all the data available on protein trafficking to the vacuole, gathered over many years of research, it is clear that it is a flexible and highly coordinated network [52][60]. As such, it is not surprising that, under abiotic stress, this tight balance can be altered to face the cell's needs and, ultimately, the plant, in order to adapt and to be able to prosper.

The alterations in the vacuolar trafficking as a cellular response to stress have not been characterized yet, and only a few studies approach this theme. Nevertheless, some isolated observations and reports are worth mentioning, as they may open the door for more focused research. In a recent study, Neves and collaborators [6] evaluated how different abiotic stresses affect the endomembrane system in *A. thaliana* by studying the expression of several endomembrane system effectors. The authors show that *AtRMR1, AtVSR1, AtSYP51* and *AtVTI12* genes, involved in the PSV sorting, are positively regulated under abiotic stress, while genes involved in the LV sorting, such as *AtVTI11* and *AtVSR2,* are downregulated. These findings enable the authors to create a hypothesis where the PSV route would be enhanced under abiotic stress conditions, in detriment of the LV pathway. Despite being very preliminary, this study points to several important genes in the vacuolar route that may be useful to fully understand how the cell copes with adverse conditions. One example is the v-SNAREs *VTI12* and its homologue *VTI11*, which function in different vesicle transport pathways, mediating the transport to different vacuolar types [61]. *VTI12*, however, has broader roles, participating in the docking and fusion of autophagic vesicles [62]. It is also part of a protein complex, together with SYP61 and SYP41, localized at the TGN. SYP61 has been implicated in osmotic stress responses [63], and it is thought that it may also be involved in stress-responsive transport mechanisms, similar to what has been described for SYP121 at the plasma membrane [10]. Being in a complex with SYP61, it is possible that *VTI12* may also participate in this mechanism. In fact, It has been shown that, in Arabidopsis plants grown under abiotic stress, *VTI12* expression is 20–30-fold higher than in control conditions [6], which is indicative of a putative role in cells' adaptation or response to stress. Furthermore, the VSR's implicated in the trafficking to the PSV also seem to respond to stress. Recently, Wang and collaborators [64] proposed a novel role for AtVSR1 in

osmotic stress tolerance and in the regulation of abscisic acid (ABA) biosynthesis, which is an important regulator of the signalling pathways induced by osmotic stresses. The authors used a *vsr* mutant and showed that the vacuolar trafficking mediated by *VSR1* was necessary for a response, in terms of ABA biosynthesis and to attain osmotic stress tolerance. In another study, Arabidopsis plants overexpressing *AtRabG3e* showed increased tolerance to salt and osmotic stress, along with a reduction in the accumulation of reactive oxygen species [65]. The Rab GTPases consist of a large family of proteins with a role in regulating vesicle targeting and specificity [66], and *AtRabG3e* participates in membrane fusion between the PVC and the vacuole, reinforcing the role of this pathway in adaptations to stress. Apart from the conventional route, the endocytic route to the vacuole has also been implicated in plant salt stress tolerance. This was shown in a work by Leshem and collaborators [67], where the suppression of the v-SNARE *AtVAMP7C*, essential for endosomal vesicle fusion with the tonoplast, had a positive impact in improving plant salt tolerance by inhibiting the fusion of H_2O_2-containing vesicles with the vacuole.

5. Unconventional Vacuolar Routes, or a Way to Get to the Vacuole Faster

In the past years, several studies have characterized proteins and vacuolar signals that do not follow the conventional sorting route. Some alternative sorting pathways, such as AP-3 and dense vesicles sorting, require the Golgi apparatus, but others also appear to be Golgi-independent [52]. Very little is known about the relationship between unconventional sorting routes and stress, but these alternative routes might be activated under stress to better match the plant's specific needs at the cellular level. In fact, direct ER-to-vacuole pathways appear to be linked to autophagy-related processes, which can be caused by multiple types of environmental disturbances. In recent years, several proteins or vacuolar sorting determinants have been described to follow an alternative, ER-to-vacuole, route [68][69][70]. Among them, cardosin A Plant Specific Insert (PSI) is an interesting case, as other related domains do not have this ability [71]. It is thought that other undescribed unconventional routes similar to the PSI-mediated vacuolar sorting act when plants face stress situations, providing the option to sort proteins through the conventional pathway or through a direct ER-to-vacuole transport. In fact, a recent study published in *Conference Proceedings* [72] showed that overexpression of PSIB in *Arabidopsis thaliana* correlates with salt and osmotic stress conditions, in some cases improving plant fitness. A different type of proteins that also appear to relate with salt stress are cysteine proteases that accumulate in long ER bodies, both in seedlings (as seen in *Vigna mungo* [73] and *Ricinus communis* [74]) or vegetative tissues' epidermis (*Arabidopsis thaliana* [75]) that eventually fuse with the vacuole. These proteins, along with vacuolar processing enzymes, are responsible for the degradation of storage proteins during plant development, but observations that ER bodies' direct fusion with the vacuole may be triggered by stress [75] enable a new perspective on the importance of this type of transport.

6. A Role for the Cytoskeleton in Keeping Cell Homeostasis under Stress

The cytoskeleton concept has been changed from a static supportive structure to a dynamic process in energetic equilibrium that adapts its functions to driving changes and stress responses in a fine-tune time and space

resolution [76]. Myosin motors along with actin filament bundles predominantly drive intracellular transport in plant cells. Changes in the rate of actin remodelling also affect its functionality, as observed by alteration in Golgi body motility [77]. Both remodelling of the ER and Golgi movement are inhibited by depolymerization of actin, demonstrating the importance of the actin cytoskeleton [78][79]. Mutant knock-out analysis of four members of the Myosin XI family (xi-k, xi-1, xi2 and xi-i) demonstrate that these proteins are important for normal whole-organism and cellular growth as well as Golgi body dynamics [80]. However, microtubules are thought to be essential during critical stages of plant cell development [81]. Considering that stress is a condition quite challenging to the cell, the hypothesis that the cytoskeleton network also has to adapt needs to be tested, since the interaction with membranes is critical for the self-organization of the cell. In a review of the complexity of organelle movement within the plant secretory pathway, Brandizzi and Wasteneys [81] argue that the actin-centric view of the motility of secretory organelles has been challenged by recent advances and revisited reports that support the relevant role of microtubules in plant cell development, positioning of Golgi stacks, involvement in cellulose synthesis and auxin polar transport. A milestone in the elucidation of the connection between endomembrane trafficking and microtubules was the work of Ambrose and collaborators [82] that, using hybrid and in vivo bimolecular fluorescence complementation techniques, discovered that microtubule-associated protein CLASP interacts with the retromer, facilitating the association between TGN/early endosomes and cortical microtubules via an interaction with sorting nexin1 (SNX1). SNX1 is a component of the retromer protein complex responsible for recycling the plasma membrane auxin efflux carrier PIN2, and thus controlling auxin transport.

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