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E3 ubiquitin ligases regulating plant stress responses: an overview

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Abstract. Post-translational modifications are emerging as a key regulatory component of many important cellular mechanisms. Among these ubiquitination, a multistep reaction, sequentially involving three enzymes named E1, E2 and E3, labels proteins with ubiquitin and led to 26S-mediated degradation. Protein ubiquitination plays a key role in a wide variety of cellular processes such as hormone signaling, DNA repair, biotic and abiotic stress response, cell cycle regulation to name few. In *Arabidopsis* more than one thousands of genes code for E3 ubiquitin ligase enzymes that specifically recognise target proteins. This suggests that a large amount of targets might be regulated by ubiquitination.

Keywords. Abiotic stress – Ubiquitination – E3 ligase – Protein protein interaction – Wheat.

Les E3 ubiquitines ligases régulant les réponses au stress de la plante : un aperçu

Résumé. *Les modifications post-traductionnelles sont en train de devenir un élément clé de régulation de nombreux mécanismes cellulaires importants. Parmi ces ubiquitinations, une réaction en plusieurs étapes, impliquant successivement trois enzymes nommées E1, E2 et E3, étiquette les protéines avec l'ubiquitine et conduit à la dégradation médiée par le 26S. L'ubiquitination de la protéine joue un rôle clé dans bon nombre de différents processus cellulaires tels que la signalisation hormonale, la réparation de l'ADN, la réponse au stress biotique et abiotique, la régulation du cycle cellulaire, pour n'en citer que quelques-uns. Chez Arabidopsis, plus d'un millier de gènes codent pour des enzymes E3 ubiquitines ligases qui reconnaissent spécifiquement les protéines cibles. Cela nous incite à avancer qu'une grande quantité de cibles pourrait être régulée par l'ubiquitination.*

Mots-clés. *Stress abiotique – Ubiquitination – Ligase E3 – Interaction protéine-protéine – Blé.*

I – Introduction

Abiotic and biotic stresses result in major constrains in growth and therefore productivity in crops. Understanding how plants respond to such stresses is of key importance to ensure and improve agricultural yield.

Post-translational modifications (PTMs) of proteins are deeply involved in the regulation of cellular processes in all organisms and also in adaptation to environmental changes including biotic and abiotic stress responses in plants. Ubiquitination, the attachment of ubiquitin to a protein substrate, has emerged as a key PTM involved in all aspects of plant physiology (Vierstra, 2009). Ubiquitin, discovered 30 years ago, is a 76 amino acid protein highly conserved in all eukaryotes, which is covalently attached to a target substrate. Ubiquitination was firstly described in the labeling of proteins for degradation through the 26S-proteasome (Hershko and Ciechanover, 1998). Protein ubiquitination is mediated by the sequential action of three enzymes, namely ubiquitin activating enzymes (E1), ubiquitin conjugating enzymes (E2) and E3 ubiquitin ligases (Vierstra 2009). Ubiquitinated proteins can undergo of several fates that include changes in their activity, re-localization within the cell or proteasome-mediated proteolysis. Ubiquitination in plants has been shown to regulate several processes including hormonal responses (Liu and Stone, 2011), light response (Roberts *et al*., 2011), control of the circadian rhythm (Cui *et al*., 2013), flowering process (Pineiro and Jarillo, 2013), pathogen resistance (Trujillo *et al*., 2008), tolerance to abiotic stress (Guerra *et al*., 2012; Cho *et al*. 2008), sugar response (Huang *et al*., 2010), intracellular trafficking and vacuole biogenesis (Isono *et al*., 2010) among others. The functional diversity of the ubiquitin/26S proteasome system (UPS) pathway is reflected by the high number of proteins involved in the UPS occupying approximately 6% of the total proteins encoded by the Arabidopsis genome with about 1600 genes.

This review will present the current status on knowledge about the role of ubiquitination pathway in relation to plant stress response with special attention to the role of E3 ubiquitin ligases.

II – E3 ubiquitin ligases and stress response

As described above, the E3 enzymes are responsible of recruiting the target proteins, conferring specificity to the selection of the entire ubiquitination process. There are different types of E3 ubiquitin ligases, including monomeric ones such as RING, U-Box or HECT proteins, and multisubunit E3s such as cullin-RING ligases (CRLs).

RING E3s have been found to regulate specific molecular responses by targeting critical elements. In Arabidopsis the RING ubiquitin ligase DEHYDRATION RESPONSIVE ELEMENT BINDING PROTEIN2A (DREB2A)-Interacting Protein1 (DRIP1) acts as negative regulator of drought response mediating the ubiquitination and the degradation of DREB2A (Qin *et al*., 2008). Moreover the well characterized RING-finger protein high expression of osmotically responsive gene (HOS1) is induced during cold exposure to exert a negative control of the stress response through the ubiquitination of the key transcription factor Inducer of CRT/DRE-binding factor Expression1 (ICE1; Dong *et al*., 2006). SDIR1 a H2-type zinc finger-protein is a positive regulator of ABA signaling, acting upstream of the main transcriptional regulators of the ABA molecular response (Zhang *et al*., 2007). Indeed, the ectopic expression of SDIR1 gene greatly enhances ABA-induced stomatal closure resulting in increased drought tolerance. In cross-complementation experiments, the ABA-insensitive phenotype of the sdir1-1 mutant can be rescued by several transcription factor genes acting in the ABA pathway (ABI5, ABF3 and ABF4).

The Arabidopsis RING finger E3 ligase RHA2a is also a positive regulator of abscisic acid signaling during seed germination and seedling development. Moreover, RHA2a negatively regulates seed germination on salt medium (Bu *et al*., 2009). In recent work (Lee *et al*., 2009), another drought stress-induced RING finger protein, Rma1H1 for RING membrane-anchor 1 homolog 1, was isolated in *Capsicum annuum* and characterized through cross transformation in *A. thaliana*. Expression analysis on Rma1H1 showed a clear induction during drought stress with a major amount of transcript in leaf tissue. Rma1H1 is also induced by cold stress, mechanical wounding, high salinity and ethylene but not by ABA. Moreover Rma1H1 confer drought tolerance when overexpressed in Arabidopsis plants (Lee *et al*., 2009). In addition, there are several examples of E3 ligases with a pivotal role in the regulation of stress response, as well as in processes related to growth and development, thus ensuring the connections among different pathways. For instance, Delayed Seed Germination1 (OsDSG1) participates both in stress response and seed germination (Park *et al*., 2010). BTH-induced RING finger protein1 (OsBIRF1) is a rice (Oryza sativa) RING protein with pleiotropic effects on growth and defense response against multiple abiotic and biotic stresses (Liu *et al*., 2008).

Often E3 ligases represents a connecting point between different signaling pathways. The already mentioned RING ligase HOS1 is responsible for the 26S-mediated degradation of two transcription factors, ICE1, the master regulator of cold response, and CONSTANS, the central component of the flowering pathways (Dong *et al*., 2006; Lazaro *et al*., 2012). The RING-finger E3 ligase TdRF1 (*Triticum durum* RING-finger protein 1) represents an interesting example (Guerra *et al*., 2012). TdRF1 is induced upon exposure to low temperatures and dehydration.

TdRF1 was shown to be phosphorylated by the kinase TdWNK5 (With No Lysine [K]5) a member of the Arabidopsis WNK family of MAP kinases involved in flowering time and circadian clock regulation (Wang *et al*., 2008). Moreover TdRF1 interacts with another E3 ligase, WVIP2 (Wheat Viviparus1 Interacting Protein2) showing a strong up-regulation upon cold treatment and sharing high amino acid similarity with the wild oat VIP2 (Jones *et al*., 2000). Finally TdRF1 was shown to degrade *in vivo* the transcription factor WBLH1 (Wheat Bel1-Type Homeodo-main1), a previously described protein belonging to KNOX (Knotted1-like homeobox) gene family (Mizumoto *et al*., 2011). Finally the over-expression of TdRF1 increases tolerance of barley cells to dehydration, suggesting it as a positive regulator of plant response to drought and freezing conditions. In a recent work AtPUB22 U-box ligase, an Arabidopsis E3, was shown to negatively regulate immunity response and drought stress in *A. thaliana* (Trujillo *et al*., 2008, Cho *et al*., 2008). Indeed AtPUB22 shows a clear induction in response to several abiotic stresses such as cold, drought and salinity stresses but not ABA treatment. Finally PUB22 was demonstrated to interact and ubiquitinate RPN12a, a subunit of the 19S regulatory particle (RP) in the 26S proteasome (Cho *et al*., 2008). In a recent work the group of Dr. Trujillo discovered another protein target of AtPUB22, the exocytic machinery component Exo70B2 (Stegmann *et al*., 2012). The authors demonstrated that AtPUB22 undergoes proteasomal degradation by autocatalytic activity and by contrast it is stabilized upon pathogen elicitors treatment (Stegmann *et al*., 2012). Finally the authors propose the following action mechanism: in standard conditions AtPUB22 regulates itself by autoubiquitination, flg22 perception by FLS2 stabilize AtPUB22 allowing it to interact and ubiquitinate Exo70B2 finally leading to an attenuation of PAMP-induced signaling. Interestingly PUB22 was shown to coordinately co-operate with other PUBs in both abiotic and biotic stress responses indicating a certain degree of redundancy.

III – Conclusions

The emerging picture indicates the E3 enzyme as the hub point connecting regulatory proteins of different cellular processes, e.g., the response to abiotic and biotic stress and various aspects of plant development. Different E3 ligases can play a role in the same process by targeting the same substrates or they can regulate each other’s besides other targets. All these scenarios create cross-talking between different signaling pathways or cellular processes. Otherwise a single E3 can regulate several aspects of plant life cicle simply by mediating degradation of different target proteins in a different temporal window. Thus plants can exploit ubiquitination to coordinate the functioning of different processes according to environmental and cellular conditions.

Finally an E3 is able to regulate all these processes mediating degradation of other proteins, thus identification of E3 targets is a crucial step to completely unravel ubiquitination role in regulating plant life cycle.

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