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*Novel cellular pathways mediating root responses to salt stress and phosphate starvation*

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Chapter 2

Regulation of mRNA decay in plant responses to salt and osmotic stress*

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Abstract

Plant acclimation to environmental stresses requires fast signaling to initiate changes in developmental and metabolic responses. Regulation of gene expression by transcription factors and protein kinases acting upstream are important elements of responses to salt and drought. Gene expression can be also controlled at the post-transcriptional level. Recent analyses on mutants in mRNA metabolism factors suggest their contribution to stress signaling. Here we highlight the components of mRNA decay pathways that contribute to responses to osmotic and salt stress. We hypothesize that phosphorylation state of proteins involved in mRNA decapping affect their substrate specificity.

Keywords: osmotic stress, salinity, mRNA decay, mRNA decapping, mRNA stability, 5’->3’ exoribonucleases, SnRK2 kinases, protein phosphorylation, posttranscriptional regulation, P bodies

Emerging role of posttranscriptional level regulation in osmotic stress responses

Plant survival under suboptimal conditions relies on the perception of environmental signals followed by signal transduction pathways leading to changes in gene expression to switch on protective mechanisms. Among the abiotic stresses with a deleterious effect on plant development, salinity and osmotic stress are huge constrains for agriculture worldwide (Fita et al., 2015). The decrease in osmotic potential of the soil that occurs both in saline soils and during drought leads to a reduction in water uptake by the plant. Plant adaptations to dehydration include stomatal closure, regulation of water fluxes and biosynthesis of osmoprotectants. To cope with salinity, in addition ion homeostasis needs to be adjusted (Golldack et al., 2014; Hasegawa, 2013). Both salt stress and low water availability lead to alteration in shoot and root growth (Julkowska and Testerink, 2015; Pierik and Testerink, 2014). These physiological changes are guided through complex signal transduction pathways including Ca$^{2+}$, reactive oxygen species and lipid signaling, abscisic acid (ABA) formation, sucrose-non-fermenting 1-related protein kinase 2 (SnRK2) and mitogen activated protein kinase (MAPK) activation, and transcription factor regulation (Golldack et al., 2014; Julkowska and Testerink, 2015; McLoughlin and Testerink, 2013). Recent research has identified the regulatory networks of the osmotic stress-induced AREB/ABFs and DREB2A transcription factors (reviewed in Fujita et al., 2013 and Yoshida et al., 2014), and transcriptomic studies have identified many transcriptionally regulated candidates genes for osmotic and salt stress responses (Kreps et al., 2002; Rasheed et al., 2016). However, abundance of mRNA depends not only on the control of transcription, but also on transcript degradation rate. Salt induced changes in Arabidopsis transcript abundance do not always correlate with
observed changes in the proteome and most of the mRNAs for which abundance decreases on dehydration also show lower assembly with polysomes, suggesting posttranscriptional regulation of osmotic and salt stress responses (Jiang et al., 2007; Kawaguchi et al., 2004). Besides translation, another fate of transcribed mRNAs is to be subjected to mRNA decay or quality control pathways (Belostotsky and Sieburth, 2009). Increasing knowledge about factors regulating mRNA turnover in plants suggests their contribution to developmental processes (Goeres et al., 2007; Xu and Chua, 2011; Xu et al., 2006) as well as stress responses (Maldonado-Bonilla, 2014). Altered sensitivity to osmotic stress or salinity of mRNA decay machinery mutants (Maldonado-Bonilla, 2014), together with the short half-life time of transcripts involved in osmotic stress responses (Narsai et al., 2007), suggests mRNA decay regulation to be an additional, important level of osmotic and salt stress signaling. Moreover SnRK2 and MAP kinases known to be involved in salt and osmotic responses seem to interfere with mRNA stability pathways (Stecker et al., 2014; Umezawa et al., 2013; Wang et al., 2013; Xu and Chua, 2012). Here, we summarize current knowledge on mRNA metabolism contribution to drought and salt stress signaling and put these results into perspective of the action of already well-known players in these pathways (Fig. 1).

Global mRNA stability under osmotic stress

Many stress responsive genes, including ones involved in osmotic stress in human cells (Fan et al., 2002), yeast (Gowrishankar et al., 2005; Greatrix and van Vuuren, 2006; Hilgers et al., 2006; Romero-Santacreu et al., 2009) and plants (Maldonado-Bonilla, 2014; Perea-Resa et al., 2016; Steffens et al., 2015) has been shown to be regulated by their mRNA stability. In yeast, the dynamics of global transcriptional rate after osmotic stress does not always match the changes in dynamics of mRNA abundance, which can be explained by global transcript destabilization (Romero-Santacreu et al., 2009). Stability of mRNA was shown to depend on stress severity. Mild stress in general caused mRNA decay, while most osmotic stress-responsive mRNAs were stabilized, suggesting that regulation of mRNA turnover can affect specific subsets of transcripts. Severe osmotic shock resulted in global mRNA retention in processing bodies (P bodies), cytoplasmic foci where mRNA can be degraded or withheld from translation (Romero-Santacreu et al., 2009). Decay of osmotic stress-induced mRNAs in yeast has been proposed to be essential in the recovery phase, to return to the base level of these mRNAs (Molin et al., 2009).

In Arabidopsis, mRNAs coding for proteins involved in responses to osmotic stress have been found among rapidly degraded transcripts (Narsai et al., 2007). Around 40% of mRNAs encoding sodium transporters were found in their uncapped version, implying that 5’->3’ mRNA decay can also regulate responses to salt (Jiao et al., 2008). Global analysis of the transcriptome and degradome of Setaria italica revealed that also in this monocot species 5’->3’
mRNA degradation is one of the mechanisms of responses to drought (Yi et al., 2015). While control of mRNA stability in response to osmotic stress is receiving increasing attention, our knowledge of the mechanisms behind observed changes is still fragmentary.

Figure 1: Contribution of mRNA metabolism processes to plant responses to salinity and osmotic stress. Control of splicing occurs in the nucleus and is controlled by CBP20, CBP80 and the LSM2-8 complex. Targeting of specific transcript subsets to P bodies is hypothesized to be guided by TZF proteins. Cytoplasmic mRNA decay starts with deadenylation. After shortening the poly(A) tail, transcripts can be degraded from their 3' end via the exosome complex or undergo 5' cap removal in a process of decapping followed by 5'→3' decay catalyzed by XRN4. Proteins marked in blue and green are involved in responses to salt and osmotic stress, respectively, purple color denotes factors involved in salt, osmotic and ABA signaling, while yellow indicates proteins responding to osmotic stress and ABA. Proteins for which function is only hypothetical are marked with dashed circles.
mRNA decay pathways in Arabidopsis
Control of mRNA stability is an essential component of gene expression regulation. The rate of transcript degradation correlates with encoded function, and abundance of the transcript, number of introns and conserved sequences in the 5’ and 3’ UTR (Narsai et al., 2007). Arabidopsis mRNAs coding for transcription factors and protein kinases as well as transcripts targeted by microRNAs, and low abundance transcripts are in general unstable. Presence of at least one intron increases mRNA stability and multiple stabilizing motifs within the UTRs sequences have been identified (Narsai et al., 2007). Most transcripts appear in complex with proteins involved in translation initiation or mRNA decay and are constantly shifted between these two processes. Factors that activate the first steps of mRNA degradation in general repress translation and vice versa (Coller and Parker, 2005; Schwartz and Parker, 1999). The mRNA decay machinery can target defective transcripts with premature stop codons or long 3’UTR in nonsense-mediated decay (NMD), mRNA molecules without a stop codon in the process of non-stop decay (NSD), as well as transcripts that did not disassociate from ribosomes, by a mechanism known as non-go decay (NGD; reviewed in Doma and Parker, 2007; Garneau et al., 2007). Besides degradation of abnormal transcripts, the mRNA decay machinery can also target mRNAs with a correct structure, serving as a regulatory step in gene expression (Parker and Song, 2004). The same enzymes are involved in mRNA degradation in specialized and general pathways (Fig. 1), but the recognition of substrates follows different mechanisms (Parker and Song, 2004).

First step- deadenylation
In all eukaryotic organisms mRNA degradation starts by shortening the 3’ poly(A) tail (Doma and Parker, 2007). This critical and rate-limiting step is guided by the deadenylases PARN, PAN and the CCR4/CAF1/NOT complex (Abbasi et al., 2013). The poly(A) ribonuclease AtPARN catalyses deadenylation of a subset of embryonic transcripts in Arabidopsis, and lack of functional AtPARN results in embryo lethality (Chiba et al., 2004; Reverdatto et al., 2004). Expression of AtPARN is induced by ABA, osmotic and salt stress, while several AtPARN substrates were found to be degraded in both stress and control conditions (Nishimura et al., 2005). Two Arabidopsis homologs of CARBON CATABOLYTE REPRESSOR4 (AtCCR4a and AtCCR4b) were shown to be involved in starch and sucrose metabolism (Dupressoir et al., 2001; Suzuki et al., 2015). Eleven Arabidopsis CAF1 (CCR4-ASSOCIATED FACTOR1) genes have been found, among which AtCAF1a and AtCAF1b, which are associated with responses to multiple environmental stimuli, in most cases by non-redundantly targeting different mRNA subsets (Walley et al., 2010). A mutant in AtCAF1a was able to germinate in the presence of salt and one third of the transcripts upregulated in
this mutant were salt-inducible, including one aldehyde dehydrogenase, ALDH7B4, which upon overexpression led to the same phenotype as the caf1a mutant (Kotchoni et al., 2006; Walley et al., 2010). Important to note is that the paralogous deaminase AtCAF1b did not share a function in responses to salt, suggesting that similar enzymes with the same function can target different transcripts and thereby regulate other stress responses (Walley et al., 2010). Neither the poly(A) nuclease PAN, that functions in deamidation in humans and yeast, or other components of the CCR4/CAF1/NOT complex have been identified in Arabidopsis (Abbasi et al., 2013; Parker and Song, 2004).

3’->5’ degradation
Transcripts deprived of their poly(A) tail can be targeted to 3’end decay catalyzed by the exosome, a multisubunit complex involved in cytoplasmic mRNA degradation, and maturation of rRNA, snRNA and snoRNA in the nucleus (Chlebowski et al., 2013). The exosome is comprised of nine core subunits and associated cofactors including RNA-binding proteins and RNA helicases (Parker and Song, 2004). On the contrary to yeast and humans, the plant exosome core complex is self-activated through the phosphorolytic function of AtRRP41 (Arabidopsis thaliana homolog of Saccharomyces cerevisiae exosome subunit RRP41p; Chekanova et al., 2000). Mutations in exosome proteins result in early growth, female gametogenesis and embryogenesis defects (Chekanova et al., 2007; Chekanova et al., 2000; Yang et al., 2013), but so far no evidence has been reported for a role of the exosome machinery in osmotic stress signaling.

5’->3’ degradation starts with decapping
Deadenylated transcripts can be also directed to 5’->3’ decay, where 5’cap removal is the first step (Belostotsky and Sieburth, 2009). In Arabidopsis, this process is catalyzed by DCP2 (DECAPPING 2), acting in a complex with its activator DCP1 (DECAPPING 1), which does not have decapping activity itself. The DCP2-DCP1 interaction is crucial for 5’cap removal and depends on VCS (VARICOSE) as a scaffolding protein (Xu et al., 2006). Impaired function of decapping complex components results in severe developmental phenotypes, including cotyledon deformation or improper vein patterning, and lethality of homozygous knock-out (KO) mutants (Deyholos et al., 2003; Goeres et al., 2007). Decapping and further degradation take place in processing bodies (P bodies), dynamic cytoplasmic foci concentrating mRNA decay enzymes, which depend on the supply of mRNA for their formation (Goeres et al., 2007; Sheth and Parker, 2003; Xu et al., 2006). Besides the decapping machinery, another protein complex, consisting of LSM1-7 proteins, has been shown to serve as a decapping activator. Eight highly conserved SM-Like (LSM) proteins have been identified in Arabidopsis, which via specific interactions with each other, form two separate heptameric complexes:
cytoplasmic LSM1-7 and nuclear LSM2-8, the latter functioning in splicing events (Golisz et al., 2013; Perea-Resa et al., 2012). LSM 2, LSM3, LSM4, LSM5, LSM6 and LSM7 are present both in cytoplasm and nucleus, while LSM1 and LSM8 localization is restricted to cytoplasm and nucleus, respectively (Perea-Resa et al., 2012). Two duplicated functionally redundant genes, \textit{LSM1A} and \textit{LSM1B}, encode for the LSM1 protein (Golisz et al., 2013; Perea-Resa et al., 2012). In \textit{lsmla lsm1b} double mutant decapping and decay rates of transcripts was decreased (Perea-Resa et al., 2016; Perea-Resa et al., 2012). Another protein carrying an LSM domain, DCP5, was shown to promote decapping without affecting DCP2 activity (Xu and Chua, 2009). The formation of P bodies was altered in both \textit{dcp5} and \textit{lsmla lsm1b} mutants, suggesting that DCP5 and the LSM1-7 complex may indirectly activate decapping via P body assembly (Perea-Resa et al., 2012; Xu and Chua, 2009). Two other LSM proteins, LSM4 and LSM5/SAD1 (SUPERSENSITIVE TO ABA AND DROUGHT 1), have been described only for their role in splicing processes so far (Cui et al., 2014; Golisz et al., 2013; Zhang et al., 2011).

While proper functioning of the decapping complex is required for basal plant development, an increasing amount of evidence suggests it may also be involved in stress responses (Perea-Resa et al., 2016; Perea-Resa et al., 2012; Steffens et al., 2015; Xu and Chua, 2012). Stability of osmotic and salt-responsive transcripts appears to be regulated via DCP1 and its interaction with other proteins. Within 15 minutes upon dehydration, MAP kinase 6 (MPK6) is activated and phosphorylates DCP1. Phosphorylation of DCP1 then triggers DCP1 dimerization and association with DCP2 and DCP5. Increased assembly and activity of the decapping complex stabilizes mRNAs encoding for signal transduction components, transcription factors and nucleic acid binding factors and induces decay of transcripts involved in lipid metabolism and nutrient balance (Xu and Chua, 2012). Among the stabilized transcription factors, DREB2b is also induced by osmotic stress and high salinity (Liu et al., 1998), indicating that dehydration not only triggers expression of DREB2b transcript, but also protects it from the decay. DCP1 has also been shown to physically interact with the BEACH (beige and Chedial Higashi) domain protein SPIRRIG (SPI) (Steffens et al., 2015). Arabidopsis SPI, known previously to participate in maintenance of membrane integrity, was recruited to P bodies upon interaction with DCP1 (Saedler et al., 2009; Steffens et al., 2015). A \textit{spi} mutant showed less P body formation upon salt stress, and was hypersensitive to salt (Steffens et al., 2015). SPI triggered the recruitment of a subset of salt-responsive mRNAs and ribonucleoprotein (RNP) assembly critical for P body formation. Together this suggests that under stress conditions activity of DCP1 can be modulated by interaction with MPK6 and SPI.

LSM1, one of the components of the decapping activator complex LSM1-7, was also found to guide responses to salt and drought stress. The \textit{lsmla lsm1b} mutant has altered levels of both hormonal signaling and stress
defense transcripts (Golisz et al., 2013). Interestingly, *lsm1alsm1b* plants showed a higher survival after drought in soil experiments and higher fresh weight and number of lateral roots on osmotic stress applied in agar media (Perea-Resa et al., 2016). On the other hand, in the presence of salt, overall growth of *lsm1alsm1b* seedlings was severely inhibited, suggesting their hypersensitivity to salt. Transcriptome analysis revealed that in the *lsm1alsm1b* mutant exposed to osmotic stress, transcripts encoding positive factors for drought tolerance were stabilized (e.g. *ABR1*, *ANAC019*, *ERF53*), while salt stress led to stabilization of mRNAs of negative regulators of salinity tolerance (e.g. *ANAC092*, *AHK5*, *ATGSTU17*). One of the mRNAs stabilized specifically on salt was the ABA biosynthesis gene *NCED3*, indicating involvement of the decapping machinery in ABA biosynthesis in response to salinity. LSM1 is also crucial for formation of P bodies in osmotic or salt stress conditions. By guiding its target mRNAs to P bodies, activating their decapping and thus exposing them to degradation, LSM1 regulates sensitivity to osmotic stress and salinity (Perea-Resa et al., 2016). Via the same processes, LSM1 regulates not only responses to drought and salinity, but also to cold stress (Perea-Resa et al., 2016). Different stress signaling pathways target different mRNAs, yet the mechanism of this selectivity remains unknown.

5′→3′ cleavage

The naked mRNA molecule is an easy target for 5′→3′ exoribonucleases. While three of these have been identified in Arabidopsis, only XRN4 localizes to the cytoplasm and can target decapped mRNAs (Kastenmayer and Green, 2000). Surprisingly only a small subset of transcripts has an altered decay rate in the *xrn4* mutant and none of the previously identified highly unstable mRNAs were targets of XRN4, suggesting existence of other plant 5′→3′ exoribonucleases (Narsai et al., 2007; Souret et al., 2004). Substrate selectivity of XRN4 was linked to the presence of hexamer motifs in the 5′ end of its targets (Rymarquis et al., 2011). Decapped transcripts can be also targets of RNA-dependent RNA polymerases and in this way induce gene silencing (Gazzani et al., 2004). A subset of XRN4 substrates appears to be derived from miRNA cleavage, suggesting the existence of two mechanisms of XRN4 action: via general cytoplasmic 5′→3′ decay and by decay of miRNA degradation products (Souret et al., 2004). On the contrary to mutants in decapping machinery components, *xrn4* mutants do not have any developmental alterations, but are insensitive to ethylene, show higher tolerance to heat stress and exhibit decreased sensitivity to auxin with respect to lateral root formation (Chen and Xiong, 2010; Nguyen et al., 2015; Potuschak et al., 2006). In *xrn4* mutants, levels of *EBF1* and *EBF2* mRNA, encoding F-box proteins that participate in degradation of the major transcriptional regulator of ethylene signaling EIN3, was decreased. *EBF* RNA decay rates were not altered, suggesting they are indirect targets (Olmedo et al., 2006; Potuschak et al., 2006). In addition, abundance of many other transcripts
was decreased in the *xrn4* mutant, suggesting that XRN4 can affect transcripts level not only via its exoribonucleic activity, but also indirectly (Rymarquis et al., 2011).

Through direct degradation of mRNA of heat shock factor A2 (*HSFA2*), XRN4 is involved in suppression of heat stress responses after return to normal temperature (Nguyen et al., 2015). While the *xrn4* mutant was more tolerant to short and severe heat stress, a very low level of thermotolerance was observed to moderately high temperatures, possibly caused by separate subsets of mRNAs being targeted at different stress intensities (Merret et al., 2013; Nguyen et al., 2015). Interestingly, upon heat stress XRN4 interacts with LARP1, a homolog of human RNA-binding protein, and in this complex XRN4 is directed to polysomes (Bousquet-Antonelli and Deragon, 2009; Merret et al., 2013). A proportion of the transcripts rapidly degraded upon heat stress is subjected to XRN4-dependent 5′-3′ decay while still being associated to elongating polysomes, supporting the concept of co-translational 5′-3′ decay, suggested previously to occur in yeast and Arabidopsis (Hu et al., 2009; Merret et al., 2013; Sement et al., 2013). Co-translational mRNA decay was shown to be involved in salt stress signaling in yeast, and this function might be conserved in plant responses to salinity (Merret et al., 2015; Sweet et al., 2012).

**Role of nuclear mRNA metabolism regulators in salt and osmotic stress signaling**

In parallel to regulators of cytoplasmic RNA metabolism, nuclear pre-mRNA processing enzymes also appear to be involved in responses to osmotic stress. Hypersensitivity to salinity of *sad1/lsm5* and *lsm4*, mutants in the nuclear LSM2-8 complex for intron removal, suggests involvement of the splicing machinery in salt stress responses (Cui et al., 2014; Xiong et al., 2001; Zhang et al., 2011). SAD1 is essential for accurate splice site recognition and splicing efficiency. Overexpression of SAD1/LSM5 led to an increase in properly spliced transcripts of *CIPK3*, *ABF3*, and *DREB2A*, and improved salt tolerance. Aberrant splicing events of transcripts involved in salinity responses were found in *sad1/lsm5* plants exposed to salt stress (Cui et al., 2014). A *sad1/lsm4* point mutation did not affect global gene expression, but expression of many transcripts of ABA biosynthesis and signaling pathway factors decreased (Xiong et al., 2001). Two nuclear cap-binding proteins CBP80 and CBP20 have been shown to regulate alternative splicing of transcripts coding for the auxin response factors ARF10 and ARF17, as well as for proline and sugar metabolism factors (Kong et al., 2014). The *cbp80* and *cbp20* mutants both had higher sensitivity to salt and ABA and *cbp20* showed decreased stomatal conductance and sensitivity to water withdrawal (Kong et al., 2014; Papp et al., 2004). *Cbp80/abh1* mutants also had decreased levels of *PP2C* phosphatase transcripts involved in ABA signal transduction, which could explain its ABA hypersensitivity (Hugouvieux et al., 2001). Abscisic acid (ABA) is
a phytohormone with a crucial role in salt and drought signaling (Zhu, 2002). Both ABA biosynthesis and its signaling pathways seem to be regulated at the level of pre-mRNA splicing (also reviewed in Kuhn and Schroeder, 2003). Another nuclear protein, the mRNA capping enzyme family protein At5g28210, was mapped recently as a putative gene involved in root growth in the presence of a combination of salt and phosphate starvation (Kawa et al., 2016).

Small RNA-mediated regulation of mRNA decay
Another level of regulation of gene expression in plants is RNA interference (RNAi) in which a micro RNA (miRNA), through the RNA-induced silencing complex (RISC) can guide the cleavage of a target mRNA (Iwakawa and Tomari, 2015). Products of miRNA cleavage are then degraded by XRN4 (Souret et al., 2004). On the other hand, XRN4 acts antagonistically with RNAi processes because endonucleolytic cleavage of uncapped mRNA removes template for RNA-dependent RNA polymerases (Gazzani et al., 2004). Accumulation of these transcripts in an xrn4 mutant leads to excessive gene silencing (Gazzani et al., 2004). Nuclear 5'->3’ exoribonucleases XRN2 and XRN3 suppress posttranscriptional gene silencing by degradation of the loops that are excised from a pri-miRNA during its maturation (Gy et al., 2007). Moreover, numerous miRNAs are involved in responses to osmotic stress or salinity (Covarrubias and Reyes, 2010; Ding et al., 2013) and multiple proteins participating in miRNA biogenesis are also implicated in responses to salt, osmotic stress or ABA (Zhang et al., 2008).

TZF proteins
Another group of proteins putatively involved in regulation of salt and osmotic stress responses at the posttranscriptional level are tandem CCCH zinc finger proteins (TZFs; Bogamuwa and Jang, 2014; Guo et al., 2009). Mammalian TZFs are able to bind to ARE elements, AU-rich sequences at the 3’end of mRNA molecules, and this interaction recruits enzymes involved in deadenylation, decapping and exonucleolitic cleavage (Fabian et al., 2013; Lykke-Andersen and Wagner, 2005). Evidence increases that plant TZF proteins may be also involved in mRNA turnover control. Arabidopsis TZF1 and TZF9 and rice OsTZF1 possess RNA binding capacity (Jan et al., 2013; Pomeranz et al., 2010), while TZF2 and TZF3 exhibit RNAse activity (Lee et al., 2012). So far the contribution of plant TZFs to mRNA decay has been shown only for TZF1, which can induce degradation of ARE-containing transcripts (Qu et al., 2014).

Expression of Arabidopsis TZF1 is triggered by salt only, while TZF2, TZF3, TZF10 and TZF11 are induced by salinity, osmotic stress and ABA (Lee et al., 2012; Sun et al., 2013). Overexpression of TZF10 or TZF11 led to increased salt tolerance and decreased expression of RD29A and KIN1 (Sun et al., 2007). Overexpression lines of TZF2 and TZF3 showed higher drought
tolerance and reduced water loss than wild type plants (Lee et al., 2012). Moreover, induction of TZF3 resulted in decreased expression of ABI2, while a tzf3 knock-out mutant was hypersensitive to both salt stress and ABA (Huang et al., 2012). Rice OsTZF1 relocalized to P bodies upon salt stress or ABA treatment and its overexpression conferred faster recovery from salinity or drought (Jan et al., 2013). Osmotic stress-induced cotton GhZFP1 is involved in adaptation to salinity and its overexpression led to high potassium/sodium ratio. Similar to mammalian TZF s, plant TZF s can shuttle between nucleus and cytoplasm and localize to P bodies and stress granules (Bogamuwa and Jang, 2016; Franks and Lykke-Andersen, 2007; Jan et al., 2013; Pomeranz et al., 2010). Importantly, altered expression of TZF s affects usually a subset of transcripts (Huang et al., 2012; Lee et al., 2012; Sun et al., 2013). Specificity of mRNA metabolism processes that occur in P bodies may rely on TZF proteins binding to cis-elements in mRNA (Maldonado-Bonilla, 2014). Interestingly, Y2H analysis revealed that TZF5 interacts with MARD1 and RD21A, proteins involved in ABA and dehydration responses, but not with any of the components of mRNA decay machinery (Bogamuwa and Jang, 2016). This may suggest that additional interactions with stress-induced proteins may be also required for osmotic and salt regulation at the level of mRNA metabolism.

**Emerging role of protein kinases in mRNA stability regulation**

The aforementioned factors involved at different stages of mRNA stability can guide responses to multiple stresses by selective targeting of subsets of transcripts (Perea-Resa et al., 2016; Steffens et al., 2015; Xu and Chua, 2012). The mechanism of this stress-dependent control of differential gene expression remains unclear, but increasing recent evidence suggests that decapping factors are phosphorylated upon stress exposure. In response to osmotic stress, human DCP1a is phosphorylated by c-Jun N-terminal kinase (JNK) and targeted to P bodies (Rzeczkowski et al., 2011). In glucose-deprived yeast, the protein kinase Ste20 phosphorylates DCP2 and stabilizes the DCP1-DCP2 interaction (Yoon et al., 2010). Yeast mRNA stability upon exposure to osmotic stress is dependent on the MAP kinase Hog1, via an unknown mechanism (Romero-Santacreu et al., 2009; Fig. 2).

Another member of the decapping complex, VCS, was also differentially phosphorylated upon osmotic and salt stress, but not after ABA treatment (Stecker et al., 2014; Umezawa et al., 2013). The phosphorylated version of VCS was downregulated in a triple KO mutant of ABA-dependent protein kinases, the snrk2.2/2.3/2.6 mutant, while VCS phosphorylation was lower upon ABA treatment, suggesting it could be an indirect target of SnRK2.2/SnRK2.3/SnRK2.6 (Umezawa et al., 2013). Phosphorylation of multiple individual Ser residues of VCS was either enhanced or inhibited by mannitol treatment, reflecting the complicated nature of this regulation (Stecker et al., 2014). Together, this evidence suggests that differential phosphorylation of decapping factors upon osmotic or salt stress could enable
them to target only a subset of transcripts. Since cytoplasmic 5’-3’mRNA decay, starting with 5’cap removal, occurs also in control conditions it is plausible that under osmotic stress conditions decapping activity and substrate selectivity is regulated in a different way. Evidence is accumulating for a role of post-translational modifications, including phosphorylation, in regulation of this process.

Recent phosphoproteomics studies (Stecker et al., 2014; Umezawa et al., 2013) have revealed upregulation of phosphorylated forms of SnRK2.1/4/5/6, MAP3K Raf18, MAP4Kα1 and DCP2 within 5 minutes after osmotic stress treatment. Nine out of 10 Arabidopsis SnRK2 protein kinases are rapidly activated by osmotic stress, while activity of SnRK2.2, SnRK2.3 and SnRK2.6 is also regulated by ABA (Boudsocq et al., 2004). SnRK2.6 has been shown to phosphorylate the anion channel SLAC1 (Geiger et al., 2009; Lee et al., 2009), potassium channel KAT1 (Sato et al., 2009), AtrbohF NADPH oxidase (Sirichandra et al., 2009), transcription factor AREB3 (ABA-RESPONSIVE ELEMENT BINDING PROTEIN 3; Wang et al., 2013) and aquaporin PIP2;1 (PLASMA MEMBRANE INTRINSIC PROTEIN; Grondin et al., 2015). All SnRK2 subclass 2 and 3 can phosphorylate the transcription factor AREB1 (Furihata et al., 2006). Contrary to ABA-dependent SnRK2s, targets of SnRK2 subclass 1 protein kinases, remain to be identified. Whether these kinases or MAP3Ks/MAP4Ks can directly or indirectly target DCP2 remains unknown (Fig. 2).

Figure 2: Involvement of protein kinases in regulation of mRNA decapping processes. In human cells protein kinase JNK phosphorylates DCP1a and through this regulates P body formation. In yeast, upon glucose deprivation Ste20 phosphorylates DCP2 to stabilize its interaction with DCP1. In Arabidopsis MPK6 is activated by drought and phosphorylates DCP1, thus inducing DCP1 interactions with DCP2 and DCP5. Upon salt stress, SPI protein binds to DCP1 and facilitate its recruitment to P bodies. Phosphorylation of DCP2 and VCS is triggered by osmotic stress, but kinases functioning upstream remain still unknown. Phosphoproteomic profiling studies may suggest involvement of SnRK2 or MAP kinases in this process.
It is unknown whether activity of plant DCP2 depends on its phosphorylation status, or is possibly triggered by phosphorylation of DCP1 or VCS, and finally whether phosphorylation of DCP1, DCP2 and VCS influences their relocalisation to P bodies or assembly of decapping complex. To address these questions, protein kinases targeting these proteins need to be identified. For SnRK2.6, no subcellular localization data are available, but the subclass 1 SnRK2.4 and a Medicago SIMKK (stress induced MAP2K) have been shown to localize to punctate structures after exposure of roots to salt (McLoughlin et al., 2012; Ovecka et al., 2014). Yet, it remains to be established whether these would be P bodies or in case of SnRK2.4, would reflect lipid binding capacity (Julkowska et al., 2015). Finally, it should be noted that phosphorylated versions of DCP1 and VCS have also been detected in control conditions, so any putative regulation by protein kinases in response to osmotic stress may have a quantitative character (Stecker et al., 2014; Xu and Chua, 2012).

Several proteins involved in pre-mRNA processing and splicing have been found as putative targets of ABA-dependent SnRK2 kinases (de la Fuente van Bentem et al., 2006; Umezawa et al., 2013; Wang et al., 2013), implying that proteins involved in nuclear mRNA metabolism can be also regulated by phosphorylation. Altered ABA sensitivity of sad1/lsm5, lsm4, cbp20, cbp80/abh1, and ahg2-1 mutants suggests they function via adjustment of hormonal signaling pathways (Hugouvieux et al., 2001; Kong et al., 2014; Nishimura et al., 2005; Xiong et al., 2001; Zhang et al., 2011).

The mRNA decapping step takes place in P bodies, meaning that salt- and osmotic-dependent mRNA degradation relies on proper assembly of RNP particles and recruitment of all their components to P bodies. So far it is not known how mRNAs targeted for degradation relocate to P bodies and whether the process would involve interactions with RNA-binding proteins, decapping factors or other stress-induced proteins. The salt responsive protein SPI does not possess RNA-binding capacity but seems to regulate uptake of mRNA into P bodies and RNP formation via an unknown mechanism (Steffens et al., 2015). The LSM1 protein physically interacts not only with members of the LSM1-7 complex, but was also co-purified with some stress-responsive proteins without RNA-binding properties (Golisz et al., 2013). It would be of interest to investigate whether osmotic- and salt stress can induce interaction of decapping factors with SnRK2 kinases, MAP4Kα1, MAP3K Raf18, MPK6 or SPI and how this would modify their function.

Another possibility is that substrate selectivity of the decapping machinery depends on the abundance of targeted mRNAs, as was shown for some of the transcripts for which degradation is triggered by LSM1 (Perea-Resa et al., 2016). Finally, specificity could also depend on the mobility of selectively targeted mRNAs. Regulation of mRNA transport from nucleus to cytoplasm is relatively well known, but the mechanism of transcript mobility in the cytoplasm and recruitment to P bodies still remains unknown (Kohler and
Hurt, 2007). RNA binding capacity of TZFs that shuttle between nucleus, cytoplasm and P bodies together with their role in tolerance to osmotic and salt stress (Bogamuwa and Jang, 2014) suggests that their interaction with a specific subset of transcripts could be a possible mechanism to regulate their loading to P bodies (Huang et al., 2012; Jan et al., 2013; Maldonado-Bonilla, 2014; Pomeranz et al., 2010; Sun et al., 2007).

Conclusions
Several factors controlling mRNA metabolism are involved in osmotic or salt stress responses of plants. Most of these are involved in mRNA decapping and as a consequence in 5'->3' decay of transcripts. The contribution of other factors may be indirect, for example CAF1a could function by promoting deadenylation, inducing decapping in a similar way as in yeast (Tarun and Sachs, 1996). Interestingly, most of the factors discussed here are involved in regulation of multiple stresses, but target only a condition-specific subset of transcripts (Maldonado-Bonilla, 2014; Nishimura et al., 2005; Perea-Resa et al., 2016; Potuschak et al., 2006). Unraveling the mechanism of this selectivity would be of high importance. Here, we propose posttranslational modification of mRNA decay factors as a possible mechanism of substrate selectivity. Multiple phosphorylation sites have been identified in several mRNA metabolism regulators, which appear to be phosphorylated in response to osmotic and salt stress in a quantitative way. Further characterization of osmotic- and salt-induced targets of 5'->3' decay pathways is also necessary. We propose here that changes in gene expression upon osmotic and salt stress should be studied not only at the transcriptome level, but should be extended to the posttranscriptional level.

References


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