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Stress-induced tRNA fragments take action in alternative splicing in Arabidopsis

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Transfer RNAs (tRNAs) are the most abundant RNA molecules (in moles) in living cells (Pan 2018). In the hypothetical RNA world, tRNAs are regarded as the pillar molecules that support the origin of life (Muller et al. 2022). As amino acid carriers, tRNAs precisely decipher the genetic code of the mRNA codons during protein translation. However, our understanding of tRNA biology is not complete despite extensive research. Recent discoveries of natural tRNA-derived fragments in cells opened a new line of research in RNA biology (Fig. 1A).

It has been increasingly evident that these fragments are no tRNA degradation debris. Some tRNA-derived fragments are involved in regulating mRNA levels of specific genes through an ARGONAUTE (AGO)-dependent gene silencing mechanism (Gu et al. 2022). It has also been reported that 5′ fragments of $tRNA^{Ala}$ inhibit protein synthesis with association to polyribosomes in Arabidopsis (*Arabidopsis thaliana*) (Lalande et al. 2020). High-throughput small RNA sequencing technologies have allowed the identification of numerous tRNA-derived fragments in eukaryotic and prokaryotic cells. Recently, these fragments are proposed to be systematically named as tRNA-derived RNAs (tDRs) (Holmes et al. 2023).

Some tDRs exhibit tissue-specific accumulation in a developmental stage-dependent manner (reviewed by Chery and Drouard [2023]). In Arabidopsis, the tDR populations were highly dynamic in response to environmental stresses (Cognat et al. 2017). Elucidating the mechanisms by which tDRs function is a new frontier of RNA biology. Progress in this field has brought exciting changes to the paradigm of RNA world. In this issue of *Plant Physiology*, Li et al. (2023) found new functions of a plant tDR in stress responses via interacting with the mRNA splicing factor SERINE-ARGININE RICH PROTEIN 34 (SR34).

Small RNAs derived from the 5′ fragment of $tRNA^{Ala}$ (tDR-Ala-5D), which show increased accumulation in response to biotic and abiotic stresses, have been extensively studied (Cognat et al. 2017; Gu et al. 2022). Li et al. (2023) found that a tDR-Ala-5D (with a standard name of tDR-1:20-Ala-CGC-1-M2 in the consensus nomenclature, hereafter tDR-Ala-5D for short) showed increased accumulation in Arabidopsis in response to toxic arsenite. Overexpression of *tDR-Ala-5D* resulted in an increased sensitivity to abscisic acid (ABA) and NaCl stresses. In contrast, the knockdown lines of *tDR-Ala-5D* (*tDR-Ala-5D*-kd) were more resistant to ABA and NaCl stresses compared with the wild-type (WT) plants.

The expression levels of the tRNA-Ala were not altered in the transgenic lines despite substantial changes in the abundance of tDR-Ala-5D. Thus, the changes in the sensitivity to ABA and NaCl stresses likely resulted from the difference in tDR-Ala-5D abundance. To investigate how tDR-Ala-5D is involved in the regulation of stress resistance, Li et al. (2023) identified proteins interacting with tDR-Ala-5D using the RNA interactome capture technology. The biotin-labeled tDR-Ala-5D was incubated with total proteins extracted from Arabidopsis seedlings. Affinity purification using streptavidin beads in conjunction with quantitative mass spectrometry identified 482 proteins that are potential partners of tDR-Ala-5D (Li et al. 2023).

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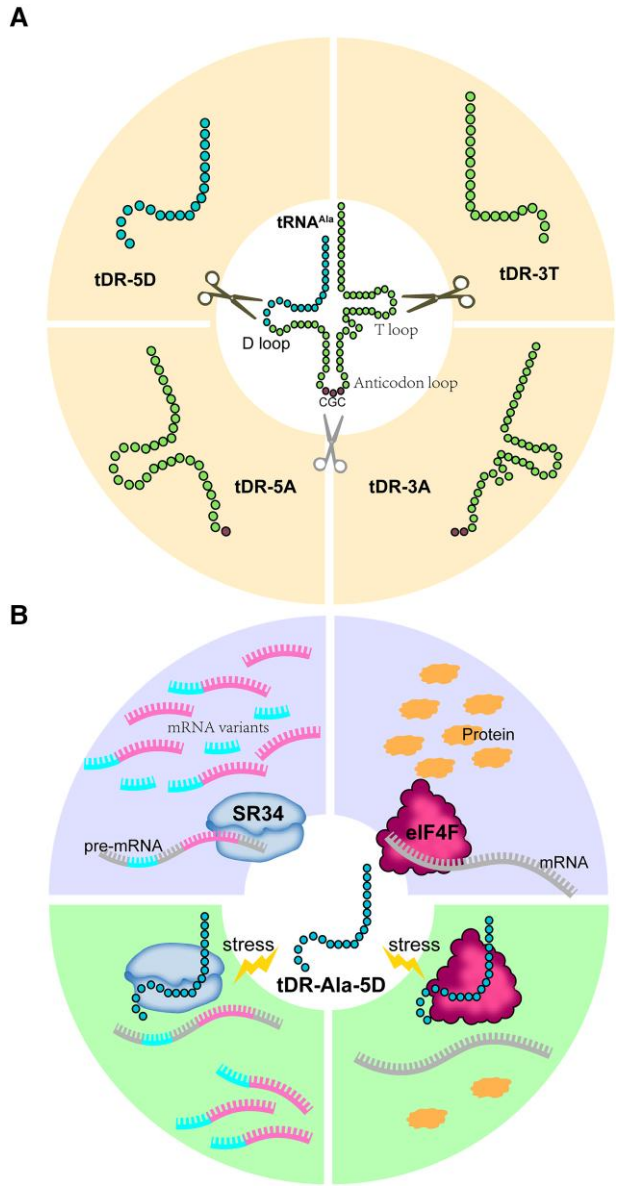

Figure 1. Stress-induced tRNA-derived fragments are involved in regulation of mRNA splicing and translation. **A)** Production of tRNA-derived fragments. The cloverleaf-like structure of tRNAs consists of 4 arms, which are designated as the acceptor stem, dihydrouridine (D) stem–loop, anticodon stem–loop, and TψC(T) stem–loop, where ψ represents pseudouridine. Nucleolytic cleavage of tRNAs at different positions produces various RNA fragments. When cleavage occurs in the anticodon region, 2 tRNA halves will be generated as tDR-5A and tDR-3A. Cleavage in the D region results in a 5′ tRNA-derived RNA called tDR-5D, while cleavage in the T region results in a tDR-3 T. The example shows the cloverleaf-like structure of $tRNA^{Ala}$, and in the upper left is tDR-5D produced from it, namely tDR-Ala-5D. **B)** Stress-induced tDR-Ala-5D plays roles in regulating mRNA splicing and protein translation. Li et al. (2023) show that tDR-Ala-5D interacts with the splicing factor SR34 and interferes with precursor mRNA alternative splicing of crucial genes associated with stress responses. A previous work by Ivanov et al. (2011) demonstrated that tDR-Ala-5D also binds to the translation initiation complex eIF4F, which results in attenuated protein synthesis in response to stress. Thus, tDR-Ala-5D plays multiple roles in regulating gene expression during stress response. The question remains whether the 2 pathways would interact with each other in stress response. The figure is created by Xiaofei Yang.

The putative tDR-Ala-5D–interacting proteins were mostly localized in the cytoplasm (436 proteins), and some were also predicted to localize in the nucleus, chloroplasts, and mitochondria. Among the candidates, eIF4E1 (a subunit of the eIF4F complex responsible for translation initiation) was a known interactor of tDR-Ala-5D (Ivanov et al. 2011). It has been demonstrated that stress-induced tDR-Ala-5D binds to the eIF4F complex, which causes its dissociation from m^7G caps of mRNAs and inhibits translation initiation in mammalian cells (Ivanov et al. 2011).

Gene Ontology (GO) enrichment analyses suggested that these candidate tDR-Ala-5D–interacting proteins were mostly associated with stress response, DNA and RNA metabolism, transport, and energy metabolism (Li et al. 2023). These results suggested that tDR-Ala-5D also might be involved in other mechanisms regulating stress responses besides interfering translation via interacting with the eIF4F complex. Proteins associated with transcriptional and post-transcriptional regulation were particularly interesting. The authors chose an mRNA splicing factor SR34 for further analyses.

SR proteins are a type of splicing factor that bind to specific motifs and mediate alternative splicing events on precursor mRNAs (Hartmann et al. 2018). SR34 contains 2 RNA-recognition motifs in the N terminus and 1 serine-arginine–rich domain in the C terminus. An RNA electrophoresis mobility shift assay revealed a direct interaction between SR34 and tDR-Ala-5D (Li et al. 2023). The interaction between SR34 and tDR-Ala-5D was further validated using multiple technologies, including pull-down and RNA immunoprecipitation assays.

An RNA sequencing analysis revealed distinct gene expression patterns between the *tDR-Ala-5D*-overexpressed lines (*tDR-Ala-5D*-OE) and WT plants (Li et al. 2023). There were 1017 genes displaying significantly lower expression levels (fold-changes >1, $P < 0.05$) in the *tDR-Ala-5D*-OE compared with the WT plants. Interestingly, some of these downregulated genes in *tDR-Ala-5D*-OE plants were known to be targets of SR34. Further GO enrichment analyses found that the downregulated genes were enriched in functions related to responses to stresses and phytohormones (Li et al. 2023).

The RNA sequencing data revealed that 786 genes exhibited a total of 1102 altered alternative splicing events in the *tDR-Ala-5D*-OE compared with the WT plants (Li et al. 2023), suggesting that tDR-Ala-5D is involved in regulation of alternative splicing. Among these 786 genes, 318 were identified as SR34 targets with known SR34-binding motifs recognized. GO enrichment analyses demonstrated that these genes with altered alternative splicing events were mostly enriched in biological functions related to stress responses. Li et al. (2023) further validated these splicing events using PCR experiments.

To investigate how tDR-Ala-5D is involved in SR34-mediated alternative splicing, Li et al. (2023) tested whether tDR-Ala-5D affects the binding affinity of SR34 to the transcripts. Constructs carrying green fluorescent protein–labeled SR34 were transfected into protoplasts of *tDR-Ala-5D*-OE, *tDR-Ala-5D*-kd, and WT plants. Lower levels of SR34 target transcripts were detected in the *tDR-Ala-5D*-OE plants than in the WT, while higher levels were detected in the *tDR-Ala-5D*-kd plants. These results suggested that the binding affinity of SR34 to the target transcripts was lower in the presence of tDR-Ala-5D. Another RNA electrophoresis mobility shift assay experiment showed that tDR-Ala-5D could effectively compete with SR34 for binding to the RNA probe with cognate motif.

Therefore, Li et al. (2023) provided evidence that stress-induced tDR-Ala-5D could interfere with the binding of SR34 to its targets and alter the subsequent alternative splicing processes. In stress responses in Arabidopsis, tDR-Ala-5D was found to interact with both SR34 and eIF4E1. It will be interesting to investigate whether SR34-tDR-Ala-5D and eIF4E1-tDR-Ala-5D affect the same set of downstream genes or whether they behave rather differently in response to different types of stress. Further research on tRNA fragments will help illustrate new roles of tRNAs (Fig. 1B).

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