CellPress

Trends in
Biochemical Sciences

Spotlight

Helicases clear hurdles during plant defense protein translation

Liyuan You [1], Cuilan Shi [1], Daowen Wang [1,*] and Zheng Qing Fu [2,*]

Plants undergo translational reprogramming when they are under attack by pathogens. Xiang *et al.* recently revealed that plant helicases induced by pathogen recognition unwind RNA hairpins upstream of the main open reading frames (mORFs), thus allowing ribosomes to bypass the upstream ORFs (uORFs) and translate downstream defense proteins, a mechanism that is also found in mammals.

Plants provide precious food and rich nutrients for human beings. Unfortunately, in nature, they can also be infected by a wide range of pathogens including fungi, oomycetes, bacteria, and viruses. Plants go through both transcriptional and translational reprogramming to allow efficient expression of defense proteins to combat pathogen infection. The former has been extensively studied [1], whereas our understanding of the latter remains poor. A key question concerns how plants translate defense proteins efficiently only when they are under attack by plant pathogens. A recent paper in *Nature* by Xiang *et al.* sheds light on this fundamental question [2].

Translational regulation plays an essential role in cellular differentiation as well as in responses to growth cues and to abiotic and biotic stresses including pathogen infections. Translation of mRNAs in eukaryotic cells can be regulated through different mechanisms at the initiation, elongation, and termination stages. uORFs with upstream AUGs (uAUGs) are found in the 5′ leader sequences of ~64% of human mRNAs and 54% of *Arabidopsis thaliana* mRNAs [3], and their presence typically hinders the translation of downstream mORFs starting from the main AUGs (mAUGs) [4].

To defend themselves against pathogen infection, plants have evolved pattern recognition receptors (PRRs) that recognize conserved molecules termed microbe-associated molecular patterns (MAMPs) [5]. The MAMP EF-Tu in plant bacterial pathogens is recognized by its receptor EFR and coreceptor BAK1, leading to the induction of pattern-triggered immunity (PTI) in *Arabidopsis* plants [6]. To investigate the underlying mechanism of uAUG-mediated translational regulation during plant pathogen infection, Xiang *et al.* performed global ribosome sequencing of *Arabidopsis* seedlings following treatment with the EF-Tu epitope peptide elf18 to induce PTI responses [2]. By comparing elf18-treated samples to controls, the authors found that, among a total of 13 051 expressed transcripts, 1157 and 1150 exhibited increased or decreased translation, respectively. Interestingly, the authors found that translated uAUGs were over-represented in transcripts whose translation was upregulated by elf8. Furthermore, in these transcripts elf18 treatment was followed by a significant decrease in the association of uAUGs with ribosomes, suggesting that uAUGs modulate the translation of downstream plant defense proteins.

Next, Xiang *et al.* examined the role of RNA secondary structure in translation start-codon selection [2]. They performed RNA structure analysis using selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) [7]. The authors found lower SHAPE reactivity adjacent to the uAUGs of transcripts whose translation was increased by elf8, indicating that double-stranded RNA (dsRNA) structures are present [2]. These data suggest that dsRNA structures associated with uAUGs might prevent the translation of downstream plant defense proteins. Through modeling using deep learning, Xiang *et al.* provided strong evidence that dsRNA structures associated with uAUGs play important roles in uORF translation and in inhibiting the translation of downstream mORFs under normal conditions.

What is the effect of elf18 treatment on RNA secondary structure? Xiang *et al.* observed increased SHAPE reactivity (more single-stranded RNA) in regions adjacent to uAUGs following elf18 treatment, especially in transcripts whose translation was increased by elf18, suggesting that elf18 promotes the unwinding of RNA hairpins in these regions [2].

The next question concerns how the RNA hairpins associated with uAUGs are unwound in response to elf18 treatment. Based on the literature, DEAD-box RNA helicases, which have been shown to work in conjunction with the translation initiation complex, not only unwind RNA hairpins but also promote protein translation [2,8]. Xiang *et al.* identified three cytosolically localized RNA helicases (RH11, RH37, and RH52) in *Arabidopsis* which are induced by elf18 (Figure 1B) [2]. To validate the function of these RNA helicases in alleviating the inhibitory effect of the uAUG-associated hairpin on translation of the downstream mORF, Xiang *et al.* first showed that dexamethasone (Dex)-inducible expression of these RNA helicases enhanced the translation of TBF1, which is a key transcription factor involved in plant growth-to-defense transition. Importantly, in the *rh37 rh52* double mutant generated by CRISPR technology, elf18-induced RNA structural opening of uAUG-associated regions was diminished, and the mutant plants exhibited significantly compromised elf18-induced immunity accompanied by more bacterial growth [2]. These findings confirmed that

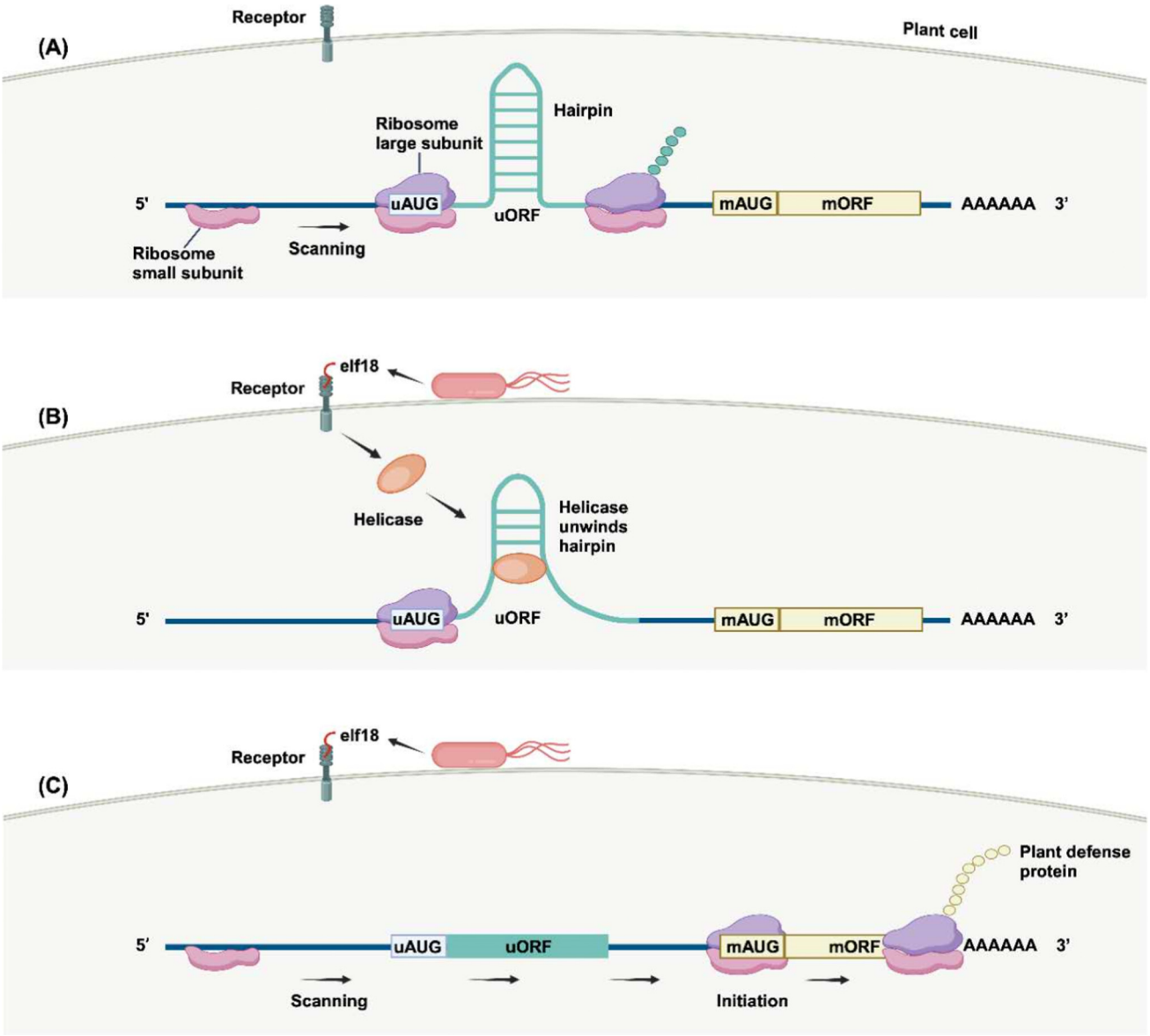

Figure 1. Schematic model illustrating dynamic regulation of protein translation by RNA secondary structure and pathogen-induced helicases during pattern-triggered immunity (PTI). (A) Under normal conditions, a hairpin structure adjacent to the upstream AUG (uAUG) interferes with the movement of ribosomes to the main AUG (mAUG), thus inhibiting the translation of the main open reading frame (mORF) and favoring translation of the upstream ORF (uORF) from the uAUG. (B) Upon pathogen infection, elf18 produced from bacterial EF-Tu binds to its receptor, which induces helicases to unwind the RNA hairpin structure adjacent to the uAUG. (C) Once the hairpin is unwound by a helicase, ribosomes can migrate to the mAUG and start translation of the mORF, resulting in the efficient synthesis of defense proteins to combat pathogen infection. Figure created with BioRender.

RH37 and RH52 regulate elf18-induced PTI by unwinding hairpins associated with uAUGs, which removes inhibition of mORF translation and leads to more efficient translation of downstream defense proteins (Figure 1B,C).

The presence of RH11, RH37, and RH52 homologs DDX3X in humans and Ded1p in yeast suggests that similar regulation could also take place in these organisms. Indeed, Xiang *et al.* found that the presence of an uAUG and its adjacent dsRNA hairpin inhibited the translation of *ATF4*, a well-investigated stress-responsive gene [2,9]. In addition, translation inhibition of *BRCA1 mRNA*, encoding a mutant version of the tumor-suppressor BRCA1 which is present in breast cancer tissues [10], is likely due to

dsRNA structures adjacent to uAUG2 and uAUG3 because SHAPE reactivity was found to be lower in these regions than in upstream regions [2]. In the future, a deeper study of the uAUGs/uORFs and their downstream harpins-mediated translational regulation of *ATF4* and *BRCA1* could contribute to a better understanding of the molecular basis of stress responses as well as of breast cancer in human.

Given that uAUGs are present in more than half of genes in humans and *Arabidopsis* plants, and that similar regulation also takes place in human cells [2,3], it will be important to determine to what extent this uAUG–hairpin–helicase regulatory module dictates dynamic protein translation across different species under diverse conditions. Future studies may also focus on how the three RNA helicases (RH11, RH37, and RH52) are induced by elf18 to promote PTI, and whether they have different and complementary substrate specificities in unwinding hairpins associated with uAUGs. In conclusion, these new insights into translational regulation by uAUGs and their associated hairpins may enable the design of novel gene regulation strategies for agricultural and therapeutic applications.

Acknowledgments

This work is supported by a grant from the National Science Foundation (IOS-2207677) to Z.Q.F.

Declaration of interests

The authors declare no competing interests.

[1]State Key Laboratory of Wheat and Maize Crop Science, College of Agronomy, and Center for Crop Genome Engineering, Henan Agricultural University, Zhengzhou 450002, China
[2]Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

*Correspondence:
dwwang@henau.edu.cn (D. Wang) and
zfu@mailbox.sc.edu (Z.Q. Fu).

https://doi.org/10.1016/j.tibs.2023.10.007

Published by Elsevier Ltd.

References

1. Chen, J. *et al.* (2020) Reprogramming and remodeling: transcriptional and epigenetic regulation of salicylic acid-mediated plant defense. *J. Exp. Bot.* 71, 5256–5268
2. Xiang, Y. *et al.* (2023) Pervasive downstream RNA hairpins dynamically dictate start-codon selection. *Nature* 621, 423–430
3. Zhang, H. *et al.* (2021) Determinants of genome-wide distribution and evolution of uORFs in eukaryotes. *Nat. Commun.* 12, 1076
4. Calvo, S.E. *et al.* (2009) Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. *Proc. Natl. Acad. Sci. U. S. A.* 106, 7507–7512
5. Schwessinger, B. and Zipfel, C. (2008) News from the frontline: recent insights into PAMP-triggered immunity in plants. *Curr. Opin. Plant Biol.* 11, 389–395
6. Zipfel, C. *et al.* (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* 125, 749–760
7. Siegfried, N.A. *et al.* (2014) RNA motif discovery by SHAPE and mutational profiling (SHAPE-MaP). *Nat. Methods* 11, 959–965
8. Pisareva, V.P. *et al.* (2008) Translation initiation on mammalian mRNAs with structured 5'UTRs requires DExH-box protein DHX29. *Cell* 135, 1237–1250
9. Vattem, K.M. and Wek, R.C. (2004) Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proc. Natl. Acad. Sci. U. S. A.* 101, 11269–11274
10. Sobczak, K. and Krzyzosiak, W.J. (2002) Structural determinants of BRCA1 translational regulation. *J. Biol. Chem.* 277, 17349–17358