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Spotlight

Translating while under attack: Plant defense mRNAs find a way

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In plants, pattern-triggered immunity shuts down global translation while allowing the translation of defense mRNAs. Wang et al. (2022) describe a previously unknown mechanism for how elements in the 5′ UTR of these mRNAs can recruit the translation machinery to initiate protein synthesis.

Organisms constantly need to defend themselves against invading pathogens. To do so, they must be able to recognize these pathogens, transduce the recognition signal into effector factors to reprogram gene expression, and ultimately produce responsive elements to resolve the challenge. For both plants and animals, recognition of pathogens is carried out via pattern recognition receptors (PRRs) (Dardick and Ronald, 2006). These PRRs monitor for the presence of molecules that are broadly conserved across pathogens, known as pathogen-associated molecular patterns (PAMPs) (Hou et al., 2019). In plants, perception of a PAMP by PRRs triggers a signaling pathway known as pattern-triggered immunity (PTI), which leads to a multitude of transcriptional and translational changes to increase host defenses (Hou et al., 2019). While PTI-mediated transcriptional control and signaling cascades through MAP kinases have been well studied, translational reprogramming during PTI has only recently begun to be uncovered (Xu et al., 2017). The work by Wang et al. provides details on some of the mechanisms utilized in translation control during PTI.

Our understanding of translation-mediated changes to gene expression in response to stress comes from a wealth of studies in other eukaryotes. For example, in yeast and animals the primary response pathway is the integrated stress response (ISR) (Pakos-Zebrucka et al., 2016). The system comprises various kinases that monitor distinct stresses and, upon activation, phosphorylate the α subunit of translation initiation factor eIF2. Phosphorylation of eIF2α then leads to rapid global repression of translation but a simultaneous increase in translation of stress-response genes. Key among these genes are transcription factors that act as key regulators of the downstream response, such as *GCN4* in yeast and *ATF4* in mammals. Interestingly, the regulatory mechanism of *GCN4* and *ATF4* is conserved and occurs primarily at the translational level; the genes harbor upstream open reading frames (uORFs) in their 5′ UTRs that repress translation under normal conditions but enable translation under stress conditions (Hinnebusch et al., 2016).

A similar system appears to be conserved in plants. eIF2 and a homolog of the nutrient-deprivation-sensing kinase, GCN2, are conserved in plants, as is a downstream transcription factor, TBF1. The *TBF1* transcript also contains uORFs and appears to be under eIF2α-phosphorylation-mediated translational control (Pajerowska-Mukhtar et al., 2012). However, GCN2-mediated signaling does not appear to be necessary under several stress conditions, including some that activate PTI, suggesting that *TBF1* and other stress-response genes have evolved alternative means of translation. Previous work by the group shed light on such a mechanism (Xu et al., 2017). There it was found that upon PTI induction in response to the PAMP elf18 (an epitope from the bacterial translation elongation factor Tu), the translation of certain transcripts was increased without a comparable increase in their transcription. The upregulated translation of these transcripts was dependent on sequences in their 5′ UTRs that were composed almost entirely of purines, which were termed R-motifs. Initial observations suggested that translational control by R-motifs was mediated via association with polyA-binding proteins (PABPs), but details of the molecular mechanism by which this pathway was carried out were lacking. The new work by Wang et al. uncovers important details about these mechanisms and provides insight into the role of R-motifs in translational reprogramming in response to stress.

In their work, Wang and colleagues showed that global mRNA decapping by DCP2 plays a major role during PTI induction. By accelerating mRNA decay and inhibiting canonical cap-dependent translation, PTI-induced decapping leads to general repression of gene expression. Although mechanistically distinct, the result is functionally similar to ISR induction via eIF2α phosphorylation. However, decapping by DCP2 appears indiscriminate, similarly targeting both growth and defense mRNAs, necessitating a distinct mechanism for initiating translation on defense mRNAs. Here the authors found that R-motifs in the 5′ UTRs of these mRNAs are sufficient to stimulate translation in response to elf18 induction of PTI. More importantly, using various reporters *in planta* and *in vitro*, they showed that the R-motifs can drive translation in the absence of a cap structure. In addition, they showed that binding of the PABP PAB8 to R-motifs is important for translation of the transcript *in vivo* and that artificial recruitment of PAB8 to mRNAs is sufficient to drive translation.

Using co-immunoprecipitation and split-luciferase complementation assays, the authors also identified other factors involved in PABP-mediated translation of defense mRNAs. Two factors that stood out were the translation initiation factor

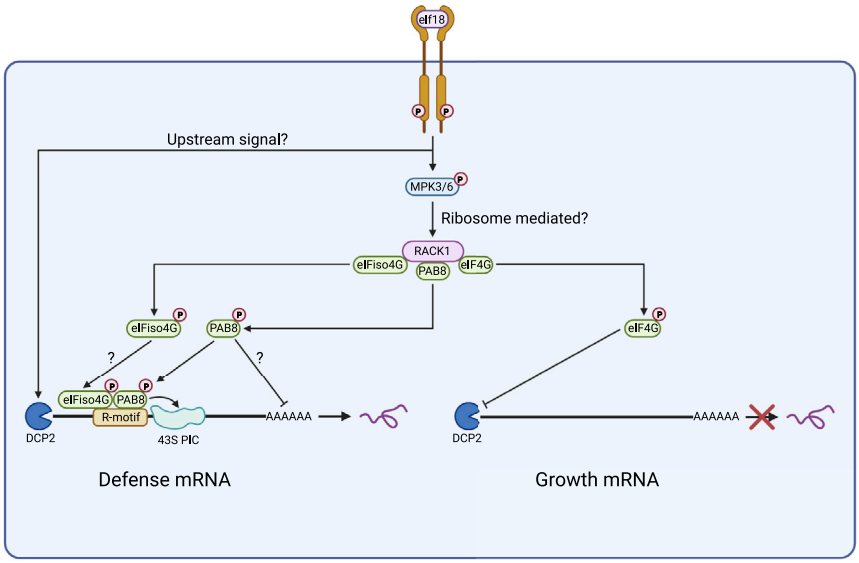

Figure 1. Defense mRNAs are translated during PTI through the recruitment of phosphorylated PAB8 and eIFiso4G proteins to the R-motif found in their 5′ UTR

Proposed model by Wang et al. (2022) for selective translation of R-motif-bearing mRNAs in response to elf18 induction. The upstream signal for increased decapping of mRNAs by DCP2 in response to elf18 induction is still unknown. The role of RACK1 in mediating phosphorylation of eIFiso4G, PAB8, and eIF4G is unclear, as is whether or not this interaction further requires the ribosome. How phosphorylation of these factors alters their binding preferences has yet to be determined.

eIF4G, which plays an important role as a scaffolding factor in canonical translation, and its plant-specific isomer, eIFiso4G1. In particular, the authors found that PABPs preferentially interacted with eIFiso4G over eIF4G in response to elf18 induction of PTI. Moreover, loss of eIFiso4G repressed translation of R-motif-containing reporters and compromised elf18-triggered protection to the bacterial pathogen *Pseudomonas syringae* pathovar *maculicola*, in contrast to the loss of eIF4G, suggesting a mechanism whereby altered interaction of PABPs with eIF4G and eIFiso4G drives selective translation of R-motif-bearing transcripts.

The PTI-induced switch of PABP binding preference from eIF4G to eIFiso4G can explain the mechanism by which R-motif-containing mRNAs are translated in response to PTI induction, but not the regulatory mechanism that controls PAB8 recruitment to R-motifs and/or eIFiso4G. Given that PTI induction activates the MAP kinases MPK3/6 (Hou et al., 2019), which in turn interact with RACK1, a factor that interacts with PABPs, eIF4G, and eIFiso4G, the authors hypothesized that phosphorylation was the regulatory signal for translational reprogramming. Indeed, the authors found not only that MPK3/6 were important for translation of R-motif mRNAs, but that PAB8, eIF4G, and eIFiso4G are also substrates for the kinases. Furthermore, the authors observed that PAB8 phosphorylation by MPK3/6 enhances its association with R-motifs, while phosphorylation of eIF4G and eIF4isoG had opposing effects; phosphorylation of the former inhibits its function during canonical translation, whereas phosphorylation of the latter enhances its activity during cap-independent initiation. From their data, the authors proposed a model in which elf18-induced MPK3/6 phosphorylation of PABP, eIF4G, and eIFiso4g enables plants to reprogram their translational output (Figure 1).

Still, several questions remain regarding the mechanism of R-motif-mediated translational control. One is how phosphorylation of eIFiso4G and PABPs alters their binding characteristics. The observation that *eif4g eif4e1* double mutants do not appear to show a phenotype, whereas *eifiso4g1 eifiso4g2* double mutants show a reduced stature, suggests that eIFiso4G also functions in basal translation. Phosphorylation of eIFiso4G would then necessitate a shift in function from a cap-binding mode to a cap-independent one. Similarly, it is unclear how phosphorylation of PAB8 targets the factor to bind R-motifs, as presumably polyA tails are also available for binding. More work will need to be done to understand if these interactions are mediated via RACK1, possibly with ribosome involvement, as well as other *trans*- and *cis*-acting elements of R-motif-bearing transcripts that enable their selective translation under stress. Regardless, the work by Wang et al. sheds new light on how plants activate innate immunity mechanisms through reprogramming of global translation.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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