nature portfolio

Peer Review File

A nuclear tRNA-derived fragment triggers immunity in Arabidopsis

Corresponding Author: Professor Hong-Gu Kang

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Communications Biology.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This manuscript is a transfer from Nature Communications. Based on the rebuttal letter, one of the reviewers raised additional concerns, which I will address after summarizing the manuscript and its findings.

Overall Summary
The authors demonstrated that the dcl1-7 mutant supports significantly higher levels of Pst_avrRpt2 (ETI) compared to the wild type (WT) at 3 days post-inoculation (dpi). They performed RNA-seq across multiple timepoints using WT and dcl1-7 mutants, focusing on the 6-hour timepoint, which revealed unique GO terms. The study then investigated nuclear small RNAs (sRNAs) dependent on DCL1, ultimately focusing on tRNA-derived sRNAs (tRFs). Among these, 31-nt tRFs were predominantly derived from a few tRNAs, with AspGTC being the most abundant. Other ETI inducing effectors similarly induced tRF31Asp2. The authors found that AGO2, one of the ten AGO proteins in Arabidopsis, associates with tRF31Asp2 in the nucleus. Synthetic tRF31Asp2 RNA—but not tRF31SerGCT or tRF31ValCAC—induced PR1 expression. Pretreatment with tRF31Asp2 RNA reduced pathogen growth, particularly with avirulent pathogens. Interestingly, this reduction was even more pronounced in tRF31Asp2-pretreated dcl1-7, suggesting that tRF31Asp2 may effectively complement DCL1 deficiency. To further characterize tRF31Asp2, the authors performed mutagenesis on its stem-loop structure. Transcriptome analysis revealed 810 differentially expressed genes (DEGs) in response to tRF31Asp2. ChAP-seq analysis identified associations with Gypsy and Copia superfamilies of LTR retrotransposons. They concluded that tRF31Asp2 may facilitate the activation of defense genes heavily suppressed under non-stressed conditions.

Evaluation
This is a well-developed and mature story. The authors have addressed the concerns raised by the reviewer to a satisfactory extent. I find the newly raised issues unsubstantiated and disagree with the reviewer’s comments. The authors' responses are thorough, and their data convincingly support their conclusions.

We want to thank all the editors and reviewers who contributed to improving this MS. Initially submitted to Nature Communications on Jan 31, 2022, this MS underwent three rigorous rounds of review for nearly three years. Since the current review references the previous round from Nat Comm, we believe sharing the final round review would be beneficial for the audience. Thus, we included this last feedback from Nat Comm, with the reviewer's comments highlighted in red and our responses in blue. Please note that only one reviewer remained at the final review stage. Again, we appreciate the community's efforts in refining the MS and ensuring the scientific rigor.

Reviewer comments from the final round of Nature Communications review:

In this new version of the manuscript the authors have tried to address some of my previous concerns. Despite this, I am very concerned about the potential correlational nature of the data.
A correlational observation could be, for instance, where a gene increases when a biological cue is applied and decreases when the cue is removed. Indeed, data from our sRNA-seq and RIP-seq (Fig. 2) reflect correlational relationships (e.g., infection vs. control). However, this is precisely why we included the data in Figures 3 and 4—to demonstrate causal and mechanistic insights beyond correlation.

1. The tRF treatment alone triggers defense responses (Fig. 3a, Fig. 4a), which is genetically dependent on the AGO2 clade (Fig. 3h, Fig. 4a).
2. The sequestration of tRF leads to compromised defense responses through two independent methods: antisense and genetic (STTM) sequestration (Fig. 3f, Fig. 3g).

In Figures 3 and 4, we actively added and removed the tRF from the system and observed direct, measurable changes in defense responses, demonstrating causality. Furthermore, the ChAP-seq data (Fig. 4b–4e) provide a mechanistic explanation for how the tRF induces defense responses. By analyzing genomic interactions, we identified specific mechanisms underlying these observations where the tRF interacts with the target genomic regions. If the findings were merely correlational, this level of mechanistic insight would not be possible with experimental data presented.

As shown in Extended (Supplementary) Figure 4 a and b, the tRF31Asp2 fragment accumulates to extremely high levels in mock samples, where there is no detection of DEGs.
While we agree there is a basal level of this tRF, the DCL1-dependent increase (please compare WT vs dcl1 in this figure) is CLEAR, strongly supporting that DCL1-dependent tRFs are responsible for triggering defense genes.

Additionally, the confirmation of the nuclear activity of AGO2 would need a much stronger evidence and several additional experiments.
We addressed this question by performing a ChAP-seq experiment in ago2/3/7. The NGS experiment in Extended Data (Supplementary) Fig. 11 showed that tRF interactions in the nucleus are significantly reduced in ago2/3/7. This observation

supports the conclusion of AGO2 nuclear activity in addition to other supporting data in the MS. We felt that the MS did not emphasize AGO2, and the current evidence is relevant to support the conclusions. Further research on AGO2 could be performed in the future. The main conclusion was best shown in the TITLE.

In view of this I can not recommend the publication of this manuscript.
We wish the reviewer had suggested a constructive path for publishing this MS, especially after our revision effort for almost three years.