Cell Host & Microbe
Previews

After the trap snaps in the plant immune response

Morgan Carter[1,*]
[1]Department of Biological Sciences, University of North Carolina at Charlotte, North Carolina, 28223, USA
*Correspondence: morgan.carter@uncc.edu
https://doi.org/10.1016/j.chom.2023.01.015

In this issue of *Cell Host and Microbe*, Chen et al. report that global translation is increased upon plant pathogen detection by intracellular resistance proteins. To achieve this, the conserved protein CDC123 promotes translation initiation complex assembly during the early hours of a defensive programmed cell death in *Arabidopsis*.

Plant immunity relies on extracellular and intracellular monitoring for pathogen presence to initiate an immune reaction. Invaders may be detected by the damage they cause, by conserved motifs identifying them as nonself, and by effector molecules they secrete directly into the plant host to promote infection and hinder resistance. The intracellular line of defense is largely made of up NLRs, NOD-like receptor proteins also called nucleotide-binding leucine-rich repeat receptors, which play a role in animal immunity as well. NLRs have a variety of mechanisms by which they can indirectly or directly detect pathogen effectors within a plant cell.[1] In only the past few years, considerable progress has been made on the downstream signaling begun by the activation of an NLR and leading to programmed cell death.[2] Notably, activated NLRs swap ADP for ATP and oligomerize to form a structure referred to as the resistosome. A major influx of calcium occurs, at least in some cases, due to formation of a cation channel by certain types of NLRs.[3] Additionally, a plant cell undergoing effector-triggered immunity (ETI) has a shift in transcription and translation but not a sharp reduction as seen in the programmed cell death of other organisms. How and whether protein production is modified in concert with ETI has been a gap in our understanding of plant immune signaling.

In considering that gap, Chen et al.[4] ask whether there is an activator responsible for translational reprogramming during plant defense. Their previous findings showed that relevant metabolic enzymes were coregulated transcriptionally and translationally during ETI.[5] In this study, a dual luciferase reporter is used to show a general increase in protein synthesis in response to a bacterial effector protein, AvrRpt2. A constitutive promoter was used to drive expression of a transcript with three open reading frames (ORFs) in the model plant *Arabidopsis thaliana*. Two upstream ORFs (uORF) act as translational regulators of the ORF for firefly luciferase during typical translation by a single ribosome on the transcript. Increased translational activity through multiple ribosomes occupying the transcript yields more functional luciferase protein. Though the expression of luciferase mRNA remained consistent, luciferase activity and polysome-to-monosome ratio increased when challenged with AvrRpt2. A follow-up experiment evaluating labeled peptide incorporation confirmed the global increase in translation during ETI.

Finding that known stress regulators were not responsible for ETI-mediated translation changes, the authors sought to find the regulator involved. A random mutagenesis screen of their luciferase reporter line yielded a mutant that did not increase luciferase translation during ETI. The mutant line had reduced ETI and supported bacterial growth equivalent to the control line missing the cognate NLR that detects AvrRpt2. Through backcrossing and sequencing, the causal mutation was identified in the cell division cycle 123 (CDC123) gene and could be complemented with a functional copy of CDC123. An ATP-grasp protein, CDC123 has been studied in humans and yeast for its association with disease and as an interactor with eukaryotic translation initiation factor 2 (eIF2). Also known as BICE1 and EDA35, *Arabidopsis* CDC123 can interact with prereplication complex machinery affecting DNA synthesis, embryogenesis, and pollen development.[6,7] To identify relevant CDC123-interacting proteins in *Arabidopsis* for the translational reprogramming seen during ETI, CDC123 was pulled down from transgenic plants with and without AvrRpt2 present and was subjected to mass spectrometry. The most abundant interactor was eIF2γ, which was confirmed to be an interactor by split luciferase complementation analysis and yeast two-hybrid. The other two eIF2 subunits, eIF2α and eIF2β, also indirectly or transiently interacted with CDC123.

The initial observation of an increased number of polysomes and a global increase in translation during an immune response would require increased assembly of eIF2 complexes. A role for eIF2γ in ETI-mediated translational changes is further supported by the ETI-impaired line having a mutation in the CDC123 ATP-binding site that prevents interaction with eIF2γ. Maneuvering around the difficulty of studying an essential gene like eIF2γ, the authors employed an inducible RNAi silencing strategy to inhibit eIF2γ in mature plants. Similar to the CDC123 mutant line, eIF2γ knockdown blocked ETI. Furthermore, the entire eIF2 complex has increased assembly during ETI, likely with CDC123 serving as a chaperone.

How exactly does CDC123 go about enhancing eIF2 complex assembly? And in what way does that intersect with other changes occurring in the plant cell during ETI? The authors show that the intracellular ATP concentration increases once challenged with effectors that trigger immunity via either major class of NLR proteins. Conversely, inhibiting ATP-dependent enzyme activity reduces eIF2 assembly. The initial CDC123 mutant line still undergoes an increase in ATP but not in eIF2 assembly, showing that the elevated ATP concentration alone is not improving interaction between eIF2γ, eIF2α, and eIF2β. Activation of NLRs to form the resistosome also requires ATP, but this occurs before CDC123 induction

upon NLR activation. The resistosome model has been informed by the mammalian inflammasome, which is actively being researched for how it is impacted by intracellular ATP levels.[8] Perhaps the most interesting question highlighted by the findings of this study is the cause and other repercussions of elevating cellular ATP *in planta* during an immune response. Aligning the timetables of intracellular ATP levels, transcriptional and translational reprogramming, calcium influx,[3] and other relevant cellular states could inform a better step-by-step model of what happens post-NLR activation.

Ultimately, the authors present a model in which the increased cellular levels of ATP enable CDC123 to chaperone the assembly of three subunits into full eIF2 complexes that increase translation initiation as part of global reprogramming during the ETI response. This largely aligns with the characterization of human and yeast CDC123 as an interactor of eIF2γ that positively impacts eIF2 assembly[9,10]. This is not the first observation of the conservation of CDC123 between plants and animal; CDC123 from *Arabidopsis* was able to be partially complemented by CDC123 from humans but not from yeast.[6] Further analysis of CDC123 and its roles across plant development and immunity will likely inform human biology and vice versa. Mutational analysis of CDC123 may shed light on how this protein can be involved in such different, crucially important processes as DNA replication and translation.

REFERENCES

1. Kourelis, J., and van der Hoorn, R.A.L. (2018). Defended to the Nines: 25 years of Resistance Gene Cloning Identifies Nine Mechanisms for R Protein Function. Plant Cell *30*, 285–299. https://doi.org/10.1105/tpc.17.00579.

2. Margets, A., Rima, S., Helm, M., and Carter, M. (2021). Molecular Mechanism & Structure—Zooming in on Plant Immunity. Mol. Plant Microbe Interact. *34*, 1346–1349.

3. Jacob, P., Kim, N.H., Wu, F., El-Kasmi, F., Chi, Y., Walton, W.G., Furzer, O.J., Lietzan, A.D., Sunil, S., Kempthorn, K., et al. (2021). Plant "helper" immune receptors are Ca2+-permeable nonselective cation channels. Science *373*, 420–425.

4. Chen, T., Xu, G., Mou, R., Greene, G.H., Liu, L., Motley, J., and Dong, X. (2023). Cell Host Microbe *31*, 334–342.

5. Yoo, H., Greene, G.H., Yuan, M., Xu, G., Burton, D., Liu, L., Marqués, J., and Dong, X. (2020). Translational Regulation of Metabolic Dynamics during Effector-Triggered Immunity. Mol. Plant *13*, 88–98.

6. Long, Y.-P., Xie, D.-J., Zhao, Y.-Y., Shi, D.-Q., and Yang, W.-C. (2019). Bicellular pollen 1 is a modulator of DNA replication and pollen development in Arabidopsis. New Phytol. *222*, 588–603.

7. Pagnussat, G.C., Yu, H.-J., Ngo, Q.A., Rajani, S., Mayalagu, S., Johnson, C.S., Capron, A., Xie, L.-F., Ye, D., and Sundaresan, V. (2005). Genetic and molecular identification of genes required for female gametophyte development and function in Arabidopsis. Development *132*, 603–614.

8. Billingham, L.K., Stoolman, J.S., Vasan, K., Rodriguez, A.E., Poor, T.A., Szibor, M., Jacobs, H.T., Reczek, C.R., Rashidi, A., Zhang, P., et al. (2022). Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation. Nat. Immunol. *23*, 692–704.

9. Perzlmaier, A.F., Richter, F., and Seufert, W. (2013). Translation initiation requires cell division cycle 123 (Cdc123) to facilitate biogenesis of the eukaryotic initiation factor 2 (eIF2). J. Biol. Chem. *288*, 21537–21546.

10. Panvert, M., Dubiez, E., Arnold, L., Perez, J., Mechulam, Y., Seufert, W., and Schmitt, E. (2015). Cdc123, a Cell Cycle Regulator Needed for eIF2 Assembly, Is an ATP-Grasp Protein with Unique Features. Structure *23*, 1596–1608. https://doi.org/10.1016/j.str.2015.06.014.

Getaway car: Fungal HscA steers human phagosomal p11 into an escape route

Emma Camacho[1] and Carolina Coelho[2,*]
[1]W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA
[2]MRC Centre for Medical Mycology at University of Exeter, Exeter, EX4 4QD Devon, UK
*Correspondence: c.coelho@exeter.ac.uk
https://doi.org/10.1016/j.chom.2023.02.006

In this issue of *Cell Host & Microbe*, Jia and colleagues discover how the human p11 (s100A10)-Anxa2 heterodimer drives sorting of microbial phagosomes into recycling versus degradative pathways. In a remarkable evolutionary arms race, the *Aspergillus fumigatus* protein HscA latches to p11 to steer its phagosome away from fungal killing.

Phagocytosis and endocytosis are cargo-sensitive, tightly regulated processes. For example, within the same dendritic cell, when distinct particles are ingested, they are segregated and processed differently for antigen presentation.[1] In macrophages and monocytes, phagosomes are used to kill pathogens, which leads to an evolutionary arms race by pathogens to manipulate phagocytic killing, allowing escape and/or persistence, and serendipitously providing researchers with insights on how these endocytic processes work.

Healthy individuals inhale hundreds of *Aspergillus fumigatus* spores, which are normally cleared by professional