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COMMENTARY

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Release brake and set plant defense in motion

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Plants deploy elegant strategies to defend themselves against pathogen infection.[1] To prevent plant pathogens from causing serious disease symptoms, plants must be able to recognize these pathogens. Facing challenges from bacterial, fungal, oomycete, and nematode pathogens, plants utilize cell surface-localized pattern recognition receptors (PRRs) to perceive conserved molecules in these plant pathogens, activating pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI).[2] On the other hand, plant pathogens deliver effectors into plant cells to suppress PTI, resulting in effector-triggered susceptibility. To defeat these highly virulent pathogens, plants develop a second layer of defense termed effector-triggered immunity (ETI), in which plants rely on nucleotide-binding leucine-rich repeat receptors to detect pathogen effectors directly or the activities of these effectors indirectly, ultimately leading to a rapid localized cell death called hypersensitive response.[3] The activation of PTI and/or ETI during a primary infection can further induce long-lasting and broad-spectrum systemic acquired resistance in the entire plant against a secondary pathogen infection.[4]

Constitutive activation of plant immune responses can have detrimental effects on plant growth.[5] Therefore, it makes sense that plants have developed delicate control mechanisms to allow themselves to launch effective defense only when they are under attack by plant pathogens. However, it remains obscure how plants make this crucial decision. Recently, a fascinating article published in *Nature* provided fresh insights on these important dual regulations.[6]

Both rice (*Oryza sativa*) and *Arabidopsis thaliana* plants can recognize fungal PAMP chitin and bacterial peptidoglycan via the PRR chitin elicitor receptor kinase 1 (CERK1), which forms a protein complex with several other proteins to initiate downstream PTI responses (Figure 1).[6] In rice, chitin binds to and actives the kinase activity of its receptor OsCERK1, which in turn triggers a phosphorylation cascade, increases cytosolic calcium levels, and induces a burst of reactive oxygen species (ROS), ultimately leading to enhanced resistance to fungal pathogens (Figure 1).[7]

To investigate the regulation of OsCERK1-mediated rice immunity, Wang et al. performed co-immunoprecipitation assays followed by mass spectrometry analysis to identify OsCERK1-FLAG associated

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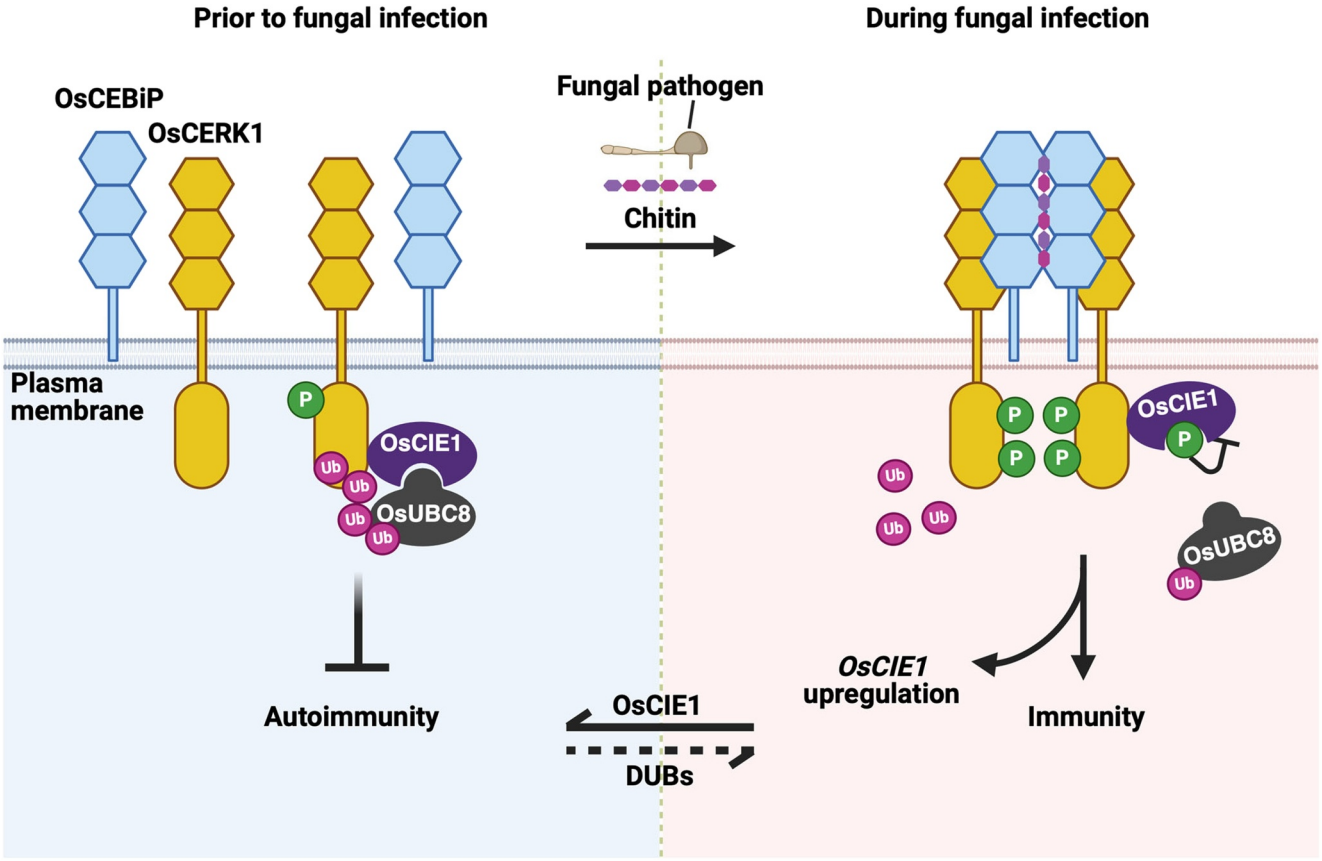

FIGURE 1 A simplified model illustrating dual regulations of chitin-triggered PTI before and after rice plants are exposed to a fungal pathogen. Prior to a fungal infection, the E3 ligase OsCIE1 recruits its E2 conjugase OsUBC8 to ubiquitinate OsCERK1. Once OsCERK1 is poly-ubiquitinated by OsCIE1, the kinase activity of OsCERK1 is inhibited. Consequently, OsCERK1 cannnot phosphorylate its downstream target protein(s) to activate PTI. In the presence of a fungal pathogen, the PAMP chitin from the fungal pathogen is perceived by the rice OsCEBiP-OsCERK1 complex. Presumably, some deubiquitinases will remove the ubiquitin chain from OsCERK1, which allows OsCERK1 to subsequently phosphorylate OsCIE1, blocking its interaction with OsUBC8 to inhibit the E3 ligase activity of OsCIE1. Therefore, PTI will be activated to fight against pathogens. Activation of plant immunity upregulates the expression of OsCIE1, which will dampen PTI when the pathogen infection is alleviated. CERK1, chitin elicitor receptor kinase 1; DUBs, deubiquitinase; OsCIE1, OsCERK1-interacting E3 1; PAMP, pathogen-associated molecular pattern-triggered immunity (PTI).

proteins in rice protoplast.[6] OsCERK1-interacting E3 1 (OsCIE1), which is an ortholog of *Arabidopsis* U-box type E3 ubiquitin ligase 14 (PUB14) in rice, was identified as a candidate interactor of OsCERK1 (Figure 1). The interaction between OsCIE1 and OsCERK1 was then confirmed by yeast two-hybrid, in vitro pull down, co-immunoprecipitation, and split luciferase assays.

OsCIE1 was then found to be a negative regulator of OsCERK1-mediated rice immunity.[6] Wang et al. first discovered that chitin-induced ROS burst was significantly increased in *Oscie1* mutants. Consistent with this observation, Wang et al. observed increased expression of *OsRbohH* encoding, an NADPH oxidase involved in ROS generation, and reduced expression of *OsCATA* encoding, a catalase responsible for ROS elimination, in *Oscie1* mutants in the presence and absence of chitin.[6] Importantly, the *Oscie1* mutants exhibit enhanced resistance to both the rice blast pathogen *Magnaporthe oryzae* and the rice bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*).

To find out whether the *Oscie1*-mutant phenotypes are dependent on OsCERK1, the authors generate *Oscerk1-1 Oscie1-1* double mutants.[6] Wang et al. found that chitin-induced ROS burst and resistance to pathogens in the *Oscerk1-1 Oscie1-1* double mutants were largely restored to wild-type levels, indicating that the *Oscie1*-mutant phenotypes are OSCERK1 dependent.

How does OsCIE1 suppress OsCERK1-mediated rice immunity? As a predicted E3 ligase with a U-box, OsCIE1 poly-ubiquitinates OsCERK1 in vitro and in vivo (Figure 1).[6] The authors then wondered whether the poly-ubiquitination of OsCERK1 promotes the degradation of OsCERK1. Consequently, the authors examined the stability of OsCERK1 protein through the treatment of the protein translation inhibitor

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cycloheximide in the presence of wild-type or E3 ligase inactive mutant of OsCIE1 in the presence or absence of chitin. Surprisingly, it turned out that the half-life of OsCERK1 was similar in all these treatments, suggesting that OsCIE1 does not promote the degradation of OsCERK1. What is the purpose of poly-ubiquitination of OsCERK1 by OsCIE1? Consistent with their data that OsCIE1 preferentially poly-ubiquitinates kinase-active OsCERK1, OsCIE1 inhibits the kinase activity of OsCERK1 (Figure 1). OsRLCK185 is an OsCERK1 substrate that plays a key role in the activation of downstream chitin signaling.[8] To test the kinase activity of ubiquitinated OsCERK1 versus non-ubiquitinated OsCERK1, Wang used OsRLCK185(K108E) kinase-dead protein as a substrate of OsCERK1. The levels of phosphorylated OsRLCK185(K108E) were greatly reduced after the incubation of ubiquitinated OsCERK1 compared to non-ubiquitinated OsCERK1. Consistent with these findings, the OsCERK1–Flag isolated from *Oscie1-1* mutant protoplasts showed higher kinase activities on the universal substrate myelin basic protein and OsRLCK185(K108E). Consequently, the basal MAPK activation, an early output of chitin-mediated PTI, was detected even in the absence of chitin treatment in the *Oscie1-1* background *OsCERK1* complementary rice seedlings. Taken together, these data indicate that OsCIE1-mediated poly-ubiquitination of OsCERK1 suppresses the kinase activity of OsCERK1, thereby inhibiting autoimmunity in the absence of pathogens (Figure 1).

Upon infection by a fungal pathogen, the PAMP chitin from the pathogen is recognized by the chitin receptor CERK1 to activate PTI (Figure 1).[9,10] All the aforementioned data explain well how plants keep PTI inactive in the absence of chitin. How rice plants activate PTI in the presence of chitin is still an open question. To answer this important question, Wang et al. wondered whether OsCERK1 as a positive regulator can inhibit the negative regulator OsCIE1 to initiate chitin-mediated PTI.[6] They found that OsCERK1, as an active kinase, phosphorylates OsCIE1 to dampen OsCIE1's E3 ligase activity in the presence of chitin (Figure 1). Consequently, OsCIE1 can neither poly-ubiquitinate OsCERK1 nor inhibit its kinase activity. As an active kinase, OSCERK1 will then be able to phosphorylate its target protein OsRLCK185, turning on chitin-mediated PTI signaling cascade (Figure 1). To determine how the phosphorylation of OsCIE1 by OCERK1 affects rice immune responses, the authors complemented the *Oscie1* mutant with wild-type *OsCIE1*, *OsCIE1(4D),* or *OsCIE1(4A)*.[6] *OsCIE1 (4D)* carries a phosphorylation-mimetic mutation, while *OsCIE1(4A)* harbors a mutation, which makes it impossible to be phosphorylated by OsCERK1. Moreover, the authors found that both *OsCIE1* and *OsCIE1 (4A)* reduced the ROS burst and blast resistance of the *Oscie1* mutant while *OsCIE1(4D)* did not.

Structural studies have been highly beneficial in gaining insights into protein functions, especially for enzymes. The crystal structure of the binary complex of the U-box domain of OsCIE1 and its E2 conjugase OsUBC8 revealed that the OsCIE1-U-box interacts OsUBC8 mainly through two surface patches, namely surface patch A and surface patch B.[6] Residues 233–239 in surface patch A form a short loop called loop A. OsCIE1-U-box residues Arg233 and Glu239 in loop A form salt bridges with OsUBC8 residues Asp12 and Lys8, respectively. Serine 237 in loop A is one of the four OsCIE1 residues that are phosphorylated by OsCERK1. Wang et al. found that unphosphorylated Serine 237 in OsCIE1 stabilizes loop A, which is essential for the interaction between OsCIE1 and OsUBC8, while phosphorylation-mimetic OsCIE1-U-box(S237D) prevents its interaction with OsUBC8. Intriguingly, the phosphorylation site S237 in the U-box domain and its adjacent amino acid sequences in OsCIE1 are remarkably conserved in E3 ligases from plants to animals, hinting at a shared regulatory mechanism among these E3 ligases.

A previous study has shown that OsCIE1 (PUB14) paralogs in *Arabidopsis* PUB12 and PUB13 not only polyubiquitinate FLS2 but also promote flg22-induced degradation of FLS2.[11] Protein ubiquitination manifests in several forms: mono-ubiquitination, multi-mono-ubiquitination, branched-chain ubiquitination, mixed-chain ubiquitination, and poly-ubiquitination.[1] Each type of ubiquitination yields distinct outcomes. For instance, mono-ubiquitination is involved in regulating DNA repair and gene expression. In contrast, K48-linked and K11-linked poly-ubiquitination typically targets proteins for proteasomal degradation, while K36-linked ubiquitin chains play roles in signaling and endocytosis. In fact, the poly-ubiquitin chain can be formed through many lysine residues including K6, K27, K29, K33, K48, and K63.[12] Hence, future investigations on the identification of lysine residues, which mediate the formation of poly-ubiquitination chain on FLS2 and OsCERK1, will help us find out why PUB12 and PUB13 facilitate both the poly-ubiquitination and degradation of FLS2, while OsCIE1 only catalyzes the poly-ubiquitination of OsCERK1 without affecting the stability of OsCERK1. Future studies could also focus on dual regulations of PTI triggered by other PAMPs from diverse plant pathogens.

Inappropriate activation of plant defense in the absence of plant pathogens could incur significant fitness cost and even cell death. On the other hand, plants must turn on effective defense responses to keep plant pathogens from causing severe diseases. In this paper, Wang et al. uncovered crucial regulation mechanisms which allow rice plants to activate defense only when rice plants recognize chitin in rice fungal pathogens.[6] The findings from this research may pave the way for innovative approaches to managing plant diseases.

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AUTHOR CONTRIBUTIONS

Huan Wang: Conceptualization; writing—original draft; writing—review & editing. **Ruize Zhang**: Conceptualization; writing—original draft; writing—review & editing. **Ziming Zhang**: Writing—original draft; writing—review & editing. **Daowen Wang**: Writing—original draft; writing—review & editing. **Zheng Qing Fu**: Writing—original draft; writing—review & editing; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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REFERENCES

1. Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323–329. https://doi.org/10.1038/nature05286
2. Boller, T., & Felix, G. (2009). A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology*, *60*(1), 379–406. https://doi.org/10.1146/annurev.arplant.57.032905.105346
3. Wu, L., Chen, H., Curtis, C., & Fu, Z. Q. (2014). Go in for the kill: How plants deploy effector-triggered immunity to combat pathogens. *Virulence*, *5*(7), 710–721 [corrected]. https://doi.org/10.4161/viru.29755
4. Fu, Z. Q., & Dong, X. (2013). Systemic acquired resistance: Turning local infection into global defense. *Annual Review of Plant Biology*, *64*(1), 839–863. https://doi.org/10.1146/annurev-arplant-042811-105606
5. Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth-defense tradeoffs in plants: A balancing act to optimize fitness. *Molecular Plant*, *7*(8), 1267–1287. https://doi.org/10.1093/mp/ssu049
6. Wang, G., Chen, X., Yu, C., Shi, X., Lan, W., Gao, C., Yang, J., Dai, H., Zhang, X., Zhang, H., Zhao, B., Xie, Q., Yu, N., He, Z., Zhang, Y., & Wang, E. (2024). Release of a ubiquitin brake activates OsCERK1-triggered immunity in rice. *Nature*, *629*(8014), 1158–1164. https://doi.org/10.1038/s41586-024-07418-9
7. Akamatsu, A., Wong, H. L., Fujiwara, M., Okuda, J., Nishide, K., Uno, K., Imai, K., Umemura, K., Kawasaki, T., Kawano, Y., & Shimamoto, K. (2013). An OsCEBiP/OsCERK1-OsRacGEF1-OsRac1 module is an essential early component of chitin-induced rice immunity. *Cell Host & Microbe*, *13*(4), 465–476. https://doi.org/10.1016/j.chom.2013.03.007
8. Wang, C., Wang, G., Zhang, C., Zhu, P., Dai, H., Yu, N., He, Z., Xu, L., & Wang, E. (2017). OsCERK1-mediated chitin perception and immune signaling requires receptor-like cytoplasmic kinase 185 to activate an MAPK cascade in rice. *Molecular Plant*, *10*(4), 619–633. https://doi.org/10.1016/j.molp.2017.01.006
9. Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., & Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(49), 19613–19618. https://doi.org/10.1073/pnas.0705147104
10. Wan, J., Zhang, X. C., Neece, D., Ramonell, K. M., Clough, S., Kim, S. Y., Stacey, M. G., & Stacey, G. (2008). A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. *The Plant Cell*, *20*(2), 471–481. https://doi.org/10.1105/tpc.107.056754
11. Lu, D., Lin, W., Gao, X., Wu, S., Cheng, C., Avila, J., Heese, A., Devarenne, T. P., He, P., & Shan, L. (2011). Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. *Science*, *332*(6036), 1439–1442. https://doi.org/10.1126/science.1204903
12. Chen, Y., Ren, W., Wang, Q., He, Y., Ma, D., & Cai, Z. (2022). The regulation of necroptosis by ubiquitylation. *Apoptosis*, *27*(9–10), 668–684. https://doi.org/10.1007/s10495-022-01755-8

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