action of the increased T6P levels is still puzzling, and application in more variable field conditions might prove more challenging with densely growing plants and the associated reduced accessibility and shading of target tissues.

A Powerful New Research Tool

However, it is clear that controlled chemical intervention is an important new addition to the more elaborate genetic modification technologies used for basic research, which can also be applied in non-model crops that typically have more complex genomes and low transformation efficiencies. The possibility of temporal and spatial control (using the application of short pulses in specific conditions, tissues, and developmental stages) can also significantly reduce unwanted ectopic effects. In combination with cellular assays and other novel research tools, such as plant-optimized genetically encoded sensors for monitoring *in vivo* T6P dynamics and SnRK1 activity, this approach will enable the more direct investigation and elucidation of the molecular mechanisms and targets of T6P signaling. However, it is vital that physiologically relevant levels of active compounds (and activating light conditions) are used to avoid confounding effects.

So, while T6P remains an elusive molecule, controlled chemical intervention, when used appropriately, provides a powerful new tool to investigate its functions and the mechanisms involved. This recent study illustrates the potential of this strategy and also confirms T6P signaling as a key regulatory mechanism in crop species. Analogous strategies, targeting specific physiological processes, will undoubtedly accelerate future biological research and discoveries.

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References

1. Lastdrager, J. *et al.* (2014) Sugar signals and the control of plant growth and development. *J. Exp. Bot.* 65, 799–807
2. Sheen, J. (2014) Master regulators in plant glucose signaling networks. *J. Plant Biol.* 57, 67–79
3. Griffiths, C.A. *et al.* (2016) Chemical intervention in plant sugar signalling increases yield and resilience. *Nature* 540, 574–578
4. Avonce, N. *et al.* (2006) Insights on the evolution of trehalose biosynthesis. *BMC Evol. Biol.* 6, 109
5. Lunn, J.E. (2007) Gene families and evolution of trehalose metabolism in plants. *Funct. Plant Biol.* 34, 550–563
6. Eastmond, P.J. *et al.* (2002) Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for *Arabidopsis* embryo maturation. *Plant J.* 29, 225–235
7. Schluepmann, H. *et al.* (2003) Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 6849–6854
8. Lunn, J.E. *et al.* (2006) Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana*. *Biochem. J.* 397, 139–148
9. Yadav, U.P. *et al.* (2014) The sucrose-trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signalling by Tre6P. *J. Exp. Bot.* 65, 1051–1068
10. Figueroa, C.M. and Lunn, J.E. (2016) A tale of two sugars: trehalose 6-phosphate and sucrose. *Plant Physiol.* 172, 7–27
11. Zhang, Y. *et al.* (2009) Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiol.* 149, 1860–1871
12. Nuccio, M.L. *et al.* (2015) Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. *Nat. Biotechnol.* 33, 862–869

Spotlight

Bodyguards: Pathogen-Derived Decoys That Protect Virulence Factors

Judith K. Paulus,[1] Jiorgos Kourelis,[1] and Renier A.L. van der Hoorn[1,*]

Recent studies on plant-pathogen interactions have exposed a new strategy used by plant pathogens: decoy effectors that protect virulence factors. Examples of these "bodyguards" include the recently discovered PsXLP1 from *Phytophthora sojae* and truncated TALEs from *Xanthomonas oryzae*. These examples suggest important roles for seemingly non-functional effector proteins in distracting the host.

The Xyloglucan-specific Endo Glucanase (*Ps*XEG1) acts as an important virulence factor of the soil-borne oomycete pathogen *Phytophthora sojae* [1]. During infection, *Ps*XEG1 is secreted into the apoplast of its host soybean (*Glycine max*) to macerate the host cell wall. However, *Ps*XEG1 is recognized by the plant's recognition machinery as a Pathogen-associated Molecular Pattern (PAMP), independent of its enzymatic activity [1]. *P. sojae* is able to suppress PAMP-triggered immune responses caused by *Ps*XEG1, presumably by injecting multiple intracellular RxLR effectors [1]. But in addition to being recognized as a PAMP, *Ps*XEG1 is targeted by a secreted endoglucanase inhibitor (*Gm*GIP1) of soybean. *Gm*GIP1 binds to *Ps*XEG1 and strongly inhibits XEG1-catalyzed depolymerisation of xyloglucan, reducing the virulence role of *Ps*XEG1 for *P. sojae* [2].

The pathogen, however, protects *Ps*XEG1 from host inhibition by secreting a more tightly binding, truncated paralog of *Ps*XEG1, called *Ps*XLP1. *Ps*XLP1 has lost one of the residues critical for enzyme activity and has no known function in pathogenicity, other than to intercept *Gm*GIP1 [2]. This leads to a 'bodyguard' model in which *Ps*XLP1 has evolved as a paralogous decoy to neutralize *Gm*GIP1 and prevent inhibition of *Ps*XEG1 (Figure 1A). The conservation of *Ps*XLP1 paralogs across *Phytophthora* species suggests that these oomycete pathogens use these paralogous decoys frequently to bodyguard *Ps*XEG1-like effectors [2].

The publication of Ma *et al.*, 2017 [2] strengthens this exciting new area in the field of host-pathogen interactions. The described pathogen decoy is distinct from the decoy function of host-derived factors as described in the Decoy Model, where decoys act as co-receptors or sensors with immune receptors [3]. Both host- and pathogen-derived decoys are specialized, often paralogous forms of a functional effector target or effector, respectively. But in contrast to host-derived decoys that trap effectors to mount effector-triggered immunity (ETI), pathogen decoys protect virulence factors as bodyguards.

Two recent publications describe a distinct but similar bodyguard strategy used by the gram-negative bacterium *Xanthomonas oryzae* [4,5]. *X. oryzae* pathovars *oryzae* (*Xoo*) and *oryzicola* (*Xoc*) cause devastating bacterial blight (BB) and bacterial leaf streak (BLS) in rice (*Oryza sativa*), respectively. *Xanthomonas* strains express a variety of Type-III effectors, including many Transcription activator-like effectors (TALEs) [6,7]. TALEs are major virulence determinants that act by *trans*-activating host genes in the plant cell nucleus by binding to promoter elements (Effector-binding Elements, EBEs) in a sequence-specific manner.

TALEs contain several nuclear localization signals (NLSs) that target these effectors to the host cell nucleus. TALEs also carry a C-terminal acidic activation domain (AD) that activates host gene expression, and a central repeat domain that directly binds to the EBE in the DNA. Resistance to *Xoo* has mostly been attributed to the action of TALEs, either by polymorphisms in EBEs that prevent the induction of susceptibility (*S*) genes, or by the induction of executor genes that carry EBEs embedded in their promoter and induce immune responses as dominant *R* genes [8].

However, some TALEs are recognised by classical *R* genes that encode NLRs (Nod-like Receptors). The TALE AvrBs4 of *X. euvesicatoria* is recognized by the *Bs4*-encoded NLR protein in tomato [9], whilst all tested, full length TALEs of *Xoo* are recognized by the *Xa1*-encoded NLR in rice [4]. Interestingly, however, both the Yang (Iowa State University, USA) and Bogdanove (Cornell University, USA) laboratories found a new class of truncated TALEs that could suppress TALE recognition by NLRs.

The Yang group demonstrated that TALE perception by the NLR Xa1 is suppressed by two groups of truncated TALE variants that interfere with Xa1, which they coined "iTALEs". The frequent occurrence of iTALEs could explain the narrow resistance spectrum of Xa1, even though it appears to perceive most, if not all TALEs [4]. Similarly, the Bogdanove group found that *Xo1* resistance in a different rice variety, which is also mediated by the perception of TALEs [11], can be blocked by truncated TALEs – "truncTALEs" - such as Tal2h from *Xoc* strain BLS256. In contrast to *Xa1*, *Xo1* has not been cloned yet, but the *Xo1* locus is allelic to *Xa1*, confers resistance to both *Xoc* and *Xoo*, and contains six candidate *R* genes encoding NLRs, indicating that *Xo1* encodes an NLR conferring TALE perception [4,10,11].

The truncated Tal2h TALE lacks N- and C-terminal regions that are important for DNA binding and this implies that Tal2h does not act by binding host DNA [5]. Both iTALEs and truncTALEs were previously annotated as pseudogenes due to their N- and C-terminal deletions. Both studies conclude that the truncated TALE proteins bind to TALE-recognizing NLR proteins to block the TALE binding site without activating the NLR receptor, to prevent the recognition of full length TALEs that act as virulence factors (Figure 1B). Even though there is strong genetic evidence, this hypothesis still requires experimental support to demonstrate a direct interaction of TALEs with the Xa1/Xo1 NLRs and that truncated

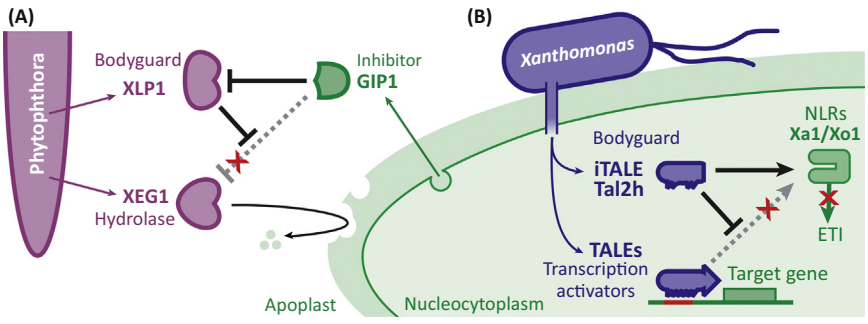

Figure 1. Model of Two Bodyguards at Work. (A) Effector decoy XLP1 prevents host protein GIP1 from inhibiting glucanase XEG1. (B) Truncated TALEs prevent TALE effectors from being recognized by immune receptors Xa1/Xo1. Abbreviations: ETI, effector-triggered immunity; GIP1, glucanase inhibitor protein; TALE, Transcription activator-like effectors; Xa1, *R* gene in rice; XEG1, Xyloglucanase; XLP1, XEG1-Like Protein; Xo1, *R* gene in rice.

TALEs interfere with this interaction by having a higher affinity for NLRs. These are notoriously challenging experiments as NLR proteins are difficult to produce and purify.

Both XLP1 and truncated TALEs illustrate that paralogous decoys can evolve to protect important virulence factors to prevent their inactivation or recognition. Theoretically, these bodyguards have no other function in disease development, meaning that they have no role in the absence of the virulence factor they are mimicking, or in the absence of host proteins that inactivate or recognize this virulence factor. These bodyguard effectors probably have a higher affinity to the host protein and/or higher abundance when compared to the corresponding virulence factor. Both mechanisms allow the virulence factor to remain unaffected or undetected.

The evolution of a bodyguard that protects a single virulence factor may seem very costly. From an evolutionary standpoint, it might seem more efficient for a pathogen to develop effectors that interfere broadly with immune signalling. However, this bodyguard strategy might be worthwhile for critical effectors. In addition, the example of truncated TALEs illustrates how one bodyguard can simultaneously protect a larger class of virulence factors.

The production of decoy effector proteins by two unrelated pathogens highlights a common strategy of pathogens to protect virulence factors. These observations encourage new searches in pathogen genomes to investigate truncated or seemingly inactive effector proteins that were previously considered pseudogenes. Increased knowledge on these mechanisms will enable us to engineer the host immune system to circumvent manipulation by bodyguard effectors.

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References

1. Ma, Z. *et al.* (2015) A *Phytophthora sojae* glycoside hydrolase 12 protein is a major virulence factor during soybean infection and is recognized as a PAMP. *Plant Cell* 27, 2057–2072
2. Ma, Z. *et al.* (2017) A paralogous decoy protects *Phytophthora sojae* apoplastic effector *Ps*XEG1 from a host inhibitor. *Science* 335, 710–714
3. Van der Hoorn, R.A.L. and Kamoun, S. (2008) From guard to decoy: a new model for perception of plant pathogen effectors. *Plant Cell* 20, 2009–2017
4. Ji, Z. *et al.* (2016) Interfering TAL effectors of *Xanthomonas oryzae* neutralize *R*-gene-mediated plant disease resistance. *Nat. Commun.* 7, 13435
5. Read, A.C. *et al.* (2016) Suppression of *Xo1*-mediated disease resistance in rice by a truncated, non-DNA-binding TAL effector of *Xanthomonas oryzae*. *Front. Plant Sci.* 7, 1516
6. Kay, S. and Bonas, U. (2009) How *Xanthomonas* type III effectors manipulate the host plant. *Curr. Opin. Microbiol.* 12, 37–43
7. Boch, J. and Bonas, U. (2010) *Xanthomonas* AvrBs3 family-type III effectors: discovery and function. *Annu. Rev. Phytopathol.* 48, 419–436
8. Hutin, M. *et al.* (2016) Ectopic activation of the rice NLR heteropair RGA4/RGA5 confers resistance to bacterial blight and bacterial leaf streak diseases. *Plant J. Cell Mol. Biol.* 88, 43–55
9. Schornack, S. *et al.* (2004) The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. *Plant J.* 37, 46–60
10. Kawahara, Y. *et al.* (2013) Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. *Rice* 6, 4
11. Triplett, L.R. *et al.* (2016) A resistance locus in the American heirloom rice variety Carolina Gold Select is triggered by TAL effectors with diverse predicted targets and is effective against African strains of *Xanthomonas oryzae* pv. *oryzicola*. *Plant J. Cell Mol. Biol.* 87, 472–483

Forum

Are Circular RNAs New Kids on the Block?

Sang-Moo Lee,[1,2,3] Hyun Gi Kong,[1,3] and Choong-Min Ryu[1,2,*]

Circular RNAs (circ-RNAs), a novel class of noncoding RNAs, are a popular topic in animal research because they have potential as post-transcriptional regulators and diagnostic markers. Research in plants is only now emerging, but indicates that circ-RNAs could also be a crucial class of noncoding regulators.

In animals, many thousands of circ-RNAs have been identified, representing a relatively unexplored class of **noncoding RNAs** (see Glossary) compared to the better-studied **microRNAs** (miRNAs) and small interfering RNAs (siRNAs) [1]. The unique structure of circ-RNAs, a covalently closed continuous loop, means that they are more resistant to exonuclease attack than are linear RNAs [1]. circ-RNAs were initially thought to be the result of splicing errors, but more recently it has been shown that circ-RNAs can act as **miRNA sponges** or as sponges for RNA-binding proteins, and regulate post-transcriptional events [2]. A close association with human diseases, including cancers and neurologic diseases such as Alzheimer disease, has been demonstrated, and circ-RNAs are currently being explored as diagnostic biomarkers and therapeutic targets in such diseases [2,3]. Recently, transcriptome-wide sequencing studies have revealed that circ-RNAs are highly conserved and many circ-RNAs have also been identified in plants [4], but insight into their function is only now emerging. We highlight here the distinct features of plant circ-RNAs and propose possible functions.

Genesis and Exodus from the Nucleus

The regulation and function of circ-RNAs in plants were largely unknown until recently, whereas circ-RNAs in human cells were identified as far back as 1994 [5]. In plants, **viroids** – pathogenic viruses without capsids that were identified in the 1980s – replicate using a rolling-circle mechanism and self-cleavage [6]. The circular structure and replication