Fungal pathogenesis

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Fine-tuning fungal effector secretion

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The rice blast fungus *Magnaporthe oryzae* uses transfer RNA modifications to fine-tune effector protein secretion into host plant cells.

Plants and fungi have been closely entwined throughout the evolution of modern life on Earth, beginning with their critical partnership that supported land colonization by plants over 450 million years ago, transforming the Earth's surface and laying the foundations for the immense biodiversity we see today[1]. Yet even the earliest land plants were exposed to a diverse array of fungal organisms from the highly beneficial to the extremely pathogenic. Today, parasitic fungi blight our food production systems, frequently causing large-scale disease outbreaks due to the widespread use of genetically uniform monoculture crops; between 10% and 23% of crops are lost every year to fungal diseases[2]. The extraordinary success of these fungal pathogens lies in their ability to evade the innate defence systems that plants have evolved to protect themselves from attack. Central to the strategies fungal pathogens use to successfully colonize their host plants is the secretion of effectors, which are secreted proteins that interfere with host defence processes and reprogramme the host plant to support the pathogens' own survival[3]. Yet how fungi regulate the secretion of these effector proteins, often using unconventional mechanisms, remains poorly understood.

Now writing in *Nature Microbiology*, Li et al.[4] deciphered a previously unknown relationship between messenger RNA (mRNA) translation and unconventional secretion of cytoplasmic effector proteins by the devastating rice blast fungal pathogen, *Magnaporthe oryzae* (Fig. 1). This fungus is considered the greatest threat to rice production worldwide and *M. oryzae* alone is responsible for destroying enough rice to feed 60 million people globally each year[5]. In *M. oryzae*, effector proteins are secreted through two routes largely dependent on the proteins' ultimate site of action in the host plant; apoplastic effectors that are destined for the extracellular space are secreted via the conventional endoplasmic reticulum–Golgi secretory pathway, whereas cytoplasmic effectors that are ultimately translocated into the host cell cytoplasm are secreted via alternative unconventional routes that remain ambiguous[3]. As with all proteins, effector proteins begin their journey in the ribosomes, the protein factories of cells, where mRNAs bring the effectors' genetic code from the nucleus to the site of protein synthesis in the cytoplasm. Here, transfer RNAs (tRNAs) then act to 'read' the genetic code contained in codon triplets in mRNA, ensuring the corresponding amino acids are brought to the ribosome in the correct order for protein synthesis[6]. To modulate the speed of protein synthesis, eukaryotes often extensively chemically modify tRNAs. Any deficiencies in tRNA modification abilities can lead to protein misfolding, which in humans has been linked to an array of life-threatening diseases[7].

Among the wealth of chemical modifications that can occur in tRNAs, one modification that is conserved across the three domains of life is the thiolation of uridine at position 34 (U_{34}) in the anticodon of tRNAs[8]. Modification of tRNAs, particularly at U_{34}, which interacts

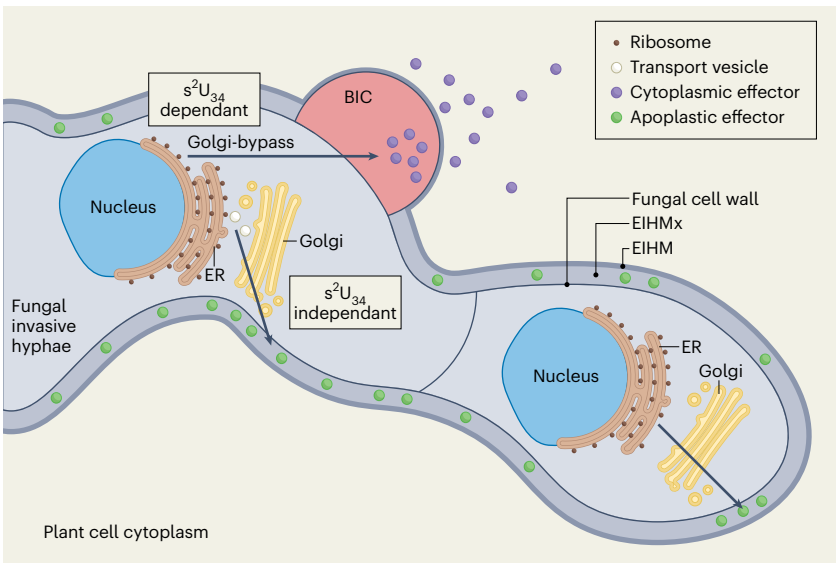

Fig. 1 | Unconventional secretion of *M. oryzae* cytoplasmic effectors is linked to thiolation of U_{34} in the anticodon of tRNAs. In *M. oryzae*, effector proteins are secreted through two routes: (1) apoplastic effectors transported into the extra-invasive hyphal matrix (EIHMx) are secreted via the conventional endoplasmic reticulum (ER)–Golgi secretory pathway that occurs independently of U_{34} modification of tRNAs; (2) cytoplasmic effectors that accumulate initially in the biotrophic interface complex (BIC) and are translocated across the extra-invasive hyphal membrane (EIHM) and into the host cell cytoplasm are secreted via alternative unconventional routes that are dependent on U_{34} modification of tRNAs. s^2U_{34}, 2-thiolation of U_{34} in tRNA.

with the third position of mRNA codons, can have a profound impact on tRNA stability and the ultimate decoding speed of mRNA transcripts[9]. In the yeast *Saccharomyces cerevisiae*, disruption of thiolation of U_{34} has been shown to slow the translation of mRNA transcripts specifically enriched in AAA, GAA or CAA codons, as tRNAs that decode these codons are typically thiolated[10]. In the current study, Li et al. found a remarkable role for tRNA modifications and codon usage in fine-tuning effector protein secretion in *M. oryzae*. Focusing first on the *M. oryzae* cytoplasmic effector Pwl2, the authors showed that disrupting one of the key proteins (Uba4) required for thiolation of U_{34} prevented Pwl2 secretion. Yet, treating with a chemical that promotes the binding of near-cognate tRNAs that do not quite match the AA-ending codons, or recoding Pwl2 to swap AA-ending codons for AG-ending codons, completely restored Pwl2 secretion in the Δ*uba4* deficient mutant. At least two other cytoplasmic effectors, AVR-Pita and AVR-Pik, were then shown experimentally to also require Uba4 for translation. By contrast, disrupting thiolation of U_{34} had no effect on secretion of the apoplastic effectors Bas4 and Slp1. These findings suggest that the disruption of tRNA thiolation has a unique effect on translation of cytoplasmic effector proteins secreted using unconventional pathways, likely due to ribosomal pausing at AA-ending codons.

The link Li et al. uncovered between cytoplasmic effector secretion in *M. oryzae* and tRNA modifications was further supported by the discovery that cytoplasmic effectors in *M. oryzae* are specifically enriched in AA-ending codons. Thus, this suggests that the unconventional secretion system used by cytoplasmic effectors in *M. oryzae* is less tolerant to changes in mRNA decoding speeds than the conventional endoplasmic reticulum–Golgi secretory pathway[4]. Although the current study is limited to *M. oryzae*, if codon usage bias for cytoplasmic effector proteins secreted via unconventional systems is shown in future to be ubiquitous across fungal and fungus-like (oomycete) pathogens, this could have wide-reaching implications for accelerating the genomics-led search for cytoplasmic effectors for the hundreds of different species of fungi and oomycetes that currently inflict disease on the world's most important crops[2]. Deciphering these vast catalogues of cytoplasmic effectors will provide insight into the ancient conflict at the fungi–plant interface. However, as Li et al. also conclude, their discovery may not be limited to fungi or even diseases of plants. With so many pathogens of animals and humans also using unconventional secretion systems to deliver virulence proteins, it will be fascinating to see in future if the regulatory mechanism Li et al. uncovered for modulating protein secretion could have much broader relevance across the diverse array of plant, human and animal health threats.

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Competing interests

The author declares no competing interests.