Your package has arrived! Trans-Golgi Network component ECHIDNA regulates plant defenses

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To successfully parasitize a plant cell, a filamentous fungus must overcome two layers of defense: penetration resistance and post-invasion resistance (Lipka et al., 2005). Penetration resistance consists of a physical and chemical barrier built with cell wall polysaccharides and proteins, as well as anti-microbial metabolites and proteins that form at the infection site (Hückelhoven, 2007; Underwood and Somerville, 2008; Meyer et al., 2009). The production, sorting, and packaging of the materials needed to build this barrier follow the conventional secretion pathway (CSP). In the CSP, products are made in the endoplasmic reticulum (ER) or in the cytosol and then translocated to the ER. The next stop is the Golgi apparatus, followed by the trans-Golgi Network (TGN) until the products reach the cell surface. However, little is known about the molecular players that link the CSP with the defense against filamentous fungi.

In this issue of Plant Physiology, Liu et al., 2022 uncover the role of ECHIDNA (ECH), a component of the TGN (Gendre et al., 2011), in the regulation of plant immunity and stress response. To determine how ECH participates in the distribution of different cargos, the authors fused membrane or apoplastic proteins to GFP. The constructs were expressed in wild-type Arabidopsis (Arabidopsis thaliana) plants and in an ech knockout line, and protein location was followed by microscopy. In the ech background, the cargoes including the antimicrobial proteins PLANT DEFENSIN 1.2A (PDF1.2A) and PATHOGENESIS-RELATED GENE 1 (PR1) were found in cytoplasmic aggregates. Furthermore, when they followed the distribution of POWDERY MILDEW RESISTANT 4 (PMR4), an enzyme that produces a defense polysaccharide known as callose (Jacobs et al., 2003; Nishimura et al., 2003), the authors observed that PMR4 aggregates co-localized with intracellular callose deposition. Altogether, these results suggested that the loss of ECH compromises the delivery of various cargos to the plasma membrane and the apoplast.

The impaired transportation system in the ech line could compromise penetration resistance. To test this hypothesis, the authors challenged plants with the non-adapted powdery mildew fungus Blumeria graminis f.sp.hordei (Bgh). In the wild type, most of the Bgh penetration attempts were blocked, and only 13% of the infection sites showed formation of feeding structures known as haustorium. In the ech knockout mutant, penetration success was around 38%, suggesting that ECH loss facilitated pathogen infection (Fig. 1).

If a pathogen manages to overcome the first resistance barrier, it will face a second layer of defense, known as post-invasion resistance (Lipka et al., 2005). This defense mechanism is associated with the hypersensitive-response (HR) that triggers restricted cell death at the infection site (Lipka et al., 2005; Wen et al., 2011; Zhang et al., 2015). To evaluate a role of
ECH in post-invasion resistance, the authors challenged plants with a different fungus, the adapted powdery mildew *Erysiphe cichoracearum* (*Ec*) that bypasses penetration resistance. Unexpectedly, the *ech* line was more resistant to *Ec*, as *Ec* conidia grew fewer hyphal branches and haustoria (Fig. 1). The enhanced post-invasion resistance in the *ech* plants coincided with a stronger HR that was related with a high accumulation of salicylic acid (SA), a plant hormone that promotes defense against biotrophic pathogens (Glazebrook, 2005; Pieterse et al., 2012). Besides infection-triggered cell death, the *ech* lines showed spontaneous cell death that occurred in absence of the pathogen. However, in contrast to the HR, spontaneous cell death was independent of SA, and the mechanism and regulators behind this phenomenon remain to be elucidated.

Finally, the authors analyzed if the loss of ECH impacted ER function or structure. RNAseq analysis of uninfected *ech* plans revealed that genes related with protein processing in the ER, such as chaperons, were upregulated compared to the wild type. The *ech* lines were also more sensitive to the effects of ER-stress-induced compounds, such as DTT. When observed by transmission electron microscopy, the ER of *ech* cells showed altered morphology and dilated tubules. These results suggest that ECH is required to maintain normal function of the ER and that the loss of ECH induces chronic ER stress. ER stress has been reported to cause cell death (Wan and Jiang, 2016), which could also explain the spontaneous cell death observed in the *ech* lines.

To summarize, the study of Liu et al. 2022 addresses the role of ECHIDNA protein in plant defense against powdery mildew, as well as in maintaining ER function (Fig. 1). During the first phase of resistance, ECH is required to deliver several anti-microbial proteins to the plasma membrane and the apoplast, and ECH loss causes reduced resistance to the non-adapted powdery mildew fungus *Bgh*. In contrast, in the second phase of resistance, ECH loss enhanced resistance to the adapted powdery mildew fungus *Ec*. The enhanced post-invasion resistance was associated with stronger HR-triggered cell death that depended on the hormone SA. ECH was also necessary for normal ER function, as its loss caused severe ER stress, possibly leading to spontaneous cell death. The work of Liu et al. 2022 provides a beautiful example of how the integrity of the endomembrane system influences physiology in eukaryotic organisms.

**Figure legend**

*Fig. 1. A trans-Golgi network component ECHIDNA (ECH) regulates defense, cell death, and Endoplasmic Reticulum (ER) stress in plants.* ECH is required to deliver several anti-microbial proteins to the plasma membrane (PM) and the apoplast, and the disruption of ECH leads to the formation of intracellular protein aggregates. ECH loss also causes ER stress, as evidenced by altered ER morphology, and reduced penetration resistance to the non-adapted powdery mildew fungus *Bgh*. In contrast, ECH loss enhanced post-invasion resistance against the adapted powdery mildew fungus *Ec*, which was associated with a stronger HR-triggered cell death. ECH loss also led to spontaneous cell death. H=haustorium. Created with BioRender.com.
References


