

Barley RIPb Opens the Gates for Epidermal Fungal Penetration

The ascomycete *Blumeria graminis* f. sp. *hordei* (*Bgh*) is a powdery mildew causal agent, specifically adapted to barley (*Hordeum vulgare*), wheat (*Triticum aestivum* and *Triticum turgidum*), and triticale (\times *Triticosecale*). *Bgh* can cause up to 40% of yield losses (Draz et al., 2019) and has become a model in which to study the interactions between plants and leaf fungal pathogens.

On wheat leaves, *Bgh* forms specialized hyphae called haustoria that penetrate through epidermal cells and subsequently inject virulent factors in plant tissues, while starting nutrient uptake. As a countermeasure, the invaded cell's morphology changes drastically: thanks to cytoskeleton reorganization, the nucleus and other organelles (e.g. mitochondria and the vacuole) relocate to the site of invasion to favor the apposition of callose and other insulating molecules (Chowdhury et al., 2014). Therefore, studying the cytoskeletal dynamics is essential to explain plant defense mechanisms.

One of the most studied cytoskeleton remodelers in *Arabidopsis* (*Arabidopsis thaliana*) is RACB, a RHO GTPase influencing the stability of actin filaments and microtubules (Opalski et al., 2005). During *Bgh* infection, a constitutively active version of the barley RACB protein favors mildew spread; conversely, down-regulation of RACB leads to a lower fungal penetration rate (Schultheiss et al., 2002; Hoefle et al., 2011). However, RAC proteins are part of a complex signaling pathway. Previous studies have revealed that the modulation of RACB interactors can lead to unexpected outcomes in terms of fungal penetration rates (Nottensteiner et al., 2018; Hoefle et al., 2020).

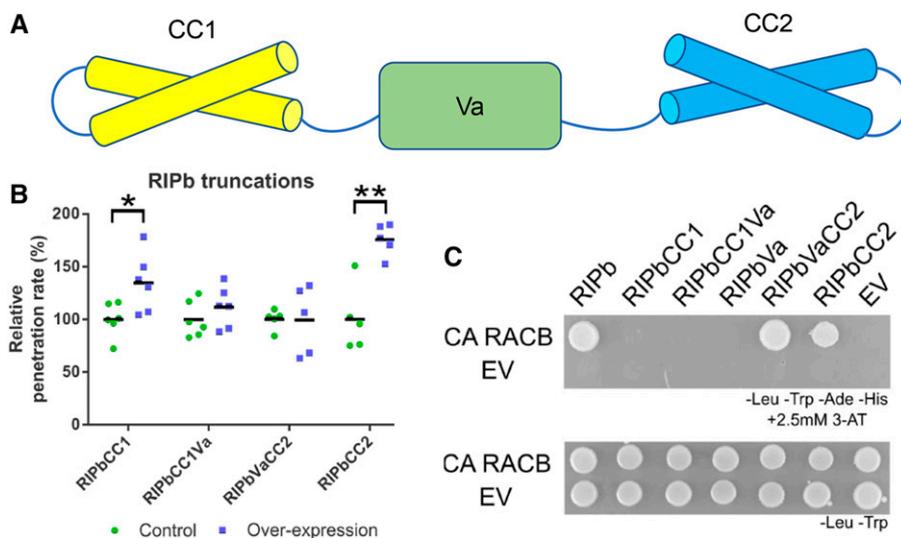
In this issue of *Plant Physiology*, McCollum et al. (2020) identify barley ROP Interactive Partner b (RIPb)

as another modulator of *Bgh* virulence. Indeed, the study showed that transient RIPb overexpression in barley leaves was able to increase the *Bgh* penetration rate by 22%.

To understand the molecular mechanism by which RIPb promotes fungal penetration, McCollum and colleagues (2020) observed the subcellular localization of RIPb in barley epidermal cells by confocal microscopy. YFP-RIPb was present in the cytosol, at the cell periphery (likely the plasma membrane), but also in contact with microtubules. Interestingly, YFP-RIPb coexpression with a constitutively active RACB version (but not with a constitutively inactive RACB) triggered RIPb relocalization from the cytoplasm to the cell periphery. Therefore, RIPb localization appears to be dynamic and dependent on RACB activation status, suggesting a physical interaction between RIPb and RACB.

Yeast two-hybrid assays confirmed the physical interaction between RIPb and RACB. By testing various RIPb truncations, the authors revealed that the RIPb CC2 domain is essential for RACB interaction, while a central variable domain is important for RIPb-RIPb oligomerization (Fig. 1). These results are in accordance with previous work that identified the conserved QWRKAA amino acid sequence, located in the CC2 domain, as the motif responsible for RIP-RAC interactions (Lavy et al., 2007). The same truncations monitored *in vivo* by confocal microscopy further revealed that the RIPb-RIPb interaction takes place mainly at microtubules and only in the presence of both the variable domain and the CC1 domain, while the RIPb CC2 domain is essential for the relocation of the

Figure 1. RIPb interacts with RACB. A, Schematic representation of RIPb domains: the N-terminal coiled-coil domain (CC1), the central variable region (Va), and the C-terminal coiled-coil domain (CC2). B, Effect of RIPb truncations on the interaction of barley and *Bgh* based on biolistic transformation of epidermal cells of 7-d-old barley leaves and determining the penetration rate of *Bgh* into the transformed cells 24 h after inoculation. C, Yeast two-hybrid assays for the interaction of RIPb truncations with constitutively active RACB (CA RACB). As controls, the interaction with the respective empty vector (EV) was tested. B and C were modified from McCollum et al. (2020).



protein at the plasma membrane in the presence of constitutively active RACB. In addition, the CC2 domain is necessary and sufficient to determine the increase in fungal penetration rate observed when RIPb is overexpressed.

To explore RACB-RIPb interaction in the context of fungal attack, RIPb and RACB-expressing leaves were inoculated with *Bgh* conidia. Ring-like accumulation of both proteins appeared at the sites of infection, especially around the haustorial neck of successful penetration sites, but also, in some cases, at sites of repelled fungal attempts.

A phylogenetic analysis revealed that barley possesses three RIP proteins (RIPa, RIPb, and RIPc), which are likely conserved in grasses (e.g. rice [*Oryza sativa*] and *Brachypodium distachyon*). There is, however, very little sequence similarity between RIPs from monocots and dicots. In particular, while the CC2 domain is well conserved, the CC1 domain is more divergent between the two groups. This difference suggests that dicot RIPs probably also interact with RAC proteins at the plasma membrane, but their regulation might be very different.

In conclusion, this study identified RIPb as a RACB-interacting protein that is recruited at the periphery of epidermal cells under fungal attack, via the RIPb CC2 domain. To better understand the role of RIPb in fungal pathogenesis, it will be important to identify additional RACB and RIPb interactions. Because of their high evolutionary divergence, it will also be important to undertake functional studies of RIPs from dicot species and assess similarities and differences in their roles and regulatory mechanisms with respect to monocots.

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