

Membrane microdomains and the cytoskeleton constrain AtHIR1 dynamics and facilitate the formation of an AtHIR1-associated immune complex

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SUMMARY

Arabidopsis hypersensitive-induced reaction (AtHIR) proteins function in plant innate immunity. However, the underlying mechanisms by which AtHIRs participate in plant immunity remain elusive. Here, using VA-TIRFM and FLIM-FRET, we revealed that AtHIR1 is present in membrane microdomains and co-localizes with the membrane microdomain marker REM1.3. Single-particle tracking analysis revealed that membrane microdomains and the cytoskeleton, especially microtubules, restrict the lateral mobility of AtHIR1 at the plasma membrane and facilitate its oligomerization. Furthermore, protein proximity index measurements, fluorescence cross-correlation spectroscopy, and biochemical experiments demonstrated that the formation of the AtHIR1 complex upon pathogen perception requires intact microdomains and cytoskeleton. Taken together, these findings suggest that microdomains and the cytoskeleton constrain AtHIR1 dynamics, promote AtHIR1 oligomerization, and increase the efficiency of the interactions of AtHIR1 with components of the AtHIR1 complex in response to pathogens, thus providing valuable insight into the mechanisms of defense-related responses in plants.

Keywords: AtHIR1, membrane microdomain, cytoskeleton, lateral mobility, single-particle analysis, *Arabidopsis thaliana*.

INTRODUCTION

The plant immune system has evolved two branches to fight against pathogen attack: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl, 2006). Plant cells undergoing ETI often show a hypersensitive response (HR) of programmed cell death (Block and Alfano, 2011). Hypersensitive-induced reaction (HIR) proteins, members of the Proliferation, Ion and Death (PID) superfamily, participate in the HR in response to pathogen attack, and in the development of spontaneous hypersensitive lesions in leaves of *Capsicum annuum*, rice, and wheat (Zhou *et al.*, 2010; Choi *et al.*, 2011; Duan *et al.*, 2013). The *Arabidopsis thaliana* genome contains four HIR family genes (*AtHIR1–4*), and all the HIR family genes encode proteins that contain a stomatin/prohibitin/flotillin/HflK/C (SPFH) domain (Qi *et al.*, 2011).

Plasma membranes (PMs) are highly dynamic structures that provide a barrier to extracellular components and have important functions in the recognition of extracellular

factors (Yáñez-Mó *et al.*, 2009). Microdomain-dependent and cytoskeleton-based processes affect PM organization and the dynamic status of membrane proteins (Lenne *et al.*, 2006). Membrane microdomains are specific compartments with distinct characteristics from those of the surrounding PM, such as differences in lipid and protein composition and organization (Fan *et al.*, 2015). The cytoskeleton is a dynamic, three-dimensional network of filamentous proteins. Microdomains and the cytoskeleton function in a number of vital cellular processes (Lenne *et al.*, 2006), but few studies have reported the effects that microdomains and the cytoskeleton have on dynamic behaviors of proteins at the PM or their influence on plant immunity.

Single-particle tracking analysis and *in vivo* protein labeling techniques offer a new way to probe the dynamics of membrane proteins and examine the correlation between protein dynamics and signal transduction