Plant Signaling & Behavior 6:8, 1205-1206; August 2011; © 2011 Landes Bioscience

The Arabidopsis *hit1-1* mutant has a plasma membrane profile distinct from that of wild-type plants at optimal growing temperature

Lian-Chin Wang,* Kai-Yu Chang, Yi-Ting Ke, Hao-Yu Huang and Shaw-Jye Wu*
Department of Life Sciences; National Central University; Jhong-li City, Taoyuan, Taiwan

High temperatures alter the physical properties of the plasma membrane and cause loss-of-function in the embedded proteins. Effective membrane and protein recycling through intracellular vesicular traffic is vital to maintain the structural and functional integrity of the plasma membrane under heat stress. However, in this regard, little experimental data is available. Our characterization of the Arabidopsis *hit1-1* mutant, linking a subunit of a vesicle tethering complex to plasma membrane thermostability, provided valuable information to this end. We further dissected the effect of the *hit1-1* mutation on plasma membrane properties and found that even at optimal growth temperature (23°C), the *hit1-1* mutant exhibited a plasma membrane protein profile distinct from that of wild-type plants. This result implies that the *hit1-1* mutation essentially alters vesicle trafficking and results in changes in the plasma membrane components under non-stress conditions. Such changes do not affect normal plant growth and development, but is significant for plant survival under heat stress.

Key words: GARP complex, heat stress, heat intolerant, HIT1, membrane trafficking, vesicle tethering factor, Vps53

Abbreviations: DGDG, digalactosyldiacylglycerol; GARP, golgi-associated retrograde protein; HIT, heat intolerant; MGDG, monogalactosyldiacylglycerol; TGN, trans-golgi network

Submitted: 04/15/11

Accepted: 04/15/11

DOI: 10.4161/psb.6.8.15842

*Correspondence to: Lian-Chin Wang and Shaw-Jye Wu;
Email: dcmotoya@yahoo.com.tw and jyewu@cc.ncu.edu.tw

Addendum to: Wang LC, Tsai MC, Chang KY, Fan Ys, Yeh CH, Wu SJ. Involvement of the Arabidopsis HIT1/AtVPS53 tethering protein homologue in the acclimation of the plasma membrane to heat stress. J Exp Bot 2011; 62:3609–20; PMID: 21398432; DOI: 10.1093/jxb/err060

The plasma membrane is indispensable to all living cells. It serves as a barrier separating the interior and exterior of the cell, and consists of a variety of proteins that accomplish vital biological functions. High temperature can fluidize the plasma membrane and damage the functions of its embedded proteins. Severe heat stress may even disrupt the integrity of the plasma membrane, causing escape of essential cytoplasmic constituents and leading to cell death.[1,2] Although it is predictable that effective vesicle trafficking machinery, which is involved in the removal of proteins and lipid molecules from and the delivery of freshly synthesized components to the plasma membrane, is important for plasma membrane rejuvenation and should participate in plant thermotolerance,[3] little empirical data has come to light to elucidate the protective mechanism by which vesicle trafficking improves plant tolerance to heat stress.

The Arabidopsis *hit1-1* mutant was originally isolated by its heat-intolerant phenotype.[4] Map-based cloning led to the identification of *HIT1* as a homolog of yeast Vps53p,[5] which is a subunit of the Golgi-associated retrograde protein (GARP) tethering complex that is involved in vesicle trafficking from the endosome to the trans-Golgi network (TGN).[6] Taking advantage of the available *hit* mutants, Wang et al. investigated the causality between HIT1 and plasma membrane thermostability,[7,8] and demonstrated that, while the anti-oxidative capability of *hit1-1* plants was equal to that of wild-type plants, significantly more electrolyte leakage from *hit1-1* leaves was detected after long term heat exposure (37°C for 24 h). Furthermore, *hit1-1* was not sensitive to sudden heat shock (44°C for 30 min).[7] These findings demonstrated that there is indeed a vesicle trafficking mediator of plasma membrane thermal adaptation, and this adaptation is probably more involved in remodeling than in repair. Such remodeling enables the membrane to withstand elevated temperatures, circumvent heat-induced damage, and thus is specifically significant for tolerance of long-term heat stress.[7]

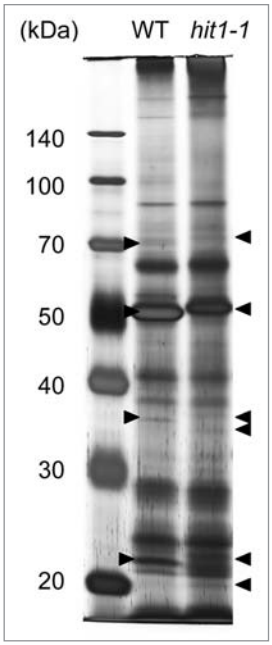

Figure 1. One-dimensional SDS gel electrophoretic banding patterns of plasma membrane proteins from 4-wk-old, 23°C-grown wild-type (WT) and *hit1-1* plants. Plasma membrane proteins were prepared as previously described in reference 13, with minor modification, and then separated on a 12.5% SDS-PAGE gel, and the protein bands were detected by silver staining. Equal amounts of proteins were loaded in each lane. Arrows indicate some protein bands showing noticeable differences in staining intensity, reflecting that these proteins were present in different abundances in the wild-type and *hit1-1* plasma membranes. Molecular mass markers are shown on the left.

Since the *hit1-1* allele is a point mutation leading to a Ser-to-Tyr amino acid substitution,[5] one may suspect that the temperature-sensitive phenotype of *hit1-1* plants is produced by the thermolabile properties of the hit1-1 protein. Nevertheless, because *hit1-1* plants are more sensitive than wild-type to osmotic and saline stress inhibition during seedling germination and development,[4,5,7] it is unlikely that the heat-intolerant phenotype of *hit1-1* is derived from a simple alternation in the thermal stability of a gene product. To answer this question and to provide further insight into the roles of HIT1 in plasma membrane acclimation to heat stress, electrophoretic patterns of plasma membrane proteins were analyzed. Even at the optimal growth temperature (23°C), wild-type, and *hit1-1* plants have different plasma membrane protein profiles (**Fig. 1**). This result suggests that, regardless of growing temperature, the *hit1-1* mutation essentially alters regular recycling of plasma membrane components, and this alteration does not affect plant growth and development under non-stress conditions, but is unfavorable or even lethal for plants growing under certain stress conditions.

Modification of lipid saturation levels is a well known mechanism for membrane acclimation to heat,[1] and is more significant for plant tolerance of longer-term heat stress than heat shock.[9] The *hit1-1* heat-sensitive phenotype correlates with this notion. Meanwhile, mutants that have a defect in a gene that encodes digalactosyldiacylglycerol (DGDG) synthase become thermosensitive. This thermosensitivity is associated with an inability to increase the ratio of DGDG to monogalactosyldiacylglycerol (MGDG) upon exposure to high temperature.[10] DGDG is normally a plastid-specific lipid but has been found in the plasma membrane upon phosphate deprivation.[11,12] These data suggest that modification of membrane lipids for high temperature adaptation may not be restricted to changes in fatty acid saturation level. How vesicle trafficking machinery participates in this global reorganization of membrane lipids and to what extent it affects the heat tolerance are largely unclear, and the *hit1-1* mutant holds great potential to provide novel insight to these questions.

Acknowledgments

We gratefully acknowledge the National Science Council (Taiwan) for financial support (97-2311-B-008-001-MY3 and 99-2324-B-008-001-MY2). We also thank Chin-Hui Yeh and Aislin Lee for feedback on the manuscript.

References

1. Upchurch RG. Fatty acid unsaturation, mobilization and regulation in the response of plant to stress. Biotechnol Lett 2008; 30:967-77.
2. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: an overview. Environ Exp Bot 2007; 61:199-233.
3. Levine A. Regulation of stress responses by intracellular vesicle trafficking? Plant Physiol Bioch 2002; 40:531-5.
4. Wu SJ, Locy RD, Shaw JJ, Cherry JH, Singh NK. Mutation in Arabidopsis *HIT1* locus causing heat and osmotic hypersensitivity. J Plant Physiol 2000; 157:543-7.
5. Lee CF, Pu HY, Wang LC, Sayler RJ, Yeh CH, Wu SJ. Mutation in a homolog of yeast Vps53p accounts for the heat and osmotic hypersensitive phenotypes in Arabidopsis *hit1-1* mutant. Planta 2006; 224:330-8.
6. Conibear E, Stevens TH. Vps52p, Vps53p and Vps54p form a novel multisubunit complex required for protein sorting at the yeast late Golgi. Mol Biol Cell 2000; 11:305-23.
7. Wang LC, Tsai MC, Chang KY, Fan YS, Yeh CH, Wu SJ. Involvement of the Arabidopsis HIT1/AtVPS53 tethering protein homolog in the acclimation of the plasma membrane to heat stress. J Exp Bot 2011; 62:3609-20; PMID: 21398432; DOI: 10.1093/jxb/err060.
8. Wu SJ, Wang LC, Yeh CH, Lu CA, Wu SJ. Isolation and characterization of the Arabidopsis *hit-intolerant 2 (hit2)* mutant reveal the essential role of the nuclear export receptor EXPORTIN1A (XOP1A) in plant heat tolerance. New Phytol 2010; 186:833-42.
9. Falcone DL, Ogas JP, Somerville CR. Regulation of membrane fatty acid composition by temperature in mutants of Arabidopsis with alterations in membrane lipid composition. BMC Plant Biol 2004; 4:17.
10. Chen J, Burke JJ, Xin Z, Xu C, Velten J. Characterization of the Arabidopsis thermosensitive mutant *atts02* reveals an important role for galactolipids in thermotolerance. Plant Cell Environ 2006; 29:1437-48.
11. Andersson MX, Larsson KE, Tjellstrom H, Liljenberg C, Snadelius AS. The plasma membrane and the tonoplast as a major targets for phospholipid-to-glycolipid replacement and stimulation of phospholipase in the plasma membrane. J Biol Chem 2005; 280:27578-86.
12. Andersson MX, Stridh MH, Larsson KE, Liljenberg C, Snadelius AS. Phosphate-deficient oat replaces a major portion of the plasma membrane phospholipids with the galactolipid digalactosyldiacylglycerol. FEBS Lett 2003; 537:128-32.
13. Santoni V. Plant plasma membrane protein extraction and solubilization for proteomic analysis. Method Mol Biol 2007; 335:93-109.