A forward genetic screen on chemicals that disrupt the actin cytoskeleton uncovers a novel regulator of auxin efflux carrier trafficking in Arabidopsis

J. Alan Sparks[1], Taegun Kwon[1], and Elison B. Blancaflor[1]
Plant Biology Division, The Samuel Roberts Noble Foundation

To discover new proteins that function in actin–dependent cellular processes in plants, we isolated *Arabidopsis thaliana* mutants that were resistant or hypersensitive to latrunculin B (LatB), a potent chemical inhibitor of the actin cytoskeleton. We isolated 3 non-allelic mutants that had enhanced sensitivity to, and 4 non-allelic mutants that were tolerant to the growth inhibitory effects of LatB. One recessive mutant, *hypersensitive to latrunculin B 1*(*hlb1*), was disrupted in a gene (*AT5G41950*) encoding a tetratricopeptide repeat (TPR) domain-containing 565 amino acid protein of unknown function. Nanomolar concentrations of LatB induced more profound alterations of seedling growth and F-actin organization in *hlb1* compared to wild type. Surprisingly, *hlb1* was also hypersensitive to the actin stabilizing chemical Jasplakinolide. In addition to its heightened sensitivity to LatB, *hlb1* had aberrant root hair shape and mild primary root growth defects. Further, *hlb1* had reduced vegetative growth under short day conditions. A functional HLB1-GFP fusion colocalized with *trans*-Golgi Network (TGN)/early endosome (EE) markers through its conserved C-terminal domain. Recycling of the auxin efflux carrier, PIN- formed 2 (PIN2) to the plasma-membrane was disrupted in *hlb1*. Co-immunoprecipitation identified the TGN/EE-localized Brefeldin A (BFA)-visualized endocytic trafficking defective 1 (BEN1) as a putative HLB1 interactor. Interestingly, *ben1* mutants were hypersensitive to LatB to the same extent as *hlb1* mutants. Genetic interaction studies and live imaging of HLB1-GFP in the *ben1* mutant background indicate that BEN1 is crucial for HLB1 localization to the TGN/EE. Based on these data, we propose that HLB1 together with BEN1 form a complex with actin to modulate trafficking of PIN2 at the intersection of the exocytic and endocytic pathways. Taken together, our results demonstrate the feasibility of forward genetic screens on actin-disrupting chemicals to uncover novel protein regulators that mediate interactions between the cytoskeleton and endomembrane trafficking (Supported by NASA grant NNX12AM94G).