GTR Seminar

Multi-Knock - a multi-targeted genome-scale CRISPR toolbox to overcome functional redundancy in plants

Speaker: Prof. Eilon Shani
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Date/Time: 29 May, 2023 (Mon) 15:00--16:00

Room: NU Agricultural Building lecture room #7

Abstract: Plant genomes are characterized by large and complex gene families that often result in similar and partially overlapping functions. This genetic redundancy severely hampers current efforts to uncover novel phenotypes, delaying basic genetic research and breeding programs. To address the problem of masked phenotypic variation due to functional redundancy, we developed and validated Multi-Knock, a novel genetic approach in plants that combines forward-genetics with dynamically targeted genome-scale CRISPR/Cas9 tools. A total of 59,129 multi-targeted sgRNAs, divided into 10 functional sub-libraries targeting 16,152 genes in *Arabidopsis,* were designed, synthesized, and cloned into a genome-editing intronized Cas9 vector. From this collection, 5,635 sgRNAs targeting 1,123 of the 1,327 transporters (TRP) in *Arabidopsis* were cloned into four different Cas9 vectors generating independent CRISPR libraries, wherein each sgRNA was designed to target closely related homologues within sub-clades in transporter families. A proof of concept forward-genetic screen using over 3,500 CRISPR lines targeting the plant transportome recovered multiple known phenotypes in *Arabidopsis*, demonstrating the validity of the approach. Moreover, our screen allowed us to uncover novel transporters whose function has been hidden due to genetic redundancy. Specifically, we identified a homologous subfamily of three previously unstudied genes with partially overlapping function, *PUP7*, *PUP21*, and *PUP8*. We discovered that while all three proteins biochemically function as cytokinin transporters, PUP8 localizes to the plasma membrane, while PUP7 and PUP21 are localized to the tonoplast. We show that these proteins regulate meristem size, phyllotaxis, and plant growth, revealing complex redundant activity within this sub-family and providing a demonstration of the power of the Multi-Knock approach to discover new biological functions.

References: ***Nat. Plants***, doi: 10.1038/s41477-023-01391-3 (2023); ***Annu Rev Plant Biol***, doi: 10.1146/annurev-arplant-070722-015329 (2023); ***Nat. Plants***, 9: 572-587 (2023); ***Sci. Adv.,*** 7: eabf6069 (2021); ***Nat. Commun.***, 12: 1657 (2021)

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