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Grant Title: A pipeline to determine GT functions by probing the sub-Golgi interactome

Objectives: The complexity of cellular glycan structures requires an enormous number of glycosyltransferases (GTs) since thousands of glyosidic linkages are found in nature. A comprehensive understanding of the identity and selectivity of GTs involved in glycan synthesis is vital to unravelling the roles that these enzymes play. However, it is not always simple to infer GT functions from sequence data alone and thus only a small proportion of GT enzymes have been functionally characterized to date. Here we established a novel pipeline for the discovery and characterization of GT functions. Our previous work has developed a biochemical assay to characterize nucleotide sugar transporters (NSTs) that supply the sugar substrates to the Golgi-localized GTs and its successful application led to the identification of transporters for all major nucleotide sugars required for glycan assembly in the model plant Arabidopsis. Our unpublished results further indicated that these NSTs can physically interact with GTs, presumably to channel substrates directly to specific enzymes and subsequently specific polymers. Therefore, the main objective of this study was to probe the interactome of NSTs and GTs to identify new candidate GTs that are able to interact with the recently identified UDP-arabino*furanose* transporters (UAfTs). Further objectives of the proposal included localizing NSTs, candidate GTs and their interaction domains at sub-Golgi resolution and testing GT activity.

Methods / Results: We used a targeted approach employing a membrane-bound two hybrid assay in yeast (MbY2H) using the four members of the Arabidopsis UAfT family as baits and a cDNA library that was produced exploiting the Arabidopsis GT clone collection as prey. This screen revealed interactions between the UAfTs and ~80 members of different GT families listed in the Carbohydrate active enzymes (CaZy) database as well as non-CaZy GTs. The first step was to reduce the number of putative interactors and eliminate false positives. We performed pairwise MbY2H assays to validate the NST-GT interactions that were observed in the large-scale interaction screen. In addition, since most techniques studying protein interactions are prone to detect false positives, complementary to the MbY2H assays we performed Bi-fluorescence complementation (BiFC) assays with selected candidates. However despite great efforts the application of BiFC assays has proven difficult in producing reliable data. For that reason, we decided to use an alternative technique and are currently in the process of performing co-immunoprecipitation assays to validate GT candidates and prove interactions. Leveraging the GT clone collection, we are also generating clones expressing GT candidates under the CaMV 35S promoter transiently in tobacco to verify that they localize to the Golgi. We, furthermore, fluorescently tagged the four UAfTs and expressed them under their native promoter both stable in Arabidopsis and transiently in tobacco to examine their distinct localization within the different Golgi compartments. For that we have generated genetic crosses between Arabidopsis lines expressing fluorescently tagged UAfTs and a set of well-established sub-Golgi marker lines, which we will use to examine their localization using a quantitative live cell imaging. Besides, constructs and a first sample set for immunoTEM have been generated to further dissect the localization of the UAfTs.