Quantitative Analysis of Endomembrane Dynamics in Tobacco Pollen
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Abstract / Introduction
Endomembrane trafficking is a dynamic process that is required for essential biological processes such as signaling, stress response, defense, development, and polar growth. Recently, the intersection of synthetic chemistry and biology has lead to the use of chemical genomics as a tool to study biological processes. In this study, tobacco pollen was used in a semi-automated high-throughput imaging process to identify novel compounds that disrupt membrane cycling and, thus, pollen germination and morphogenesis. Of more than 46,000 compounds screened, 390 were found to be bioactive in pollen leading to inhibition of germination or growth. In examining the endomembrane trafficking of a specific protein in tobacco pollen, seven compounds were found to cause mislocalization in the localization or movement of RAB2/GFP (Cheung et al., 2002, Plant Cell 14:6945), an endomembrane marker for ER to Golgi trafficking, and were termed “RAB2 effectors.” The most common RAB2/GFP phenotype observed was the formation of RAB2 agglomerations or “RAB2 bodies.” The size and movement of these bodies was quantified using an existing image analysis software package. Taken together, these techniques demonstrate the utility of this high-throughput screen in identifying compounds that alter the movement or localization of endomembrane proteins, the translatability of this method between systems and the advantage of using chemical genomics to dissect dynamic processes.

Chemical Libraries Show a High Rate of Translatability

Bioactive Compounds Cause Developmental Defects That Fall into Eight Classes

Four RAB2 Effectors Cause Changes in Localization and Transport of RAB2:GFP

Figure 3. Six compounds caused either a change in localization or movement of RAB2:GFP and were designated “RAB2 effectors.” Mislocalization of RAB2:GFP manifested in larger than normal RAB2 vesicles, thought to be agglomerations of multiple vesicles termed “RAB2 bodies.” Some RAB2 effectors arrested the normal movement of RAB2:GFP while others appeared to halt movement altogether.

Figure 4. Although many compounds inhibited germination, trafficking of RAB2:GFP was not visibly affected. Initial inspection found that RAB2 effectors 1 and 2 slowed movement of RAB2:GFP and caused small agglomerations. RAB2 effectors 5, 6, and 7 caused larger bodies while appearing to halt all movement; however, video analysis shows a slight movement which is quantified below.

Quantitative Analysis Reveals Discrete Differences in RAB2:GFP Localization and Speed

Figure 5. To confirm and quantify the differences in size and movement of the RAB2 bodies, image and video analysis was used on still and time-lapse movies. Compound-induced RAB2 bodies were up to seven times the diameter of wild-type organelles but moved at 5% of the speed of wild-type RAB2 organelles. Quantification shows similar RAB2 body sizes and speeds of RAB2 bodies for effectors 6 and 7. Such variations may indicate distinct targets or modes of action.

Conclusions

- This high-throughput quantitative method is an efficient way of screening chemical libraries for compounds affecting trafficking.
- The tested libraries exhibit a high rate of translatability from pollen to seedlings.
- Six RAB2 effectors were discovered that perturbed RAB2:GFP localization and movement.
- The size and movement of the resulting RAB2 bodies were quantified and indicated that while RAB2 effectors 1, 2.5, and 6 induced relatively small RAB2 bodies, RAB2 effectors 6 and 7 induced relatively large RAB2 bodies. Likewise, RAB2 effectors 1 and 2 induced RAB2 bodies that traveled more rapidly than RAB2 effectors 5, 6, and 8.
- Possible bioactive functional groups were identified.
- RAB2 effectors are not directly altering microtubules or actin.