Unconventional trafficking pathways in BY-2 cells

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Abstract

Plants of Nicotiana tabacum are common models, used for genetic engineering and biotechnological purposes over the last decades. Vacuolar trafficking and sorting pathways were successfully validated on N. tabacum plants to identify different pathways towards the vacuole: a conventional and an unconventional sorting route still poorly described. The classical protein sorting begins in the endoplasmic reticulum (ER), passes through the Golgi, and the prevacuolar compartment (PVC). However, an unconventional pathway, which bypasses the Golgi, has been reported as an exception to the traditional pathway. In order to discern the different mechanisms involved in these two pathways, we have been investigating the role of PSI (Plant Specific Insert) - a 100 amino acid domain isolated from cardosins A and B, responsible for determining vacuolar sorting. Regardless the similarities between the two, PSI-A mediated sorting bypasses the Golgi while PSI-B follows the conventional pathway to the vacuole. The use of whole plant tissues brings some limitations, such as low multiplication rate of cells and multiple responses due to changes in cultivation conditions. N. tabacum BY-2 cells line has numerous advantages to overcome whole plants restraints. BY-2 suspension cultures can be grown under constant and specific conditions, have a high multiplication rate and are easily transformed by Agrobacterium tumefaciens. Our goal in this study is to validate BY-2 cells as a model of endomembrane trafficking, particularly in the case of PSI mediated sorting and explore in more detail the mechanisms involved in unconventional trafficking. For this purpose, BY-2 cells were transformed by co-culture with Agrobacterium tumefaciens. Both PSI-A and PSI-B were tagged with the fluorescent protein mCherry in order to localize and follow these sorting signals. We expect to validate this model by comparison to our common model Nicotiana tabacum for further experiments and overcome whole plants limitations.

Introduction

The cell sorting system is characterized by two pathways: the conventional pathway (ER–Golgi – PVC – Vacuole) and the unconventional pathway (Golgi bypass). Proteins are synthesized in the ER then are directed to the vacuole following one of those pathways.

Preliminary Results

We successfully obtained the recombinant plasmids with endomembrane markers SYP23 and SYP51 tagged with fluorescent protein mEos by using the Gibson Assembly method.

Future Prospects

The goal of this project is to successfully validate our new model Nicotiana tabacum BY-2 cell line, in order to overcome whole plant tissues limitations in endomembrane trafficking. To achieve that we will transform the N. tabacum BY-2 cell cultures by different methods with endomembrane reporters and markers for further localize and distinguish the pathways used by these sorting signals.

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