ENCONTRO COM A CIÊNCIA E TECNOLOGIA EM PORTUGAL
28 a 30 JUNHO 2021
#ciencia2021PT

GREENUPORTO Sustainable Agrifood Production Research Centre

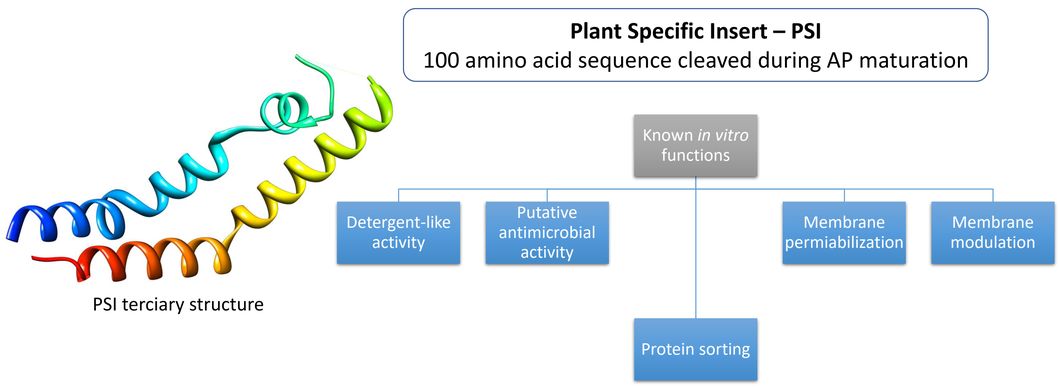
INTERACTION NETWORK OF UNCONVENTIONAL VSDs

Miguel Sampaio[1], João Neves[1], Susana Pereira[1], José Pissarra[1] and Cláudia Pereira[1]

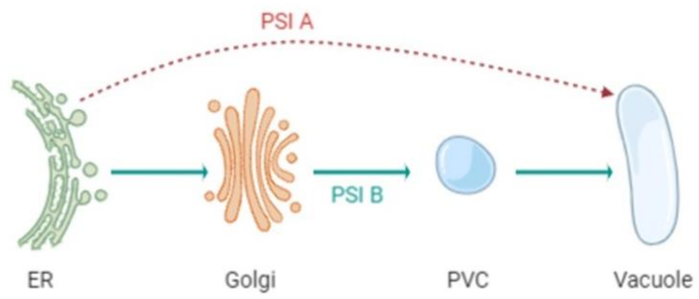
[1] GreenUPorto-Sustainable Agrifood Production Research Centre & Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, s/nº, 4169-007 Porto, Portugal

Introduction

In plants, there are several thousands of different types of proteins with different functions that must be correctly located to a specific subcellular compartment. The conventional vacuolar sorting route is already well described, and research teams are now more interested in understanding mechanisms behind how unconventional sorting routes work. Our laboratory has been working with a 100 amino acid domain showed to be both sufficient and necessary for correct vacuolar sorting, the plant specific insert (PSI).

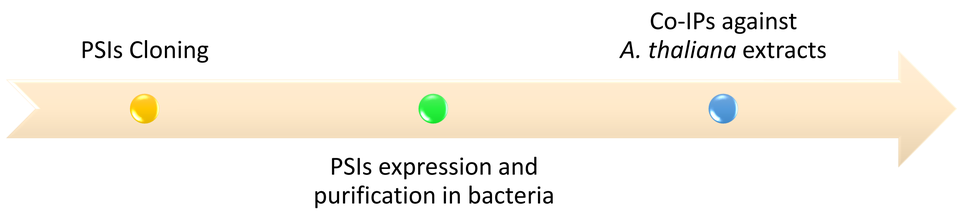


Even though different, yet related, PSI domains (PSI A and PSI B) present high similarity, they mediate different routes: PSI A is able to Golgi bypass, directly delivering proteins from the Endoplasmic Reticulum to the Vacuole; while PSI B mediates a conventional ER – Golgi - Vacuole pathway. PSI A mediates an unconventional route to the vacuole, that still lack characterization.

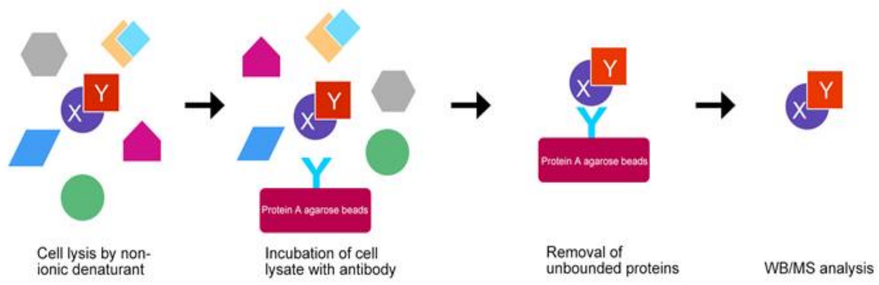


The main goal of this study was to identify intermediate players in PSIs sorting processes, particularly involved in the unconventional pathway to the vacuole.

Methodology

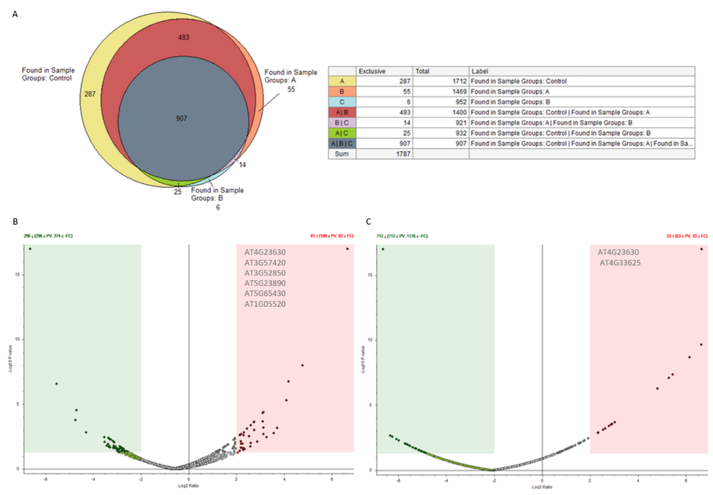


Co-immunoprecipitation (Co-IP) assays were performed using PSI purified protein and total protein extracts from *A. thaliana*. The main goal of this assay was to detect PSI interacting proteins through a wide search approach. Co-immunoprecipitation (Co-IP) assays were performed using PSI purified protein and total protein extracts from *A. thaliana*. The main goal of this assay was to detect PSI interacting proteins through a wide search approach.



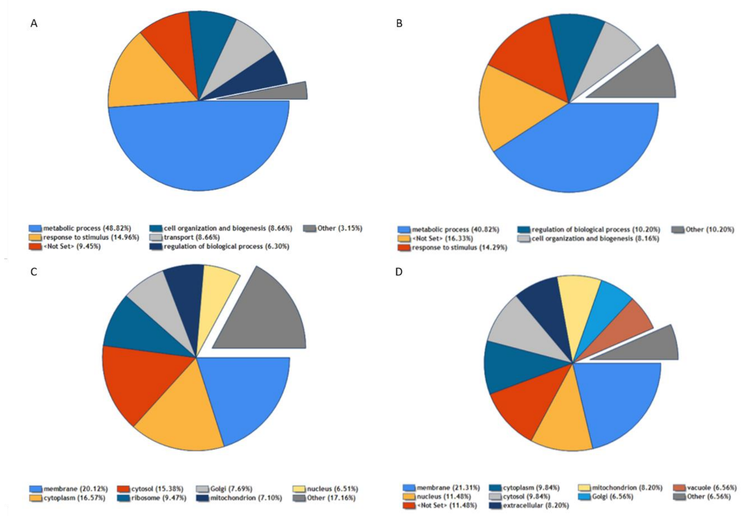
Results and Discussion

The results obtained from the PSI A/B – *A. thaliana* proteins Co-IP analysis were very good, presenting a wide range of detected proteins with different functions and cellular processes. Indeed, as shown, 2411 (1459 for PSI A and 952 for PSI B) proteins were identified for interaction with PSI A and PSI B. For PSI A seven proteins were chosen to be studied: Reticulon-like protein B1 (AT4G23630), Probable glycosyltransferase STELLO2 (AT3G57420), Vacuolar sorting receptor 1 (AT3G52850), GPI-anchored adhesin-like protein (AT5G23890), 14-3-3-like protein GF14 kappa (AT5G65430) and transport protein SEC23 (AT1G05520). Regarding PSI B, two interacting proteins were studied: RTNLB1 (AT4G23630) and Vacuole protein (AT4G33625).



	Exclusive	Total	Label
A	287	1712	Found in Sample Groups: Control
B	55	1469	Found in Sample Groups: A
C	6	952	Found in Sample Groups: B
A ∩ B	493	1400	Found in Sample Groups: Control ∩ Found in Sample Groups: A
B ∩ C	14	921	Found in Sample Groups: A ∩ Found in Sample Groups: B
A ∩ C	25	932	Found in Sample Groups: Control ∩ Found in Sample Groups: B
A ∩ B ∩ C	907	907	Found in Sample Groups: Control ∩ Found in Sample Groups: A ∩ Found in Sa...
Sum	1787		

Co-IP Results: A - Veen diagram representing detected proteins in control (Yellow), PSI A (Orange) and PSI B samples (Blue). B, C - Volcano plot comparing control and PSI A (B) and PSIB (C) Co-IPs. Proteins marked in red are significantly found in higher amounts in the Co-IP while proteins marked in green are significantly found in higher amounts in the control. Accession numbers in grey correspond to significative hits with relevance for the PSI study. Data analyzed using: Proteome Discoverer v2.4 (Thermo Scientific).



Distribution of positive hits from PSI A and PSI B Co-IPs regarding their physiological role and subcellular localization. A – Physiological role of proteins detected in PSI A Co-IP. B - Subcellular localization of proteins detected in PSI A Co-IP. C – Physiological role of proteins detected in PSI A Co-IP. D - Subcellular localization of proteins detected in PSI A Co-IP. Data analyzed using: Proteome Discoverer v2.4 (Thermo Scientific).

This study allowed to disclose several putative interactors for both PSI A and PSI B and, most interestingly, the majority of them do not overlap, indicating different interactions in the cell.

Conclusion and Future Perspectives

This study was a very preliminary assay towards the mapping of PSI A and PSI B network of interactions and the uncovering of the mechanisms underneath their function as vacuolar sorting domains.
Regarding the PSI A interactions, it was interesting to see that a high number of the proteins detected are related to protein sorting to different locations in the cell. However, for PSI B, the coverage obtained was not as significant, not allowing to draw any conclusions.

Overall, this pilot study provided a lot of information that still needs further analysis beyond what is discussed in this topic but represents a good starting point towards our goals.

Acknowledgements

This research was conducted in the frame of the scientific project PTDC/BIA-FBT/32013/2017 and supported by national funds through FCT - Foundation for Science and Technology within the scope of UIDB/05748/2020 and UIDP/05748/2020

U.PORTO FACULDADE DE CIÊNCIAS UNIVERSIDADE DO PORTO

Correspondence:
Cláudia Pereira – cpereira@fc.up.pt
Miguel Sampaio – up201503801@edu.fc.up.pt