

Studying the sRNA trafficking in transgenic barley using Rab7 GTPase marker

Shaoshuai Liu, Karl-Heinz Kogel, Jafargholi Imani

Workshop Molekulare Züchtung

05.-6.09.2019

Geisenheim, Germany

Contents

- Introduction
- Purpose of the study
- Methods
- Results
- Conclusion
- Acknowledgements

RNA-interference

- RNA-interference, natural biological process in eukaryotes
- RNA molecules inhibit gene expression or translation process by degradation of mRNA
- siRNA and miRNA have central role in the RNAi process



Mocellin & Provenzano (2004)

Cross-kingdom communication



From plant to pathogen

- Interaction between plants and pathogen requires specific bidirectional sRNAbased communication.
- Plants and fungi can use
 conserved RNA interference
 machinery to regulate each
 other's gene expression



From pathogen to plants

Kerry Grens, 2017

Cross-kingdom sRNA transportation between plant and pathogen





Communication involves processing of RNA and its transport across the cellular boundaries.

REPORTS

Cite as: Q. Cai et al., Science 10.1126/science.aar4142 (2018).

Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes

Qiang Cal,¹ Lulu Qiao,^{1,3} Ming Wang,¹ Baoye He,¹ Feng-Mao Lin,³ Jared Palmquist,¹ Hslen-Da Huang,³ Halling Jin¹⁺ ¹Department of Microbiology and Plant Pathology. Center for Plant Cell Biology, Institute for Integrative Genome Biology. University of California, 900 University Avenue, Riverside, CA 92521, USA. ²Department of Plant Protection, Nanjing Agriculture University, Nanjing 210095, China. ³Department of Biological Science and Technology. National Chiao Tung University, Hsin-Chu 300, Taiwan.

Exosomes

Fit to deliver small RNA

Anoek Zomer, Tineke Vendrig, Erik S. Hopmans, Monique van Eijndhoven, Jaap M. Middeldorp and D. Michiel Pegtel Department of Pathology; Cancer Center Amsterdam; VU University Medical Center; Amsterdam, The Netherlands

Cai *et al.* 2018

Rab proteins



- "Rab" is an abbreviated form of "Ras-associate binding" proteins. For instance **Rab5**, **Rab7** etc
- Rab4, **Rab5** function in the early endocytosis
- **Rab7** functions in the late endocytic pathway (Ueda *et al.,* 2001; Sohn *et al.,* 2003)
- Rab7 is possibly involved in membrane
 trafficking steps (Mazel *et al.*, 2004)

Rab7 as a late endosme marker



- Rab belongs to the Ras small
 GTPase superfamily which can
 regulate many steps of membrane
 trafficking and membrane fusion
- Rab7 is necessary for lysosomal biogenesis, positioning and functions, trafficking and degradation of receptors, signal transduction.

Chavrier et al. 1990, Bucci et al. 2000, Nina et al. 2018

Purpose of the study

- Identification of the sub-cellular localization of Rab7GTPase in barley plant
- Extracellular vesicle isolation to track sRNA pathway between plant and pathogen interaction.

Methods

1. Plasmid construction

2. Genetic transformation

3. Initial screening of plants through PCR and western blotting

4. Fluorescence microscopy

5. Vesicle isolation

6. Confocal laser scanning microscopy(CLSM) of isolated apoplastic fluid

Confirmation of GFP tagged *Rab7* construction



Western blot to detect fusion protein 35S:GFP-HvRab7 in Barley



Localization study of *HvRab7*-GFP in barley leaf cells



Microscopic analysis and confirmation of GFP tagged Rab7GTPase expression in isolated barley apoplastic fluid



Microscopic images of isolated vesicle with 35S-HvRab7-GFP expression in apoplast fluid.

Conclusion

Rab7GTPase is mostly localized on the stomota and plasma membrane in barley

Presence of membrane trafficking gene or Rab7 protein (Rab7GTPase) in the barley apoplastic fluid isolation

Acknowledgements



Prof. Dr. Karl-Heinz Kogel



Dr. Jafargholi Imani



Ms.c Moammar Hossain



TA Eugen Swidtschenko



....and all institue members

Thank you for your attention !