# Investigating the Role of RING Peroxins in Establishing Peroxisome Membrane Structure

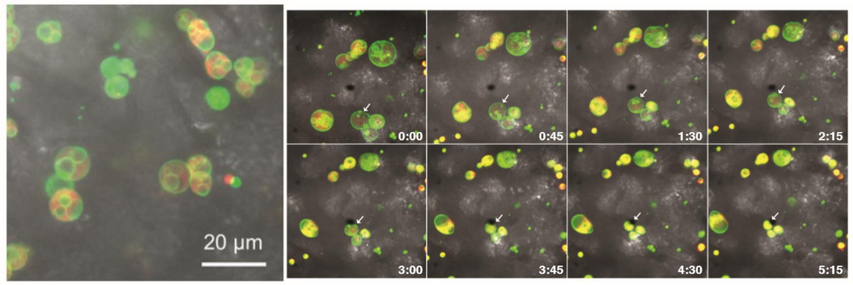
Chihiro Akiyama[1], Sarah E. Bradford[2], Zachary J. Wright[2], Bonnie Bartel[2]

1. Department of Agriculture, Tohoku University, Sendai 980-8573, Japan 2. Department of Biosciences, Rice University, Houston, TX 77005, USA

## Peroxisomes have densely packed internal membranes

While peroxisomes have long been thought to have only a single-membrane, recent data has revealed uncharacterized inner peroxisomal membrane structure.

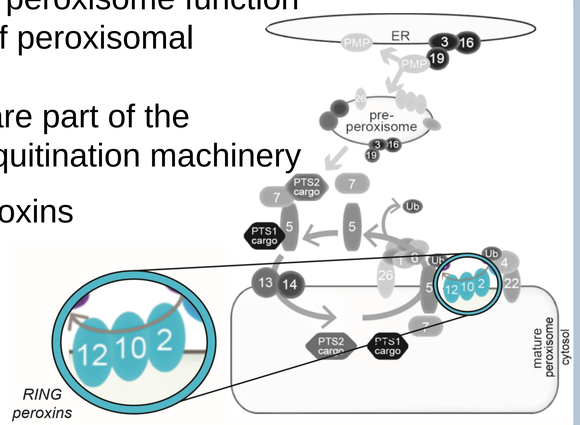
A) 3-d-old cotyledons of seedling expressing mNeon-Green-PEX26TM an mRuby3-PTS1 show intralumenal vesicles
B) Time-lapse imaging of cotyledon of 3-d-old seedlings expressing mNeon-Green-PEX26TMD and mRuby3-PTS1 shows peroxisome size decrease as inner membrane density increases over time




A B

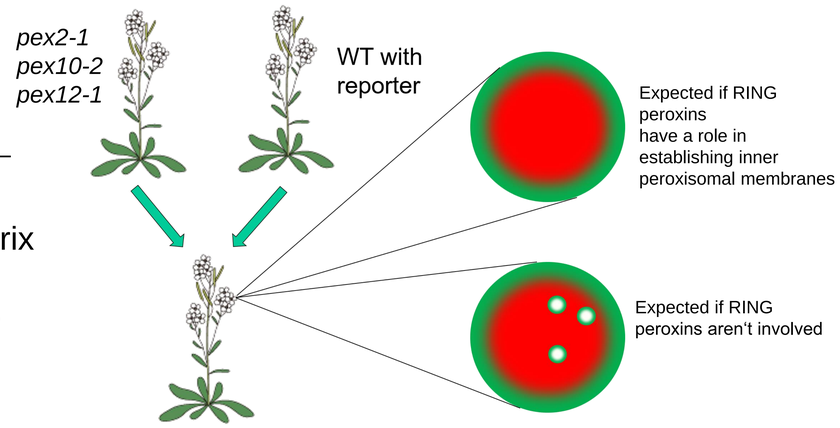
## Peroxins are proteins responsible for peroxisome biogenesis and function

- Peroxins control peroxisome function and the import of peroxisomal proteins
- RING peroxins are part of the peroxisomal ubiquitination machinery
  - Do RING peroxins function in establishing Internal membranes?

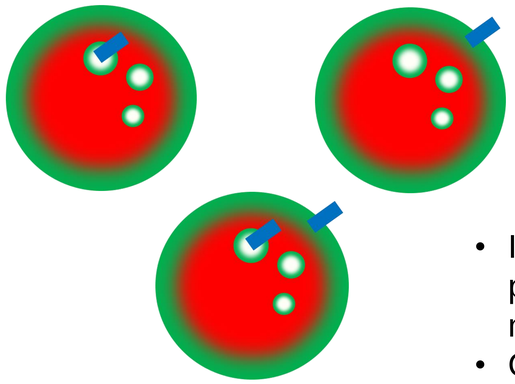



## Investigating the role of the RING peroxins in establishing peroxisomal inner membrane structure

- Cross RING peroxin mutants to a di-fluorescent reporter line marking peroxisome membrane/matrix
- Isolate homozygous crosses
- Observe impact on inner peroxisomal membrane function using confocal microscopy




## How are the RING peroxins localized on peroxisomal membranes?



| Reporter | Target |
|---|---|
| mNeonGreen-PEX26TMD | Peroxisomal membrane |
| mRuby3-PTS1 | Peroxisomal matrix |
| PEX-mTagBFP2 | PEX2, PEX10, PEX12 |

- How proteins are distributed across the inner and outer peroxisomal membrane is unknown
- Do the RING peroxins localize differently across these membranes?
- Investigate by fusing PEX2, PEX10 and PEX12 to a blue fluorescent protein in a vector with the PEX26TMD peroxisomal membrane marker and the mRuby4-PTS1 matrix reporter
- Observe RING fusion protein localization using confocal microscopy

## Future Plans

- Finish isolating homozygous RING peroxin mutants expressing di-fluorescent reporter line
- Complete cloning of tri-fluorescent reporter line and transform plants
- Observe the results of both using confocal microscopy
- Investigate other parts of the peroxisomal ubiquitination machinery, PEX4 and PEX22

## Acknowledgements

- This research was conducted as a part of The Nakatani RIES: Research & International Experiences for Students Fellowship with support from Nakatani Foundation(more detail is available on http://nakatani-ries.rice.edu/).
- Special thanks to all the members of the Bartel Lab for their excellent research guideline, kindness, and patience, and to Prof. Junichiro Kono, Sara Phillips, Kenji Ogawa and Aki Shimada for warm support and making this program possible