# Identification of novel peroxisome functions in yeast

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*DOI:*
10.33612/diss.99106402

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The studies presented in this thesis were performed in the research unit Molecular Cell Biology of the Groningen Biomolecular Sciences and Biotechnology Institute (GBB) of the University of Groningen, The Netherlands.

This project was supported financially by the EU Marie Skłodowska-Curie Innovative Training Network (ITN) program, PerFuMe.



ISBN printed version: 978-94-034-2050-9
ISBN digital version: 978-94-034-2049-3

Layout and Printing by: Off Page, Amsterdam

university of groningen

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PhD thesis

to obtain the degree of PhD at the
University of Groningen
on the authority of the
Rector Magnificus, Prof. Dr. C. Wijmenga
and in accordance with
a decision by the Doctorate Board.

This thesis will be defended in public on
Friday 01 November 2019 at 09:00 hours

by

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*To Ma and Papa*

*for their love, support and prayers*

# Acknowledgements

I would like to take this opportunity to express my gratitude for the wisdom, support, and lessons taught by the people who crossed my Ph.D. path which contributed to the preparation of this thesis.

I would like to begin by expressing my sincere gratitude to Professor Ida van der Klei for selecting me and giving me the opportunity to conduct my doctoral research at the Molecular Cell Biology group back in 2013. Thank you Ida, for believing in me, for your constructive feedback that helped me to refine my work, for making the time to meet with me every Friday, for being so closely involved in helping me with the research progress and for ceaselessly supporting me along the way. Thank you for everything.

I take this opportunity to thank my second supervisor Professor Sigrun Reumann for joining Ida in guiding my Ph.D. course, for your support and invaluable feedback to all my writings, be it project reports, posters or presentation slides.

I extend my special thanks to the members of the reading committee Prof. A.J.M. Driessen, Prof. B.M. Bakker and Prof. H.A.B. Wosten for taking time to evaluate this thesis and giving valuable suggestions.

I would like to thank Marie Curie Initial Training Network, PerFuMe (PERoxisome Formation, Function, Metabolism) for funding my Ph.D. position and giving the opportunity to work with leading academic institutions and learn.

I take this opportunity to thank Srishti and Renate for accepting to be my paranymphs and for their help in the preparation of my defense day. Thank you, Renate, for helping with the Dutch translation of my thesis summary.

A giant "thank you" to our lab secretary Jannet for her help with all official procedures, for the kind support and for all the good times. Jannet, thank you for your friendship and for being there along the way. It means a lot.

I would like to thank Arjen and Rinse for all the help with the experiments whenever it was needed. Thank you very much for your time and kind help.

My special thanks to Runa and Sanjeev for going out of the way to help me in moving and settling down in Groningen, and for always being there. I will be forever grateful for this and for your friendship.

My sincere thanks to Adam for his support and supervision during the early days of my Ph.D. Thank you for all the technical training, and hours spent at the office explaining everything.

I had the pleasure to supervise and teach some great students during my Ph.D. Harshitha, Bohdan, Gwen, and Maartje thank you for all your amazing work and dedication.

I would like to also extend my appreciation to my colleagues who have been there along the way. I would like to thank Sanjeev, Selva, Malgosia, Chris, Kevin, Terry and Anita for their help and support in different phases of my Ph.D.

Most importantly, thank you to the lovely lab-mates and my dearest friends Arman, Ann, Srishti, Justyna, and Natasha. Thank you for the great time spent over coffees, trying to diffuse Ph.D. related frustrations, sharing our little victories along the way and giving reasons to smile and carry on. I thank you guys for making our lab a delightful place to come to and work in. I would also like to thank Fei (My peng you), Huala (Lady gaga), Chen and Renate (Banana) for being the sweetest people around. Thank you for the fun times and get-togethers (especially the hotpot dinners lady gaga). For the new lab members, best wishes!

Ann and Srishti, words will fall short if I start writing for you both. So, I would just say thank you from the bottom of my heart. Thank you for sticking with me through thick and thin, for helping me to overcome everything life threw at me, for being there, and for lifting me up. I cannot imagine this journey without you both. I am deeply beholden.

Special thanks to my very first neighbour, my lab bench-mate, my fashion guru, and my travel companion, Justynka. I will always cherish our work trips to different places (thanks to PerFuMe), dinner dates (Thai Jasmine), bike rides to home from work, and endless nonsense conversations. Thank you for being that particular person who makes me laugh like crazy and live just a little better. You made me meet my second mamus (cutest mama). I will be forever grateful for this and for your backing and friendship while our paths crossed. You are the best. Thank you pani for brightening up my life.

To my dearest friends Ritu (and Archit), Neha, Ritika, Shonali, Mansi, Samvartika Di, and Jaishree….Thank you for your constant support, encouragement, and love over the years. You are all wonderful.

A warm “thank you” to my sweetest Bue who cares too much. Thank you for being you bue.

My heartfelt regards to Mom and Papa for the love and moral support during my thesis writing days. I feel blessed to be part of the family.

I am fortunate to have awesome parents and the best siblings. Although we are apart, a family is close at heart. Bhai, thank you for your unbelievable support and belief in me. Happy, thank you for doing nothing. Lol. Thank you champ for being my constant and for always encouraging me to push my limits. I am blessed to have you both.

Maa..my love for you is ineffable. The best in me I owe to you. Thank you, papa, for always motivating us to do our best. I would not have made it here had it not been for you. This book is a culmination of your support and love. I don't know how I will ever thank you for everything you have done for me. This thesis is for you, Ma and Papa. I hope this book makes you proud.

Finally, I would like to thank my husband, my sidekick, Vikalp, for his constant encouragement and backing. You empower me, you help me grow as a person and bring meaning to my life day in, day out. Thank you for all the love, joy and laughter you bring into my life every single day. Thank you for making me more than I am.

*Ritika*

# Table of Contents

# Aim and Outline

Peroxisomes are organelles occurring in most eukaryotic cells. They are involved in wide range of metabolic and non-metabolic functions. Despite the extensive research since their discovery in the fifties of the previous century, our knowledge on peroxisomal functions is incomplete. The research described in this thesis aimed to identify and characterize novel peroxisome proteins and functions in yeast.

**Chapter 1** gives an overview on our current knowledge on peroxisomes focussing on peroxisome function, redox-regulation, and proliferation in yeast.

Using an organelle proteomic approach using peroxisomal fractions isolated from *Hansenula polymorpha* cells exposed to ethanol stress, we identified 6 putative peroxisomal peroxiredoxins **(Chapter 2)**. Two of them, named C8BNF3 and C8BNF4, contain a putative peroxisomal targeting signal 1 (PTS1). Although C8BNF3 contains a PTS1, it is localized to the mitochondria, whereas C8BNF4 partially localizes to the peroxisomes in glucose-grown cells, but not when cells were grown on methanol. That absence of C8BNF4 did not result in a growth defect or enhanced sensitivity to any of the stress conditions tested.

Both Pnc1 (nicotinamidase) and Gpd1 (glycerol-3-phosphate dehydrogenase) are stress-related peroxisomal proteins. Gpd1 was previously reported to relocalize to the cytosol upon exposure of cells to osmotic stress. In **Chapter 3** we show that Pnc1 is transported to peroxisomes by piggy-backing on Gpd1 We show that the levels of both peroxisomal and cytosolic Gpd1 and Pnc1 increased when the cells were exposed to stress. Our quantitative analysis of the distribution of Gpd1 and Pnc1 over the cytosol and peroxisomes revealed that both proteins are predominantly localized to peroxisomes. The non-stress related peroxisomal protein thiolase, when produced under the control of *GPD1* promoter, displayed a similar behaviour indicating that the presence of peroxisomal matrix proteins in the cytosol of cells exposed to stress is a result of reduced matrix protein import efficiency and not relocalization.

Mammalian PXMP2 has been indicated to function as a non-selective pore in the peroxisomal membrane. A homologous protein in *Neurospora crassa*, Wsc, is involved in the formation of Woronin bodies from peroxisomes. In **Chapter 4** we analysed all four Pxmp2 proteins of *H. polymorpha*. One of these proteins, designated Pex37, localizes to peroxisomes. Deletion of *PEX37* resulted in a reduction in peroxisome numbers and a defect in peroxisome segregation in cells grown at peroxisome repressing conditions (glucose). This phenotype could be partially complemented by human PXMP2, suggesting that PXMP2 is a functional homologue of Pex37.

*Saccharomyces cerevisiae* Vac8 plays among others a role in vacuole inheritance and fusion. In addition it is a component of nucleus-vacuole junctions (NVJ). Organelle proteomics revealed that Vac8 also occurs in peroxisomal fractions isolated from *S. cerevisiae* or *H. polymorpha* (Chapter 2). In **Chapter 5** we show that HpVac8 is also required for NVJ formation and vacuole inheritance, but not for vacuole fusion. The absence of HpVac8 had no effect on peroxisome function, number and distribution indicating that a role of Vac8 in peroxisome biology is very unlikely.