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**Identification of novel proteins that potentially
are in complex with Yih1 and that are required
for promoting Gcn2 function**

A thesis submitted in partial fulfilment of the requirements
for the degree of Master of Philosophy in Biochemistry

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November 2015

The ability of organisms to respond to various stress conditions is important for life. Under amino acid starvation conditions the protein Gcn2 is activated and phosphorylates the translation initiation factor eIF2 α . This leads to a downregulation of general protein synthesis and an upregulation of the synthesis of proteins that are involved in helping the cell overcome starvation, a process called General Amino Acid Control (GAAC). It is important that the GAAC is only switched on when necessary and for this Gcn2 needs to be regulated. For instance, the protein Gcn1 is needed for Gcn2 activation. Another protein, Yih1, inhibits Gcn2 activity by competing with Gcn2 for Gcn1 binding. However, the balance between Gcn2 activation by Gcn1 and Gcn2 inhibition by Yih1 is not well understood. Actin was already identified as a Yih1-binding protein and modelling exercises strongly suggests that additional proteins bind Yih1.

The aim of this project was to identify novel proteins that are in a complex with Yih1 (Yih1-binding proteins, YBP) and to then discover which are required for Gcn2 activation. For the first aim, YBP were ascertained from published large-scale protein-protein interactions studies and from data generated in-house.

19 different strains deleted for one putative YBP exhibited an impaired growth under starvation conditions. Of those, four deletion mutants showed a reduced Gcn2 activity. One protein was Spc72 which is involved in mitochondrial organisation. Another protein was Idh2, an enzyme of the citric acid cycle. The growth defect of strains deleted for *SPC72* or *IDH2* was complemented with a plasmid containing *SPC72* or *IDH2*, respectively, and other genes. This suggested their involvement in Gcn2 activation.

Elongation factor eEF1A was found as a putative YBP and as a co-precipitator of Yih1, supporting previous unpublished observations. eEF1A was found to bind Gcn2 in previous studies and this suggested that Yih1-eEF1A interaction may regulate Gcn2 activation.

Another putative YBP, the heat shock protein Hsc82, is needed for Gcn2 maturation. Strains deleted for *HSC82* showed an impaired growth under starvation conditions and this was reversed by deleting *YIH1*. This suggested that Yih1 may regulate Hsc82-Gcn2 interaction and thus Gcn2 activity.

This study was a step to further advance our understanding of Yih1-binding proteins and Gcn2 activity. In addition, this further emphasised the idea of Yih1 as an important regulator inside the cell.

Acknowledgments

Firstly, I would like to thank my supervisor Dr Evelyn Sattlegger for giving me the opportunity to study in New Zealand, for advice, providing resources and guidance throughout my research. I would like to thank Dr Jane Allison for taking the job as my co-supervisor. Thank you for both for a thorough reading and valuable feedback on my thesis. I would like to thank Professor Dianne Brunton for support in the late stages of my studies and for providing me with a quiet space to write my thesis.

I would like to acknowledge that this project was funded by the Auckland Medical Research Foundation and The Maurice and Phyllis Paykel Trust. I would like to thank the New Zealand Lottery Grants Board for financial support in the form of a scholarship. I also would like to thank the Institute of Natural and Mathematical Sciences for financial support at the beginning of my studies and to attend conferences where I presented my work.

I would like to acknowledge Associate Professor David Greenwood from the Auckland University Proteomics Facility as well as Dr Torsten Kleffmann and Manya Sabherwal from the Otago University Centre for Protein Research for sample analysis and helpful feedback about the results.

I am unbelievably grateful to Su Jung Lee for the uncountable number of ways she supported me inside the laboratory and outside of it. I thank fellow group member Rashmi Ramesh and visiting doctoral student Richard Cardoso da Silva for helpful comments on my thesis. I want to thank other present and past members of the Sattlegger group Renuka Shanmugam, Viviane Jochmann, Rangachari Krishnan, Shweta Pandya, Hee Jun Lee and Yuting (Tiena) Liang for friendly support in the lab. I would like to thank Mathias Joachim for helping me with the growth assays, western blots and sporulation experiments. I want to thank past group members Dr Jyothsna Visweswaraiah, Dr Martina Dautel and Dr Andrew Cridge for introducing me into lab and to the Sattlegger group. I want to say thanks to all the present and previous people in Building 11 that made the lab work a pleasant experience. Special thanks to Colleen van Es for helping to keep everyone in Building 11 sane. Another special thanks goes to Yogesh Dalvi for being a friend.

Acknowledgments

I want to thank Austen Ganley for access to the dissection microscope and Daniela Quintana Rincon for teaching me how to use it and for providing chemicals. Thanks to Jarod Young for letting us borrow equipment. I want to thank Helen Matthews, Laura Nigon and Erin Moffet for being available whenever I needed access to the metal storage container.

I am highly indebted to everyone who contributed to yeastgenome.org – without it this thesis would not have been possible.

I want to thank Associate Professor Peter Lineham and Kaye McGregor for their much needed moral support.

Finally, I want to thank my family for giving me the freedom to choose my own path.

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List of abbreviations

| | |
|------------------|--|
| 3AT | 3-amino-1,2,4-triazole |
| APS | ammonium persulfate |
| AGC | automatic gain control |
| ATP | adenosine triphosphate |
| CID | collision induced dissociation |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| dNTP | deoxyribonucleotide triphosphate |
| DTT | dithiothreitol |
| EDTA | ethylenediaminetetraacetic acid |
| eIF2 | elongation initiation factor 2 |
| F-actin | filamentous actin |
| FTMS | Fourier transform mass spectrometry |
| GAAC | general amino acid control |
| G-actin | globular (monomeric) actin |
| Gcn | general control non-derepressible |
| GDP | guanosine diphosphate |
| GST | glutathione S-transferase |
| GTP | guanosine triphosphate |
| HEPES | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid |
| His ₆ | hexahistidine tag |
| HSP | heat shock protein |
| IMPACT | imprinted and ancient |
| IPTG | isopropyl β -D-1-thiogalactopyranoside |
| KCl | potassium chloride |
| kDa | kilodalton |
| LB | lysis broth |
| LC-MS/MS | liquid chromatography-tandem mass spectrometry |
| mRNA | messenger RNA |
| NaCl | sodium chloride |
| OD | optical density |
| ORF | open reading frame |
| PAGE | polyacrylamide gel electrophoresis |
| PEG | polyethylene glycol |
| PIC | pre-initiation complex |
| PMSF | phenylmethylsulfonyl fluoride |
| PVDF | polyvinylidene difluoride |
| RNA | ribonucleic acid |
| RNase | ribonuclease |
| rpm | revolutions per minute |
| RWD | RING finger, WD repeat, yeast DEAD-like helicase |
| SD | synthetic dextrose |
| SDS | sodium dodecyl sulfate |

| | |
|-----------------|---------------------------------|
| SM | sulfometuron methyl |
| SM ^s | SM sensitivity |
| TAP | tandem affinity purification |
| TBS | Tris-buffered saline |
| TBS-T | TBS-Tween20 |
| TOR | target of rapamycin |
| Tris | tris(hydroxymethyl)aminomethane |
| tRNA | transfer RNA |
| v/v | volume/volume |
| w/v | weight/volume |
| WCE | whole cell extract |
| YBP | Yih1-binding protein(s) |
| Yih1 | Yeast Impact Homolog 1 |
| YPD | yeast extract peptone dextrose |
| YPG | yeast extract peptone glycerol |

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