Characterization of *Arabidopsis thaliana* CPR5 via the Elucidation of Interacting Protein Partners

A thesis presented in partial fulfilment of the requirements for the degree of

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Fiona (Shane) Chiem 2015
Abstract

The *Arabidopsis thaliana* Constitutive expresser of pathogenesis related genes5 (*CPR5*) has previously been suggested to play a role in the regulation of disease resistance, plant and cell proliferation, development and death. Analysis of *cpr5* mutant alterations to hormone and hormone-like signalling mechanisms have provided evidence that abolishment of *CPR5* involvement within these hormone signalling pathways, results in many of the stunted growth, early senescence and constitutive expression of pathogen defense phenotypes observed. Despite the pleiotropic effect that *cpr5* mutants have on the plant system, it is unclear whether *CPR5*-dependent pathways are due to a direct interaction with *CPR5* or due to a more indirect association. *CPR5* has been proposed to be a regulator of a multitude of different pathways, including reactive oxygen species (ROS), cell wall biosynthesis, and transcription but evidence of these proposals are limited to the effects that *cpr5* mutants have on downstream targets.

In an attempt to address the involvement of *CPR5* in *Arabidopsis* plant processes, a series of studies were conducted to determine the protein interacting partners of *CPR5*. Proteins were identified via 2 independent yeast 2 hybrid (Y2H) screening of an *Arabidopsis* transcriptome library. Ten proteins of interest were identified via two independent screenings using two truncated forms of *CPR5*. Functional involvement of *CPR5* with the identified proteins was further explored using the Y2H pairwise interaction system. *CPR5* was found to interact with 3 full length proteins identified.

To explore the possibility that *CPR5* interacts with multiple protein partners in different locations within the cell, Bifluorescence molecular complementation assays were performed to determine the localization and interaction of *CPR5* with the ten identified genes as well as 3 previously identified genes. Several novel interactions were identified that occur within the nucleus and outside of the nucleus. Not only was *CPR5* confirmed to have an interaction with KRP2 within the nucleus, *CPR5* exhibited interaction with FSD1, CRK4, PATL3, PATL5, and PATL6, outside of the nucleus.

In the final set of experiments, several double mutant lines were produced that did not yield any observable phenotypes that differ from *cpr5*-2 single mutant plants. In order to determine the effects these double mutants have on various plant processes affected by *cpr5*-2 single mutant; qRT-PCR was performed to determine the expression pattern of pathogen related genes (*PR1* and *PDF1.2*) known to be significantly upregulated in *cpr5*-2 plants. qRT-PCR analysis revealed that *cpr5*-2 *fsd1* exhibits a down-regulation of *PDF1.2*. 
PR1 regulation was found to be down-regulation in cpr5-2 bzip61 and up-regulated in cpr5-2 patl3 compared to cpr5-2.

Sugar and dark treatment of the cpr5-2 double mutant lines yielded several alterations to hypocotyl length, root length, and apical hook curvature by several of the double mutant lines, indicating a connection between CPR5 and the knocked out gene of interest. None of the double mutants were able to completely rescue the sugar-induced morphological phenotypes exhibited by cpr5-2, and some double mutant lines exhibited more pronounced effects indicating an additive effect by sugar treatment.

Together this data suggests that CPR5 interacts with various proteins involved in different plant processes in various locations throughout the cell. Further research of these proteins and a more direct analysis of the interaction that may occur between CPR5 and these proteins will be required to provide a foundation for more direct characterization the CPR5 molecular function; and ultimately to determine the role that CPR5 plays within the hormone and hormone like signalling pathway and their effects on major plant processes.
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I would like to thank my supervisor Dr. Paul Dijkwel for giving me this opportunity and for helping me complete my Masters degree. Paul has been an exemplary supervisor providing me with the guidance I required when help was asked and for challenging me. I thank you for the patience you have shown me and for providing me with the independence I required to succeed. Thank you especially for your confidence in my abilities to successfully accomplish the goals we set forth and for allowing me to carry out these goals in my own unorthodox fashion.

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Abbreviations

minutes
seconds
Ade Adenine
aa amino acids
amp Ampicillin
BiFC BiFluorescence Molecular Complementation
BLAST Basic logical alignment search tool
bp Base-pair
cDNA DNA synthesized from an mRNA template
C-terminus (at the) carboxy-terminal end of a polypeptide chain
CPR5 CPR5 wild-type gene
CPR5 CPR5 wild-type protein
cpr5 CPR5 mutant gene
cpr5 CPR5 mutant protein
cpr5-2 cpr5 mutant line with mutation at aa420 (W->stop)
DAPI A DNA binding fluorescent stain ((4',6-diamidino-2-phenylindole)
DNA Deoxyribonucleic acid
DNase Deoxyribonuclease
dNTP 2'-deoxynucleotide 5' triphosphate
dH2O distilled water
ddH2O double distilled water
E. coli Escherichia coli
EDTA Ethylenediaminetetraacetic acid
FW Fresh weight
g Gram
gDNA Genomic DNA
Gen Gentamycin
h Hour
His Histidine
IPTG Isopropyl-β-D-thiogalactopyranoside
kan kanamycin
kb Kilo base-pair
kD(a) Kilo daltons
L Litre
LB Luria-Bertani (media or broth)
Leu Leucine
M Molarity (moles per litre)
MCS Multiple cloning site
mg Milligram
Milli-Q-water Water purified by Milli-Q-ion exchange chromatography
### Definition of Terms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml</td>
<td>Milliters</td>
</tr>
<tr>
<td>mol</td>
<td>Mole (Avagadro's number)</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>MS</td>
<td>Murashige &amp; Skoog Media</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>OD600</td>
<td>optical density at 600nm (measured in a spectrophotometer)</td>
</tr>
<tr>
<td>°C</td>
<td>Degree celsius</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>pH</td>
<td>-Log (H+)</td>
</tr>
<tr>
<td>psi</td>
<td>a unit of pressure (pounds per square inch)</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Reverse transcriptase-polymerase chain reaction</td>
</tr>
<tr>
<td>RE</td>
<td>Restriction Enzyme</td>
</tr>
<tr>
<td>Rnase</td>
<td>Ribonuclease</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse osmosis</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SALK</td>
<td>Arabidopsis T-DNA insertion lines from the SALK Institute, a non-profit research organization</td>
</tr>
<tr>
<td>SD</td>
<td>Synthetic Defined (media)</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium Dodecyl Sulfate</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error mean</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris base, acetic acid, and EDTA buffer</td>
</tr>
<tr>
<td>TAIR</td>
<td>The Arabidopsis Information Resource</td>
</tr>
<tr>
<td>TE</td>
<td>Tris base, EDTA buffer</td>
</tr>
<tr>
<td>Tet</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature at which DNA strands separate prior to annealing</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris (hydroxymethyl) aminomethane</td>
</tr>
<tr>
<td>Trp</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Tween-20</td>
<td>Polyoxyethylenesorbitan monolaurate</td>
</tr>
<tr>
<td>U</td>
<td>Unit (based on enzyme activity)</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μl</td>
<td>Microlitre</td>
</tr>
<tr>
<td>μM</td>
<td>Micromolar</td>
</tr>
<tr>
<td>V</td>
<td>Volt</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume per volume</td>
</tr>
<tr>
<td>w/v</td>
<td>Weight per volume</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight per weight</td>
</tr>
<tr>
<td>X-α-Gal</td>
<td>X-α-Gal is a chromogenic substrate used to detect α-galactosidase activity</td>
</tr>
<tr>
<td>Y2H</td>
<td>Yeast-2-hybrid</td>
</tr>
<tr>
<td>YFP</td>
<td>Yellow fluorescent protein</td>
</tr>
<tr>
<td>YPDA</td>
<td>yeast peptone dextrose adenine (media/agar)</td>
</tr>
</tbody>
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