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

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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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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 (terminal)	(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	Miligram
Milli-Q-water	Water purified by Milli-Q-ion exchange chromatography

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

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