FUNCTIONAL CHARACTERISATION of *CONSTITUTIVE EXPRESSER* of *PATHOGENESIS-RELATED GENES 5*

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MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Muhammad Faisal

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ABSTRACT

As reported previously, CPR5 negatively regulates the onset of leaf death, hypersensitive response, disease resistance and early leaf senescence. *cpr5* plants contain aberrant trichomes and higher levels of ROS, SA and JA. Cell-cycle, JA/ET, ABA and sugar signalling are also affected in *cpr5* plants. These results suggest that CPR5 is a master regulator of multiple processes. However, how CPR5 manages to exert pleiotropic effects is still poorly understood. The first objective of the current study was the purification of the CPR5 protein to solve its crystal structure. Extensive *in silico* analyses were carried out and the results showed that CPR5 is predicted to be a membrane protein with 4 or 5 transmembrane (TM) domains. Additionally, CPR5 contains intrinsically disordered regions (IDRs) at its N-terminus. Proteins containing IDRs and TM domains are often difficult to purify for crystallization studies. Therefore, the undesirable regions of CPR5 such as, IDR and TM domains were deleted and a set of 24 constructs were developed. Despite several efforts, none of the CPR5 recombinant proteins were isolated. In addition to predicting IDR and TM domains, *in silico* results also predicted three NLS-encoding clusters, casein kinase phosphorylation sites, multiple start codons, coiled-coil domains and glycine motifs. To find out the roles of these putative structural elements on CPR5 functions, firstly a *CPR5* cDNA was synthesised and termed as *SynCPR5*. Subsequently, predicted sites or motifs were mutated in *SynCPR5* through site-directed mutagenesis and a set of 25 mutated *CPR5* transgenes (cDNA constructs) were developed. Using a complementation strategy, all the constructs were transformed into *cpr5-2* plants. The results show that the complementation of *cpr5-2* plants with *SynCPR5*, fully restored HR-like lesions, wildtype-like trichomes and leaves on *SynCPR5* plants. Further physiological characterization such as, transcript abundance of *SynCPR5*, *PR1*, *PR5* and *PDF1.2*, leaf area measurements and ploidy levels showed that *CPR5* regulates some of its functions and phenotypes quantitatively as well as qualitatively. When compared with the wildtype, better growth (larger leaves) but enhanced disease susceptibility was found in *metCPR5* transgenic lines (in which putative start codons were mutated), indicating that CPR5 regulates a balance between growth and resistance. Functional characterization of NLS mutants (*nlsCPR5*) showed that NLS-encoding clusters are important for CPR5 proper

functions. However, current evidence is insufficient to relate their role in CPR5 localization. Moreover, *in silico* results show that putative NLS clusters are present in the region of CPR5 which were annotated as intrinsically disordered region (IDR). Similar phenotypes shown by both *nlsCPR5* and *Del63CPR5* (in which the first 63 amino acids of CPR5 including putative NLS were deleted), indicate that the putative NLS clusters could be part of IDR and may have dual functions. Loss-of-function phenotypes shown by coiled-coil domain mutants (*ccdCPR5*) reinforce the role of coiled-coil domains in CPR5 homo-dimerization. Moreover, in contrast to previous reports, the downregulation of *PDF1.2* in the majority of *CPR5* complementation lines proposes CPR5 to be a positive regulator of *PDF1.2*. Based on the results presented in the current study, putative CPR5 IDRs and coiled-coil domains are proposed to facilitate CPR5 dimerization in order to restrict the entry of deregulated cargos into the nucleus. Moreover, these results uncover a novel role of CPR5 in the regulation of balance between plant growth and resistance. Furthermore, this study, for the first time, reports evidence of the requirement of NLS clusters for CPR5 functions.

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MUHAMMAD FAISAL

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LIST OF ABBREVIATIONS

aa	Amino acid
ABA	Abscisic acid
ABI5	ABA-INSENSITIVE 5
acd6	*ACCELERATED CELL DEATH 6*
APC/C	anaphase-promoting complex/cyclosome
BRI	BRASSINOSTEROID-INSENSITIVE
BRs	Brassinosteroids
Ca	Calcium
CaM 35S promoter	Cauli Mosaic Virus 35S promoter
CC-domains	Coiled-coil domains
CDC*20*	*CELL DIVISION CYCLE 20*
CDH	*CDH HOMOLOG 1*
CDK	Cyclin-Dependant-Kinase
CK	Casein kinase
CKI	CDK inhibitor
CNGC	*Cyclic Nucleotide Gated Channels*
COI1	*CORONATINE-INSENSITIVE 1*
Col-0	*Arabidopsis* ecotype Columbia
CPR5	*CONSTITUTIVE EXPRESSER of PATHOGENESIS-RELATED GENES 5*
DAS	Days after sowing
EDR1	*ENHANCED DISEASE RESISTANCE 1*
EDS5	*ENHANCED DISEASE SUSCEPTIBILITY 5*
EIN2	*ETHYLENE INSENSITIVE 2*
ET	Ethylene
ETI	Effector-Triggered Immunity
FZR	*FIZZY-RELATED*
GA	Gibberellin
GeBP	*GL1 ENHANCER BINDING PROTEIN*
GFP	Green Fluorescent Protein
GIG1	*GIGAS cell 1*
GPLs	*GeBP*-like proteins
GST	Glutathione-S-transferases
H_2O_2	Hydrogen Peroxide
HR	Hypersensitive Response
HXK	*Hexokinase*
HYS1	*HYPERSENESCENCE1*
IDP	Intrinsically Disordered Protein
IDR	Intrinsically Disordered Region
JA	Jasmonic Acid
JAR1	*JASMONATE RESISTANT 1*
JAZ1	*JASMONATE-ZIM-DOMAIN PROTEIN 1*

K	Potassium
KRPs	*KIP-RELATED PROTEINS*
L*er*-0	*Landsberg erecta*
LOX	Lipoxygenases
LSD	*LESIONS SIMULATING DISEASE*
MeJA	Methyl jasmonate
MoRFs	Molecular Recognition Features
mRNA	Messenger RNA
NDGA	Nordihydroguaiaretic acid
NE	Nuclear Envelope
NLS	Nuclear Localization Signal
NO	Nitric oxide
NPC	Nuclear Pore Complex
NPR1	*NON-EXPRESSER of PATHOGENESIS-RELATED GENES 1*
OE lines	Overexpression lines
OLD1	*ONSET OF LEAF DEATH1*
OSD1	*OMISSION of SECOND DIVISION 1*
PAD4	*PHYTOALEXIN DEFICIENT 4*
PAMPs/ MAPMs	Pathogen- or microbe-associated molecular patterns
PCD	Programmed Cell Death
PCR	Poly Chain Reaction
PDF1.2	*PLANT DEFENSIN 1.2*
PIF	*PHYTOCHROME INTERACTING FACTOR*
PR1	*PATHOGENESIS-RELATED 1*
PRRs	Pathogen Recognition Receptors
PstDC3000	*Pseudomonas syringae* pv *DC3000*
PTI	PAMP-Triggered Immunity
qRT-PCR	Quantitative Reverse-Transcriptase Poly Chain Reaction
R proteins	Resistance proteins
RB	Retinoblastoma
ROS	Reactive Oxygen Species
RPM1	*RESISTANCE TO PSEUDOMONAS SYRINGAE PV MACULICOLA1*
RPS2	*RESISTANCE TO PSEUDOMONAS SYRINGAE*
SA	Salicylic Acid
SAGs	*SENESCENCE-ASSOCIATED GENEs*
SAR	Systemic Acquired Resistance
SIM	*SIAMESE*
SMR	*SIAMESE-RELATED*
SOD	Superoxide Dismutase
TFs	Transcription Factors
TM	Transmembrane
tRNA	Transfer RNA
UVI4	*UV INSENSITIVE 4*