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Characterisation of the interactions of RGL1; a negative regulator of gibberellin signalling

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

Doctor of Philosophy
in
Biochemistry

Massey University, Palmerston North
New Zealand.

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2010

Abstract

The gibberellins are a family of phytohormones that promote many aspects of plant development. Central to the function of gibberellins are the DELLA regulatory proteins. The DELLA proteins actively repress cell differentiation and elongation, but are degraded upon perception of gibberellin, thus relieving repression of gibberellin responses. The GID1-family gibberellin receptors and DELLA-specific F-box proteins are essential for the gibberellin-induced degradation of the DELLA proteins. Importantly, the direct interaction between gibberellin-bound GID1-family gibberellin receptors and the N-terminal domain of DELLA proteins is a prerequisite for proteasomal degradation through recruitment of the F-box proteins. To increase understanding of gibberellin signalling, I have characterised a gibberellin-dependent GID1-DELLA-F-box protein signalling switch in *Arabidopsis thaliana*. First, I have characterised a suite of anti-DELLA antibodies for detection of four endogenous *A. thaliana* DELLA proteins, GIBBERELLIC ACID-INSENSITIVE (GAI), REPRESSOR OF GA1-3 (RGA), RGA-LIKE-1 (RGL1), and RGA-LIKE-2 (RGL2). Using these monoclonal antibodies against the conserved motifs of DELLA proteins, I showed that residues Asp/Glu/Leu/Leu within the signature DELLA motif are not essential for interaction of RGL1 with GID1A. Further, *in vitro* interaction assays allowed modelling a two-step conformational change within the N-terminal domain of RGL1 upon interaction with gibberellin-bound GID1A. Together with interaction assays in yeast two- and three-hybrid systems, these experiments provided three clues to the mechanism of GID1A-RGL1-SLY1 gibberellin signalling switch: i) N- to C- inter-domain interactions of RGL1 regulate its accessibility to SLY1; ii) the N-terminal domain of RGL1 undergoes conformational rearrangement upon interaction with gibberellin-GID1A; iii) the conformational changes of the N-terminal domain of RGL1 primes the C-terminal domain for the recruitment of SLY1. I have also isolated two novel RGL1-interacting proteins, the myrosinase THIOGLUCOSIDE GLUCOHYDROLASE-2 (TGG2) and GERMIN-LIKE-PROTEIN-1 (GLP1), through affinity-purification from nuclear extract and mass spectrometry fingerprinting. Neither protein has yet been implicated in gibberellin signalling. Therefore, the identification of these novel components may help resolve several uncharacterised aspects of gibberellin signalling.

Acknowledgments

I would like to thank my supervisors, Dr Jasna Rakonjac, Dr William Jones, and Dr Toshi Foster for their advice and guidance. I would also like to thank the other members of both the Helipad lab and the Plant and Food Research Immunology group for their helpful support.

I am grateful to Julian Spagnuolo who assisted in the optimisation of quenching conditions used for deuterium exchange mass spectrometry. I also thank members of Prof. Nicholas Harberd's research group at the John Innes Centre for supplying *A. thaliana* plant material and reagents for the determination of monoclonal antibody specificity.

This project was supported by grants from the Foundation for Research Science and Technology (No. C06X0207) and the Agricultural and Marketing Research and Development Trust (No. 20585).

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Abbreviations

3-AT	3-amino-1,2,4- trizol
ABA	abscisic acid
cDNA	complementary deoxyribonucleic acid
cGMP	cyclic guanosine monophosphate
Col-0	Columbia-0
CPS	<i>ent</i> -copalyl diphosphate synthase
CTR1	<i>Arabidopsis thaliana</i> CONSTITUTIVE TRIPLE RESPONSE-1
D1	<i>Oryza sativa</i> DWARF-1
D27	27 residue synthetic DELLA motif peptide
DMSO	dimethyl sulfoxide
DTT	1,4-dithiothreitol
EDTA	ethylene-diamine-tetra-acetic acid
EIN3	<i>Arabidopsis thaliana</i> ETHYLENE INSENSITIVE-3
GA ₁	gibberellin A1
GA ₃	gibberellin A3
GA ₄	gibberellin A4
GA1	<i>Arabidopsis thaliana</i> GA REQUIRING 1 (CPS)
GA2ox	GA2-OXIDASE
GA3ox	GA3-OXIDASE
GA20ox	GA20-OXIDASE
GAI	<i>Arabidopsis thaliana</i> GIBBERELIC ACID-INSENSITIVE
GARE	gibberellic acid responsive element
LC-ESI-MS/MS	liquid chromatography electrospray ionisation quadrapole-time-of-flight coupled mass spectrometry
LC-MS	liquid chromatography coupled mass spectrometry
GID1	<i>Oryza sativa</i> GIBBERELLIN-INSENSITIVE DWARF-1
GID1A	<i>Arabidopsis thaliana</i> GID1-LIKE-A
GID1B	<i>Arabidopsis thaliana</i> GID1-LIKE-B
GID1C	<i>Arabidopsis thaliana</i> GID1-LIKE-C
GID2	<i>Oryza sativa</i> GIBBERELLIN-INSENSITIVE DWARF-2
GFP	<i>Aequorea victoria</i> GREEN FLUORESCENT PROTEIN

GLP1	<i>Arabidopsis thaliana</i> GERMIN-LIKE-PROTEIN-1
GLP1 ^{ΔSS}	GLP1, lacking N-terminal secretion signal sequence
GST	<i>Schistosoma japonica</i> GLUTATHIONE S-TRANSFERASE
GUS	<i>Escherichia coli</i> β-D-GLUCURONIDASE
HA	<i>Influenza</i> HAEMAGGLUTININ epitope tag
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGP	heterotrimeric G-protein
HIS3	<i>Saccharomyces cerevisiae</i> IMIDAZOLEGLYCEROL-PHOSPHATE DEHYDRASE
HSIMYB	<i>Hordeum vulgare</i> SPY INTERACTING MYB
HSINAC	<i>Hordeum vulgare</i> SPY INTERACTING NAC
IAA	indole-acetic acid
IPTG	isopropylthio-β-D-galactoside
Ler	Landsberg <i>erecta</i>
MBP	<i>Escherichia coli</i> MALTOSE BINDING PROTEIN
MBP-β-gal	MBP- fusion to β-GALACTOSIDASE-α
MG132	proteasome inhibitor Z-Leu-Leu-Leu-al
O-GlcNAc	O-linked N-acetyl glucosamine
OD _{420/600}	optical density measured at either 420 or 600 nm
ONPG	O-nitrophenyl-β-D-galactopyranoside
PAGE	poly-acrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG-4000	poly-ethylene glycol-4000
PHOR1	<i>Solanum tuberosum</i> PHOTOPERIOD-RESPONSIVE-1
PIF3	<i>Arabidopsis thaliana</i> PHYTOCHROME INTERACTING FACTOR-3
PIF4	<i>Arabidopsis thaliana</i> PHYTOCHROME INTERACTING FACTOR-4
PMSF	phenyl-methyl-sulfonyl-fluoride
RGA	<i>Arabidopsis thaliana</i> REPRESSOR OF GA1-3
RGL1	<i>Arabidopsis thaliana</i> RGA-LIKE-1
RGL1 ^{ΔDELLA}	17 residue DELLA motif deletion of RGL1
RGL1 ^{ΔTVHYNP}	18 residue TVHYNP motif deletion of RGL1

RGL1 ^{Q272R}	glutamine 272-arginine replacement of RGL1
RGL2	<i>Arabidopsis thaliana</i> RGA-LIKE-2
RGL3	<i>Arabidopsis thaliana</i> RGA-LIKE-3
SCF	SKP-CULLIN-F-BOX E3 Ubiquitin ligase complex
SDS	sodium dodecyl sulfate
SHI	<i>Arabidopsis thaliana</i> SHORT-INTERNODES
SLN1	<i>Hordeum vulgare</i> SLENDER-1
SLR1	<i>Oryza sativa</i> SLENDER RICE-1
SLY1	<i>Arabidopsis thaliana</i> SLEEPY-1
SLY1 ^{E138K}	glutamic acid 138 lysine replacement of SLY1
SPY	<i>Arabidopsis thaliana</i> SPINDLY
T21	21 residue synthetic TVHYNP motif peptide
TGG2	<i>Arabidopsis thaliana</i> THIOGLUCOSIDE GLUCOHYDROLASE-2
TRIS	tris (hydroxymethyl) aminomethane
Tween-20	polysorbate-20
YFP	YELLOW FLUORESCENT PROTEIN (GFP mutant)