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The DELLA Protein Family and Gibberellin Signal Transduction

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Abstract

Gibberellins (GA) are plant hormones that promote important aspects of growth such as seed germination, leaf expansion, trichome initiation, transition to flowering, stem elongation, flower and fruit development. Genetic and molecular data indicate that growth and development are a default state and that the DELLA proteins are key repressors of GA-mediated growth and development. Mutant analysis indicates that GA does not directly promote growth; rather it overcomes the repression of the DELLA proteins by causing them to be degraded. The N-terminal domain of the DELLA proteins is involved in the perception of the GA signal and the C-terminal domain mediates the repression of GA responses. A GA-bound receptor recognises the DELLA proteins and interacts with an F-box E3 ligase, the DELLA proteins are then poly-ubiquitinated and degraded through the ubiquitin-26S proteasome system. However, the DELLA proteins are also post-translationally modified which affects their activity. It is believed the DELLAs are modified both with *O*-linked N-Acetyl glucosamine for stability and phosphate groups to mark them for F-box recognition. However, the precise nature, and role of these modifications is yet to be shown. DELLA-repressive action is mediated by interaction with other proteins and not through direct DNA binding. Few DELLA-interacting proteins are known.

Apple and Kiwifruit *DELLA* repressor, *GID1* GA receptor and *SLY1*/ *GID2* F-box orthologues were identified in their respective sequence databases. Relative amount and location of the orthologous transcript sequences was examined through qPCR and reporter gene experiments. Apple qPCR experiments indicated relatively high levels of *DELLA* transcripts in developmentally arrested tissues. Kiwifruit experiments present a more complicated picture, with high relative levels of *DELLA*s in the actively expanding tissues, however, concomitant with this were high relative levels of the *GID1* and *GID2* transcripts. Each transcript was found in every tissue studied and indicated complex developmental transcriptional control.

Both direct and indirect immunoprecipitation experiments utilising a novel tag were performed in GA-deficient plant backgrounds in order to isolate DELLA proteins and their interacting proteins likely targeted by DELLA repressive function. Proteins from these experiments were identified from their peptides in mass spectrometry analysis and database

query. Several transcription factors, kinases, proteins involved in RNA processing and protein components involved in hormonal signalling pathways other than GA were present.

DELLA repression complex formation was also investigated with two-dimensional electrophoresis and western blotting, and indicated a dominant repressive complex at approximately 160 kDa, with additional multiple larger complexes of up to 600 kDa.

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Abbreviations

2-MEA	2-Mercaptoethanolamine.HCl .
ABA	Abscisic acid.
Bar	Bialaphos Resistance.
bHLH	Basic Helix-Loop-Helix
BMV	Brome Mosaic Virus.
BNE	Blue Native gel Electrophoresis.
bp	Base pairs
BR	Brassinosteroid.
BSA	Bovine Serum Albumin.
cDNA	complementary Deoxyribosenucleic acid.
CDS	Coding Sequences.
ChIP	Chromatin Immunoprecipitation.
C-terminal	Carboxyl end/ portion of polypeptide.
DDM	n-dodecyl β-D-maltoside.
DNA	Deoxyribose Nucleic Acid.
dNTP	Deoxynucleotide triphosphates.
DTT	1,4-Dithiothreitol.
EDTA	Ethylene-diamine-tetra-acetic acid.
EGTA	Ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N'*,*N'*-tetraacetic acid.
EMCS	Maleimidocaproyloxy]succinimide ester.
EST	Expressed Sequence Tag.
FSB	Frozen Storage Buffer.
GA	Gibberellin.
GA3	Gibberellin A3.
GDR	Genome Database for Rosaceae.
GSP	Gene Specific Primer.
IEF	Isoelectrical Focus.
IgG	Immunoglobulin G
IP	Immunoprecipitation.
IPG	Immobilised pH Gradient.
IPTG	Isopropyl β-D-1-thiogalactopyranoside.

JA	Jasmonic acid.
LB	Luria Broth.
LG	Linkage Group
mAb	monoclonal Antibody.
MBP	Maltose Binding Protein.
MG132	Z-Leu-Leu-Leu-al.
miRNA	micro Ribose Nucleic Acid.
mRNA	messenger Ribose Nucleic Acid.
N-terminal	Amino end/ portion of polypeptide.
PAC	Paclobutrazol
PBS	Phosphate Buffered Saline.
PBST	Phosphate Buffered Saline supplemented with Tween 20™ (0.1 % polysorbate-20).
PCR	Polymerase Chain Reaction.
PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid).
qPCR	quantitative PCR.
RACE	Rapid Amplification of cDNA Ends.
RE	Restriction Endonuclease type II.
RNA	Ribose Nucleic Acid.
RT	Reverse Transcription.
SAM	Shoot Apical Meristem
SDS	Sodium Dodecyl Sulphate.
SDS-PAGE	Sodium Dodecyl Sulphate Poly-Acrylamide Gel Electrophoresis.
Silwet® L-77	84 % polyalkylencoxide modified heptamethyltrisiloxane 16 % allyloxypolyethyleneglycol methyl ether.
TEMED	Tetramethyl-ethylene-diamine.
TEV	Tobacco Etch Virus.
Tris	Tris (hydroxymethyl) aminomethane.
Tween™ 20	Polysorbate-20.
uidA	β-D-Glucuronidase gene.
X-gal	5-bromo-4-chloro-3-indolyl galactopyranoside.
X-gluc	β-D-glucuronide.
YEB	Yeast Extract Broth.