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# **Interactions of AtRGL1, a Negative Regulator of Gibberellic Acid Signalling**

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## Abstract

*Arabidopsis thaliana* AtRGL1 (repressor of *ga1-3* like-1) is a negative regulator of the signal transduction pathway of the plant hormone gibberellin. AtRGL1 belongs to the DELLA subfamily within the GRAS family of plant regulatory proteins. There are four other DELLA proteins, including AtRGA (repressor of *ga1-3*) and AtRGL2, encoded by the *A. thaliana* genome. Previous studies provided evidence that the DELLA proteins are nuclear localised and are functionally divided into N- and C-terminal domains. The N-terminal domain perceives the gibberellin signal, while the C-terminal domain functions as a negative regulator of transcription and also as a possible dimerisation domain. Previous studies have also shown that *AtRGA*, *AtRGL1*, and *AtRGL2* function together in the regulation of the development of the inflorescence and that *AtRGL1* is primarily expressed in this tissue.

To investigate how DELLA proteins function in gibberellin signalling, I sought plant proteins that interact with AtRGL1. Two proteins, p24 (24 kDa) and p64 (64 kDa), were isolated from wild-type plant nuclear extracts by affinity to the N-terminal 121 amino acid residues of AtRGL1. The identity of these two proteins remains to be established. To investigate the interactions of the C-terminal domain of AtRGL1 an anti-AtRGL1 polyclonal antiserum was developed for co-immunoprecipitation experiments. However, AtRGL1 was not detectable in plant nuclear extracts from the inflorescence of wild-type plants, precluding this approach.

The possibility of DELLA protein dimerisation was also investigated using AtRGA, AtRGL1, and AtRGL2 in yeast 2-hybrid experiments. Yeast 2-hybrid protein interaction results suggest that AtRGA, AtRGL1, and AtRGL2 do not form homo- or hetero-dimers. Complexities encountered with this approach could make these results invalid, so these interactions require further investigation.

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## Abbreviations

|                    |  |
|--------------------|--|
| 3-AT               | 3-amino-1,2,4-triazole   |
| ABA                | Abscisic acid  |
| AD                 | GAL4 activation domain   |
| AMP                | Ampicillin   |
| AmSO <sub>4</sub>  | Ammonium sulfate   |
| AP                 | Alkaline phosphatase   |
| AtASK1             | <i>A. thaliana</i> Arabidopsis Skp like-1  |
| AtGAI              | <i>A. thaliana</i> Gibberellic acid insensitive  |
| AtGAMYB33          | <i>A. thaliana</i> Gibberellic acid induced MYB-33                                     |
| AtGAMYB65          | <i>A. thaliana</i> Gibberellic acid induced MYB-65                                     |
| AtGCR1             | <i>A. thaliana</i> G-protein coupled receptor-1  |
| AtGPA              | <i>A. thaliana</i> G-protein $\alpha$ subunit  |
| AtHDA19            | <i>A. thaliana</i> Histone deacetylase-19  |
| AtLFY              | <i>A. thaliana</i> Leafy   |
| AtLRP              | <i>A. thaliana</i> Lateral root primordium   |
| AtPKL              | <i>A. thaliana</i> Pickle  |
| Atrga $\Delta$ 17  | <i>A. thaliana</i> Repressor of <i>gal-3</i> , deletion of 17 amino acid DELLA motif   |
| AtRGL1             | <i>A. thaliana</i> Repressor of <i>gal-3-1</i>   |
| Atrgl1 $\Delta$ 17 | <i>A. thaliana</i> Repressor of <i>gal-3-1</i> , deletion of 17 amino acid DELLA motif |
| AtRGL2             | <i>A. thaliana</i> Repressor of <i>gal-3-2</i>   |
| AtRGL3             | <i>A. thaliana</i> Repressor of <i>gal-3-3</i>   |
| AtSHI              | <i>A. thaliana</i> Short internodes  |
| AtSLY1             | <i>A. thaliana</i> Sleepy-1  |
| AtSLY2             | <i>A. thaliana</i> Sleepy-2  |
| AtSNY              | <i>A. thaliana</i> Sneezzy (AtSLY2)  |
| AtSPY              | <i>A. thaliana</i> Spindly   |
| BD                 | GAL4 DNA binding domain  |
| BnSCL1             | <i>B. napus</i> Scarecrow like-1   |
| BSA                | Bovine serum albumin   |
| cDNA               | complementary deoxyribonucleic acid  |
| cGMP               | Cyclic guanosine monophosphate   |
| c-Myc              | Mammalian c-Myc oncogene epitope   |

|           |  |
|-----------|--|
| Co-IP     | Co-immunoprecipitation                                     |
| Col-0     | Columbia-0   |
| DAPI      | 4',6-Diamidino-2-phenylindole                              |
| DEAE      | Diethylaminoethyl  |
| DMSO      | Dimethyl sulphoxide  |
| DNA       | Deoxyribonucleic acid                                      |
| DTT       | 1,4-Dithiothreitol   |
| EDTA      | Ethylenediaminetetraacetic acid                            |
| GA        | Gibberellic acid   |
| GAL4      | GAL4 transcription factor                                  |
| GARE      | Gibberellic acid responsive element                        |
| GFP       | Green Fluorescent Protein                                  |
| HA        | Influenza hemagglutinin epitope                            |
| HDAC      | Histone deacetylase  |
| HEPES     | 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid         |
| HGP       | Heterotrimeric G-protein                                   |
| HRP       | Horseradish peroxidase                                     |
| HvCDPK1   | <i>H. vulgare</i> Calcium dependent protein kinase-1       |
| HvGAMYB   | <i>H. vulgare</i> Gibberellic acid induced MYB             |
| HvHSIMYB  | <i>H. vulgare</i> Spindly interacting MYB                  |
| HvHSINAC  | <i>H. vulgare</i> Spindly interacting NAC                  |
| HvSAD     | <i>H. vulgare</i> Sad                                      |
| HvSLN1    | <i>H. vulgare</i> Slender-1                                |
| HvSPY     | <i>H. vulgare</i> Spindly                                  |
| IgG       | Immunoglobulin G   |
| Imidazole | 1,3-Diaza-2,4-cyclopentadiene                              |
| IPTG      | Isopropylthio- $\beta$ -D-galactoside                      |
| KAN       | Kanamycin  |
| Ler-0     | Landsberg <i>erecta</i> -0                                 |
| MALDI TOF | Matrix assisted laser desorption ionisation time of flight |
| MBP       | Maltose-binding protein                                    |
| miR159    | Micro ribonucleic acid 159                                 |
| miRNA     | Micro ribonucleic acid                                     |
| mRNA      | Messenger ribonucleic acid                                 |

|                  |   |
|------------------|---|
| MWCO             | Molecular weight cut-off                              |
| NHS              | <i>N</i> -hydroxysuccinimide                          |
| NLS              | Nuclear localisation signal                           |
| <i>O</i> -GlcNAc | <i>O</i> -linked <i>N</i> -acetyl glucosamine         |
| ONPG             | <i>O</i> -Nitrophenol-galactoside                     |
| OsD1             | <i>O. sativa</i> Dwarf-1                              |
| OsDOF3           | <i>O. sativa</i> DNA binding with one finger-3        |
| OsGID2           | <i>O. sativa</i> Gibberellic acid insensitive dwarf-2 |
| OsSLR1           | <i>O. sativa</i> Slender rice-1                       |
| p24              | 24 kDa AtRGL1-interacting protein                     |
| p53              | Murine p53  |
| p64              | 64 kDa AtRGL1-interacting protein                     |
| PAGE             | Poly-acrylamide gel electrophoresis                   |
| PBS              | Phosphate buffered saline                             |
| PCR              | Polymerase chain reaction                             |
| PEG              | Poly-ethylene glycol                                  |
| PMSF             | Phenylmethanesulphonylfluoride                        |
| SCF              | Skp-Cullin-F-box                                      |
| SD               | Synthetic dropout                                     |
| SDS              | Sodium dodecyl sulfate                                |
| StPHOR1          | <i>S. tuberosum</i> Photoperiod-responsive-1          |
| T antigen        | SV40 large T antigen                                  |
| TBS              | Tris-buffered saline                                  |
| TBST             | Tris-buffered saline Tween-20                         |
| Tris             | Tris (hydroxymethyl) aminomethane                     |
| TrxA             | Thyroxine domain A                                    |
| UV               | Ultra violet  |

## Note on Nomenclature

Gene names and symbols are written in *italics*, and proteins are in standard text. Wild-type genes and products are in uppercase, mutants are in lower case. For plant species, the two letters preceding a gene/protein refer to the species. At, *Arabidopsis thaliana*. St, *Solanum tuberosum*. Hv, *Hordeum vulgare*. Os, *Oryza sativa*. Bn, *Brassica napus*.