

Inter- and intra-molecular interactions of *Arabidopsis thaliana* DELLA protein RGL1

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The phytohormone gibberellin and the DELLA proteins act together to control key aspects of plant development. Gibberellin induces degradation of DELLA proteins by recruitment of an F-box protein using a molecular switch: a gibberellin-bound nuclear receptor interacts with the N-terminal domain of DELLA proteins, and this event primes the DELLA C-terminal domain for interaction with the F-box protein. However, the mechanism of signalling between the N- and C-terminal domains of DELLA proteins is unresolved. In the present study, we used *in vivo* and *in vitro* approaches to characterize di- and tri-partite interactions of the DELLA protein RGL1 (REPRESSOR OF GA1-3-LIKE 1) of *Arabidopsis thaliana* with the gibberellin receptor *GID1A* (GIBBERELIC ACID-INSENSITIVE DWARF-1A) and the F-box protein *SLY1* (SLEEPY1). Deuterium-exchange MS unequivocally showed that the entire N-terminal domain of RGL1 is disordered prior to interaction with the *GID1A*;

furthermore, association/dissociation kinetics, determined by surface plasmon resonance, predicts a two-state conformational change of the RGL1 N-terminal domain upon interaction with *GID1A*. Additionally, competition assays with monoclonal antibodies revealed that contacts mediated by the short helix Asp-Glu-Leu-Leu of the hallmark DELLA motif are not essential for the *GID1A*–RGL1 N-terminal domain interaction. Finally, yeast two- and three-hybrid experiments determined that unabated communication between N- and C-terminal domains of RGL1 is required for recruitment of the F-box protein *SLY1*.

Key words: *Arabidopsis thaliana*, DELLA protein, F-box protein, gibberellin, GIBBERELIC ACID-INSENSITIVE DWARF-1A (*GID1A*), SLEEPY1 (*SLY1*).

INTRODUCTION

Bioactive gibberellins promote the development of vegetative and floral tissues of plants, and are essential for seed germination [1,2]. These biological effects are mediated by large alterations to gene expression through a highly conserved signal transduction pathway [3–6]. Nuclear proteins of the DELLA family serve as a central regulatory switch of this pathway. Upon perception of gibberellin, the DELLA proteins are degraded, relieving repression on cell responses to gibberellin [7–9]. *Arabidopsis thaliana* possesses five partially redundant DELLA protein-encoding genes: *GAI* (GIBBERELIC ACID INSENSITIVE), *RGA* (REPRESSOR OF *GAI*-3), *RGL1* (*RGA*-LIKE 1), *RGL2* (*RGA*-LIKE 2) and *RGL3* (*RGA*-LIKE 3) [8,10–14].

DELLA proteins are a subfamily of the GRAS [*GAI*, *RGA* and *SCR* (SCARECROW)] family of plant regulatory proteins [15,16]. The DELLA subfamily is further defined by two conserved motifs in their N-terminal domain referred to as the DELLA and TVHYNP [8,10,15,17]. Analyses of the in-frame DELLA protein deletion mutants of these two conserved N-terminal elements, DELLA or TVHYNP, have shown that deletion of either motif results in gibberellin-insensitive plants [17]. In contrast, mutants lacking the C-terminal GRAS domain or containing mutations in this domain show constitutive gibberellin responses [17]. There are only two exceptions of C-terminal domain mutations that result in gibberellin insensitivity:

a single glutamine-to-arginine amino acid substitution near the centrally located VHID motif, and a glycine-to-valine substitution near the SAW motif, at the very C-terminus of the protein [18,19].

Two additional proteins of the gibberellin signalling pathway are required for the inactivation of DELLA proteins upon perception of the gibberellin signal: a receptor for gibberellin and an F-box protein, both localized in the nucleus. *A. thaliana* contains three gibberellin receptors (*GID1A*–*C*), homologues of the single *Oryza sativa* receptor *GID1* (GIBBERELIN INSENSITIVE DWARF-1) [20,21]. The gibberellin receptors of *O. sativa* and *A. thaliana* have been shown to interact with DELLA proteins in the presence of bioactive gibberellins; this interaction is required for degradation of DELLA proteins and for gibberellin responses [20–23]. High-resolution structures of liganded GA (gibberellic acid) receptor (*GID1A/GA_s*) have demonstrated that bioactive gibberellins fit into the substrate pocket of this enzymatically inactive esterase, and that an N-terminal extension forms a lid covering the bound hormone, creating the DELLA interaction interface [24,25]. In addition to the gibberellin receptors, a specific F-box protein *SLY1* (SLEEPY1) of *A. thaliana* or *GID2* in *O. sativa* is required for gibberellin-induced degradation of the DELLA proteins [26,27]. This F-box protein is part of an SCF (Skp1/cullin/F-box) E3 ubiquitin ligase that targets DELLA proteins for 26S proteasomal degradation in response to gibberellins [26,28].

Abbreviations used: DXMS, deuterium exchange MS; GA, gibberellic acid; *GAI*, GIBBERELIC ACID INSENSITIVE; GFP, green fluorescent protein; *GID1A*, GIBBERELIC ACID-INSENSITIVE DWARF-1; GRAS, *GAI*, REPRESSOR OF *GAI*-3 and SCARECROW; MBP, maltose-binding protein; *RGA*, REPRESSOR OF *GAI*-3; *RGL1*, REPRESSOR OF *GAI*-3-LIKE 1; *SCR*, SCARECROW; *SLR1*, SLENDER RICE1; *SLY1*, SLEEPY1; SPR, surface plasmon resonance.

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Yeast three-hybrid experiments have revealed that *GID1A* induces an interaction between *SLY1* and the *A. thaliana* DELLA protein RGA in a gibberellin-dependent manner, providing an explanation of how gibberellin-induced DELLA protein degradation is achieved [21]. Similarly, yeast three-hybrid experiments have also been used to show that *GID1* binding is required for the interaction between *O. sativa* *GID2* and *SLR1* (SLENDER RICE1) [19]. However, the mechanism of *SLY1* recruitment is still unclear, given that *GID1A* interacts with the N-terminal domain of RGA, whereas *SLY1* binds to the C-terminal domain, and that *GID1A* and *SLY1* do not interact with each other [21,23,24]. In the absence of biochemical and structural characterization of full-length DELLA proteins, structural information has been derived from the analysis of easily expressed N-terminal domains. A high-resolution structure of the complex formed by the truncated N-terminal domain of DELLA protein GAI and gibberellin-bound *GID1A* showed that a pair of helix-loop-helix motifs, corresponding to sequences that include conserved DELLA and TVHYNP motifs, form the interaction surface with the N-terminal domain of liganded *GID1A* [24]. However, the essentiality of these loops and helices for interaction with *GID1A* in the wild-type DELLA protein N-terminal domain has not been investigated. Furthermore, the implied conformational transitions of the N-terminal domain or full-length DELLA proteins have not been characterized. These transitions are the key to understanding how the interaction of the N-terminal domain of DELLA proteins with the liganded gibberellin receptor predisposes the C-terminal domain for binding the F-box protein *SLY1*.

Using *in vitro* and *in vivo* approaches, we characterized the structure of the N-terminal domain of *A. thaliana* DELLA protein RGL1 (termed RGL1^N) and its interactions with the gibberellin receptor *GID1A*, showing that the DELL segment of the hallmark DELLA motif is not essential for formation of the RGL1–*GID1A*/GA₄ complex. We present evidence that unabated N-to-C-terminal domain interaction is required for full priming of the C-terminal domain to recruit the F-box protein *SLY1*. We propose a new model of DELLA protein conformational transitions that co-ordinate perception and transduction of the gibberellin signal.

EXPERIMENTAL

Escherichia coli strains and growth conditions

Strain TG1 [29], used for cloning recombinant plasmids, was propagated in 2YT medium [1.6% (w/v) tryptone/1% (w/v) yeast extract/0.5% NaCl; BD Biosciences] at 37°C. Protein expression strains TUNER and TUNER (DE3) (EMD Biosciences) were propagated in 25 g/l tryptone, 7.5 g/l yeast extract, 3 g/l NaCl, 2 g/l D-glucose and 0.02 M Tris/HCl (pH 7.5). Medium was supplemented with ampicillin (100 µg/ml) or kanamycin (50 µg/ml) as appropriate for transformed strains.

Plasmid construction

RGL1 (At1g66350), *GID1A* (At3g05120) and *SLY1* (At4g24210) open reading frames were PCR-amplified from wild-type *A. thaliana* Columbia genomic DNA (*RGL1* and *SLY1*) or cDNA (*GID1A*–C).

RGL1 was cloned into XmaI/SacI-cleaved pACT2 (yeast two-hybrid system GAL4 activation domain fusion vector; Clontech). *rgl1*^{ΔDELLA} (deletion of residues 32–48; [8]), *rgl1*^{ΔTVHYNP} (deletion of residues 68–85; [17]) and *rgl1*^{Q272R} (nucleotide 815A→G; [18]) were generated by ligation-mediated PCR

mutagenesis [30] and cloned into the XmaI/SacI site of pACT2. RGL1^{1–137}–GFP–RGL1^{138–511} (GFP is green fluorescent protein) (RGL^N–GFP–RGL^C) was generated by step-wise overlap extension PCR [31], cloned into pCR-blunt (Invitrogen) and subsequently into the XmaI/SacI site of pACT2. The GFP-coding sequence corresponds to mGFP-4 [32].

GID1A was cloned into the NotI site of multiple cloning site II [tertiary HA (haemagglutinin) tag fusion expression] of pBridge (yeast three-hybrid system; Clontech) and into EcoRI/BamHI-cleaved pGBKT7 (yeast two-hybrid system GAL4 DNA-binding domain fusion vector; Clontech). *sly1*^{E138K} (nucleotide 412G→A; [27,28]) was generated by PCR using a mutagenic reverse primer. *SLY1* and *sly1*^{E138K} were cloned into EcoRI/BamHI-cleaved pGADT7 (yeast two-hybrid system GAL4 DNA-activation domain fusion vector; Clontech) and pBridge multiple cloning site I (GAL4 DNA-binding domain fusion) with and without *GID1A* in cloning site II.

For expression and purification, *GID1A* was excised from pGBKT7 and cloned into EcoRI/SalI-cleaved pMALc2x [MBP (maltose-binding protein) fusion expression vector; New England Biolabs]. *GID1B* and *GID1C* were excised from pGBKT7 and cloned into EcoRI/PstI-cleaved pMALc2x. RGL1^{1–137} (N-terminal or DELLA domain, referred to as RGL1^N throughout the manuscript) was amplified by PCR and cloned into BamHI/SalI-cleaved pMALc2x. Oligonucleotides (synthesized by Invitrogen) are listed in Supplementary Table S1 (at <http://www.BiochemJ.org/bj/435/bj4350629add.htm>). All constructs were confirmed by sequencing (Massey University Genome Services, Palmerston North, New Zealand). Cloning techniques were performed as described previously [33].

Yeast two- and three-hybrid assays

Preparation of competent yeast cells (strain CG-1945; Clontech) and transformation were performed using the Frozen-EZ yeast transformation kit (Zymo Research). A modified culture preparation protocol was performed for β-galactosidase assays: overnight cultures in synthetic dropout medium (minus leucine and tryptophan; Clontech) were diluted to a *D*₆₀₀ of 0.05 in medium supplemented with 100 µM GA₃ (or 1 nM–10 µM GA₃/GA₄ for dose–response experiments) in 200 µM Hepes/KOH (pH 7.8), or 200 µM Hepes/KOH (pH 7.8) as a control. Cultures were incubated at 30°C with rotational agitation (250 rev./min) for exactly 20 h; harvested cells were used for liquid *o*-nitrophenyl β-D-galactopyranoside assays as described in the Clontech yeast two-hybrid system-3 instruction manual. Each assay had nine replicates (three cultures of three independent transformants).

Recombinant protein expression and purification

RGL1^N was purified as an MBP-fusion protein by ion-exchange chromatography and amylose affinity purification according to methods described previously [34]. *GID1A* was expressed and purified as an MBP-fusion protein as described in the pMAL system manual (New England Biolabs), with the following exceptions: expression was induced at 20°C with 100 µM IPTG (isopropyl β-D-thiogalactopyranoside) for 4 h. The cells were harvested by centrifugation and resuspended in ice-cold 0.01 M Hepes/NaOH (pH 7.5), 0.125 M NaCl, 0.1% Triton X-100, 0.1% octyl β-D-glucopyranoside, 1 mM EDTA, 1 mM DTT (dithiothreitol) and protease inhibitor cocktail (Sigma P2714, 1 vial/litre) to a *D*₆₀₀ of 50, followed by subsequent lysis by addition of 100 µg/ml chicken lysozyme (Roche), 50 µg/ml DNase I (Sigma) and 10 mM MgCl₂. The MBP (MBP–β-galactosidase-α,

expressed from the unmodified vector pMALc2x) was prepared as described in the pMAL system manual. Protein concentrations were determined by fluorimetry (Qubit, Invitrogen).

Antibody production

A rabbit polyclonal antibody (anti-MBP) was produced using purified protein as an immunogen by Immunology Services, AgResearch, Ruakura, New Zealand. Handling of animals to produce the antibody was carried out according to the AgResearch code of ethical conduct for the use of live animals for research and was approved by the AgResearch Animal Ethics Committee. Antisera were fractionated by ammonium sulfate precipitation as described previously [35], and subsequently affinity-purified using MBP immobilized on AminoLink Plus resin (5 mg/ml resin) as described in the manufacturer's protocol (co-immunoprecipitation kit; Pierce).

Mouse monoclonal antibodies against the RGL1 N-terminal domain (AB8, AD7, BC9) have been described previously [36]. The monoclonal antibody 6C8 was raised against the synthetic peptide DELLAVLGYK and the contact residues (DELL) were determined by alanine scanning of the corresponding synthetic peptide, as described previously [36].

DXMS (deuterium exchange MS)

To optimize the fragmentation conditions for maximal peptide coverage, 10 μ l of MBP-RGL1^N solution in PBS was diluted with 30 μ l of water and then quenched with 60 μ l of 0.8% formic acid containing various concentrations of guanidinium chloride (0.08, 0.8 and 1.6 M) at 0°C, and frozen on solid CO₂. The frozen quenched samples were thawed at 0°C and then immediately loaded on to an immobilized porcine pepsin column for digestion, collected using a C₁₈ column (Vydac) and then eluted out with a linear gradient of 6.4–38.4% acetonitrile over 30 min. The eluate was then transferred to a LCQ classic mass spectrometer (Thermo Finnigan) for analysis, with data acquisition in either MS1 profile mode or data-dependent MS2 mode. SEQUEST software (Thermo Finnigan) combined with DXMS Explorer (Sierra Analytics) were used to generate the peptide coverage maps for different quench conditions, and the best one was used for the ¹H/²H exchange experiment. The ¹H/²H exchange experiments were initiated by adding 10 μ l of MBP-RGL1^N stock solution into 30 μ l of deuterated water for time intervals of 10, 100, 1000 and 3000 s at 0°C. The exchange reaction was quenched by the addition of 60 μ l of 1 M guanidinium chloride and immediately frozen at –80°C. The frozen samples, along with control samples of non-deuterated and fully deuterated, were then subjected to the above DXMS apparatus for analysis. The centroids of isotopic envelopes of non-deuterated, partially deuterated and fully deuterated peptides were measured using DXMS Explorer, and then converted into the deuteration level with corrections for back-exchange [37,38]. The deuteron recovery of fully deuterated sample was, on average, 80%.

Interaction analyses using SPR (surface plasmon resonance)

BIAcore X and CM5 chips (GE Healthcare) with cross-linked anti-MBP rabbit polyclonal antibody were used for all SPR experiments. Ligands (purified MBP-RGL1^N and the MBP tag control) were captured, and the remaining MBP-binding sites were blocked by saturation with purified MBP tag. Binding and dissociation of analyte, the MBP fusion of GID1A (MBP-GID1A) to both MBP-RGL1^N and MBP tag control flow cells was

then measured. Simultaneous parallel MBP tag control binding traces were subsequently subtracted from MBP-RGL1^N binding traces. Assays were performed at 25°C in HBS-EP buffer (GE Healthcare), at a flow rate of 10 μ l/min over 7 min (total injected volume of 70 μ l). MBP tag and MBP-RGL1^N were used at 0.5 μ M; MBP-GID1A was used at 0.1–1.6 μ M (kinetic studies); MBP-GID1A was used at 0.2 μ M (binding assays) or 0.1 μ M (competition assays); and monoclonal antibodies were used at 0.5 μ M (competition assays). GA₄ was added to MBP-GID1A samples at 100 μ M in binding assays, or 5 μ M in competition assays, 30 min prior to injection. Gibberellins were absent from all other solutions.

Analysis of association/dissociation kinetics

Binding and kinetics were calculated using BiaEvaluation software version 3.1. Binding for competition experiments was determined by the mass bound at the end of the 7 min (420 s) association phase subjected to the following transformations: binding of analyte to the control flow cell (MBP tag) was subtracted, and standardization based on the mass of MBP-RGL1^N bound to the chip. Binding is expressed in RU (response units; 1 RU \approx 1 pg/mm²) and further converted into fmol/mm², using the following molecular mass values: antibodies, 150 kDa; MBP-GID1A, 81.6 kDa. Association and dissociation data were simultaneously fitted to a two-state conformational change model for interaction. Binding was determined as 1:1 through identical binding capacity of immobilized RGL1^N for recombinant GID1A/GA₄ and the monoclonal antibody BC9 (results not shown).

RESULTS

The free N-terminal DELLA domain of RGL1 is unstructured along its entire length

To initiate the present study of the DELLA protein gibberellin-triggered molecular switch, we first determined the structure of the unbound form of the N-terminal domain of RGL1, one of five *A. thaliana* DELLA proteins. The full-length *A. thaliana* DELLA proteins, including RGL1, are either insoluble or marginally soluble in most expression systems, preventing structural analysis of the complete proteins [24,36].

DELLA protein N-terminal domains have been reported to be disordered before binding to the liganded gibberellin receptors, based on their hydrodynamic properties and structure prediction programs [24,36]. These methods are either based on bioinformatic/statistical approaches or physicochemical methods that produce an averaged signal from all residues of a protein, hence it is not known whether all residues of these domains are unstructured, or if a short sequence motif (e.g. residues within the conserved DELLA or TVHYNP motif) could possibly form secondary or tertiary structures. To identify unstructured compared with structured (i.e. exposed compared with blocked) residues, the exchange of the peptide-backbone-bound hydrogen with deuterium from ²H₂O-based buffer was mapped using DXMS in the recombinant RGL1 N-terminal domain (RGL1^N). A fusion of RGL1^N with MBP (MBP-RGL1^N) was analysed, in which the MBP moiety, whose high-resolution structure is known, served as a gauge, demonstrating that overall folding of this purified recombinant protein fusion was not disturbed (Figure 1). The MBP exhibited regions of inaccessibility to solvent, consistent with its known secondary and tertiary structure [39]. In contrast, the RGL1^N (RGL1^{1–137}) moiety of this fusion underwent instantaneous and complete deuterium exchange along

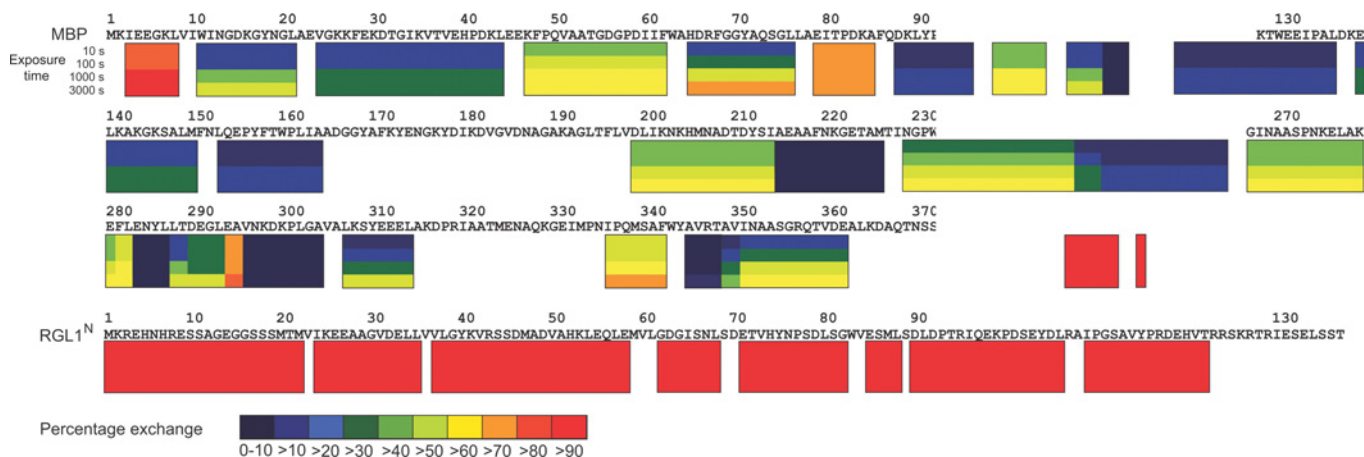


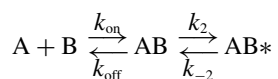
Figure 1 Solvent accessibility of the RGL1 N-terminal DELLA domain in the absence of GID1A

DXMS of the MBP–RGL^N fusion protein. The percentage of ¹H–²H exchange of the peptide backbone, as determined by MS, following exposure to deuterated water for 10–3000 s time intervals is shown.

its whole length (Figure 1). No protected regions were detected, showing that, in the absence of the C-terminal domain and the liganded GA receptor, the RGL1 N-terminal domain is completely disordered along its entire length, including the conserved motifs.

In vitro kinetics of the GID1A interaction with the N-terminal DELLA domain of RGL1

To characterize the kinetics of interaction between the N-terminal domain of RGL1 and GID1A/GA₄, association and dissociation were monitored in real-time using SPR (Figure 2A). A Scatchard plot (dR/dt against R) of the association phase, and $\ln(R_0/R)$ against time transformation of the dissociation phase were non-linear (Figures 2B–2C). Therefore this interaction does not fit Langmuir kinetics (1:1, $A+B \leftrightarrow AB$) [40–42]. Interaction curves for 100–400 nM GID1A were fitted to the following model (residual plots are shown in Supplementary Figure S1 at <http://www.BiochemJ.org/bj/435/bj4350629add.htm>):



where A is GID1A/GA₄, B is RGL1^N and * indicates a trapped conformational state (Table 1). Trivial causes of non-Langmuir kinetics were experimentally eliminated (see the Experimental section). Furthermore, kinetic characterization of the reverse interaction experiment was consistent with this model (Supplementary Figure S2 at <http://www.BiochemJ.org/bj/435/bj4350629add.htm>). The calculated k_{on} was $1.6 \times 10^5 \pm 1.6 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$ and k_{off} was $4.1 \times 10^{-3} \pm 9.6 \times 10^{-4} \text{ s}^{-1}$, whereas the constants for conformational change were $k_2 = 5.1 \times 10^{-3} \pm 5.5 \times 10^{-4} \text{ s}^{-1}$, and $k_{-2} = 3.8 \times 10^{-4} \pm 8.4 \times 10^{-6} \text{ s}^{-1}$, for the forward and reverse directions respectively (error value shows ± 1 S.D.). The overall equilibrium constant K was determined to be $5.3 \times 10^8 \pm 1.5 \times 10^8 \text{ M}^{-1}$, representing a strong interaction.

The N-terminal DELLA domains on their own are intrinsically unstructured (Figure 1), but when in complex with liganded GID1A they possess ordered secondary and tertiary structure [24]. Given that the conformation of liganded GID1A is not changed upon binding to the N-terminal domain of DELLA proteins [24,25], the conformational transition determined in the present

study by measuring RGL1^N–GID1A/GA₄ interaction kinetics is attributed to the binding-induced folding of RGL1^N.

Probing the GID1A/GA₄–RGL1 interactions by competition

Residues of DELLA proteins that mediate interaction with GID1 gibberellin receptors have, in the past, been deduced on the basis of deletion mutants of the conserved DELLA and TVHYNP motifs, or nested deletions of the DELLA proteins [21,23]. More recently, the contact residues of the N-terminal domain of DELLA protein GAI were identified in the high-resolution structure of a GID1A/GA₃–GAI^{11–113} complex [24]. However, no competition experiments have been performed yet to probe, in the context of the intact N-terminal domain, which of the contacts mediated by particular DELLA/TVHYNP motifs are required for interaction with gibberellin-liganded GID1A. We took advantage of a suite of anti-DELLA protein monoclonal antibodies specific for the DELLA and TVHYNP motifs (see the Experimental section) and applied them to *in vitro* competition assays using SPR to examine the role of the DELLA and TVHYNP motifs of RGL1 in interaction with GID1A/GA₄ (Figure 3).

Three monoclonal antibodies, 6C8, BC9 and AD7, whose epitopes overlap with GID1A contact residues, were used in these experiments (Figure 3). The antibody 6C8 binds to the Asp-Glu-Leu-Leu residues that, in GAI^{11–113} form a short GID1A/GA₃-interacting N-terminal helix αA [24]. Modelling of RGL1 using SwissModel [43] and GAI^{11–131}–GID1A/GA₃ co-ordinates predicts that, as in GAI, the Asp-Glu-Leu-Leu helix forms contacts with both the GID1A core domain and the N-terminal extension that covers the bound gibberellin (Supplementary Figure S3 at <http://www.BiochemJ.org/bj/435/bj4350629add.htm>). Our competition experiments (Figure 3) show that the antibody 6C8 has only a minor effect on binding of the liganded GID1A to RGL1, indicating that the Asp-Glu-Leu-Leu residues are not essential for the GID1A/GA₄–RGL1 interaction. The 6C8 effect is similar to that of a control RGL1-specific monoclonal antibody, AB8, which does not recognize the conserved DELLA and TVHYNP motifs of RGL1, but whose epitope lies within the N-terminal domain (Figure 3). Next, competition with the antibody BC9, which binds to highly conserved residues Val-Xaa-Xaa-Tyr-Xaa-Val-Arg immediately downstream of the Asp-Glu-Leu-Leu residues within the DELLA motif, was assayed (Figure 3).

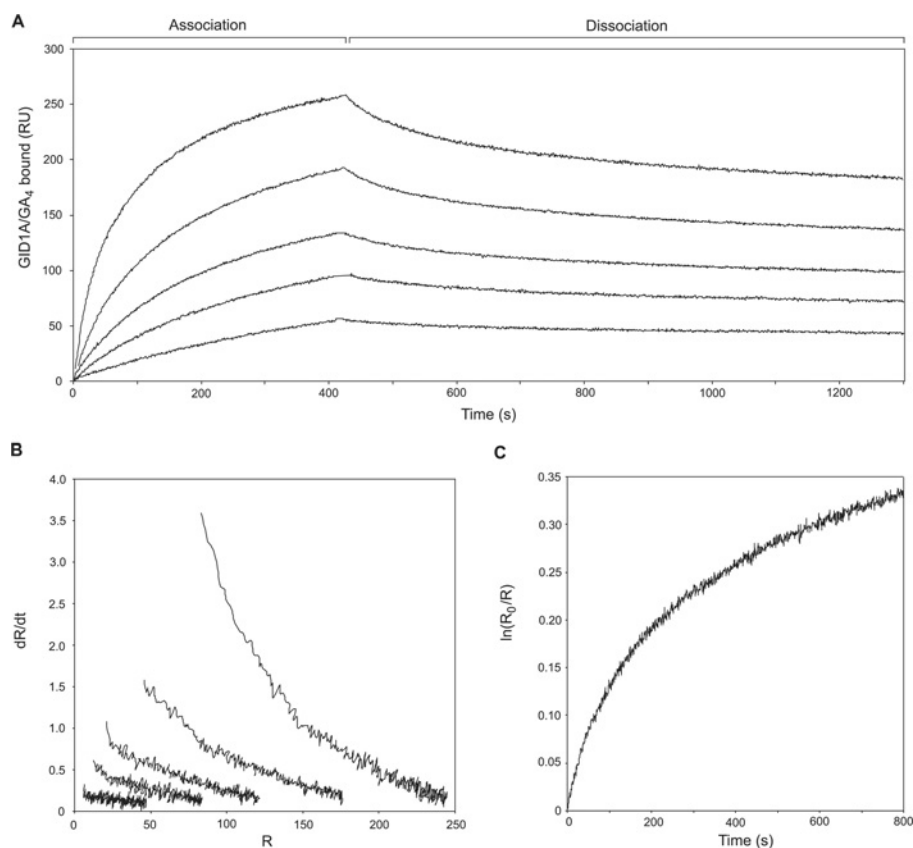


Figure 2 Kinetic characterization of the *in vitro* interaction of GID1A/GA₄ with the N-terminal domain of RGL1

(A) GA₄-dependent association (0–420 s), and dissociation (420–1300 s), of 100 nM, 200 nM, 400 nM, 800 nM and 1600 nM solutions of GID1A with RGL1^N. GA₄ (100 μM) was mixed with GID1A 30 min prior to binding and excluded during the dissociation phase. (B) Scatchard plot (dR/dt against R) of the association phase where R is response [in RU (response units)] and t is time (s). (C) $\ln(R_0/R)$ against time linearization transformation of the dissociation phase (shown for 1600 nM GID1A).

Table 1 Kinetics of the GA₄-dependent interaction between GID1A and RGL1^N

GID1A concentration (nM)	Active concentration (nM)*	k_{on} (M ⁻¹ ·s ⁻¹)†	k_{off} (s ⁻¹)†	k_2 (s ⁻¹)†	k_{-2} (s ⁻¹)†	K (M ⁻¹)†	R_{max} †	Ψ^2 ‡
100	10.5	1.6×10^5	3.7×10^{-3}	5.6×10^{-3}	3.7×10^{-4}	6.49×10^8	150	1.32
200	20.0	1.7×10^5	3.5×10^{-3}	4.5×10^{-3}	3.8×10^{-4}	5.73×10^8	160	1.26
400	36.6	1.4×10^5	5.3×10^{-3}	5.1×10^{-3}	3.9×10^{-4}	3.58×10^8	201	1.63

*Active concentration is the total concentration of dimeric and monomeric GID1A as determined by the densitometry of native electrophoresis-separated solutions.

†Association rate and other constants were obtained by simultaneous fitting of association and dissociation kinetics using BiaEvaluation software version 3.1.

‡ Ψ^2 , measure of closeness of fit; mean variance (response units) of data points from the model.

Of the BC9 epitope residues, valine, tryosine and valine are located in a loop (AB) between helices αA and αB [24]. This AB loop forms several contacts with the N-terminal extension of liganded GID1A, as determined in the high-resolution structure of the GID1A/GA₃-GAI^{I1-113} complex [24] and modelled for RGL1^N. BC9 strongly competed for binding of RGL1 to the liganded GID1A (Figure 3), as we have shown previously for endogenous GID1C extracted from *A. thaliana* [36]. The third antibody, AD7, interacts with the His-Tyr-Asn-Pro-Ser-Asp residues within the conserved TVHYNP motif (Figure 3). These epitope residues correspond to the CD loop connecting the αC and αD helices in the high-resolution structure. The proline residue of the AD7 epitope directly contacts the liganded GID1A (Supplementary Figure S3 at <http://www.BiochemJ.org/bj/435/bj4350629add.htm>)

[24]. This antibody strongly competed with binding of GID1A/GA₄, showing that the interactions mediated by epitope residues are essential for formation of the RGL1^N-GID1A/GA complex. Taken together, these competition experiments determined that contacts mediated by the TVHYNP motif (His-Tyr-Asn-Pro-Ser-Asp), which forms the CD loop, and by the distal portion of the DELLA motif (Val-Leu-Gly-Tyr-Lys-Val-Arg), which forms the AB loop, are required for the RGL1^N-GID1A/GA₄ interaction. Unexpectedly, competition with antibody 6C8 showed that the contacts mediated by the short Asp-Glu-Leu-Leu helix are not necessary for RGL1^N-GID1A/GA₄ interaction, and these could potentially be involved in communication with the C-terminal domain in regulating the access of the F-box protein SLY1.

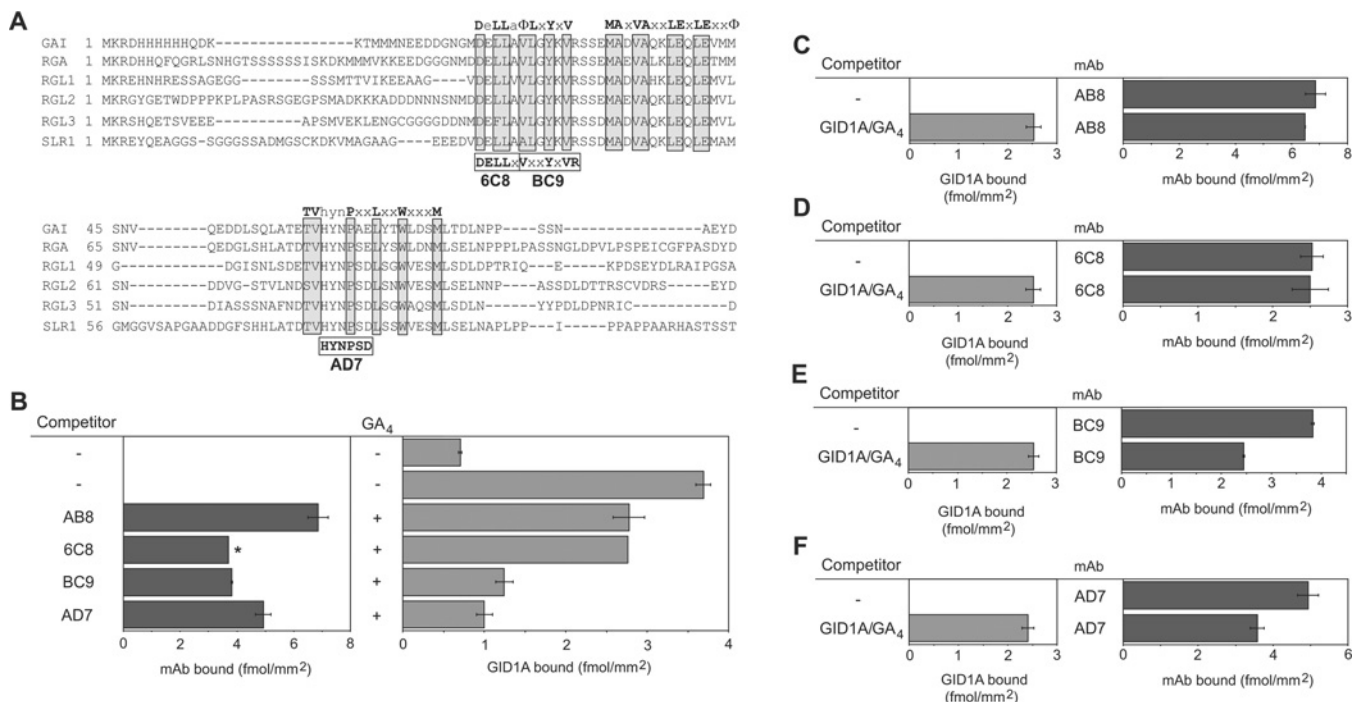


Figure 3 *In vitro* mapping of the GID1A/GA₄-interacting residues of RGL1 by monoclonal antibody competition

(A) ClustalW alignment of DELLA protein N-terminal domain primary sequences: *O. sativa* SLR1, and *A. thaliana* RGA, GAI, RGL1, RGL2 and RGL3. GID1A/GA₄-interacting residues as determined for GAI [24] are indicated above the alignment. Contact residues of the monoclonal antibodies 6C8, BC9 and AD7 are indicated below the alignments. (B) *In vitro* competition of binding of GID1A/GA₄ to immobilized RGL1^N. The mass of pre-bound anti-RGL1 monoclonal antibodies (mAb) including non-competing AB8 are shown as fmol of bound protein per mm². SPR-determined GID1A bound from solution to immobilized RGL1–mAb complexes is shown as fmol of bound protein per mm². The mass of GID1A bound from a continuous flow of 100 nM GID1A following 420 s is shown. The asterisk indicates that, owing to a low rate of association, the mAb 6C8 was pre-incubated with RGL1^N for 30 min prior to RGL1^N capture, thus, instead of direct measurement of mAb capture, the quantity of 6C8 bound was determined by the mass of RGL1^N/6C8 captured, minus the mass of RGL1^N captured in control experiments. (C–F) Reverse competition of monoclonal antibody binding to immobilized RGL1^N, in the presence or absence of pre-bound GID1A/GA₄. In all competition assays (except the no-gibberellin control) 5 μM GA₄ was mixed with GID1A 30 min prior to binding assays. Assays were performed in duplicate, and plots of simultaneous parallel experiments omitting RGL1^N were subtracted. Error bars show ± 1 S.D.

The N (DELLA) -to-C (GRAS) domain communication within RGL1 is required for recruitment of the F-box protein SLY1

Having identified conformational transitions of the RGL1 N-terminal domain upon binding to liganded GA receptor, and residues of the conserved motifs that are required for this interaction, we sought to investigate how the RGL1^N–GID1A/GA interaction primes the C-terminal domain for binding to the F-box protein SLY1 (Figure 1) [19,21]. Other full-length DELLA proteins which can be co-expressed in *Saccharomyces cerevisiae* with the gibberellin receptor GID1A and/or F-box protein SLY1 (GID1 and GID2 in *O. sativa*), have been analysed by yeast two- and three-hybrid interaction reporter systems for GID1A-gibberellin-primed recruitment of the F-box protein SLY1 to the C-terminal domain of DELLA proteins [19–23,44]. We first confirmed that RGL1 exhibits di- and tri-partite interactions with GID1A/GA₃ and SLY1 reported for other DELLA proteins in yeast two- and three-hybrid system (Figure 4 and Supplementary Figure S4 at <http://www.BiochemJ.org/bj/435/bj4350629add.htm>). Importantly, GID1A primes RGL1 for interaction with SLY1 in a GA₃-dependent manner, as has been shown for the DELLA proteins RGA and SLR1 (Figure 4) [19,21]. Furthermore, deletion mutations of the key motifs in the N-terminal domain of RGL1, ΔDELLA, a 17-amino-acid residue deletion [8,11,45], and ΔTVHYNP, an 18-residue deletion [17] prevented binding of GID1A/GA₃ and abolished the subsequent recruitment of

SLY1 to RGL1 (Figure 4). DELLA protein alignment with the indicated mutations is shown in Supplementary Figure S5 (at <http://www.BiochemJ.org/bj/435/bj4350629add.htm>).

We further investigated the recruitment of a gain-of-function mutant, sly1^{E138K}, that shows increased binding to DELLA proteins RGA and GAI in the absence of GID1A-C/gibberellin [27]. The GID1A/GA₃-dependent recruitment of the sly1^{E138K} mutant has not to date been investigated. As expected, this mutant demonstrated constitutive (GID1A/GA₃-independent) recruitment to the rg11^{ΔDELLA} mutant (it was originally isolated as a dominant gain-of-function suppressor of equivalent *A. thaliana* gai mutation [27,28,46]). Interestingly, sly1^{E138K} showed strong GID1A/GA₃-dependent recruitment to wild-type RGL1, which exceeded that of the ΔDELLA mutant in the presence of GID1A/GA₃ by a factor of 4. In contrast, its recruitment to the wild-type RGL1 in the absence of GID1A/GA₃ was less prominent than to the rg11^{ΔDELLA} mutant, suggesting an inhibitory role of the DELLA motif in sly1^{E138K} recruitment in the absence of liganded gibberellin receptor. The RGL1 ΔTVHYNP mutant could not recruit sly1^{E138K}, suggesting an essential role for the TVHYNP in sly1^{E138K} recruitment. The SLY1 Glu¹³⁸ is located in the LSL domain, which has been mapped as a DELLA protein-interacting domain [28]. Interestingly, this residue in the *O. sativa* homologue GID2 is glutamine, hence it is not conserved between SLY1 and GID2, which share only 44% sequence identity. It has been reported recently that replacement of this and an adjacent asparagine residue with alanine residues greatly

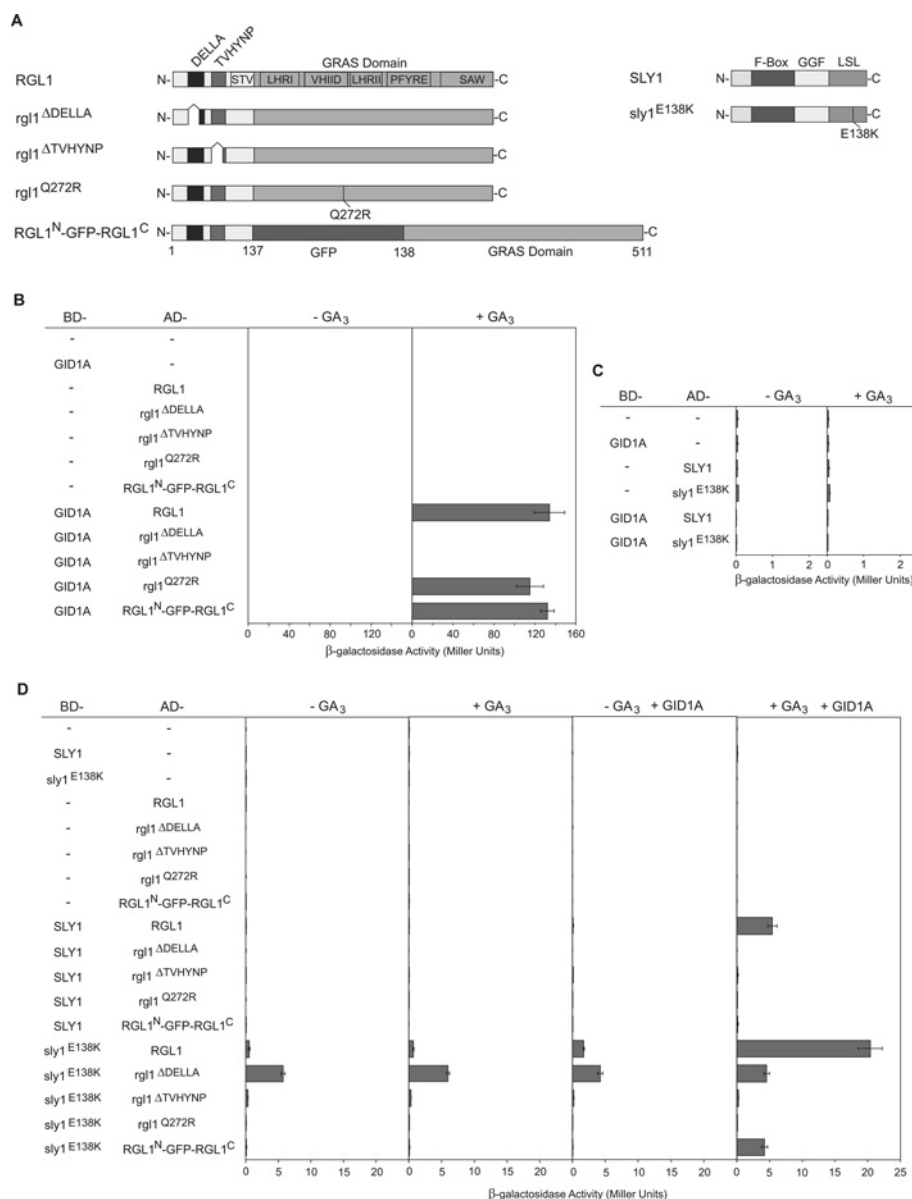


Figure 4 Mapping of gibberellin-induced interactions of GID1A and SLY1 with RGL1

(A) Schematic representation of RGL1 and SLY1 protein domain organization, including the in-frame deletion mutants RGL1^{ΔDELLA} (RGL1 Δ DELLVVLGYKVRSSDMA) and RGL1^{ΔTVHYNP} (RGL1 Δ NLSDETVHYNPSDLSGWV), the point mutants RGL1^{Q272R} and SLY1^{E138K}, and the interrupted RGL1–GFP fusion RGL1^N-GFP-RGL1^C (RGL1^{1–137}-GFP-RGL1^{138–511}). DELLA, TVHYNP and GRAS are hallmark DELLA protein motifs in RGL1; F-box and LSL are domains of SLY1. (B–D) Yeast two- and three-hybrid assays of RGL1, GID1A and SLY1, and derived mutant proteins described in (A) \pm GA₃, cultures grown in the absence or presence of 100 μ M GA₃; BD-, Gal4 DNA-binding domain fusions; AD-, Gal4 activation-domain fusions. GID1A in (D) is the bridge protein (not fused to Gal4 domains). LacZ (β -galactosidase) activity values were obtained from nine assays (triplicate assays for each of three independent transformants). Error bars show \pm 1 S.D.

decreased binding of GID2 to the rice DELLA protein SLR1 [19]. Therefore Glu¹³⁸ lies on the interaction surface, and the change to a positively charged residue highly increases the affinity of the F-box protein to its target.

The C-terminal (GRAS) domain mutations in DELLA proteins normally result in constitutive gibberellin responses, owing to failure to bind repression targets [47,48]. However, a C-terminal domain mutation, near the conserved VHIID motif, identified in the *Brassica napus* GAI protein, causes a gibberellin-insensitive phenotype characteristic of the N-terminal mutations in the conserved DELLA and TVHYNP motifs [18]. We investigated the effect of this mutation in RGL1 (rgl1^{Q272R}) on the GID1A-mediated recruitment of SLY1. When

assessed using the yeast two-hybrid system, the rgl1^{Q272R} mutation did not interfere with GA₃-dependent binding of the GID1A receptor; however, it prevented the recruitment of wild-type SLY1 (Figure 4). Furthermore, this mutation also prevented the recruitment of the dominant gain-of-function mutant sly1^{E138K}. These results demonstrate that the rgl1^{Q272R} mutation does not affect interactions with liganded GA receptor, but disrupts the interaction of RGL1 with SLY1, most probably by directly affecting contact residues between RGL1 and SLY1. Interestingly, the corresponding conserved glutamine residue of the *O. sativa* DELLA protein SLR1 was mapped by alanine scanning to the F-box protein binding segment, with double-replacement of residues leucine/glutamine to alanine/alanine resulting in failure to interact

not only with the F-box protein, but also with liganded GID1. This disagreement with our findings could be attributable to an additional Leu→Ala change in SLR1, and/or replacement of Gln²⁷² by a different residue (Gln→Arg in RGL1 as opposed to Gln→Ala in SLR1).

From the findings above, and from recently reported mutagenesis data in the *O. sativa* GID1A, DELLA and SLY1 homologues [19], it is clear that interaction of the GID1A/gibberellin with the N-terminal domain of DELLA proteins 'primes' the C-domain for interaction with SLY1. This priming event is likely to occur through communication of a signal from a GID1A/gibberellin 'anchor', the N-terminal DELLA domain, to the C-terminal GRAS domain of RGL1; however, the nature of this priming event or its mechanism are unclear. Most significantly, no direct evidence for N-to-C-terminal communication has been presented to date. Given that the N-terminal domain undergoes transition from disordered to ordered upon binding to liganded GID1A, it is likely that this transition is somehow transduced to the C-terminal domain. To test this hypothesis, we spatially separated the N- and C-terminal domains of RGL1 by inserting GFP between them, to obtain an interrupted RGL1-fusion protein RGL1^N-GFP-RGL1^C. The GA₃-dependent GID1A interaction (Figure 4) was not affected by the GFP insertion, hence N- and C-terminal domain separation did not affect the N-terminal domain interaction with GID1A/GA₃. However, GID1A/GA₃ binding to the N-terminal domain failed to recruit wild-type SLY1 to the C-terminal domain of RGL1^N-GFP-RGL1^C in the presence of GID1A/GA₃. In contrast with the wild-type SLY1, the dominant sly1^{E138K} mutant interacted with RGL1^N-GFP-RGL1^C in a GID1A/GA₃-dependent fashion, albeit with less strength than with the wild-type RGL1. Recruitment of sly1^{E138K} demonstrates that the folding of the C-terminal domain within the RGL1^N-GFP-RGL1^C fusion protein is not disrupted by insertion of GFP. Moreover, the recruitment of dominant sly1^{E138K} and failure to recruit the wild-type SLY1 suggests that the priming event in RGL1^N-GFP-RGL1^C is incomplete, and that for the complete priming the N- (DELLA) and C- (GRAS) terminal domains of RGL1 have to be in close proximity.

DISCUSSION

The gibberellin-operated DELLA protein switch recruits F-box protein through an N-to-C-terminal interdomain priming event. Recent work suggests that, although the liganded receptor binds to the N-terminal domain, the C-terminal domain may also participate in stabilization of this interaction [19]. However, given that the C-terminal domain receptor interaction is secondary to receptor binding to the N-terminal domain, the question remains of whether and how the C-terminal domain receives the information of the necessary primary event – the receptor binding the N-terminal domain. We have now provided several insights into the structure and interactions of the *A. thaliana* DELLA protein RGL1 which provide information on the N-terminal domain interactions with the liganded receptor, and demonstrated the requirement of an unabated N-to-C-terminal domain link for the priming event.

GID1A/gibberellin-RGL1 N-terminal DELLA domain interaction kinetics

The N-terminal domain of *A. thaliana* DELLA proteins in the absence of liganded GID1A has been reported to be an intrinsically unstructured protein, based on the hydrodynamic properties, NMR and CD spectra [24,36]. The methods used

thus far, however, cannot analyse the structure of the N-terminal domains at a single-residue resolution. Using DXMS, we have now shown directly that in the absence of the C-terminal domain, peptide backbone-bound protons of all residues along the N-terminal domain of DELLA protein RGL1 (RGL1¹⁻¹³⁷) instantaneously exchange with ²H ions in the solution, proving that it is a disordered protein along its whole length.

As no kinetic data has yet been available to describe the conformational changes that the N-terminal DELLA domains must undergo on binding to GID1 gibberellin receptors, we have used SPR to measure and model association/dissociation kinetics of the N-terminal domain interaction with the liganded receptor. This analysis showed that the interaction between the N-terminal DELLA domain of RGL1 and the gibberellin receptor GID1A consists of two different conformational states, suggesting that the folding of the N-terminal domain occurs after interaction with the liganded receptor.

Contacts of the DELL (αA) helix of RGL1 are not essential for an interaction with GID1A/GA₄

The high-resolution structure of the GID1A/GA₃-GAI¹¹⁻¹¹³ complex has identified the contact residues of the N-terminal fragment of DELLA protein GAI in the complex with GID1A/GA₃ [24], which correspond to the regions around two conserved N-terminal domain motifs, DELLA and TVHYNP. The gibberellin-insensitive mutations of *DELLA* genes analysed to date for interactions with the liganded GID1 receptor, or recruitment of the F-box protein SLY1 (GID2) contain mutations encompassing either the DELLA or TVHYNP motifs [15]. Our analysis using competition with monoclonal antibodies had an advantage that it could examine the essentiality of contacts determined by crystallography without mutating the DELLA proteins. This analysis confirmed that contacts by TVHYNP motif are required for the RGL1-GID1A/GA₄ interaction. In contrast, monoclonal antibodies that recognize adjacent sets of residues within the DELLA motif, Asp-Glu-Leu-Leu (6C8) and Val-Leu-Gly-Tyr-Lys-Val-Arg (BC9), showed that, whereas the Val-Leu-Gly-Tyr-Lys-Val-Arg heptapeptide that forms the AB loop is required for RGL1-GID1A/GA₄ complex formation, the αA helix (Asp-Glu-Leu-Leu) is not essential. The significance of this finding is that the αA helix may be available for interactions with unknown proteins or the C-terminal GRAS domain while part of the complex with GID1A/GA₄.

GID1A/gibberellin-dependent recruitment of SLY1 to RGL1

Given that the key effect of GID1A/GA binding to the N-terminal domain of DELLA protein is major conformational transition, the question remains as to whether these changes need to take place in the close proximity of the C-terminal domain in order to prime it for interaction with the F-box protein SLY1. To test this hypothesis, we spatially separated the N-terminal DELLA domain from the C-terminal GRAS domain by insertion of the GFP in between them (RGL1^N-GFP-RGL1^C). Binding of the liganded gibberellin receptor (GID1A/GA₃) to the N-terminus of RGL1^N-GFP-RGL1^C was not affected by separation of the C-terminal domain, confirming that the fusion contained a functional N-terminal domain and that binding to GID1A/GA₃ did not depend on the C-terminal domain. To demonstrate functionality of the C-terminal domain in the interrupted RGL1^N-GFP-RGL1^C fusion protein, we took advantage of the dominant gain-of-function SLY1 mutant sly1^{E138K}. This mutant is recruited to RGL1 that contained 17-residue DELLA motif deletion in the

absence (and independently) of liganded gibberellin receptor (GID1A/GA₃ [27,28]). The sly1^{E138K} was also recruited to RGL1^N-GFP-RGL1^C, showing that the failure to recruit the wild-type SLY1 was not due to misfolding of the C-terminal domain, but rather due to the impaired priming event. In the RGL1^N-GFP-RGL1^C construct, where the two domains of RGL1 are spatially separated, the flexible unstructured N-terminal DELLA domain is intact and probably flexible enough to stretch the additional 20 Å (1 Å = 0.1 nm) [39] to its cognate binding site of the GRAS domain. However, upon interaction of the DELLA domain with gibberellin-liganded GID1A, the formation of secondary and tertiary structure decreases the flexibility of this domain and it may no longer be able to bind efficiently to its cognate binding site within the GRAS domain to induce further putative structural changes that could fully open the SLY1-binding site.

Interestingly, recruitment of sly1^{E138K} was GID1A/GA₃-dependent for both the wild-type RGL1 and RGL1^N-GFP-RGL1^C, whereas it was constitutive for rgl1^{ΔDELLA}. Furthermore, in the absence of GID1A/GA₃, the recruitment of sly1^{E138K} to the rgl1^{ΔDELLA} was more prominent than that of the wild-type RGL1 and RGL1^N-GFP-RGL1^C. A possible explanation for both positive and negative effects of deleted residues on sly1^{E138K} recruitment by the rgl1^{ΔDELLA} mutant is that the Aα helix (Asp-Glu-Leu-Leu), which we showed not to be required for the liganded GID1A interaction, forms contacts with the C-terminal GRAS domain and induces structural changes that prevent access of SLY1 to its cognate binding site. In contrast, the downstream AB loop, missing in rgl1^{ΔDELLA}, may be required for priming of the C-terminal domain by indirectly removing the Aα helix (Asp-Glu-Leu-Leu) from the C-terminal domain upon interaction with liganded GID1A.

In contrast with the ΔDELLA mutant, deletion of the downstream N-terminal motif of RGL1, ΔTVHYNP, abolished recruitment of sly1^{E138K}, suggesting that the latter motif is absolutely required for the recruitment of the F-box protein to the C-terminal domain of RGL1, possibly through a direct interaction. Indeed, two single amino acid mutations of conserved residues within the TVHYNP motif of SLR1 have been reported to result in semi-dwarfism, yet still interact with GID1 with only partially reduced affinity [49]. The reported mutations of the TVHYNP motif must therefore affect functions or interactions of SLR1, other than binding to GID1, to cause the dwarfing phenotype. The effect of these mutations on interactions involving the F-box protein GID2 has not been tested.

Recently, a Gly→Val substitution near the SAW motif of SLR1 was shown to weaken the interaction between GID1 and SLR1 [19]. The authors of that study propose this region as an additional GID1-interaction surface; however, the SAW domain could also be a potential site of intramolecular interaction with the DELLA motif. In contrast with SLR1, the RGL1 N-terminal-domain interaction with GID1A/GA₃ is not affected by separation from the RGL1 C-terminal domain or by the Q272R mutation in the C-terminal domain of RGL, suggesting that, in RGL1, the C-terminal domain plays no, or a minimal role, in interaction with the GID1A/GA₃. Therefore the RGL1 interaction with liganded GID1A has different requirements from the SLR1 interaction with liganded GID1.

A model of DELLA protein conformational transitions

On the basis of the results of the present study we propose a model for the mechanism by which DELLA proteins are targeted for degradation in response to bioactive gibberellins (Figure 5). In this model RGL1 exists in a 'closed' state, where

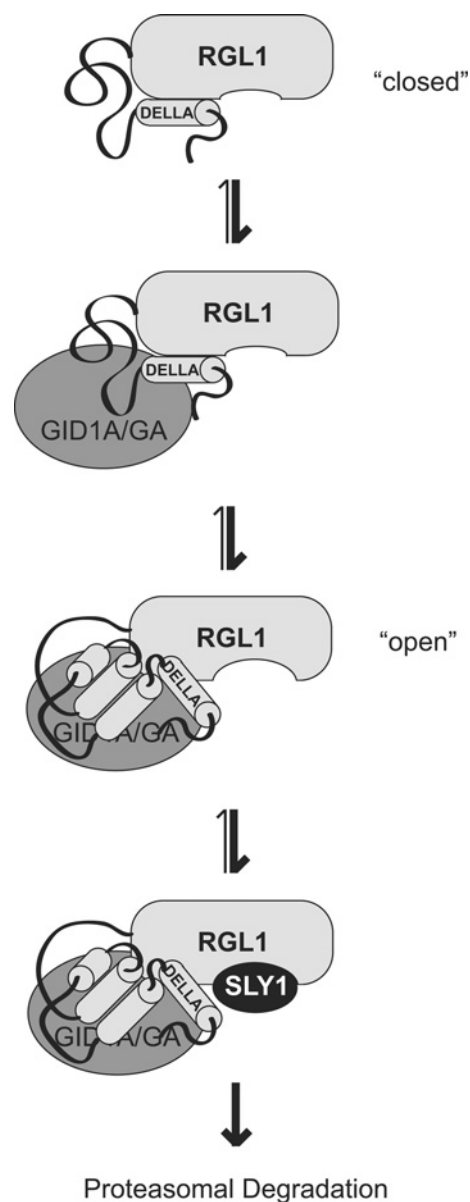


Figure 5 Model for GID1A-dependent recruitment of SLY1 to RGL1

(A) In the 'closed' state, a region of the DELLA motif of RGL1 is bound near the inaccessible SLY1-binding interface within the C-terminal GRAS domain. Upon interaction of gibberellin-liganded GID1A with the predominantly unstructured N-terminal DELLA domain, the DELLA and TVHYNP motifs undergo conformational changes. These conformational changes result in the formation of tertiary structure and transitions in the conformation of the region of the DELLA motif involved in interactions with the C-terminal GRAS domain. The transition of the structure of the DELLA motif subsequently induces structural changes within the GRAS domain, resulting in the formation of an 'open' state accessible to SLY1. The binding of SLY1 then targets the DELLA protein for proteasomal degradation.

a portion of the DELLA motif (Aα helix Asp-Glu-Leu-Leu) within the unstructured N-terminal DELLA domain is normally bound to a site on the C-terminal GRAS domain. In this state, the Aα helix Asp-Glu-Leu-Leu induces the SLY1-binding site to remain inaccessible to SLY1. The mostly unstructured N-terminal DELLA domain is freely available to interact with liganded GID1-like gibberellin receptors. Upon the binding of a liganded gibberellin receptor, the N-terminal domain of RGL1 undergoes conformational changes to form the secondary and tertiary structure elements: the AB loop (distal portion of the

DELLA motif) α B, α C, CD loop (TYHYNP motif) and α D helix. Furthermore, these structural changes within the DELLA motif translate to induced conformational changes in the SLY1-binding site on the C-terminal domain, and possibly contributing directly to the binding surface, resulting in subsequent transitions in the SLY1-binding site. This priming event forms an 'open' state and a binding surface available for binding of SLY1. In the case of DELLA proteins lacking an intact DELLA motif or those with spatially separated N- and C-terminal domains, the interaction with the GRAS domain is limited or unstable, and subsequently the priming event, formation of a high-affinity SLY1-binding site, is incomplete. This model does not exclude the possibility that DELLA proteins function as dimers, as has originally been proposed for SLR1 [17] and more recently reported for the distantly related GRAS proteins SCR and SHR (SHORT-ROOT) [50].

The phosphorylation of DELLA proteins has been implicated in their targeting for degradation [26,28]. The yeast three-hybrid assays shown in the present study do not investigate any requirement of RGL1 phosphorylation for SLY1 recruitment, given that *GID1A*, *RGL1* and *SLY1* are not predicted to function as protein kinases. The DELLA proteins *SLR1* and *GAI* extracted from plant tissue have been shown to only interact with GST (glutathione transferase) fusion protein of the F-box proteins *GID2* or *SLY1* when phosphorylated, suggesting the involvement of a kinase [26,28]. Given that DELLA protein degradation is controlled in plants by multiple signalling pathways [47,48], it is possible that phosphorylation, or indeed other post-translational modifications, may also allow or enhance transitions between the 'closed' and 'open' states of DELLA proteins proposed in the present study, in response to plant signalling molecules other than gibberellins.

In conclusion, our analyses in the present study of *GID1A/GA₃-RGL1-SLY1* interactions in a yeast two/three-hybrid system, as well as the *in vitro* structural analysis and kinetics modelling, are consistent with induction of a series of conformational changes within the N-terminal domain of *RGL1*. These changes are probably directly translated to the C-terminal domain conformational changes, forming an SLY1-binding interface. We have also shown, using competition assays with monoclonal antibodies and intact N-terminal domain of DELLA protein *RGL1*, that the contacts mediated by the AB loop and CD loop within the DELLA and TVHYNP motifs of *RGL1* are essential for interaction with *GID1A*, whereas the contacts mediated by the α A helix (Asp-Glu-Leu-Leu) within the DELLA motif are not required. The discovery of the N-to-C-terminal domain communication within *RGL1* should help elucidate interactions between current and potentially unknown binding partners of this and other DELLA proteins, important for plant development.

AUTHOR CONTRIBUTION

David Sheerin carried out most of the experimental work; Jeremy Buchanan contributed to yeast interaction assays; Chris Kirk and Xiaolin Sun contributed to recombinant work; Xiaolin Sun contributed to protein purification; Dawn Harvey, Xiaolin Sun and William Jones produced and purified monoclonal antibodies; Julian Spagnuolo, Sheng Li, Tong Liu and Virgil Woods carried out DXMS experiments; David Sheerin and Julian Spagnuolo analysed the MS data; Toshi Foster contributed to directing of the project; Jasna Rakonjac and William Jones co-directed the project. The manuscript was written by David Sheerin and Jasna Rakonjac and edited by Toshi Foster, William Jones and Julian Spagnuolo.

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REFERENCES

- Fleet, C. M. and Sun, T. P. (2005) A DELLAce balance: the role of gibberellin in plant morphogenesis. *Curr. Opin. Plant Biol.* **8**, 77–85
- Swain, S. M. and Singh, D. P. (2005) Tall tales from sly dwarves: novel functions of gibberellins in plant development. *Trends Plant Sci.* **10**, 123–129
- Cao, D. N., Cheng, H., Wu, W., Soo, H. M. and Peng, J. R. (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development in *Arabidopsis*. *Plant Physiol.* **142**, 509–525
- Hou, X. L., Hu, W. W., Shen, L. S., Lee, L. Y. C., Tao, Z., Han, J. H. and Yu, H. (2008) Global identification of DELLA target genes during *Arabidopsis* flower development. *Plant Physiol.* **147**, 1126–1142
- Nemhauser, J. L., Hong, F. X. and Chory, J. (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell* **126**, 467–475
- Zentella, R., Zhang, Z. L., Park, M., Thomas, S. G., Endo, A., Murase, K., Fleet, C. M., Jikumaru, Y., Nambara, E., Kamiya, Y. and Sun, T. P. (2007) Global analysis of DELLA direct targets in early gibberellin signaling in *Arabidopsis*. *Plant Cell* **19**, 3037–3057
- Ikeda, A., Ueguchi-Tanaka, M., Sonoda, Y., Kitano, H., Koshioka, M., Futsuhara, Y., Matsuoka, M. and Yamaguchi, J. (2001) Slender rice, a constitutive gibberellin response mutant, is caused by a null mutation of the *SLR1* gene, an ortholog of the height-regulating gene *GAI/RGA/RHT/D8*. *Plant Cell* **13**, 999–1010
- Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P. and Harberd, N. P. (1997) The *Arabidopsis* *GAI* gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* **11**, 3194–3205
- Silverstone, A. L., Ciampaglio, C. N. and Sun, T. P. (1998) The *Arabidopsis* *RGA* gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* **10**, 155–169
- Lee, S. C., Cheng, H., King, K. E., Wang, W. F., He, Y. W., Hussain, A., Lo, J., Harberd, N. P. and Peng, J. R. (2002) Gibberellin regulates *Arabidopsis* seed germination via *RGL2*, a *GAI/RGA*-like gene whose expression is up-regulated following imbibition. *Genes Dev.* **16**, 646–658
- Wen, C. K. and Chang, C. (2002) *Arabidopsis* *RGL1* encodes a negative regulator of gibberellin responses. *Plant Cell* **14**, 87–100
- Silverstone, A. L., Mak, P. Y. A., Martinez, E. C. and Sun, T. P. (1997) The new *RGA* locus encodes a negative regulator of gibberellin response in *Arabidopsis thaliana*. *Genetics* **146**, 1087–1099
- Cheng, H., Qin, L. J., Lee, S. C., Fu, X. D., Richards, D. E., Cao, D. N., Luo, D., Harberd, N. P. and Peng, J. R. (2004) Gibberellin regulates *Arabidopsis* floral development via suppression of DELLA protein function. *Development* **131**, 1055–1064
- Tyler, L., Thomas, S. G., Hu, J. H., Dill, A., Alonso, J. M., Ecker, J. R. and Sun, T. P. (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. *Plant Physiol.* **135**, 1008–1019
- Peng, J. R., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., Pelica, F. et al. (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400**, 256–261
- Pysh, L. D., Wysocka-Diller, J. W., Camilleri, C., Bouchez, D. and Benfey, P. N. (1999) The *GRAS* gene family in *Arabidopsis*: sequence characterization and basic expression analysis of the SCARECROW-LIKE genes. *Plant J.* **18**, 111–119
- Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M. and Matsuoka, M. (2002) The gibberellin signaling pathway is regulated by the appearance and disappearance of *SLENDER RICE1* in nuclei. *Plant Cell* **14**, 57–70
- Muangprom, A., Thomas, S. G., Sun, T. P. and Osborn, T. C. (2005) A novel dwarfing mutation in a green revolution gene from *Brassica rapa*. *Plant Physiol.* **137**, 931–938
- Hirano, K., Asano, K., Tsuji, H., Kawamura, M., Mori, H., Kitano, H., Ueguchi-Tanaka, M. and Matsuoka, M. (2010) Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. *Plant Cell* **22**, 2680–2696
- Nakajima, M., Shimada, A., Takashi, Y., Kim, Y. C., Park, S. H., Ueguchi-Tanaka, M., Suzuki, H., Katoh, E., Iuchi, S., Kobayashi, M. et al. (2006) Identification and characterization of *Arabidopsis* gibberellin receptors. *Plant J.* **46**, 880–889

- 21 Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z. L., Powers, S. J., Gong, F., Phillips, A. L., Hedden, P., Sun, T. P. and Thomas, S. G. (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in *Arabidopsis*. *Plant Cell* **18**, 3399–3414
- 22 Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T. Y., Hsing, Y. I. C., Kitano, H., Yamaguchi, I. and Matsuoka, M. (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. *Nature* **437**, 693–698
- 23 Ueguchi-Tanaka, M., Nakajima, M., Katoh, E., Ohmiya, H., Asano, K., Saji, S., Xiang, H. Y., Ashikari, M., Kitano, H., Yamaguchi, I. and Matsuoka, M. (2007) Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. *Plant Cell* **19**, 2140–2155
- 24 Murase, K., Hirano, Y., Sun, T. P. and Hakoshima, T. (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* **456**, 459–463
- 25 Shimada, A., Ueguchi-Tanaka, M., Nakatsu, T., Nakajima, M., Naoe, Y., Ohmiya, H., Kato, H. and Matsuoka, M. (2008) Structural basis for gibberellin recognition by its receptor GID1. *Nature* **456**, 520–523
- 26 Gomi, K., Sasaki, A., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., Kitano, H. and Matsuoka, M. (2004) GID2, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. *Plant J.* **37**, 626–634
- 27 Dill, A., Thomas, S. G., Hu, J. H., Steber, C. M. and Sun, T. P. (2004) The *Arabidopsis* F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell* **16**, 1392–1405
- 28 Fu, X. D., Richards, D. E., Fleck, B., Xie, D. X., Burton, N. and Harberd, N. P. (2004) The *Arabidopsis* mutant sleepy1(gar2-1) protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates. *Plant Cell* **16**, 1406–1418
- 29 Gibson, T. J. (1984) Studies on the Epstein-Barr Virus Genome., Ph.D. Thesis, Cambridge University, Cambridge, U.K
- 30 Ali, S. A. and Steinkasserer, A. (1995) PCR-ligation-PCR mutagenesis: a protocol for creating gene fusions and mutations. *Biotechniques* **18**, 746–750
- 31 Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K. and Pease, L. R. (1989) Engineering hybrid genes without the use of restriction enzymes: gene-splicing by overlap extension. *Gene* **77**, 61–68
- 32 Haseloff, J. and Amos, B. (1995) GFP in plants. *Trends Genet.* **11**, 328–329
- 33 Sambrook, J. and Russell, D. W. (2001) *Molecular Cloning: a Laboratory Manual*, 3rd edn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- 34 Sun, X. L., Frearson, N., Kirk, C., Jones, W. T., Harvey, D., Rakonjac, J., Foster, T. and Al-Samarrai, T. (2008) An *E. coli* expression system optimized for DELLA proteins. *Protein Expression Purif.* **58**, 168–174
- 35 Harlow, E. and Lane, D. (1988) *In Antibodies: a Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
- 36 Sun, X. L., Jones, W. T., Harvey, D., Edwards, P. J. B., Pascal, S. M., Kirk, C., Considine, T., Sheerin, D. J., Rakonjac, J., Oldfield, C. J. et al. (2010) N-terminal domains of DELLA proteins are intrinsically unstructured in the absence of interaction with GID1/gibberellin acid receptors. *J. Biol. Chem.* **285**, 11557–11571
- 37 Mendillo, M. L., Putnam, C. D., Mo, A. O., Jamison, J. W., Li, S., Woods, V. L. and Kolodner, R. D. (2010) Probing DNA- and ATP-mediated conformational changes in the MutS family of mismatch recognition proteins using deuterium exchange mass spectrometry. *J. Biol. Chem.* **285**, 13170–13182
- 38 Englander, J. J., Del Mar, C., Li, W., Englander, S. W., Kim, J. S., Stranz, D. D., Hamuro, Y. and Woods, V. L. (2003) Protein structure change studied by hydrogen–deuterium exchange, functional labeling, and mass spectrometry. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 7057–7062
- 39 Ormo, M., Cubitt, A. B., Kallio, K., Gross, L. A., Tsien, R. Y. and Remington, S. J. (1996) Crystal structure of the *Aequorea victoria* green fluorescent protein. *Science* **273**, 1392–1395
- 40 Karlsson, R., Michaelsson, A. and Mattsson, L. (1991) Kinetic-analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J. Immunol. Methods* **145**, 229–240
- 41 Morton, T. A., Myszk, D. G. and Chaiken, I. M. (1995) Interpreting complex binding-kinetics from optical biosensors: a comparison of analysis by linearization, the integrated rate-equation, and numerical-integration. *Anal. Biochem.* **227**, 176–185
- 42 O’Shannessy, D. J. (1994) Determination of kinetic rate and equilibrium binding constants for macromolecular interactions: a critique of the surface plasmon resonance literature. *Curr. Opin. Biotechnol.* **5**, 65–71
- 43 Arnold, K., Bordoli, L., Kopp, J. and Schwede, T. (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* **22**, 195–201
- 44 Willige, B. C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E. M. N., Maier, A. and Schwechheimer, C. (2007) The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of *Arabidopsis*. *Plant Cell* **19**, 1209–1220
- 45 Koornneef, M., Elgersma, A., Hanhart, C. J., Vanloenenmartinet, E. P., Vanriijn, L. and Zeevaart, J. A. D. (1985) A gibberellin insensitive mutant of *Arabidopsis thaliana*. *Physiol. Plant.* **65**, 33–39
- 46 Wilson, R. N. and Somerville, C. R. (1995) Phenotypic suppression of the gibberellin-insensitive mutant (gai) of *Arabidopsis*. *Plant Physiol.* **108**, 495–502
- 47 Harberd, N. P., Belfield, E. and Yasumura, Y. (2009) The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an “inhibitor of an inhibitor” enables flexible response to fluctuating environments. *Plant Cell* **21**, 1328–1339
- 48 Schwechheimer, C. and Willige, B. C. (2009) Shedding light on gibberellin acid signalling. *Curr. Opin. Plant Biol.* **12**, 57–62
- 49 Asano, K., Hirano, K., Ueguchi-Tanaka, M., Angeles-Shim, R. B., Komura, T., Satoh, H., Kitano, H., Matsuoka, M. and Ashikari, M. (2009) Isolation and characterization of dominant dwarf mutants, Slr1-d, in rice. *Mol. Genet. Genomics* **281**, 223–231
- 50 Cui, H., Levesque, M. P., Vernoux, T., Jung, J. W., Paquette, A. J., Gallagher, K. L., Wang, J. Y., Billou, I., Scheres, B. and Benfey, P. N. (2007) An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. *Science* **316**, 421–425

SUPPLEMENTARY ONLINE DATA

Inter- and intra-molecular interactions of *Arabidopsis thaliana* DELLA protein RGL1

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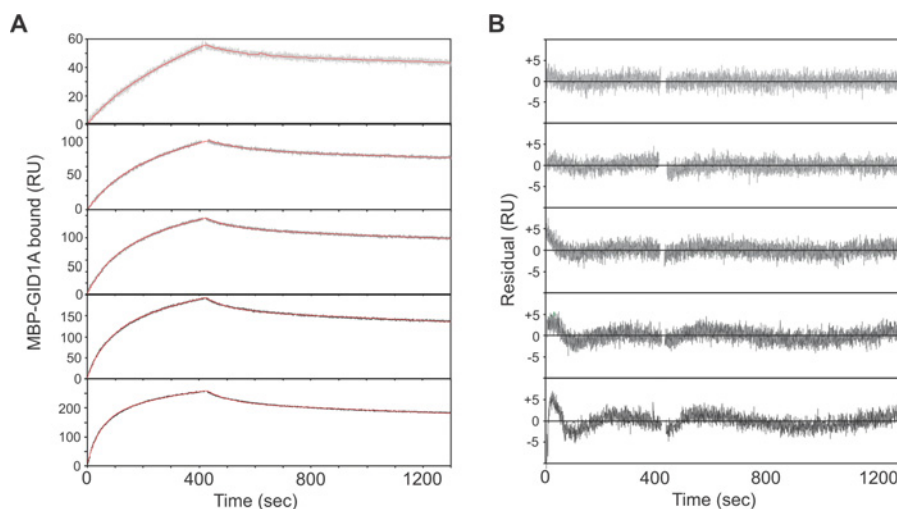


Figure S1 Conformational change kinetic modelling of the gibberellin-dependent GID1A–RGL1N interaction

(A) Gibberellin-dependent association and dissociation data for the interaction between GID1A and immobilized RGL1^N, detected by SPR. Interactions were performed for 100, 200, 400, 800 and 1600 nM solutions of GID1A (top to bottom). A calculated two-state kinetic model was fitted to individual curves, indicated in red, using BiaEvaluation software version 3.1. (B) Residual plot for variance in response units (RU), of the kinetic data from the calculated model for each GID1A concentration.

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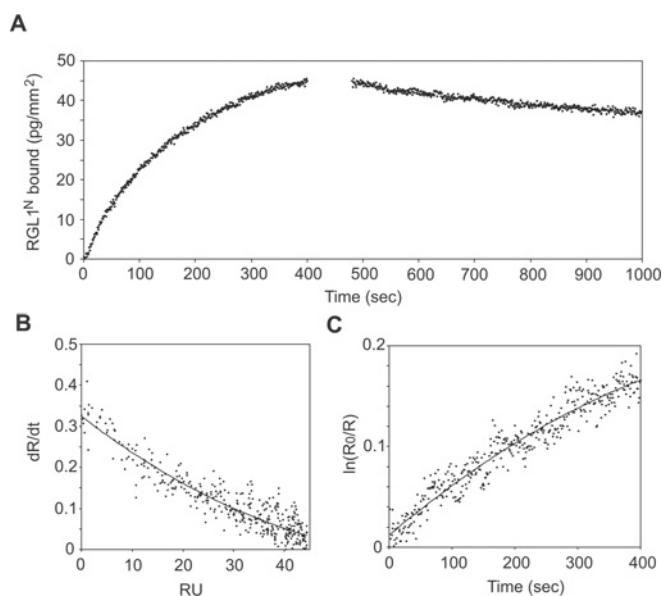


Figure S2 Gibberellin-dependent binding of RGL1^N to immobilized GID1A

(A) Binding of purified recombinant RGL1^N (residues 1–137) from a 1 μ M solution to immobilized MBP–GID1A in the presence of gibberellin in GA₄. Association, 0–420 s; dissociation, 420–1000 s. RGL1^{1–137} was prepared by rTEV protease cleavage from the MBP–fusion protein and subsequent anion–exchange chromatography as described previously [1]. Dissociation of immobilized MBP–GID1A subtracted (baseline drift, approximately -15 pg/mm² at 400 s and -28 pg/mm² at 1000 s). Gibberellin (5 μ M) was present in all solutions and running buffers. (B) dR/dt against R linearization (Scatchard plot) of the association phase. (C) $\ln(R_0/R)$ against time linearization of the dissociation phase.

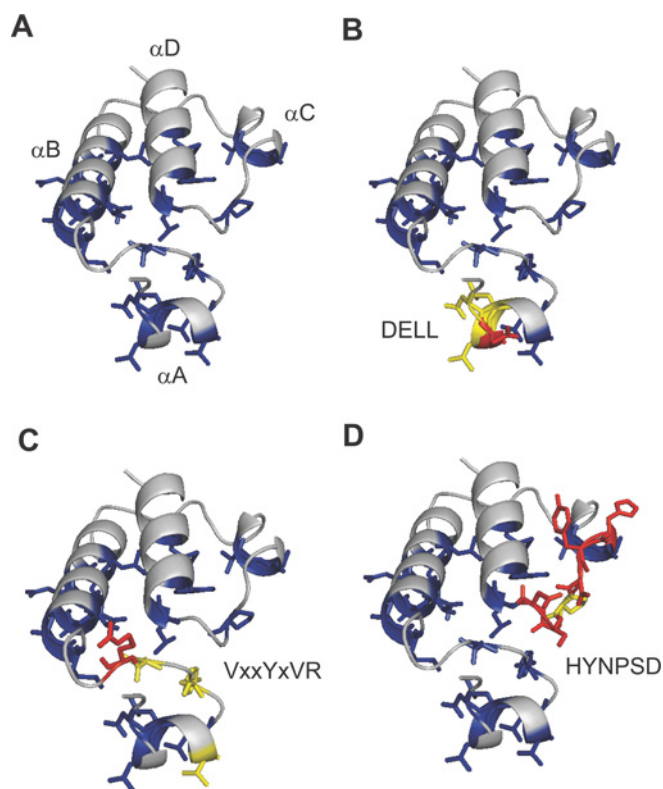


Figure S3 Structural prediction of the RGL1 N-terminal DELLA domain when in complex with GID1A

(A) Predicted RGL1^N (residues 1–137) tertiary structure, modelled from the GID1A/GA₄:GA₁^{11–113} crystal structure using SwissModel (PDB code 2ZSI) [2,3]. Conserved residues that form direct interactions between GAI and GID1A [2] are shown in blue. (B–D) RGL1^N model, indicating monoclonal antibody epitopes: 6C8 (B), BC9 (C) and AD7 (D). Antibody epitopes are highlighted in red and yellow. Yellow indicates a residue that also forms a direct GAI–GID1A interaction, whereas red residues do not.

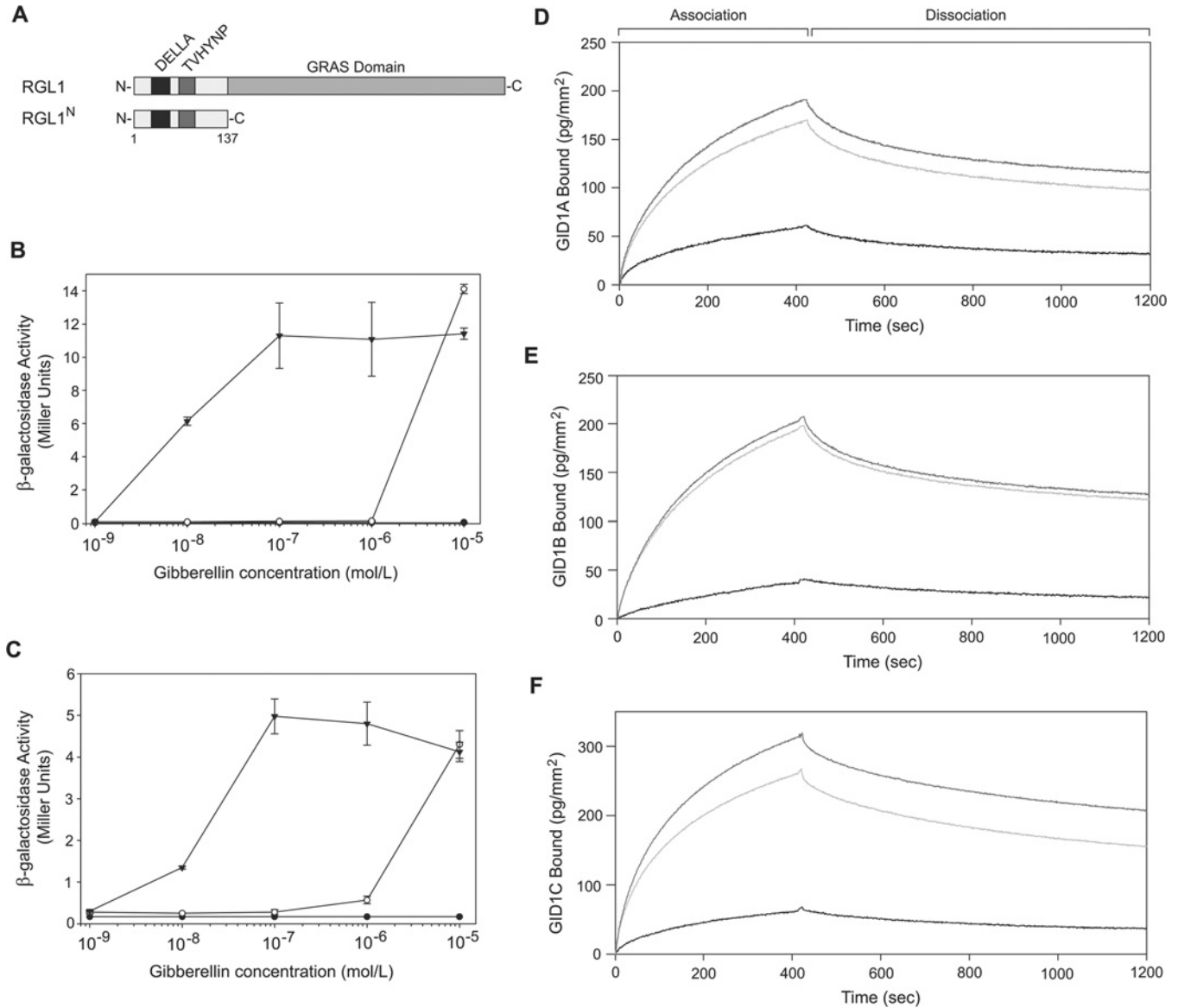


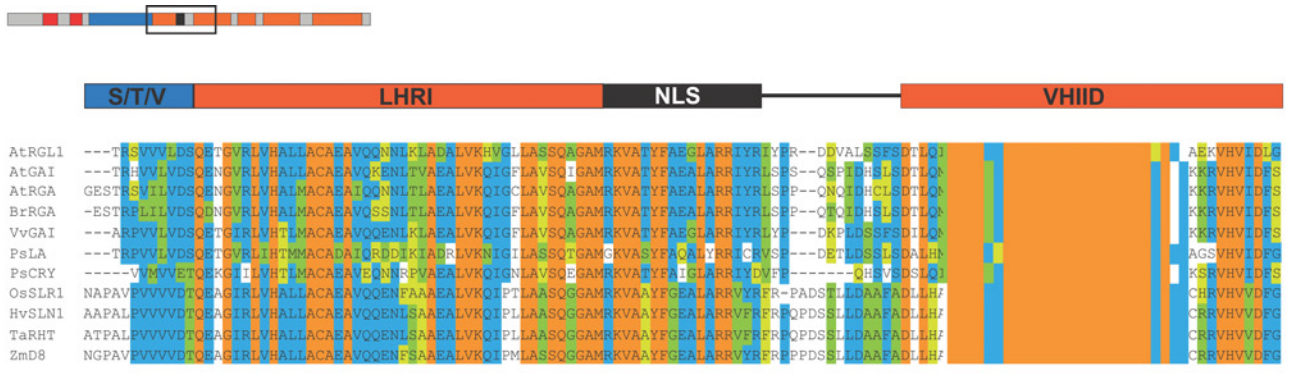
Figure S4 Comparison of the GA₃- and GA₄-dependent GID1A-RGL1^N interaction *in vivo* and *in vitro*

(A) Schematic representation of RGL1, and the N-terminal 137 residues of RGL1, RGL1^N, used in *in vitro* experiments. (B and C) Dose-response curves of yeast two- and three-hybrid assays. (B) Two-hybrid assay of the interaction between the Gal4 DNA-binding domain fusion of GID1A and the Gal4 activation-domain fusion of RGL1. (C) Three-hybrid assay of the interaction between the Gal4 DNA-binding domain fusion of SLY1 and the Gal4 activation domain fusion of RGL1 in the presence of GID1A. LacZ (β -galactosidase) reporter gene activity, from *Saccharomyces cerevisiae* grown in absence of gibberellins (EPS) and the presence of GA₃ (EPS) or GA₄ (EPS). The experiment was performed in duplicate (from two independent transformants); β -galactosidase assays were performed in triplicate for each transformant. Error bars show ± 1 S.D. (D-F) *In vitro* association and dissociation of gibberellin-saturated GID1A-C and RGL1^N, monitored using SPR. Interaction of RGL1^N with: (D) GID1A, (E) GID1B or (F) GID1C; in the absence (black), or presence of 100 M GA₃ (light grey) or 100 M GA₄ (dark grey). Gibberellins were mixed with GID1A-C 30 min prior to the binding assay and excluded from running buffer during the dissociation phase. Association, 0–420 s; dissociation, 420–1200 s. The amount of bound GID1A-C is shown as pg/mm² of surface area.



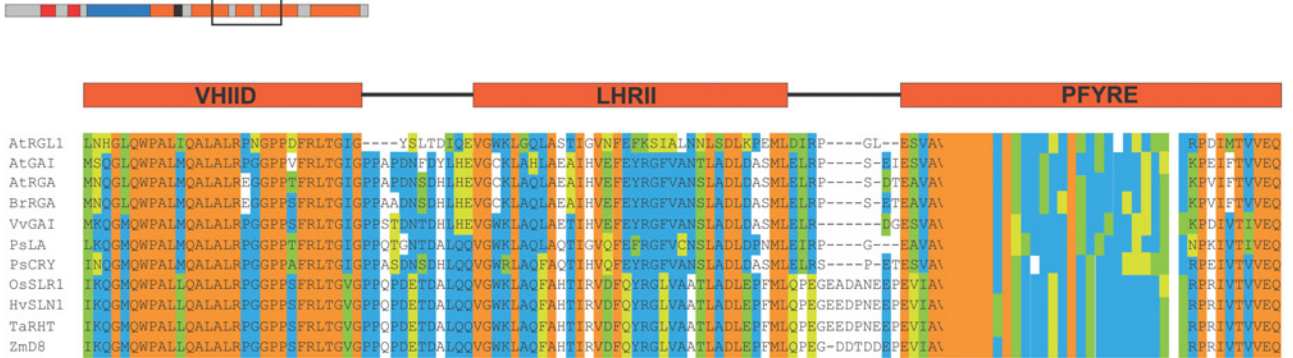
Figure S5 DELLA protein alignment

The full-length sequences for DELLA proteins from a range of plant species were aligned using AlignX (Vector NTI software, Invitrogen). Absolutely conserved residues are highlighted in orange; highly conserved residues are highlighted in blue; highly similar residues are highlighted in green; and similar residues are highlighted in yellow. The RGL1 gain-of-function mutants used in the present paper are displayed. The sequences of several DELLA gain-of function mutations are also displayed, indicating in-frame deletions or amino acid replacements. The *A. thaliana* (At) *gai-1*, *rga*^{Δ17}; grape (*Vv*, *Vitis vinifera*) *gai-1*; (*Zm*, *Zea mays*) rice (*Os*, *Oryza sativa*) *slr1w*^{ΔDELLA}, *slr1*^{ASPACE}, *slr1*^{AS/T/V}, *slr1*^{AS/T/V}; barley (*Hv*, *Hordeum vulgare*) *sln1-d*; wheat (*Ta*, *Triticum aestivum*) *rht*, *rht-B1b*, *rht-D1b*; and maize field mustard (*Br*, *Brassica rapa*) *rga1-d*; *d8-MP* have been previously described as semi-dominant gibberellin-insensitive mutations [4–11]. *Ps*, pea (*Pisum sativum*).



AtRGL1ADELLA ---TRSVVLDSEQETGVRLVHALLACAEAVQNNLKLADALVKHVGLLASSQAGAMRKVATYFAEGLARRIYRIYPR--DDVALSFSFDTLQI EKVHVIDLG
 AtRGL1ATVHYNP ---TRSVVLDSEQETGVRLVHALLACAEAVQNNLKLADALVKHVGLLASSQAGAMRKVATYFAEGLARRIYRIYPR--DDVALSFSFDTLQI EKVHVIDLG
 AtRGL1Q272R ---TRSVVLDSEQETGVRLVHALLACAEAVQNNLKLADALVKHVGLLASSQAGAMRKVATYFAEGLARRIYRIYPR--DDVALSFSI LEVFATAEKVHVIDLG

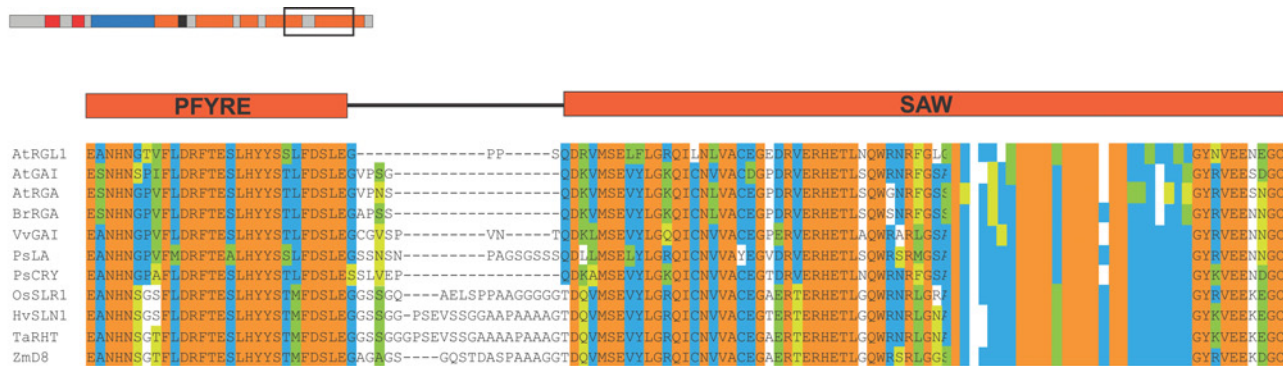
AtGAI-1 ---TRHVVLVDSQENGVRVHALLACAEAVQENLTVAEALVKQIGFLAVSQIGAMRKVATYFAEALARRIYRLSPS--QSPIDHSLSDTLQM QGKRVHVIDFS
 AtRGA17 GESTRSVILVDSQENGVRVHALLACAEAVQNNLTLAEALVKQIGFLAVSQAGAMRKVATYFAEALARRIYRLSPP--QNIQDHCSDTLQMHFYETCI VHIIDFS
 VvGAIXX ---ARPVVLDSEQETGIRLVHTLMACAEAVQENLKLAEALVKQIGFLAVSQAGAMRKVATYFAEGLARRIYRLYP--DKPLDSSFSIDLQM FEGKRVHVIDFS
 BrRGA-d -ESTRPLILVDSQDNGVRVHALLACAEAVQSSNLTAEALVKQIGFLAVSQAGAMRKVATYFAEALARRIYRLSPP--QTQIDHSLSDTLQM EGKRVHVIDFS
 OsSLR1ADELLA NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHAHFYESCJ RVHVVDVG
 OsSLR1ASPACE NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHAHFYESCJ RVHVVDVG
 OsSLR1ATVHYNP NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHA HRVHVVDVG
 OsSLR1AS/T/V ---LACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHAHFYESCJ RVHVVDVG
 Osslr1-d4 NAPAVVVVVDTQEAGIRLVHALLACAEAVQENFAAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRF-PADSTLLDAAFADLLHA FAGCRVHVVDVG
 HvSLN1-d AAPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRFPQDSSLLDAAFADLLHA AGCRVHVVDVG
 TaRht ATPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRFPQDSSLLDAAFADLLHA CRRVHVVDVG
 TaRHTXXX ATPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRFPQDSSLLDAAFADLLHA FAGCRVHVVDVG
 TaRHTXXX ATPALPVVVVDTEAGIRLVHALLACAEAVQENLSAAEALVKQIPTLAASQGGAMRKVAAYFGEALARRVYFRFPQDSSLLDAAFADLLHA FAGCRVHVVDVG
 ZmD8-MP NGPAVPPVVVDTEAGIRLVHALLACAEAVQENFSAEALVKQIPMLASSQGGAMRKVAAYFGEALARRVYFRFPDSSLLDAAFADLLHA AGCRVHVVDVG



AtRGL1ADELLA LNHLQWPALIQALALRPNPDPFRLTGIG---YSLTDIQEVGWKLGQLASTIGVNFEFKSIALNNLSDLKPEMLDIRP---GL--ESVAV PDIMTVVEQ
 AtRGL1ATVHYNP LNHLQWPALIQALALRPNPDPFRLTGIG---YSLTDIQEVGWKLGQLASTIGVNFEFKSIALNNLSDLKPEMLDIRP---GL--ESVAV PDIMTVVEQ
 AtRGL1Q272R LNHLQWPALIQALALRPNPDPFRLTGIG---YSLTDIQEVGWKLGQLASTIGVNFEFKSIALNNLSDLKPEMLDIRP---GL--ESVAVNSVFEHLRLLAHGSDIKF

AtGAI-1 MSQGLQWPALMQALALRPGGPPVFRLTGIGPPAPDNFDYHVEVGCKLAHLAEAIHVEFYRGEFVANTLADLDASMLELRP---S-EIESVAV QIKPEIFTVVEQ
 AtRGA17 MNQGLQWPALMQALALRPGGPPVFRLTGIGPPAPDNDHHEVGCKLAQLAEAIHVEFYRGEFVANSIADLDASMLELRP---S-DTEAVAVNSVFEI IFTVVEQ
 VvGAIXX MKQGMQWPALMQALALRPGGPPVFRLTGIGPPSPDNTDHLHEVGWKLQALAEAIHVEFYRGEFVANSIADLDASMLELRP-----DGESVAV KDMKPDIVTVEQ
 BrRGA-d MKQGMQWPALMQALALRPGGPPVFRLTGIGPPADNSDHLHEVGCKLAQLAEAIHVEFYRGEFVANSIADLDASMLELRP---S-ETEAVAVNSVFEHLKLLGRTGGIEKV
 OsSLR1ADELLA IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVIAVNSVFEI RIVTVVEQ
 OsSLR1ASPACE IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVIAVNSVFEI RIVTVVEQ
 OsSLR1ATVHYNP IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVIAVNSVFEI RIVTVVEQ
 OsSLR1AS/T/V IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVIAVNSVFEI RIVTVVEQ
 Osslr1-d4 IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEADANEEPEVIAVNSVFEI HAVRPRIVTVVEQ
 HvSLN1-d IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVIAVNSVFEI AVRPRIVTVVEQ
 TaRht IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVIAVNSVFEI RPRIVTVVEQ
 TaRHTXXX IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEGEEDPNEEPEVIAVNSVFEI RAVRPRIVTVVEQ
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 ZmD8-MP IKQGMQWPALLQALALRPGGPPVFRLTGIGVGPQPDETDALQVGVWKLQAFHAFTIRVDFQYRGLVAATLADLEPFMLQPEG-DDTDDEPEVIAVNSVFEI AVRPRIVTVVEQ

Figure S5 Continued



AtRGL1ADELLA	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQWRNRFGLG	YNVEENEGC
AtRGL1ATVHYNP	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQWRNRFGLG	YNVEENEGC
AtRGL1Q272R	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQW	LYAGADGYNVEENEGC

AtGAI-1	ESNHNSPIFLDRFTESLHYYSFLDSLEGVPSG-----QDKVMSEVYLGKQICNVVACDGPDRVERHETLSQWRNRFGSA	GEGYRVEESDGC
AtRGA17	ESNHNGPVFLDRFTESLHYYSFLDSLEGVPSN-----QDKVMSEVYLGKQICNVVACDGPDRVERHETLSQWRNRFGSSGLAPAH	VEESNGC
VvGAIXXX	EANHNGTVFLDRFTESLHYYSFLDSLEGCVSP-----VN-----TQDKLMSEVYLGKQICNVVACEGPERVERHETLAQWRARLGS	GGDGYRVEENNGC
BrRGA-d	ESNHNGPVFLDRFTESLHYYSFLDSLEGAPSS-----QDKVMSEVYLGKQICNVVACDGPDRVERHETLSQWRNRFGSS	GEGYRVEENNGC
OsSLR1ADELLA	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVHI	RVEEKEGC
OsSLR1ASPACE	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVHI	RVEEKEGC
OsSLR1ATVHYNP	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRA	YRVEEKEGC
OsSLR1AS/T/V	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVHI	RVEEKEGC
Osslr1-d4	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRA	GGDGYRVEEKEGC
HvSLN1-d	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ--PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGTERTERHETLGQWRNRLGNA	GDGYKVEEKEGC
TaRht	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQ--PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GYKVEEKEGC
TaRHTXXX	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQ--PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GGDGYKVEEKEGC
TaRHTXXX	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQ--PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GGDGYKVEEKEGC
ZmD8-MP	EANHNSGTVFLDRFTESLHYYSFLDSLEGAGAGS---GQSTDASPAAGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGGE	GGDGYRVEEKDGC



SAW

AtRGL1	LLLGWQTRPLIATSAWRINRVE-----
AtGAI	LLLGWHTRPLIATSAWKLSTN-----
AtRGA	LLLGWHTRPLIATSAWKLSTAA-----
BrRGA	LLLSWHTRPLIATSAWKLSAVH-----
VvGAI	LLLGWHTRPLIATSAWQLANKPALPSSTPASN
PsLA	LLLGWHTRPLIATSAWKLPSESK-----
PScRY	LLLGWHTRPLIATSAWKLAANSMVVSH-----
OsSLR1	LTLGWHTRPLIATSAWRVAAA-----
HvSLN1	LTLGWHTRPLIATSAWRLAAP-----
TaRHT	LTLGWHTRPLIATSAWRLAGP-----
ZmD8	LTLGWHTRPLIATSAWRVAAAAAP-----

AtRGL1ADELLA	LLLGWQTRPLIATSAWRINRVE-----
AtRGL1ATVHYNP	LLLGWQTRPLIATSAWRINRVE-----
AtRGL1Q272R	LLLGWQTRPLIATSAWRINRVE-----

AtGAI-1	LLLGWHTRPLIATSAWKLSTN-----
AtRGA17	LLLGWHTRPLIATSAWKLSTAA-----
VvGAIXXX	LLLGWHTRPLIATSAWQLANKPALPSSTPASN
BrRGA-d	LLLSWHTRPLIATSAWKLSAVH-----
OsSLR1ADELLA	LTLGWHTRPLIATSAWRVAAA-----
OsSLR1ASPACE	LTLGWHTRPLIATSAWRVAAA-----
OsSLR1ATVHYNP	LTLGWHTRPLIATSAWRVAAA-----
OsSLR1AS/T/V	LTLGWHTRPLIATSAWRVAAA-----
Osslr1-d4	LTLGWHTRPLIATSAWRVAAA-----
HvSLN1-d	LTLGWHTRPLIATSAWRLAAP-----
TaRht	LTLGWHTRPLIATSAWRLAGP-----
TaRHTXXX	LTLGWHTRPLIATSAWRLAGP-----
TaRHTXXX	LTLGWHTRPLIATSAWRLAGP-----
ZmD8-MP	LTLGWHTRPLIATSAWRVAAAAAP-----

Figure S5 Continued

Table S1 Oligonucleotide sequences for amplification and sequencing

Description	Sequence
RGL1 forward primer for pACT2 (XmaI)	5'-TCCCCCGGGTATGAAGAGAGAGCACAACCACC-3'
RGL1 reverse primer for pACT2 (SacI)	5'-CGAGCTCGTTATCCACACGATTGATTCGCC-3'
RGL1 internal nt93 reverse primer	5'-GACGTGGCACACAAGCTTG-3'
RGL1 internal nt145 forward primer	5'-CACTCCGGCAGCTTCTTC-3'
RGL1 internal nt213 reverse primer	5'-AGCATGCTCTCGGATCTTGAC-3'
RGL1 internal nt268 forward primer	5'-ATTCGAGATCCATACCAAGAAC-3'
RGL1 internal mutagenic primer reverse	5'-CTTTGATTGCGACTCTTGCTTTAC-3'
RGL1 internal nt830 forward primer	5'-CCGGCCATTGTAACCATGG-3'
RGL1 internal nt411 reverse primer	5'-GTTCTTCTCCTTACTCATTCCGCCACCCGTAGAGGATAACTCCGAT-3'
RGL1 internal nt412 forward primer	5'-GCATGGATGAACATACAAGGAGGGGTCGCTCTGTGGTGGTTTTGGATTC-3'
RGL1 forward for pMalc2x (BamHI)	5'-CGGGATCCATGAAGAGAGAGCACAACCACC-3'
RGL1 reverse N-term internal for pMalc2x (Sall)	5'-GACGCGTCGACTACGTAGAGGATAACTCCGATCAA-3'
GID1A forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGCGAGCGATGAAG-3'
GID1A reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGTTAACATCCGCGTTTACAAC-3'
GID1A forward primer for pBridge MCSII (NotI)	5'-ATAAGAATCGCGCCGCTATGCGTGGCGGATGAAG-3'
GID1A reverse primer for pBridge MCSII (NotI)	5'-ATAAGAATCGCGCCGCTATTAACATCCGCGTTTACAAC-3'
GID1B forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGTTGTAACGAAGT-3'
GID1B reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGCTAAGGAGTAAGAAGCACAGG-3'
GID1C forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGGAAGTGAAGAAGTT-3'
GID1C reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGTTCAATGGCATTCTGCGTTTAC-3'
SLY1 forward primer (EcoRI)	5'-CGGAATTCATGAAGCGCAGTACTACCGAC-3'
SLY1 reverse primer (BamHI)	5'-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTC-3'
SLY1 mutagenic primer reverse primer (BamHI)	5'-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTCCTCTTAGTAAACTCATCTTCTGTAG-3'
GFP forward primer	5'-ATCGAGGTATCCTCTACGGGTGGCGAATGAGTAAAGGAGAAGAAC-3'
GFP reverse primer	5'-GAATCCAAAACCACCACAGAGCGACCCCTCTTTGTATGTTCAATCCAGC-3'
pACT2 forward sequencing primer	5'-CTATCTATTGATGATGAAGATAC-3'
pACT2 reverse sequencing primer	5'-AGTTGAAGTGAACCTTGGGGTT-3'
pGBKT7 forward sequencing primer (T7)	5'-TAATACGACTCACTATAGGG-3'
pGBKT7 / pBridge MCSI reverse sequencing primer	5'-TAAGAGTCACTTAAAAATTTGTAT-3'
pBridge MCSII forward sequencing primer	5'-TTGGGAACGTGTTGGTTG-3'
pBridge MCSII reverse sequencing primer	5'-CCGTATTACCGCCTTTGAGT-3'
pGADT7 forward sequencing primer	5'-TAATACGACTCACTATAGGG-3'
pGADT7 reverse sequencing primer	5'-GTGAACCTTGGGGTTTTTCAGTATCTACGATT-3'
pMALc2x forward sequencing primer	5'-GGTCGTGAGACTGTGATGAAGCC-3'
pMALc2x reverse sequencing primer	5'-CGCCAGGGTTTTCCAGTCCACGAC-3'

REFERENCES

- Sun, X. L., Frearson, N., Kirk, C., Jones, W. T., Harvey, D., Rakonjac, J., Foster, T. and Al-Samarrai, T. (2008) An *E. coli* expression system optimized for DELLA proteins. *Protein Expression Purif.* **58**, 168–174
- Murase, K., Hirano, Y., Sun, T. P. and Hakoshima, T. (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* **456**, 459–463
- Arnold, K., Bordoli, L., Kopp, J. and Schwede, T. (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* **22**, 195–201
- Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P. and Harberd, N. P. (1997) The *Arabidopsis* GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* **11**, 3194–3205
- Peng, J. R., Richards, D. E., Hartley, N. M., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., Pelica, F. et al. (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400**, 256–261
- Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M. and Matsuoka, M. (2002) The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. *Plant Cell* **14**, 57–70
- Muangprom, A., Thomas, S. G., Sun, T. P. and Osborn, T. C. (2005) A novel dwarfing mutation in a green revolution gene from *Brassica rapa*. *Plant Physiol.* **137**, 931–938
- Hirano, K., Asano, K., Tsuji, H., Kawamura, M., Mori, H., Kitano, H., Ueguchi-Tanaka, M. and Matsuoka, M. (2010) Characterization of the molecular mechanism underlying gibberellin perception complex formation in rice. *Plant Cell* **22**, 2680–2696
- Boss, P. K. and Thomas, M. R. (2002) Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature* **416**, 847–850
- Chandler, P. M., Marion-Poll, A., Ellis, M. and Gubler, F. (2002) Mutants at the *Slender1* locus of barley cv Himalaya: molecular and physiological characterization. *Plant Physiol.* **129**, 181–190
- Weston, D. E., Elliott, R. C., Lester, D. R., Rameau, C., Reid, J. B., Murfet, I. C. and Ross, J. J. (2008) The pea DELLA proteins LA and CRY are important regulators of gibberellin synthesis and root growth. *Plant Physiol.* **147**, 199–205

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SUPPLEMENTARY ONLINE DATA

Inter- and intra-molecular interactions of *Arabidopsis thaliana* DELLA protein RGL1

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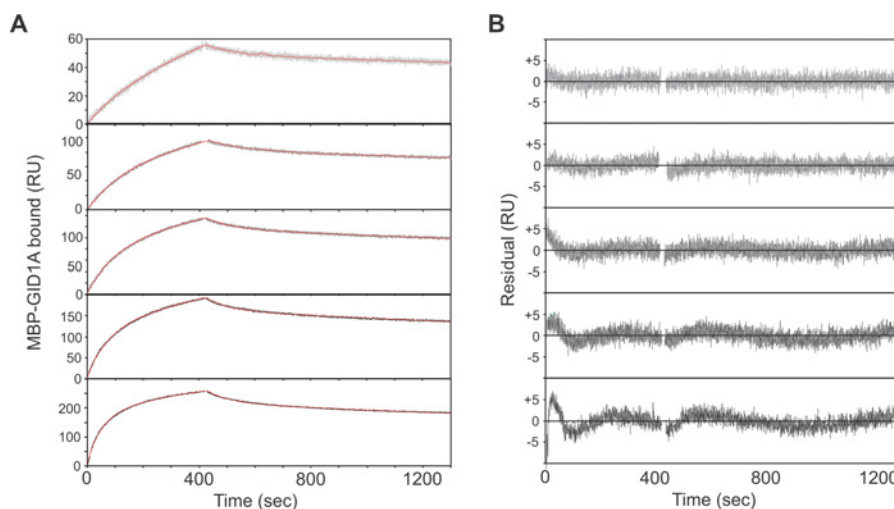


Figure S1 Conformational change kinetic modelling of the gibberellin-dependent GID1A–RGL1N interaction

(A) Gibberellin-dependent association and dissociation data for the interaction between GID1A and immobilized RGL1^N, detected by SPR. Interactions were performed for 100, 200, 400, 800 and 1600 nM solutions of GID1A (top to bottom). A calculated two-state kinetic model was fitted to individual curves, indicated in red, using BiaEvaluation software version 3.1. (B) Residual plot for variance in response units (RU), of the kinetic data from the calculated model for each GID1A concentration.

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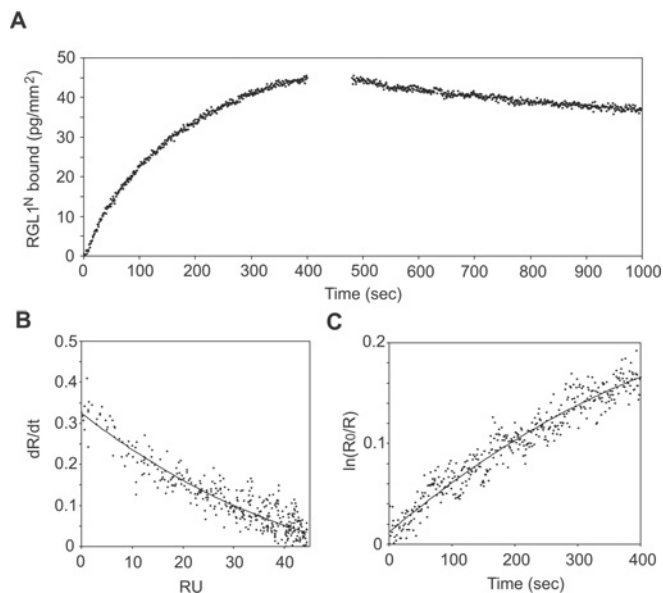


Figure S2 Gibberellin-dependent binding of RGL1^N to immobilized GID1A

(**A**) Binding of purified recombinant RGL1^N (residues 1–137) from a 1 μ M solution to immobilized MBP–GID1A in the presence of gibberellin in GA₄. Association, 0–420 s; dissociation, 420–1000 s. RGL1^{1–137} was prepared by rTEV protease cleavage from the MBP–fusion protein and subsequent anion–exchange chromatography as described previously [1]. Dissociation of immobilized MBP–GID1A subtracted (baseline drift, approximately -15 pg/mm² at 400 s and -28 pg/mm² at 1000 s). Gibberellin (5 μ M) was present in all solutions and running buffers. (**B**) dR/dt against R linearization (Scatchard plot) of the association phase. (**C**) ln(R₀/R) against time linearization of the dissociation phase.

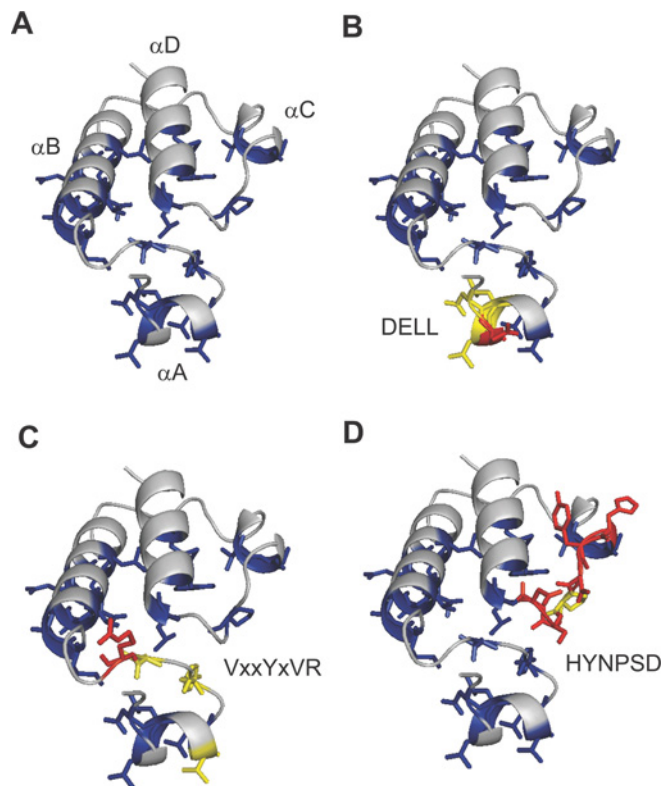


Figure S3 Structural prediction of the RGL1 N-terminal DELLA domain when in complex with GID1A

(**A**) Predicted RGL1^N (residues 1–137) tertiary structure, modelled from the GID1A/GA₄:GA₁^{11–113} crystal structure using SwissModel (PDB code 2ZSI) [2,3]. Conserved residues that form direct interactions between GAI and GID1A [2] are shown in blue. (**B–D**) RGL1^N model, indicating monoclonal antibody epitopes: 6C8 (**B**), BC9 (**C**) and AD7 (**D**). Antibody epitopes are highlighted in red and yellow. Yellow indicates a residue that also forms a direct GAI–GID1A interaction, whereas red residues do not.

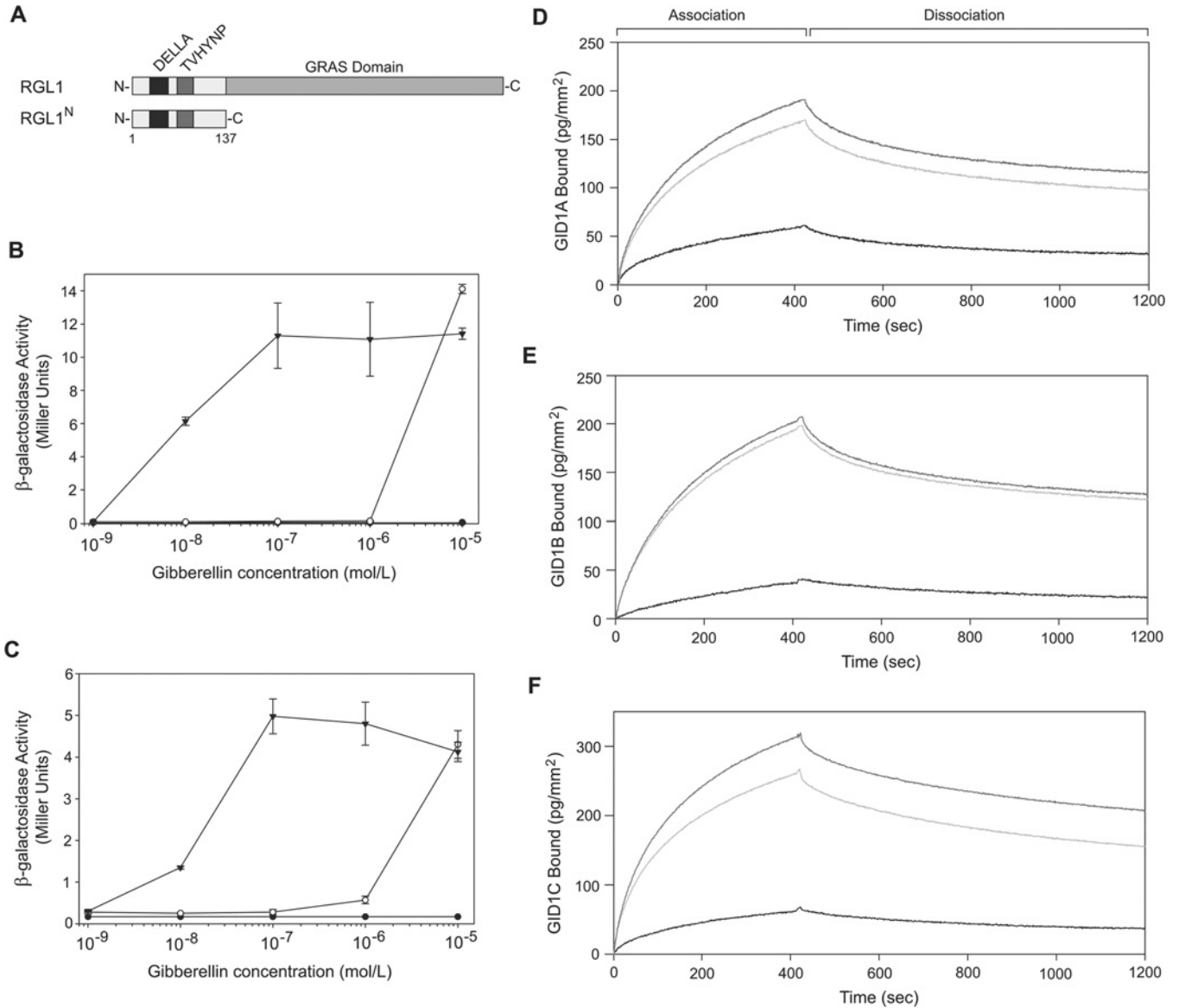


Figure S4 Comparison of the GA_3 - and GA_4 -dependent GID1A-RGL1^N interaction *in vivo* and *in vitro*

(A) Schematic representation of RGL1, and the N-terminal 137 residues of RGL1, RGL1^N, used in *in vitro* experiments. (B and C) Dose-response curves of yeast two- and three-hybrid assays. (B) Two-hybrid assay of the interaction between the Gal4 DNA-binding domain fusion of GID1A and the Gal4 activation-domain fusion of RGL1. (C) Three-hybrid assay of the interaction between the Gal4 DNA-binding domain fusion of SLY1 and the Gal4 activation domain fusion of RGL1 in the presence of GID1A. LacZ (β -galactosidase) reporter gene activity, from *Saccharomyces cerevisiae* grown in absence of gibberellins ((EPS)) and the presence of GA_3 ((EPS)) or GA_4 ((EPS)). The experiment was performed in duplicate (from two independent transformants); β -galactosidase assays were performed in triplicate for each transformant. Error bars show ± 1 S.D. (D-F) *In vitro* association and dissociation of gibberellin-saturated GID1A-C and RGL1^N, monitored using SPR. Interaction of RGL1^N with: (D) GID1A, (E) GID1B or (F) GID1C; in the absence (black), or presence of 100 M GA_3 (light grey) or 100 M GA_4 (dark grey). Gibberellins were mixed with GID1A-C 30 min prior to the binding assay and excluded from running buffer during the dissociation phase. Association, 0–420 s; dissociation, 420–1200 s. The amount of bound GID1A-C is shown as pg/mm² of surface area.



Figure S5 DELLA protein alignment

The full-length sequences for DELLA proteins from a range of plant species were aligned using AlignX (Vector NTI software, Invitrogen). Absolutely conserved residues are highlighted in orange; highly conserved residues are highlighted in blue; highly similar residues are highlighted in green; and similar residues are highlighted in yellow. The RGL1 gain-of-function mutants used in the present paper are displayed. The sequences of several DELLA gain-of function mutations are also displayed, indicating in-frame deletions or amino acid replacements. The *A. thaliana* (*At*) *gai-1*, *rga*^{Δ17}; grape (*Vv*, *Vitis vinifera*) *gai-1*; (*Zm*, *Zea mays*) rice (*Os*, *Oryza sativa*) *slr1w*^{ΔDELLA}, *slr1*^{ΔSPACE}, *slr1*^{ΔTVHYNP}, *slr1*^{ΔS/T/V}; barley (*Hv*, *Hordeum vulgare*) *sln1-d*; wheat (*Ta*, *Triticum aestivum*) *rht*, *rht-B1b*, *rht-D1b*; and maize field mustard (*Br*, *Brassica rapa*) *rga1-d*; *d8-MP* have been previously described as semi-dominant gibberellin-insensitive mutations [4–11]. *Ps*, pea (*Pisum sativum*).

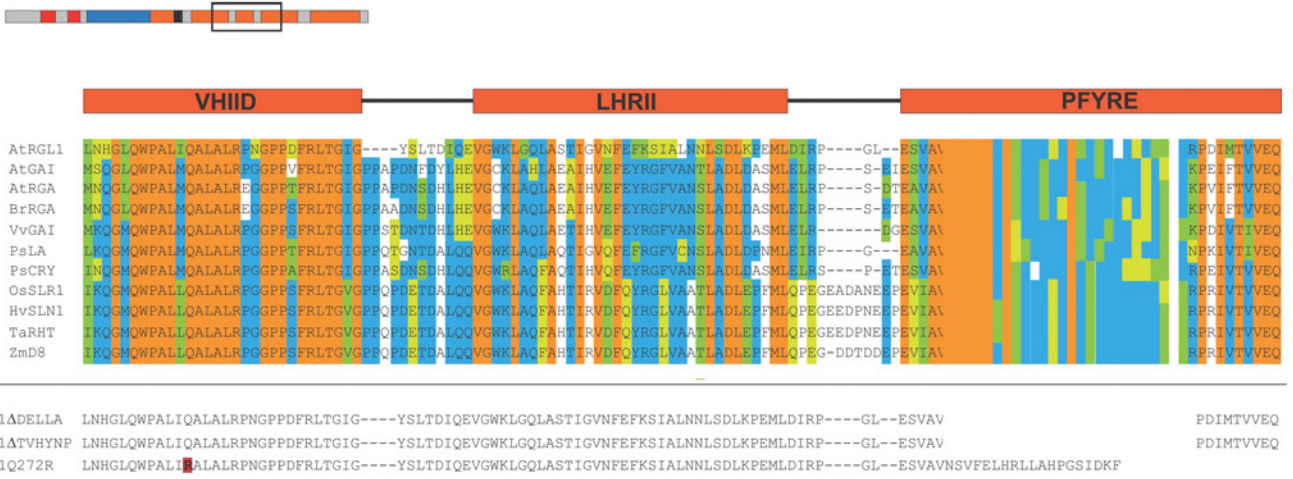
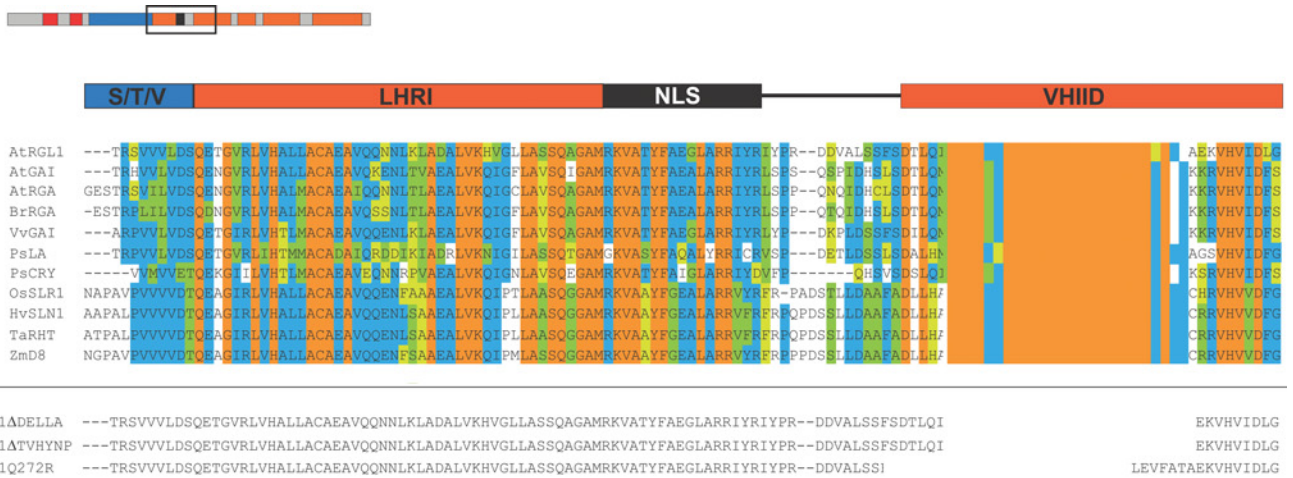
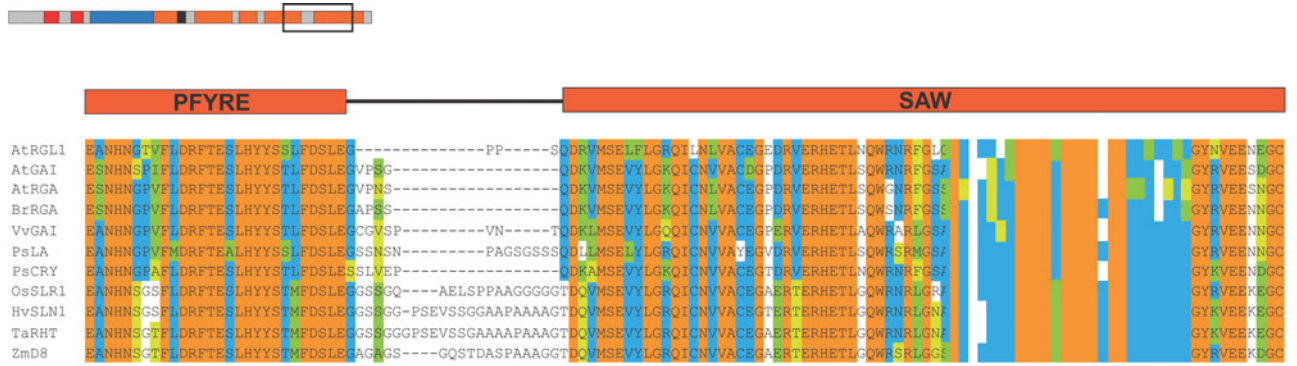


Figure S5 Continued



AtRGL1ADELLA	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQWRNRFGLG	YNVEENEGC
AtRGL1ATVHYNP	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQWRNRFGLG	YNVEENEGC
AtRGL1Q272R	EANHNGTVFLDRFTESLHYYSFLDSLEG-----PP-----SQRVMSEFLGRQILNLVACEGEDRVERHETLNQW	LYAGADGYNVEENEGC

AtGAI-1	ESNHNSPIFLDRFTESLHYYSFLDSLEGVPSG-----QDKVMSEVYLGKQICNVVACDGPDRVERHETLSQWRNRFGSA	GEGYRVEESDGC
AtRGA17	ESNHNGPVFLDRFTESLHYYSFLDSLEGVPSN-----QDKVMSEVYLGKQICNLVACEGDRVERHETLSQWRNRFGSSGLAPAH	VEESNGC
VvGAIXXX	EANHNGTVFLDRFTESLHYYSFLDSLEGCVSP-----VN-----TQDKLMSEVYLGKQICNVVACEGPERVERHETLAQWRARLGS	GGDGYRVEENNGC
BrRGA-d	ESNHNGPVFLDRFTESLHYYSFLDSLEGAPSS-----QDKVMSEVYLGKQICNLVACEGDRVERHETLSQWRNRFGSS	GEGYRVEENNGC
OsSLR1ADELLA	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ-----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVH	RVEEKEGC
OsSLR1ASPACE	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ-----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVH	RVEEKEGC
OsSLR1ATVHYNP	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ-----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRA	YRVEEKEGC
OsSLR1AS/T/V	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ-----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRAGFEPVH	RVEEKEGC
Osslr1-d4	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ-----AELSPPAAGGGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGRA	GGDGYRVEEKEGC
HvSLN1-d	EANHNSGFLDRFTESLHYYSFLDSLEGSSGQ-----PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGTERTERHETLGQWRNRLGNA	GDGYKVEEKEGC
TaRht	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQ-----PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GYKVEEKEGC
TaRHTXXX	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQ-----PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GGDGYKVEEKEGC
TaRHTXXX	EANHNSGTVFLDRFTESLHYYSFLDSLEGSSGQ-----PSEVSSGGAAPAAAAGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGNA	GGDGYKVEEKEGC
ZmD8-MP	EANHNSGTVFLDRFTESLHYYSFLDSLEGAGAGS-----GQSTDASPAAGGTDQVMSEVYLGKQICNVVACEGAERTERHETLGQWRNRLGGE	GGDGYRVEEKDGC



SAW

AtRGL1	LLLGWQTRPLIATSARINRVE-----
AtGAI	LLLGWHTRPLIATSARKLSTN-----
AtRGA	LLLGWHTRPLIATSARKLSTAA-----
BrRGA	LLLSWHTRPLIATSARKLSAVH-----
VvGAI	LLLGWHTRPLIATSARQLANKPALPSSTPASN
PsLA	LLLGWHTRPLIATSARKLPLSEK-----
PcCRY	LLLGWHTRPLIATSARKLAANSMVVSH-----
OsSLR1	LTLGWHTRPLIATSARVAAA-----
HvSLN1	LTLGWHTRPLIATSARLAA-----
TaRHT	LTLGWHTRPLIATSARLAGP-----
ZmD8	LTLGWHTRPLIATSARVAAAA-----

AtRGL1ADELLA	LLLGWQTRPLIATSARINRVE-----
AtRGL1ATVHYNP	LLLGWQTRPLIATSARINRVE-----
AtRGL1Q272R	LLLGWQTRPLIATSARINRVE-----

AtGAI-1	LLLGWHTRPLIATSARKLSTN-----
AtRGA17	LLLGWHTRPLIATSARKLSTAA-----
VvGAIXXX	LLLGWHTRPLIATSARQLANKPALPSSTPASN
BrRGA-d	LLLSWHTRPLIATSARKLSAVH-----
OsSLR1ADELLA	LTLGWHTRPLIATSARVAAA-----
OsSLR1ASPACE	LTLGWHTRPLIATSARVAAA-----
OsSLR1ATVHYNP	LTLGWHTRPLIATSARVAAA-----
OsSLR1AS/T/V	LTLGWHTRPLIATSARVAAA-----
Osslr1-d4	LTLGWHTRPLIATSARVAAA-----
HvSLN1-d	LTLGWHTRPLIATSARLAA-----
TaRht	LTLGWHTRPLIATSARLAGP-----
TaRHTXXX	LTLGWHTRPLIATSARLAGP-----
TaRHTXXX	LTLGWHTRPLIATSARLAGP-----
ZmD8-MP	LTLGWHTRPLIATSARVAAAA-----

Figure S5 Continued

Table S1 Oligonucleotide sequences for amplification and sequencing

Description	Sequence
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RGL1 reverse primer for pACT2 (SacI)	5'-CGAGCTCGTTATCCACACGATTGATTCGCC-3'
RGL1 internal nt93 reverse primer	5'-GACGTGGCACACAAGCTTG-3'
RGL1 internal nt145 forward primer	5'-CACTCCGGCAGCTTCTTC-3'
RGL1 internal nt213 reverse primer	5'-AGCATGCTCTCGGATCTTGAC-3'
RGL1 internal nt268 forward primer	5'-ATTCGAGATCCATACCAAGAAC-3'
RGL1 internal mutagenic primer reverse	5'-CTTTGATTGCGACTCTTGCTTTAC-3'
RGL1 internal nt830 forward primer	5'-CCGGCCATTGTAACCATGG-3'
RGL1 internal nt411 reverse primer	5'-GTTCTTCTCCTTACTCATTCCGCCACCCGTAGAGGATAACTCCGAT-3'
RGL1 internal nt412 forward primer	5'-GCATGGATGAACATACAAGAGGGGTCGCTCTGTGGTGGTTTTGGATTC-3'
RGL1 forward for pMalc2x (BamHI)	5'-CGGGATCCATGAAGAGAGACACAACCACC-3'
RGL1 reverse N-term internal for pMalc2x (Sall)	5'-GACGCGTCGACTACGTAGAGGATAACTCCGATCAA-3'
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GID1A forward primer for pBridge MCSII (NotI)	5'-ATAAGAATCGCGCCGCTATGGCTGCGAGCGATGAAG-3'
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GID1B forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGCTGGTGAACGAAGT-3'
GID1B reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGCTAAGGAGTAAGAAGCACAGG-3'
GID1C forward primer for pGBKT7 (EcoRI)	5'-GGAATTCATGGCTGGAAGTGAAGAAGTT-3'
GID1C reverse primer for pGBKT7 (BamHI)	5'-CGGGATCCGTTCAATGGCATTCTGCGTTTAC-3'
SLY1 forward primer (EcoRI)	5'-CGGAATTCATGAAGCGCAGTACTACCGAC-3'
SLY1 reverse primer (BamHI)	5'-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTC-3'
SLY1 mutagenic primer reverse primer (BamHI)	5'-CGCGGATCCGTTATTTGGATTCTGGAAGAGGTCCTCTTAGTAAACTCATCTTCTGTAG-3'
GFP forward primer	5'-ATCGAGGATATCCTCTACGGGTGGCAATGAGTAAAGGAGAAGAAC-3'
GFP reverse primer	5'-GAATCCAAAACCACCACAGAGCGACCCCTCTTTGTATGTTTCATCCAGC-3'
pACT2 forward sequencing primer	5'-CTATCTATTGATGATGAAGATAC-3'
pACT2 reverse sequencing primer	5'-AGTTGAAGTGAACCTTGGGGTT-3'
pGBKT7 forward sequencing primer (T7)	5'-TAATACGACTCACTATAGGG-3'
pGBKT7 / pBridge MCSI reverse sequencing primer	5'-TAAGAGTCACTTAAAAATTTGTAT-3'
pBridge MCSII forward sequencing primer	5'-TTGGGAACGTGTTGGTTG-3'
pBridge MCSII reverse sequencing primer	5'-CCGTATTACCGCCTTTGAGT-3'
pGADT7 forward sequencing primer	5'-TAATACGACTCACTATAGGG-3'
pGADT7 reverse sequencing primer	5'-GTGAACCTTGGGGTTTTTCAGTATCTACGATT-3'
pMALc2x forward sequencing primer	5'-GGTCGTGAGACTGTCGATGAAGCC-3'
pMALc2x reverse sequencing primer	5'-CGCCAGGGTTTTCCAGTCCACGAC-3'

REFERENCES

- Sun, X. L., Frearson, N., Kirk, C., Jones, W. T., Harvey, D., Rakonjac, J., Foster, T. and Al-Samarrai, T. (2008) An *E. coli* expression system optimized for DELLA proteins. *Protein Expression Purif.* **58**, 168–174
- Murase, K., Hirano, Y., Sun, T. P. and Hakoshima, T. (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature* **456**, 459–463
- Arnold, K., Bordoli, L., Kopp, J. and Schwede, T. (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* **22**, 195–201
- Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P. and Harberd, N. P. (1997) The *Arabidopsis* GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* **11**, 3194–3205
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- Itoh, H., Ueguchi-Tanaka, M., Sato, Y., Ashikari, M. and Matsuoka, M. (2002) The gibberellin signaling pathway is regulated by the appearance and disappearance of SLENDER RICE1 in nuclei. *Plant Cell* **14**, 57–70
- Muangprom, A., Thomas, S. G., Sun, T. P. and Osborn, T. C. (2005) A novel dwarfing mutation in a green revolution gene from *Brassica rapa*. *Plant Physiol.* **137**, 931–938
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- Boss, P. K. and Thomas, M. R. (2002) Association of dwarfism and floral induction with a grape 'green revolution' mutation. *Nature* **416**, 847–850
- Chandler, P. M., Marion-Poll, A., Ellis, M. and Gubler, F. (2002) Mutants at the *Slender1* locus of barley cv Himalaya: molecular and physiological characterization. *Plant Physiol.* **129**, 181–190
- Weston, D. E., Elliott, R. C., Lester, D. R., Rameau, C., Reid, J. B., Murfet, I. C. and Ross, J. J. (2008) The pea DELLA proteins LA and CRY are important regulators of gibberellin synthesis and root growth. *Plant Physiol.* **147**, 199–205

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