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Research

MEDIATOR15 destabilizes DELLA protein to promote gibberellin-mediated plant development

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Summary

- Mediator, a transcriptional coactivator, regulates plant growth and development by interacting with various transcriptional regulators. MEDIATOR15 (MED15) is a subunit in the Mediator complex potentially involved in developmental control.
- To uncover molecular functions of *Arabidopsis* MED15 in development, we searched for its interactors. MED15 was found to interact with DELLA proteins, which negatively regulate gibberellic acid (GA) signaling and positively regulate GA biosynthesis.
- Mutants and overexpressors of *MED15* exhibited multiple GA-related growth phenotypes, which resembled the phenotypes of the *DELLA* overexpressor and mutant, respectively. Consistent with this observation, DELLA protein levels were inversely correlated with MED15 protein levels, suggesting that MED15 activates GA signaling through DELLA degradation. MED15 was required not only for DELLA-mediated induction of GA-biosynthesis gene expression but also for GA-mediated degradation of DELLA. Therefore, MED15 facilitates DELLA destruction not only by promoting GA biosynthesis but also by accelerating DELLA turnover. Furthermore, MED15-mediated GA signaling was required for timely developmental responses to dark and warm conditions.
- Our results provide insight into developmental control by Mediator via precise regulation of DELLA stability. These findings are potentially useful for the generation of new crop cultivars with ideal body architecture.

Introduction

For the successful completion of its life cycle, a plant's growth and development is precisely controlled by multiple regulators. Many studies have shown that such regulators include transcription factors (TFs) and plant hormone signaling pathways (Shibata & Sugimoto, 2019; Verma *et al.*, 2022; Maple *et al.*, 2024). In crop breeding, developmental regulators have been selected as potential targets to generate cultivars with useful developmental traits to improve crop productivity. For example, the green revolution was achieved by introducing the semidwarf phenotype into high-yielding crop cultivars, in which mutations in gibberellic acid (GA)-related genes were responsible for the phenotype (Hedden, 2003). Owing to recent progress in breeding techniques, such as transformation and genome editing (Chen *et al.*, 2019; Su *et al.*, 2023), it has become possible to generate new cultivars with desired traits within a short period of time. However, these techniques require detailed information on the mechanisms of the target developmental processes. Therefore, it is important to understand how plant growth is regulated at the molecular level, which is potentially useful to develop resilient agriculture to mitigate adverse effects of global climate change.

Mediator is a critical regulator of plant growth and development via its role as a transcriptional coactivator. Mediator not only regulates specific TF activity but also affects global plant development through the modulation of plant hormone signaling (Samanta & Thakur, 2015; Buendía-Monreal & Gillmor, 2016). Although Mediator itself does not bind to DNA, it physically interacts with both TFs and RNA polymerase to activate transcription through the stabilization of the transcriptional machinery (Jeronimo & Robert, 2017; Soutourina, 2018; Richter *et al.*, 2022). Furthermore, recent studies showed that Mediator can recruit histone modifiers to modulate gene expression in *Arabidopsis* (Guo *et al.*, 2021, 2023; Shapulatov *et al.*, 2023). Because Mediator is a multi-subunit scaffolding protein complex comprising of over 30 subunits, mutants of Mediator subunits exhibit different developmental phenotypes depending on the specific role of each subunit. Through mutant analysis, previous studies have characterized Mediator subunits involved in various aspects of plant development, such as germination, organ size control, and flowering (Samanta & Thakur, 2015; Buendía-Monreal & Gillmor, 2016). MEDIATOR15 (MED15) is a subunit of the Tail module, which is the main module contributing to the interaction with TFs in the Mediator complex. In

Arabidopsis, MED15 was initially identified as a positive regulator of salicylic acid (SA) signaling and biotic stress responses (Canet *et al.*, 2012). Further study showed that MED15 is involved in fatty-acid biosynthesis through its interaction with WRINKLED1 (Kim *et al.*, 2016). In addition, the pleiotropic growth phenotypes of T-DNA insertion mutants of *MED15* implicate its involvement in developmental control since the mutant plants exhibit small stature, chlorosis, and abnormal flower structure (Canet *et al.*, 2012). The observation that MED15 is expressed in all tissues from the seedlings stage to the reproductive stage (Kim *et al.*, 2016) also implies the potential involvement of MED15 in multiple developmental processes. However, how MED15 regulates plant development remains unclear at the mechanistic level. In rice, quantitative trait locus mapping has identified a nonsynonymous single-nucleotide polymorphism in *OsMED15a*, which is associated with grain weight (Dwivedi *et al.*, 2019). Therefore, a better understanding of the molecular function of MED15 could be helpful to improve developmental traits of crops.

Gibberellic acid is a plant hormone known as a positive regulator of plant growth at various developmental stages. For example, GA accelerates germination, flowering, and elongation of petioles and stems. Hence, mutants defective in GA biosynthesis and signaling show a dwarf phenotype with delayed flowering (Sun, 2008). Unlike other plant hormone signaling, the GA signaling pathway does not have any specific TFs responsible for GA-mediated transcriptional regulation. Instead, DELLA, which cannot directly bind to DNA, functions as a negative regulator of GA signaling. In the *Arabidopsis* genome, there are five *DELLA* family members (*REPRESSOR OF ga1-3* (*RGA*), *GA-INSENSITIVE* (*GAI*), *RGA-LIKE1* (*RGL1*), *RGL2*, and *RGL3*) with overlapping functions. Through its interaction with TFs, DELLA positively or negatively modulates their activity, thereby attenuating GA signaling, which results in growth suppression (Davière & Achard, 2016). Upon GA accumulation, DELLA forms a complex with a GA-receptor, GIBBERELLIN-INSENSITIVE DWARF1 (GID1) and an SCF complex containing SLEEPY1 F-box protein (SCF^{SLY1}) resulting in DELLA degradation via the 26S proteasome pathway. Degradation of DELLA not only activates the GA signaling pathway but also functions as negative feedback regulation of the GA signaling because DELLA is an activator of GA-biosynthesis gene expression (Hedden, 2020). When recruited to promoters of GA-biosynthesis genes via its interaction with TFs, DELLA functions as a transcriptional activator. A small transactivation motif called 9aaTAD is considered to be responsible for this function (Hernández-García *et al.*, 2019). Given its transactivator function, it is possible that DELLA is associated with the Mediator complex. During the preparation of this report, Hernández-García *et al.* (2024) reported the interaction between MED15 and DELLA. However, it is still not fully understood how the MED15–DELLA interaction affects GA signaling and GA-mediated plant development.

To understand the role of MED15 in regulating plant development, we performed a yeast two-hybrid screening and identified MED15 interactors. Here, we show that MED15 functions as a positive regulator of GA signaling through its interaction with DELLA proteins. We showed the growth phenotypes of *MED15* mutants and overexpressors are similar to those of *DELLA* overexpressors and mutants, respectively. These results are consistent with the negative correlation between MED15 and DELLA protein levels. We also showed that MED15 is involved not only in DELLA-mediated induction of GA-biosynthesis gene expression but also in GA-mediated destabilization of DELLA. Therefore, as an upstream factor of DELLA, MED15 positively regulates plant growth by reducing the repressive effect of DELLA on plant growth. We further found that MED15 is required for rapid DELLA destruction under dark and warm conditions. These MED15 functions enable plants to quickly adjust DELLA protein levels to execute timely developmental responses to changing environmental conditions.

Materials and Methods

Plant materials and growth conditions

Plant materials used in this study were *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia-0 (Col-0) with the exception of the *della* pentuple mutant, which was ecotype Landsberg *erecta* (L*er*). Surface-sterilized seeds were sown on ½ Murashige & Skoog (MS) medium containing 1% sucrose and 0.8% agar. After stratification at 4°C for 3 d under dark conditions, plants were grown in a growth chamber set at 22°C. Growth conditions were 16 h : 8 h, light : dark (long-day, LD) with a light intensity of 60 ± 5 μmol m^{-2} s^{-1} unless otherwise stated. For growth assays under short-day (SD) conditions, an 8-h light photoperiod was used. For analysis of etiolated seedlings, seeds were sown and placed under continuous light conditions. After 1 d, the plates were wrapped with aluminum foil to grow seedlings (for 4 d) under dark conditions. Mutants used in this study were *med2* (SALK_023845), *med5a* (SALK_119561), *med5b* (SALK_037472), *nrb4-2* (Canet *et al.*, 2012), *nrb4-4* (SAIL_792_F02), *nrb4-5* (GABI_955_E02), *med16* (WiscDsLox_504A08), *med23* (SALK_119080), *med25* (SALK_129555), *rga-29* (SALK_089146), *ga1* (SALK_109115), and *della* pentuple mutant (Feng *et al.*, 2008). *HS::gai-1D* (Alabadí *et al.*, 2008) and *pRGA::GFP-RGA* (Silverstone *et al.*, 2001) lines were generated in previous studies. Plant transformation was performed with the floral dip method (Zhang *et al.*, 2006) using *Agrobacterium tumefaciens* strain GV3101.

Vector construction

Cloning was performed using Gateway technology (Invitrogen). *pMED15::MED15* and *GA20ox2* promoters were amplified by polymerase chain reaction (PCR) from Col-0 genomic DNA and cloned into pDONR221 (Invitrogen). Coding sequences of *MED2*, *MED15*, *RGA*, *GAI*, *RGL1*, *RGL2*, *RGL3*, and *GAF1* were amplified by PCR from Col-0 cDNA and inserted into pDONR221. Partial fragments of *MED15* and *RGA* were amplified from the full-length *MED15* and *RGA* clones using PCR. Primers used for cloning are listed in Supporting Information

Table S1. Entry clones were recombined with destination vectors listed in Table S2.

Plasmids used for the reporter assay were generated in this study. To obtain the control plasmid, the *35S-GUS-Nos*T fragment from pBI121 was inserted into the HindIII-EcoRI site of pUC19. To generate the reporter plasmid, the *35S-GUS* fragment in the control plasmid was removed by digestion with HindIII-SacI and replaced with the Gateway cassette (GW)-*ELUC* fragment from pMpGWB131 (Ishizaki *et al.*, 2015). To generate the effector plasmid, the *35S-GUS* fragment in the control plasmid was removed by digestion with HindIII-SacI and replaced by the *35S*-GW-FLAG fragment from pMpGWB111 (Ishizaki *et al.*, 2015). The *35S* promoter was replaced with a *35S* promoter fused to an omega sequence.

GA and PAC treatment for plant growth assay

To observe the effect of GA on seedling growth, seeds were directly sown on MS medium containing GA_3 (Sigma-Aldrich) and incubated for 7 d. To observe the effect of GA on flowering, 7-d-old seedlings grown on MS medium were transferred to soil. After 3 d, plants were sprayed every 2 d with water containing dimethyl sulfoxide (DMSO) or 100 μM GA until all plants started bolting. To observe the effect of paclobutrazol (PAC) on seedling growth, seeds were sown on MS medium. After incubation for 1 d, seeds were transferred to PAC-containing MS medium and grown for 6 d. To analyze hypocotyl length, plant images were taken after incubation and used for measurement of hypocotyl length using Fiji (Schindelin *et al.*, 2012).

GA and PAC treatment for RNA and protein analysis

Seeds were sown on MS medium. After incubation for 1 d, seeds were transferred to MS medium containing 1 μM PAC and grown for 6 d. Seedlings were harvested for RNA and protein extraction from PAC-treated plants. To prepare samples for the time-course immunoblot analysis, seedlings were transferred into water containing 10 μM GA, 1 μM PAC, and 200 μM cycloheximide and incubated under normal growth conditions.

Chemical treatments

Plants grown on MS medium for 7 d were used. Seedlings were transferred into water containing β-estradiol (β-EST) and incubated under normal growth conditions for 24 h. For MG132 and E-64d treatment, seedlings were transferred into water containing DMSO, 50 μM MG132, 20 μM E-64d, or both 50 μM MG132 and 20 μM E-64d and incubated under normal growth conditions for 24 h.

Germination assays

Seeds were sown on MS medium containing DMSO or 100 nM PAC and incubated under LD conditions. After 7 d, ungerminated seeds were transferred to MS medium containing 100 μM GA to assess seed viability and genotypes.

Heat, dark, and warm treatment

Plants harboring *HS::gai-1D* were grown under normal growth conditions. After incubation for 2 d, seedlings were treated with heat for 1 h using a water bath set at 37°C every day until Day 7. For dark treatment, plants were first grown under continuous light conditions. After incubation for 4 d, Petri dishes were wrapped with aluminum foil to block the light and incubated for 1 d. For warm treatment, plants were first grown under normal growth conditions. After incubation for 4 d, plants were transferred to a growth chamber set at 29°C with LD photoperiod and grown for 3 d. After the treatment, plant images were taken and used for measurement of hypocotyl length with Fiji.

Total protein extraction

Frozen plant samples were ground using TissueLyzer (Qiagen) in 2-ml microcentrifuge tubes and homogenized in 1.25× sample buffer (62.5 mM Tris–HCl (pH 6.8), 1.25% SDS, 10 M urea, and 894 mM β-mercaptoethanol). After removal of debris by centrifugation, the supernatants were collected and denatured at room temperature for 30 min. Protein samples were separated by SDS-PAGE and analyzed by immunoblots with anti-MED15 (Kim *et al.*, 2016), anti-RGA (Cat no. AS11 1630; Agrisera, Vännäs, Sweden), anti-myc (Cat no. 16286-1-AP; Proteintech, Rosemont, IL, USA), and anti-ACTIN (Abcam, Cat no. ab197345) antibodies. The band intensity was quantified using the Image Lab software (Bio-Rad).

Total RNA extraction and reverse transcription quantitative polymerase chain reaction quantitative polymerase chain reaction

Total RNA was extracted from 7- or 8-d-old seedlings using EasyPure Plant RNA Kit (TransGen Biotech, Beijing, China). cDNA was synthesized from 500 ng of total RNA using iScript cDNA Synthesis Kit (Bio-Rad). Quantitative polymerase chain reaction reactions were performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) on a CFX96 Real-Time PCR Detection System (Bio-Rad). Gene expression levels were normalized against *Actin2*. Primers used for reverse transcription quantitative polymerase chain reaction are listed in Table S1.

Yeast two- and three-hybrid assays

Full-length MED15 was used as a bait in a yeast two-hybrid screen with a transcription factor library as previously described (Mitsuda *et al.*, 2010). Yeast two-hybrid assays analyzing specific interactions between MED15 and DELLA proteins were performed as described (Ohama *et al.*, 2021). In yeast three-hybrid assays, pBridge (Clontech, Mountain View, CA, USA) was used to express GID1A and SLY1 as the GAL4 DNA-binding domain

(BD)-fused proteins and MED15 as the nuclear localization signal (NLS)-fused protein. The interaction was examined by Yeast β-Galactosidase Assay Kit (Pierce, Rockford, IL, USA).

In vitro pull-down assays

DNA constructs for MBP-MED15-myc and GST-RGA-HA were transformed into *Escherichia coli* strain BL21-CodonPlus-RIPL (Agilent Technologies, Santa Clara, CA, USA). Expression of recombinant proteins was induced with 0.5 mM isopropyl-β-D-thiogalactoside at 28°C for 3 h or at 22°C for 5 h. MBP- and GST-fused recombinant proteins were purified and used for *in vitro* pull-down assays as described previously (Park *et al.*, 2022). Pulled-down protein samples were separated by SDS-PAGE and detected by immunoblot analysis with anti-MBP (Cat no. 15089-1-AP; ProteinTech) and anti-GST (Cat no. sc-138; Santa Cruz) antibodies, respectively.

Transient expression in tobacco leaves

Cultured cells of *Agrobacterium tumefaciens* strain GV3101 harboring appropriate expression vectors and an expression vector for *p19* (Liu *et al.*, 2010) were harvested and resuspended in infiltration buffer (10 mM MES-KOH (pH 5.7), 10 mM $MgCl_2$, 200 μM acetosyringone). After incubation at room temperature for 3 h, *Agrobacterium* suspensions were infiltrated into leaves of 4-wk-old *Nicotiana benthamiana* with a needleless syringe and the leaves were analyzed 3 d later.

Co-immunoprecipitation (Co-IP) assays

YFP and MED15-YFP proteins were transiently expressed in tobacco leaves together with RGA-myc. The leaves were harvested and frozen by liquid nitrogen. Frozen samples were ground in liquid nitrogen and homogenized in Co-IP buffer (50 mM Tris–HCl (pH 7.4), 100 mM NaCl, 0.1% Triton X-100, 10% glycerol, 1 mM dithiothreitol, 20 μM MG132, 1× cOmplete protease inhibitor cocktail). After removal of debris by centrifugation, supernatants were mixed with GFP-Trap Magnetic Agarose (Chromo-Tek, Martinsried, Germany) to purify YFP protein. After five times of wash with Co-IP buffer, the beads were boiled with NuPAGE LDS Sample buffer. Proteins in the immune-precipitates were separated with SDS-PAGE and analyzed by immunoblots with anti-GFP (Cat no. 50430-2-AP; Proteintech) and anti-myc antibodies.

BiFC assays

Proteins fused to N- or C-terminal half of YFP were transiently expressed in tobacco leaves together with CFP fused to a nuclear localization signal. The YFP and CFP fluorescence signals were detected with FV3000 confocal microscope (Olympus, Tokyo, Japan).

Fluorescence observation

One-week-old seedlings expressing *GFP-RGA* and *MED15-YFP* were observed with FV3000 confocal microscope. The GA treatment was performed by placing the seedlings in GA-containing water (45-min or 2-h treatment) or on GA-containing medium (3-d treatment). The dark and warm treatments were performed as described above. The fluorescence intensity in nucleus was measured by FIJI.

Measurement of GA_4 by LC-MS/MS

GA_4 extraction was performed as described by Boonyaves *et al.* (2023) with modifications. Around 200 mg of 7-d-old seedlings was ground in liquid nitrogen and suspended in 80% methanol-1% acetic acid. Samples were shaken at 4°C for 1 h followed by overnight incubation at 20°C. After passing through Oasis Prime HLB (Waters, Milford, MA, USA) for cleanup, the supernatants were dried with a vacuum evaporator. The dried eluate was dissolved in 50% methanol-1% acetic acid. Samples were separated by UHPLC chromatography (Accucoretm Vanquish C18+ 100 mm length × 2.1 mm, ThermoFisher Scientific, Waltham, MA, USA) with 5–50% acetonitrile gradient containing 0.05% acetic acid, at 400 μl min^{-1} over 14 min. Detection of GA_4 was performed by using Q-Exactive mass spectrometry (Orbitrap detector, ThermoFisher Scientific) by MS2 mode. GA_4 concentrations were determined using standard calibration curves.

Reporter assays with Arabidopsis mesophyll protoplast

Protoplast isolation and transfection were performed according to Yoo *et al.* (2007). Reporter assays were performed as described previously (Ohama *et al.*, 2016) with modifications. Here, the luciferase (LUC) assays were performed using One-Step Luciferase Assay System (BPS Bioscience, San Diego, CA, USA). For detection of GUS and LUC activities, Spark microplate reader (TECAN) was used.

Results

MED15 interacts with DELLA

Mediator regulates gene expression through interacting with transcriptional regulators. As a first step toward understanding how MED15 regulates plant growth, we performed a yeast two-hybrid screening using full-length MED15 and a TF-specific library (Mitsuda *et al.*, 2010). Over 200 putative interactors of MED15 were isolated including three DELLA family proteins, namely RGA, GAI, and RGL2. Further work was performed with the DELLA proteins because they are important growth regulators. We verified the interactions between MED15 and all DELLA proteins and confirmed interactions among all combinations in yeast two-hybrid assays (Fig. 1a). We further examined MED15–DELLA interaction focusing on RGA, the major DELLA species in *Arabidopsis*. An *in vitro* pull-down assay showed that the MED15–RGA interaction was direct (Fig. 1b). Through narrowing down of the interaction domain in each protein, we generated a truncation series of MED15 avoiding disruption of the secondary structure predicted by JPred4 (Drozdetskiy *et al.*, 2015). Our

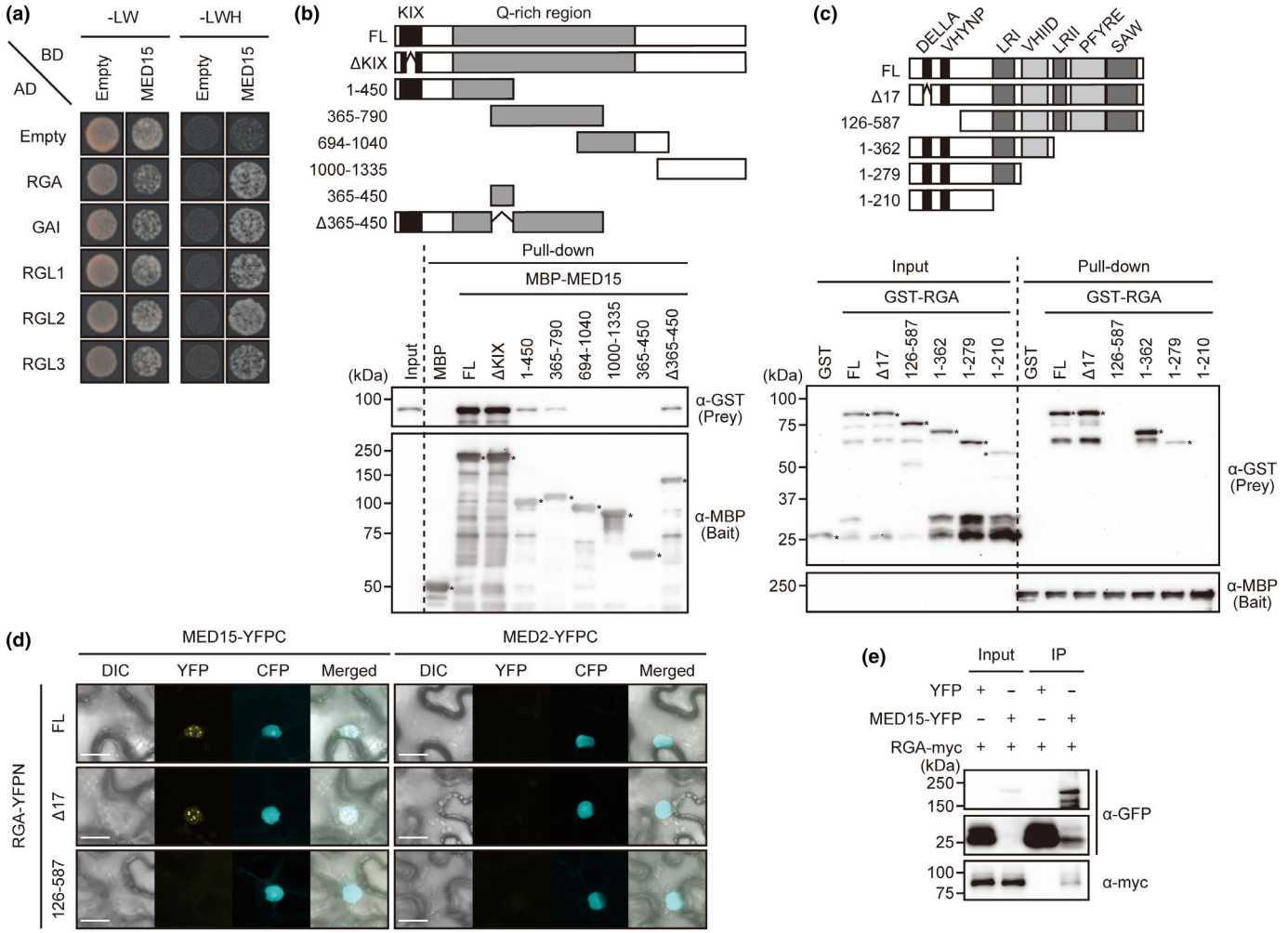

Fig. 1 MEDIATOR15 (MED15) interacts with DELLAs. (a) Yeast two-hybrid assay showing the interaction between MED15 and DELLAs. -LW, nonselection medium lacking Leu and Trp; -LWH, selection medium lacking Leu, Trp, and His; BD, bait protein fused to GAL4 DNA-binding domain; AD, prey protein fused to GAL4 activation domain. (b, c) *In vitro* pull-down assays showing MED15–RGA interaction and definition of the interaction domains. Schematic diagrams depicting structure of MED15 (b) and RGA (c). Names of truncated proteins corresponding to the N- and C- terminal amino acid residues used in each protein. In (b), GST–RGA was used as a prey and pulled down by MED15 derivatives fused to MBP. In (c), RGA derivatives fused to GST were used as preys and pulled down by MBP–MED15. Proteins were detected by immunoblots with anti-GST or anti-MBP antibodies. The positions of the full-length protein bands are indicated by asterisks. (d) Bimolecular fluorescence complementation (BiFC) assay showing MED15–RGA interaction in tobacco leaves. MED2/15 and RGA derivatives were fused to C- and N-terminal half of YFP fragment, respectively. Names of RGA derivatives correspond to those in (c). MED2 and RGA126-587 served as negative controls. CFP signal from NLS-CFP served as a nucleus marker and positive control for transformation. Bars, 20 μm. (e) Co-immunoprecipitation assay showing MED15–RGA interaction in tobacco leaves. Protein samples were extracted from tobacco leaves transiently expressing MED15–YFP and RGA-myc. Crude extracts (Input) and immunoprecipitates obtained with anti-GFP antibody (IP) were analyzed by immunoblots with anti-GFP and anti-myc antibodies.

analysis revealed that the KIX domain in MED15 was not responsible for the MED15–RGA interaction (Fig. 1b) although this domain is a well-characterized interacting domain with transcription factors in mammalian and yeast cells (Thakur *et al.*, 2014). Analysis with MED15 fragments revealed that the N-terminal half (1–450 and 365–790) interacted with RGA. Because the overlapping region between these fragments (365–450) was neither sufficient nor necessary for the interaction, a large portion of the MED15 N-terminal half is involved in the MED15–RGA interaction. However, a weaker recovery of RGA from truncated MED15 fragments implied that the entire protein including the C-terminal half might be required to achieve maximum binding affinity to RGA. About RGA, deletion of the DELLA motif (Δ17) did not affect its interaction with MED15, whereas truncation of the N-terminal region including the VHYNP motif abolished the interaction (Fig. 1c). Truncation from the C-terminus showed a necessity of the N-terminal half of RGA until the LRI motif for the interaction. A notable decrease in the recovery of the RGA_1–279 protein compared with RGA_1–362 suggests that the VHIID motif also plays a role in the MED15–RGA interaction. These results indicate that the MED15-interacting domain on RGA is different from most

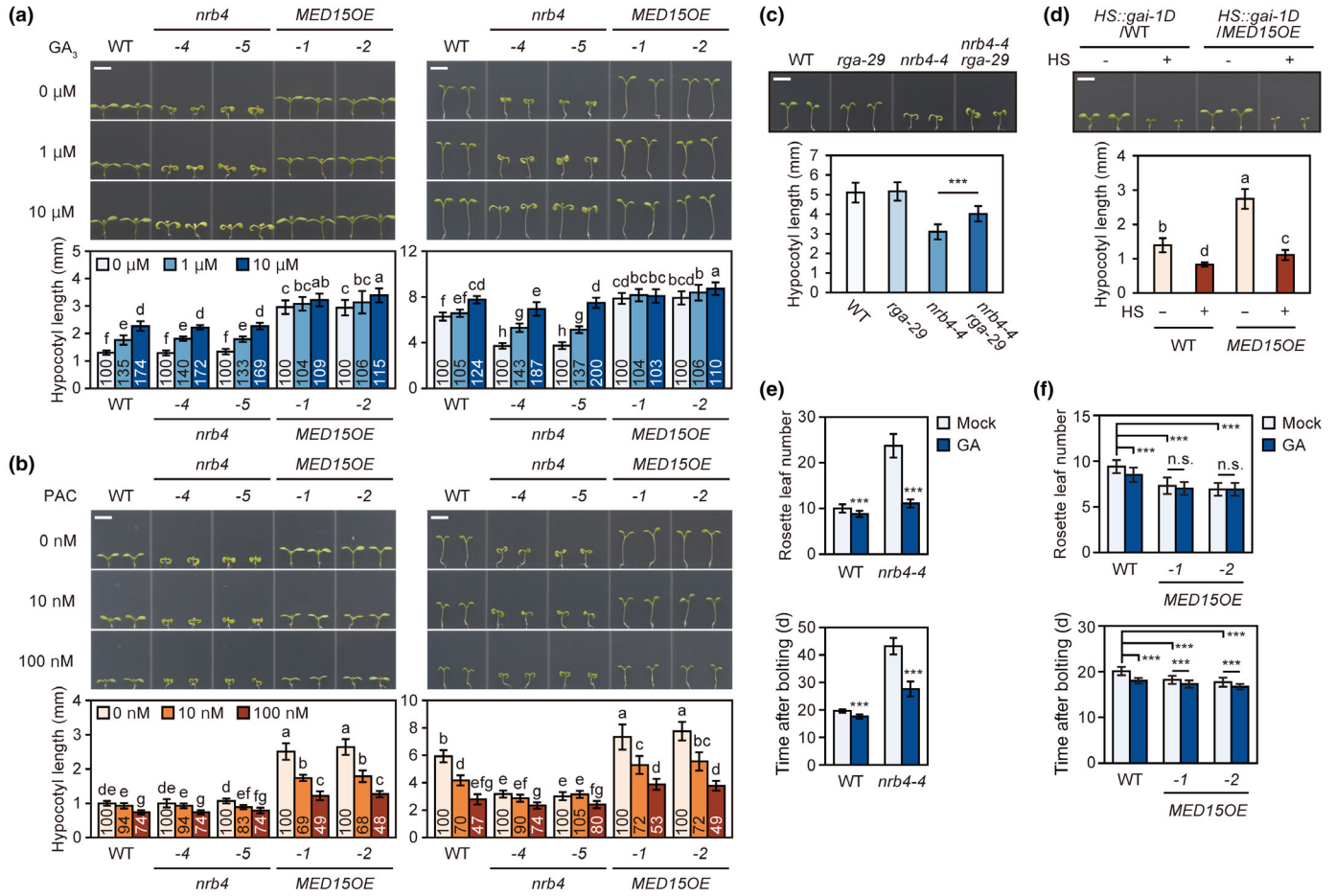

Fig. 2 MEDIATOR15 (MED15) is a positive regulator of the gibberellic acid (GA) response in *Arabidopsis*. (a, b) Hypocotyl elongation assay on GA and paclobutrazol (PAC) medium. Seedlings were grown on medium containing the indicated concentration of GA or PAC for 7 d. Left and right panels show data obtained under long-day (LD) and short-day (SD) conditions, respectively. Quantified data are shown in the lower panels. Numbers shown in bars indicate relative hypocotyl lengths compared with control seedlings. Error bars indicate standard deviation ($n \geq 15$). Bars, 5 mm. Different lowercase letters indicate statistically significant differences between means (Tukey's multiple comparison test, $P < 0.05$). (c) Hypocotyl lengths of *rga-29*, *nrb4-4*, and *nrb4-4 rga-29*. Seedlings were grown on MS medium under SD condition. Error bars indicate standard deviation ($n \geq 15$). ***, $P < 0.001$ in two-tailed Student's *t*-test. Bar, 5 mm. (d) Hypocotyl lengths of *HS:gai-1D*/WT and *HS-:gai-1D/MED15OE*. Seedlings were grown on MS medium under LD condition. After 2 d of incubation, seedlings were treated with 37°C for 1 h every day for 5 d. Error bars indicate standard deviation ($n \geq 21$). Bar, 5 mm. Different lowercase letters indicate statistically significant differences between means (Tukey's multiple comparison test, $P < 0.05$). (e, f) Rosette leaf number at flowering and days to flowering. Plants were grown under LD conditions and treated with 100 μM GA every 2 d. Error bars indicate standard deviation ($n \geq 16$). ***, $P < 0.001$ in two-tailed Student's *t*-test.

DELLA interactors, which require only the GRAS domain in the C-terminal half (Hernández-García *et al.*, 2021; Xue *et al.*, 2022). A bimolecular fluorescence complementation (BiFC) assay in tobacco leaves also confirmed the MED15–RGA interaction, whereas RGA did not interact with MED2, a subunit tightly associated with MED15 in the Tail module (Zhang *et al.*, 2004) (Fig. 1d). BiFC signals were found as speckles in the nucleus, which are similar to the subcellular localization of MED15 transiently expressed in tobacco (Huang *et al.*, 2021; Kim *et al.*, 2022). Consistent with the pull-down assay, truncation of N-terminal region abolished the BiFC signal although FL and Δ17 forms of RGA interacted with MED15. Finally, we validated the *in vivo* interaction with Co-IP assays (Fig. 1e).

MED15 positively regulates GA responses

Because DELLA is a central regulator of GA signaling, we examined whether MED15 is involved in GA responses through the analysis of T-DNA insertion mutants (*nrb4-4* and *nrb4-5* alleles) disrupted in *MED15* and of *MED15* overexpressors (*-MED15OE-1* and *-2*; Fig. S1a,b). Although *nrb4-4* and *nrb4-5* were reported as null alleles (Canet *et al.*, 2012), expression analysis indicated that both alleles were in fact strong knockdown mutants. However, as explained later, this knockdown dramatically reduced MED15 protein to undetectable levels. First, we focused on hypocotyl elongation, a hallmark of GA response. *MED15OE* showed a significantly longer hypocotyl phenotype

under LD conditions although the difference from wild-type (WT) was reduced under SD conditions (Fig. 2a). GA application on *MED15OE* gave limited effect on hypocotyl lengths, suggesting that GA signal is not limiting in *MED15OE* even under Mock conditions. On the other hand, hypocotyl length and its response to GA were similar between *nrb4* and WT under LD conditions. However, *nrb4* hypocotyls were significantly shorter than WT under SD conditions, but the mutant hypocotyls were recovered to WT lengths by GA application (Fig. 2a). GA also rescued the small plant weight of *nrb4* (Fig. S2a) but not the curling cotyledons and pale green phenotypes (Fig. 2a). We examined the effect of GA deficiency by growing plants on PAC-containing medium. Because most homozygous *nrb4* seeds could not germinate on PAC medium (Fig. S2b), we transferred seeds to PAC plates after incubation on regular medium for 1 d. Although PAC suppressed hypocotyl elongation of all lines under LD and SD conditions, the suppressive effect on *MED15OE* was larger than WT under LD conditions (Fig. 2b). By contrast, *nrb4* mutants showed small suppression effect under SD condition compared with WT although their PAC response was similar to WT under LD conditions. The hypocotyl phenotype of *MED15OE* and of *nrb4* resembles that of the *della* multiple mutant and of the *ga1* GA-biosynthesis mutant (Achard *et al.*, 2007), respectively, suggesting that MED15 promotes GA response through negative regulation of DELLA function. To genetically confirm this hypothesis, *nrb4-4* was crossed with *rga-29* and *gai-td*. Although we could not obtain *nrb4-4 gai-td* double mutant because of the close linkage of *MED15* and *GAI* on the genome, the *nrb4-4 rga-29* double mutant partially restored the short hypocotyl phenotype of *nrb4-4* under SD conditions (Fig. 2c). We also crossed *MED15OE* with the *HS::gai-1D* line, in which the GA-resistant form of *GAI* is inducible by heat stress treatment (Alabadí *et al.*, 2008). The long hypocotyl phenotype of *MED15OE* was suppressed in this line depending on *gai*-1D induction (Figs 2d, S3). These results suggest that MED15 acts genetically upstream of DELLA to positively regulate the GA response.

Next, we analyzed the GA response of adult plants. Only *nrb4-4* was used in this analysis because most *nrb4-5* seedlings could not survive on soil. We speculated that the stronger knockdown in *nrb4-5* (Fig. S1b) might have a negative effect on adaptation to soil growth conditions. Adult *nrb4-4* plants were small and pale green (Fig. S4a), which were not rescued by GA application, indicating that the primary cause of these phenotypes was not a disruption of GA signaling. By contrast, *MED15OE* plants did not exhibit significant morphological changes (Fig. S4b). As reported previously (Canet *et al.*, 2012), *nrb4-4* showed a late-flowering phenotype; however, this phenotype was recovered by GA application (Figs 2e, S4a). Furthermore, the *MED15OE* lines showed an early flowering phenotype but the effect of GA application was less pronounced compared with WT (Figs 2f, S4b). These results further suggest that MED15 functions as a positive regulator of the GA response.

Because DELLA is a negative regulator of skotomorphogenesis (Alabadí *et al.*, 2004), we analyzed the phenotype of etiolated seedlings of *nrb4* and *MED15OE*. In contrast to the above results, the contribution of MED15 to hypocotyl elongation appeared marginal (Fig. S5a). Cotyledons of *nrb4* mutants exhibited constitutive photomorphogenesis as shown by open cotyledons (Fig. S5b), which is similar to that of etiolated GA-deficient plants (Alabadí *et al.*, 2004). However, *MED15OE* also showed a weak trend toward open cotyledons. Although MED15 is involved in the developmental control of etiolated seedlings, MED15 seems to function in a GA-independent manner in the etiolated seedling as shown later.

MED15 is involved in the regulation of RGA stability

Because the GA response is controlled through the degradation of DELLA protein, we examined the relationship between MED15 and RGA protein levels. In *MED15OE* lines, the MED15 protein overaccumulated, whereas the full-length MED15 was not detected in *nrb4-4* and *nrb4-5* (Fig. 3a). Consistent with the GA-related phenotypes, RGA accumulated in *nrb4*, whereas RGA levels in *MED15OE* were lower than WT. RGA transcript levels were similar across these lines (Fig. 3b), indicating that MED15 negatively regulates RGA stability at the post-transcriptional level. The negative effect of MED15 on RGA stability was further confirmed using a β-estradiol (β-EST)-inducible *MED15* overexpressor. RGA protein levels were reduced along with MED15 induction without repression at the transcript level (Figs 3c, S6). To detect RGA levels in plants, we introduced *pRGA::GFP-RGA* (Silverstone *et al.*, 2001) into *nrb4-4* and *MED15OE-2*. Whereas the *RGA* transcript levels were similar across the lines, GFP–RGA signals were stronger and weaker in *nrb4-4* and *MED15OE-2*, respectively, than in WT (Fig. 3d). MED15 constitutes the Tail module with other subunits, whose mutants also show various growth phenotypes including delayed flowering (Buendía-Monreal & Gillmor, 2016; Zhang & Guo, 2020). Although we could not obtain a loss-of-function mutant of *MED3*, analysis of the RGA protein level in other Tail subunit mutants showed that RGA accumulation was specific to *nrb4-4* (Fig. 3e). The *nrb4-2* allele, which harbors a point mutation in the KIX domain resulting in SA insensitivity without growth defect (Fig. S1a; Canet *et al.*, 2012), did not accumulate RGA. These results suggest that MED15 is an important subunit in the Mediator complex to regulate GA responses by facilitating RGA degradation.

Because the developmental phenotype indicated that MED15 function may differ between green and etiolated seedlings (Fig. S4a,b), we analyzed the GFP–RGA signal in etiolated *nrb4-4*. Consistent with the marginal difference in hypocotyl length, the RGA–GFP signals in hypocotyl were similar between WT and *nrb4-4* (Fig. S7). However, the RGA–GFP signal in cotyledon was weaker in *nrb4-4* in spite of the constitutive photomorphogenesis phenotype, indicating that MED15 negatively regulates RGA stability specifically after greening.

We also examined the effect of GA on MED15. Immunoblot analysis showed that MED15 protein levels in the GA-biosynthesis defective mutant (*ga1*) and the *DELLA* pentuple mutant (*della*) were similar to those of the corresponding WT (Fig. S8a). The effect of GA on MED15 subcellular localization

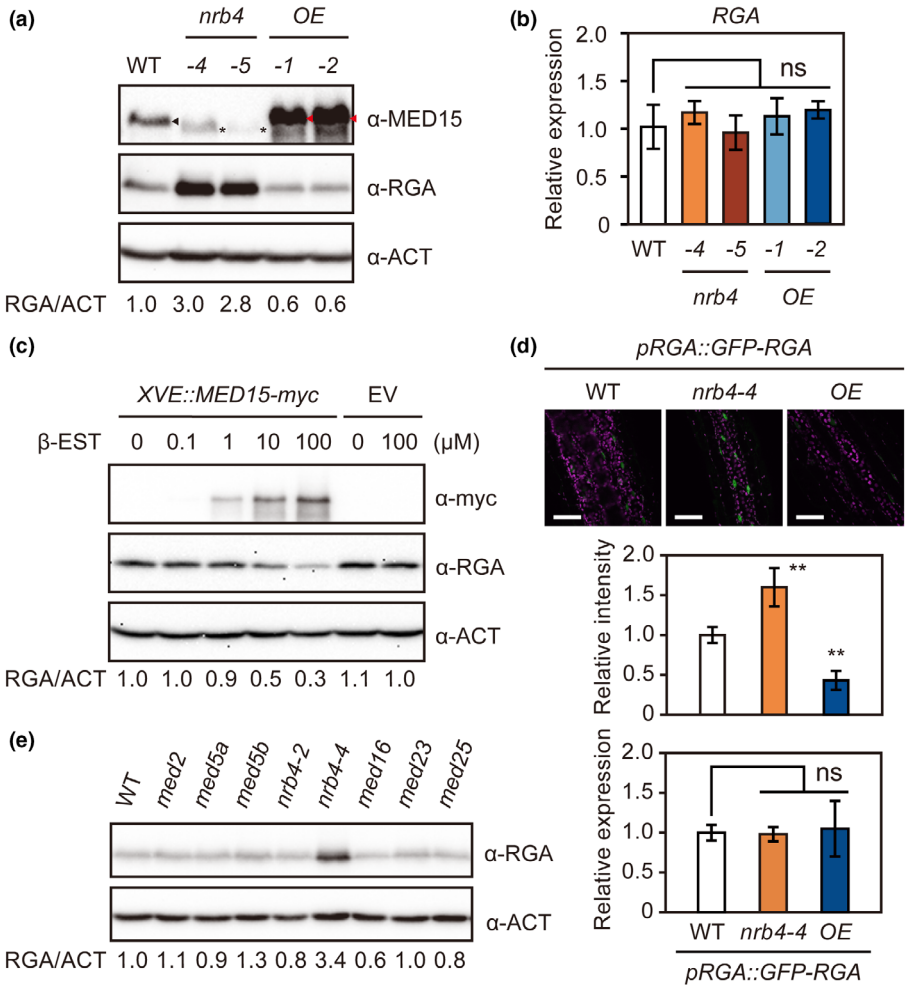

Fig. 3 MEDIATOR15 (MED15) negatively regulates *REPRESSOR OF ga1-3* (RGA) protein levels in *Arabidopsis*. (a) Protein levels of MED15 and RGA in *nrb4* and *MED15OE* lines. Total protein was extracted from whole 7-d-old seedlings and analyzed by immunoblots with anti-MED15, anti-RGA, and anti-ACTIN (ACT) antibodies. Numbers below ACTIN indicate relative RGA protein levels normalized to ACTIN levels. The band positions of MED15, MED15–YFP are indicated by a black and a red arrow head, respectively. An asterisk indicates nonspecific bands detected in *nrb4-4* and *nrb4-5* samples. (b) Transcript levels of RGA in *nrb4* and *MED15OE* lines. Error bars indicate standard deviation ($n = 3$). ns, no significant difference in Dunnett's test against WT. (c) Protein levels of MED15 and RGA in β-EST-inducible *MED15*-myc overexpressor. Total protein was extracted from seedlings after 24 h treatment with 0–100 μM β-EST and analyzed by immunoblots with anti-myc, anti-RGA, and anti-ACTIN antibodies. Numbers below ACTIN indicate relative RGA protein levels normalized to ACTIN levels. EV, a vector control line harboring the empty vector. (d) Confocal images of seedling hypocotyls expressing GFP–RGA in wild-type, *nrb4-4*, and *MED15OE-2* backgrounds. Relative GFP signal intensity and relative expression levels of *RGA* are shown in the middle and bottom panels, respectively. Error bars indicate standard deviation ($n = 6$ (GFP signal) and 3 (*RGA* expression)). **, $P < 0.01$ in two-tailed Student's *t*-test. ns, no significant difference. Bars, 50 μm. (e) RGA levels in the mutants of Tail subunits. Total protein was analyzed by immunoblots with anti-RGA and anti-ACTIN antibodies. Numbers below ACTIN indicate relative RGA protein levels normalized to ACTIN levels.

was analyzed with *pMED15::MED15-YFP*/*nrb4-4*. MED15 was constitutively localized in the nucleus regardless of GA treatment (Fig. S8b,c). In contrast to the results obtained with the tobacco transient expression system (Huang *et al.*, 2021; Kim *et al.*, 2022), our MED15-YFP signal did not exhibit clear speckles in the nucleus. These results indicate that GA affects neither the protein level nor the subcellular localization of MED15.

MED15 positively regulates GA-biosynthesis gene expression

DELLA functions as a coactivator of GA-biosynthesis gene expression in the negative feedback regulation between DELLA and GA (Hedden, 2020). Considering the Mediator function as a cofactor, MED15 may be required for transcriptional regulation via DELLA. Therefore, perturbation of the GA–DELLA feedback regulation is a potential cause of RGA overaccumulation in *nrb4*. To examine the effect of MED15 on DELLA's transactivation activity, we analyzed the expression levels of GA-biosynthesis genes (*GA20ox2* and *GA3ox1*) and a GA-signaling gene (*SCL3*), which are well-known DELLA targets, in mock- and PAC-treated plants (Zentella *et al.*, 2007; Fukazawa *et al.*, 2014; Yoshida *et al.*, 2014). In spite of the overaccumulation of RGA (Fig. S9a), the expression levels of *GA20ox2* and *GA3ox1* were significantly low in *nrb4* under the mock condition (Fig. 4a). Furthermore, PAC-inducibility of these genes was

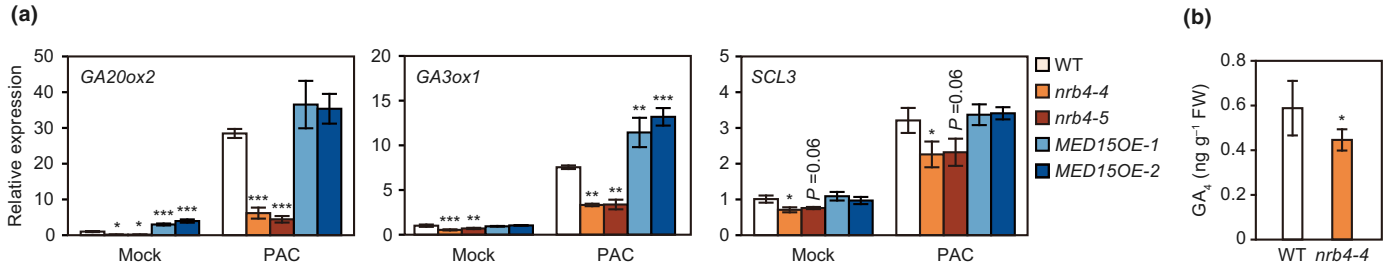

Fig. 4 MEDIATOR15 (MED15) is a positive regulator of gibberellic acid (GA)-biosynthesis gene expression in *Arabidopsis*. (a) Relative transcript levels of DELLA-target genes. RNA was extracted from 7-day-old seedlings grown on regular Murashige & Skoog (MS) plates or MS plates supplemented with 1 μM PAC. Error bars indicate standard deviation (n = 3). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ in Dunnett's test against wild-type (WT). No asterisk indicates no significant difference. (b) Quantification of bioactive GA (GA_4) in WT and *nrb4-4*. GA_4 was extracted from 7-d-old seedlings grown on regular MS plates and quantified by LC-MS/MS. Error bars indicate standard deviation (n = 5). *, $P < 0.05$ in two-tailed Student's t-test.

dampened in the mutants although the effect on *SCL3* expression was marginal. The analysis of *XVE::rga-Δ17-myc* also revealed the MED15-dependency of RGA transactivation activity. *rga-Δ17* is a GA-insensitive mutant of *RGA* lacking the DELLA motif (Dill *et al.*, 2001). Although induction of *rga-Δ17* upregulated the expression of GA-biosynthesis genes, the effect was weak in the *nrb4-4* background line (Fig. S9b). We also analyzed the *nrb4-4 rga-29* double mutant. Compared with the *nrb4-4* and *rga-29* single mutants, the double mutant exhibited lower expression of *GA20ox2* and *GA3ox1* under PAC conditions. By contrast, *SCL3* expression was not clearly affected in *nrb4-4 rga-29* (Fig. S9c). On the other hand, the GA-biosynthesis genes showed higher expression in *MED15OE* and *XVE::MED15-myc* (Figs 4a, S9d). PAC treatment increased the RGA protein to similar levels among mutant and overexpressor lines (Fig. S9a), indicating the positive effect of MED15 on the transactivation activity of DELLA. Consistent with this result, the GA_4 level in *nrb4-4* was lower than WT (Fig. 4b).

We investigated the RGA transactivation activity with a reporter assay system using the *GA20ox2* promoter as a reporter (Fig. S10a). The *GA20ox2* promoter was activated by recruitment of DELLA via GAI-ASSOCIATED FACTOR1 (GAF1) transcription factor (Fukazawa *et al.*, 2014, 2017). Although RGA and GAF1 activated reporter activity, cotransfection of MED15 did not cause a further increase in the activity (Fig. S10b). Actually, the RGA–GAF1 module could activate the reporter even in *nrb4-4*-derived protoplasts regardless of cotransfection with MED15 (Fig. S10c). These results indicate that MED15 is a positive regulator of DELLA-mediated induction of GA-biosynthesis gene expression, and it is involved in the transcriptional regulation via unknown mechanisms.

MED15 facilitates RGA degradation through the GA-dependent pathway

We further confirmed that MED15 enhances RGA degradation through GA-dependent system. GA promotes DELLA degradation via the 26S proteasome. Treatment with MG132, an inhibitor of the 26S proteasome, caused RGA accumulation in all genotypes and, in particular, resulted in RGA levels being similar between WT and *nrb4-4* (Fig. 5a). In tobacco, the RGA protein level was decreased by co-expression of MED15, which was suppressed by MG132 (Fig. S11a). Moreover, rga-Δ17 was resistant to MED15-mediated degradation (Fig. S11b), suggesting that MED15 destabilizes RGA via GA-dependent proteasomal degradation. Recent studies revealed that autophagy is important to specifically regulate the stability of some proteins (Yang *et al.*, 2019; Thirumalaikumar *et al.*, 2021; Wang *et al.*, 2021) although the relationship of DELLA and MED15 with autophagy is unknown; thus, we examined the effect of autophagy on RGA stability by treating plants with E-64d, an autophagy inhibitor. E-64d stabilized RGA and had an additive effect with MG132 on RGA stabilization (Fig. 5a), implying that RGA is degraded by both 26S proteasomes and autophagy. Interestingly, however, RGA protein levels were still higher in *nrb4-4* than that in WT when treated only with E-64d. These results indicate that RGA overaccumulation in *nrb4* is mainly caused by the decreased efficiency of 26S proteasome-mediated RGA degradation. On the other hand, simultaneous treatment with MG132 and E-64d had a limited effect on RGA accumulation in *MED15OE*. MED15 may destabilize RGA also via unknown pathways independent of 26S proteasomes and autophagy.

Although GA concentration is a major determinant of DELLA stability, many interactors modulate DELLA stability in a GA-dependent and -independent manner (Qin *et al.*, 2014; Nohales & Kay, 2019; Park *et al.*, 2020; Yan *et al.*, 2020; Blanco-Touriñán *et al.*, 2020a; Xu *et al.*, 2021; Zhong *et al.*, 2021). In the abscisic acid (ABA) response, MED25 negatively regulates ABI5 protein stability (Chen *et al.*, 2012), which led us to examine whether MED15 affects RGA sensitivity to GA. To eliminate the effect of possible differences in endogenous GA levels, seedlings were grown on the PAC-containing medium and then treated with exogenous GA. Quantified data revealed that RGA was degraded slowly in *nrb4-4* and quickly in *MED15OE* compared with WT, respectively (Fig. 5b) although the difference between WT and *MED15OE* was too small to show the clear difference in the blot image. In *XVE::MED15-myc* plants, simultaneous treatment with β-EST and PAC accumulated RGA in spite of MED15 induction; however, RGA levels were lower than plants treated with only PAC (Fig. S12a). Facilitation of RGA degradation was not observed in *XVE::MED15-myc* plants grown on PAC medium for 6 d to eliminate endogenous GA (Fig. S12b).

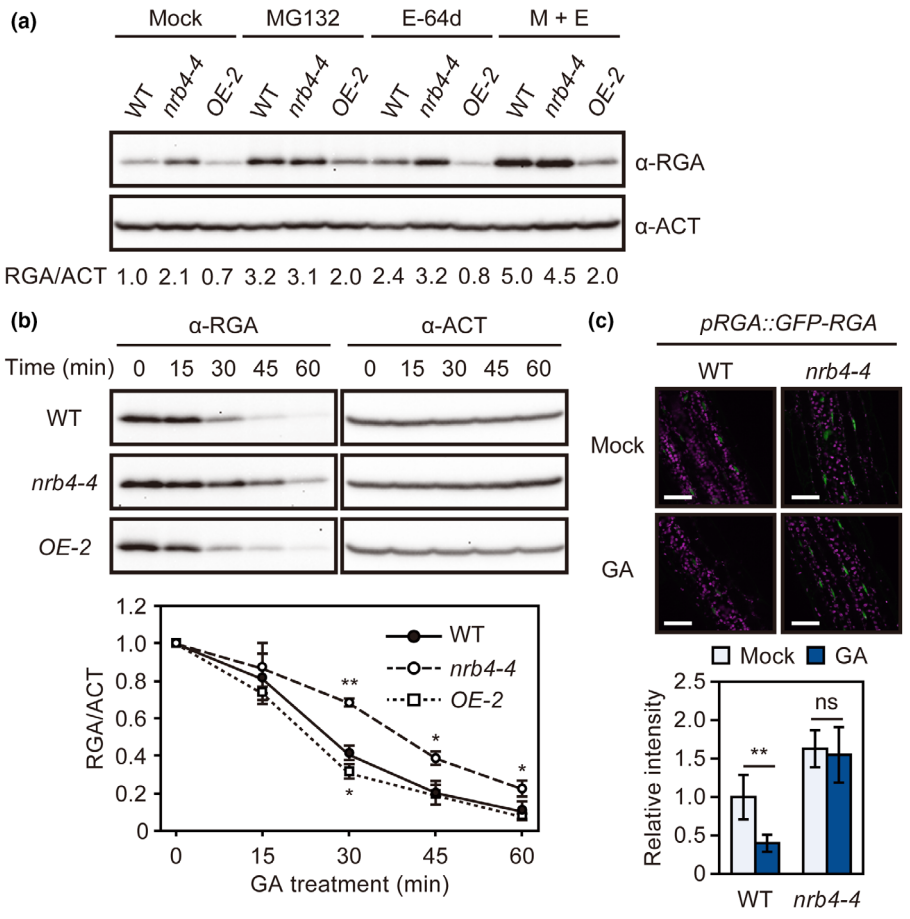

Fig. 5 MEDIATOR15 (MED15) affects the efficiency of gibberellic acid (GA)-dependent *REPRESSOR OF ga1-3* (RGA) degradation in *Arabidopsis*. (a) The effect of proteasome inhibitor (MG132) and autophagy inhibitor (E-64d) on RGA degradation. Seven-day-old seedlings were treated with either inhibitor or both (M + E) for 24 h. Total protein was analyzed by immunoblots with anti-RGA and anti-ACTIN antibodies. Numbers below ACTIN indicate relative RGA protein levels normalized to ACTIN levels. (b) Immunoblots showing GA-dependent degradation of RGA. Seven-day-old seedlings grown on plates containing 1 μM paclobutrazol (PAC) were treated with 10 μM GA, 1 μM PAC and 200 μM cycloheximide for the indicated time. The bottom panel shows the quantified RGA protein levels normalized to ACTIN levels at different times after GA treatment. Error bars indicate standard deviation (n = 3). *, $P < 0.05$; **, $P < 0.01$ in two-tailed Student's t-test. (c) Confocal images of hypocotyls of seedlings expressing GFP–RGA in wild-type and *nrb4-4* backgrounds after Mock or 100 μM GA treatment for 45 min. Relative GFP signal intensity is shown in the bottom graph. Error bars indicate standard deviation (n = 5). **, $P < 0.01$ in two-tailed Student's t-test. Bars, 50 μm. ns, no significant difference.

Therefore, MED15 induction led to RGA degradation in the presence of endogenous GA regardless of PAC inhibition of GA biosynthesis. We also examined RGA stability with live-cell imaging of GFP–RGA, which showed a slow RGA degradation in *nrb4-4* upon GA treatment (Fig. 5c). The expression of responsible factors of DELLA degradation (*GID1A/B/C* and *SLY1*) was not clearly affected in *nrb4* nor *MED15OE* (Fig. S13). These results suggest that MED15 is required to modulate RGA levels via not only GA-biosynthesis but also the rate of RGA degradation.

We elucidated how MED15 promotes DELLA degradation. First, the effect of GA on the MED15–RGA interaction was analyzed with a pull-down assay. As shown in Fig. S14, the presence of GA in the buffer did not affect the recovery of RGA protein, suggesting that GA itself is not involved in the MED15–RGA interaction. Next, the effect of MED15 on the RGA–GID1 and RGA–SLY1 interactions was examined with yeast three-hybrid assays (Fig. S15a,b). Whereas RGA–GID1A interaction was enhanced in the presence of GA, the interaction was suppressed by MED15 co-expression. Consistent with a previous report (Griffiths *et al.*, 2006), the RGA–SLY1 interaction was too weak to detect with yeast three-hybrid system. Different from co-expression of GID1 (Griffiths *et al.*, 2006), MED15 did not enhance the RGA–SLY1 interaction. These results imply that MED15 does not promote DELLA degradation by directly facilitating the DELLA–GID1–SCF^{SLY1} complex formation.

MED15 positively regulates hypocotyl elongation under dark and warm conditions via the GA pathway

The DELLA protein level is quickly adjusted upon environmental changes to optimize plant growth. In particular, light and temperature are well-studied cues affecting plant growth through the modulation of DELLA levels. The positive role of MED15 in GA-mediated RGA degradation led us to investigate the function of MED15 in growth regulation in response to environmental changes. We examined RGA levels in WT and *nrb4-4* by monitoring GFP–RGA levels under dark and warm conditions, which cause a rapid degradation of RGA in hypocotyls resulting in hypocotyl elongation (Achard *et al.*, 2007; Stavang *et al.*, 2009). Although GFP signals were decreased in both plants within 4-h dark or warm treatment, the decrease was relatively slow in *nrb4-4* (Fig. 6a,b). These treatments did not affected the MED15–YFP signal in hypocotyl (Fig. S16a,b). Consistent with this observation, hypocotyl elongation under dark and warm conditions was dampened in *nrb4* mutants (Fig. 6c,d). The hypocotyl response of *MED15OE* was normal; however, the relative difference in hypocotyl lengths between WT and *MED15OE* became smaller under dark and warm conditions. Although GA application did not cause further elongation of hypocotyls in WT and *MED15OE*, GA partially rescued the short hypocotyl phenotype of *nrb4*. This recovery was more clearly observed in petiole elongation caused by the warm treatment (Figs 6d, S17a). These

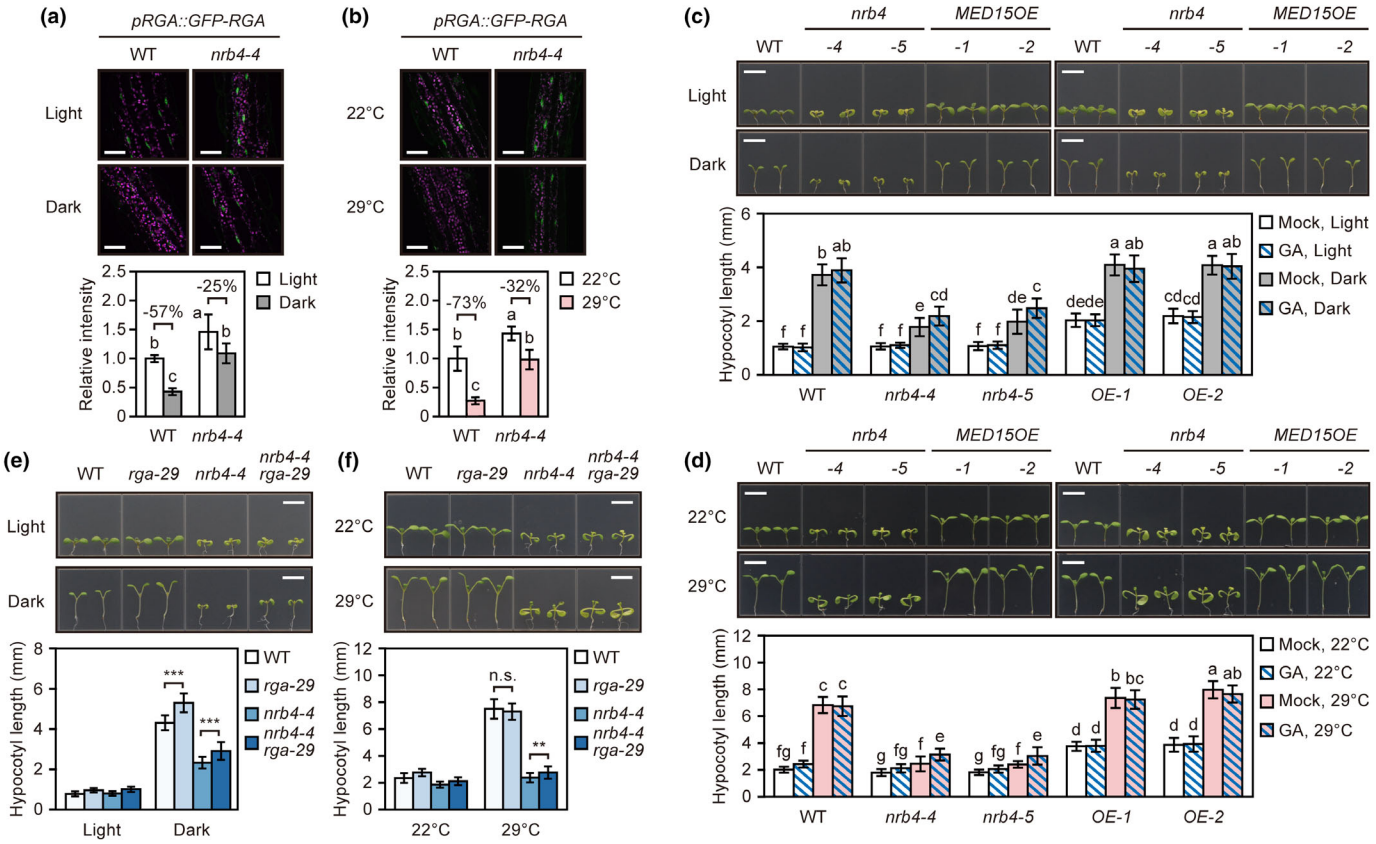

Fig. 6 MEDIATOR15 (MED15) is a positive regulator of dark- and warmth-induced hypocotyl elongation in *Arabidopsis*. (a, b) Confocal images of seedling hypocotyls expressing GFP–RGA in wild-type (WT) and *nrb4-4* backgrounds. Seedlings were treated with dark (a) or warmth (29°C) (b) for 4 h. Relative GFP signal intensity is shown in the bottom graph. Error bars indicate standard deviation ($n = 5$). Numbers above the bars indicate relative decrease in GFP signal caused by dark and warm treatment. Different lowercase letters indicate statistically significant differences between means (Tukey's multiple comparison test, $P < 0.05$). Bars, 50 μm. (c, d) Hypocotyl elongation of *nrb4* and *MED15OE* seedlings under dark and warm conditions. Four-day-old seedlings were transferred to mock (left) or 10 μM gibberellic acid-containing medium (right) and transferred to the dark for 1 d (c) or treated with 29°C for 3 d (d). Error bars indicate standard deviation ($n \geq 20$). Different lowercase letters indicate statistically significant differences between means (Tukey's multiple comparison test, $P < 0.05$). Bars, 5 mm. (e, f) Hypocotyl lengths of WT, *rga-29*, *nrb4-4*, and *nrb4-4 rga-29* under dark or warm conditions. Four-day-old seedlings were transferred to the dark for 1 d (e) or treated with 29°C for 3 d (f). Error bars indicate standard deviation ($n \geq 20$). **, $P < 0.01$; ***, $P < 0.001$ in two-tailed Student's *t*-test. ns, no significant difference. Bars, 5 mm.

results suggest that MED15 positively regulates plant growth under dark and warm conditions partially through the activation of GA pathway. Consistent with these results, the *nrb4-4 rga-29* seedling showed partial recovery of hypocotyl growth under dark and warm conditions (Fig. 6e,f) and petiole elongation under warm conditions (Fig. S17b). Therefore, our genetic interaction assays support the idea that MED15 functions upstream of GA signaling to promote plant growth in response to environmental changes.

Discussion

In this study, we have demonstrated that MED15 positively regulates plant growth through its interaction with DELLA and activation of GA signaling. Our analyses showed that mutants and overexpressors of *MED15* exhibit multiple GA-related growth phenotypes (Fig. 2a,b,e,f), which resemble the phenotypes of the DELLA overexpressor and mutant, respectively (Achard *et al.*, 2007). Consistent with this observation, RGA protein levels were inversely correlated with MED15 protein levels (Fig. 3a,c), suggesting that MED15 is involved in growth regulation through GA signaling. Furthermore, the developmental responses to GA and PAC application and the genetic interaction between *MED15* and *DELLA* clearly support the notion that MED15 positively regulates GA signaling upstream of DELLA (Fig. 2a–f). These results demonstrate that Mediator has a critical role in GA signaling via interaction with DELLA (Fig. 7). In addition to normal growth, the MED15-mediated GA signal is required for timely responses to environmental changes (Fig. 6a–f). Because of the delayed GA-mediated degradation of DELLA (discussed later), hypocotyl elongation of *nrb4* mutant plants was inhibited under dark and warm conditions, in which DELLA should be quickly removed to release growth promoters from the repression by DELLA (Achard *et al.*, 2007; Stavang *et al.*, 2009).

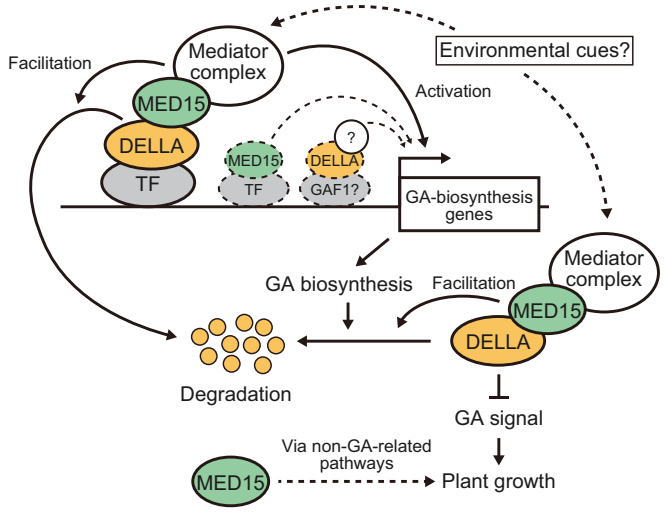

Fig. 7 Schematic model showing MEDIATOR15 (MED15)-mediated activation of gibberellic acid (GA) signal in *Arabidopsis*. DELLA induces the expression of GA-biosynthesis genes by recruiting the Mediator complex through the interaction with MED15. The regulation of GA-biosynthesis gene expression appears to be complicated as DELLA is also able to activate target genes via MED15-independent mechanisms. Furthermore, MED15 may be involved in the regulation of GA-biosynthesis through the interaction with unknown TFs. Accumulation of GA results in DELLA degradation. In addition, MED15–DELLA interaction itself facilitates DELLA degradation. At present, it is not clear whether MED15 facilitates degradation of DNA-tethered DELLA or free DELLA. GA signaling factors released from DELLA repression activate GA signal and enhance plant growth. MED15 also seems to be involved in plant growth via non-GA-related pathways. MED15-mediated DELLA degradation has important roles in plant development under both normal and stress conditions. However, it is unclear whether environmental signals directly affect the MED15–DELLA complex to control DELLA stability. Solid lines represent pathways elucidated in this study while dashed lines indicate pathways requiring further investigation. Arrows and a blunt-ended arrow denote positive and negative regulation, respectively.

In particular, the important roles of Mediator in thermomorphogenesis are characterized by recent studies focusing on MED14, MED17, and MED25 (Sun *et al.*, 2020; Agrawal *et al.*, 2022; Bajracharya *et al.*, 2022; Shapulatov *et al.*, 2023). These Mediator subunits are involved in the activation of PIF4-mediated signaling, which is located downstream of the GA signal. Because exogenous GA application could not completely recover hypocotyl elongation of *nrb4* under dark and warm conditions (Fig. 6c, d), MED15 may also be required for the activation of PIF4 and/or other thermomorphogenesis-related plant hormone signaling (Delker *et al.*, 2022). The potential involvement of MED15 in the downstream signaling of GA may explain the slightly longer hypocotyl of *HS::gai-1D/MED15OE* compared with the WT background line after heat stress treatment (Fig. 2d).

The conventional function of Mediator is modulation of transactivation activity of interacting TFs. However, MED15 positively regulates GA signaling through the modulation of not only DELLA transactivation activity but also DELLA protein stability. Our time-course immunoblot analysis and live-cell imaging of GFP–RGA clearly demonstrated a delayed degradation of RGA in *nrb4* upon exogenous GA application, indicating the positive effect of MED15 on the responsiveness of RGA protein stability to GA (Fig. 5b,c). Although this notion is further supported by the facilitation of RGA destabilization upon MED15 induction in the presence of endogenous GA (Fig. S12a), the molecular mechanism of MED15-mediated DELLA degradation still remains to be elucidated. Considering the insignificant effect of the *MED15* mutation and overexpression on expression levels of *GID1s* and *SLY1* (Fig. S13), MED15 may be required for the efficient formation of the DELLA–GID1–SCF^{SLY1} complex; however, yeast three-hybrid assays showed that MED15 does not directly promote the RGA–GID1 and RGA–SLY1 interactions (Fig. S15a,b). Instead, MED15 interfered with the RGA–GID1 interaction. These results raise the hypothesis that MED15 protects DELLA while the DELLA–Mediator complex is required for the transactivation of GA-biosynthesis genes. On the other hand, DELLA should be quickly removed to prevent overactivation of the target genes after being released from the Mediator complex. Considering the regulation of DELLA stability via phosphorylation (Dai & Xue, 2010; Qin *et al.*, 2014; Li *et al.*, 2024), we hypothesize that MED15 modulates DELLA phosphorylation status in a transactivation-coupled manner to enhance the GA sensitivity for subsequent degradation. Since Mediator recruits transcription regulators including post-translational modification enzymes (Guo *et al.*, 2021, 2023; Shapulatov *et al.*, 2023), it would be of interest to examine whether MED15 affects post-translational modifications of DELLA.

Our data also showed that MED15 is required for the DELLA-mediated transactivation of GA-biosynthesis genes, which is important to maintain optimum GA_4 levels (Figs 4a,b, S9b). To-date, only GAF1 is identified as a TF recruiting DELLA to the *GA20ox2* promoter (Fukazawa *et al.*, 2017). However, we could not obtain evidence that GAF1 is the TF responsible for recruiting the DELLA–MED15 complex to the *GA20ox2* promoter. Actually, the GAF1–DELLA complex seems to activate the *GA20ox2* promoter without MED15 (Fig. S10c). Therefore, it is likely that there are unknown TFs that recruit DELLA–MED15 complex to the *GA20ox2* promoter. This speculation implies the existence of both MED15-dependent and -independent transactivation mechanisms for DELLA, which is consistent with the additive effect of *nrb4-4 rga-29* double mutation on the GA-biosynthesis gene expression (Fig. S9c). Although many genes have been identified as DELLA targets (Zentella *et al.*, 2007; Marín-de la Rosa *et al.*, 2015), our knowledge about DELLA recruitment to the promoters is very limited. Further promoter analysis of GA-biosynthesis genes will shed light on the mechanism by which MED15 induces the expression of these genes. As yeast two-hybrid screening has identified many putative MED15 interactors, it is also possible that MED15 regulates GA biosynthesis via DELLA-independent pathways. During the preparation of this report, Hernández-García *et al.* (2024) also reported the interaction between MED15 and DELLA focusing on the coactivator function of MED15. However, we showed that the MED15–DELLA interaction also plays an important

role in DELLA degradation. Because regulation of the DELLA protein amount affects all aspects of GA signaling, our study emphasizes the global functions of MED15 in plant developmental regulation via GA signaling.

Because Mediator is a flexible protein complex, not all subunits are necessary for its function as a coactivator complex. However, the involvement in a specific signaling pathway requires specific subunits for the interaction with key TFs in the pathway. Our results showed that MED15 is a special subunit involved in GA signaling in the Tail module. Tail subunits often have several shared functions in the control of plant development and biotic/abiotic stress responses (Hemsley *et al.*, 2014; Yang *et al.*, 2014; Wang *et al.*, 2016; Dolan *et al.*, 2017; Dolan & Chapple, 2018; Lee *et al.*, 2021). For example, MED15 contributes to the activation of defense-related plant hormone signals, which also requires MED14, MED16, and MED25 (Wang *et al.*, 2016). However, RGA accumulation was observed only in *nrb4-4* among Tail mutants, suggesting that MED15 is specifically required to activate GA signaling pathway (Fig. 3e). MED15 has the most highly disordered structure among the Tail subunits, which enables flexible interactions with partner proteins (Nagulapalli *et al.*, 2015). Since DELLA forms protein complexes with a variety of interactors, its associated Mediator subunit is required to flexibly recognize DELLA in diverse context. The flexible structure of MED15 may allow it to interact with DELLA in a context-dependent manner. Due to the flexible interaction between MED15 and DELLA, it is likely to be difficult to identify specific regions responsible for the interaction in pull-down assays (Fig. 1b,c). We also found that the RGA protein level in *nrb4-2* was similar to that in WT (Fig. 3e). This suggests that the mutated amino acid in the KIX domain in *nrb4-2* is not involved in the interaction between MED15 and RGA, whereas this amino acid is critical for the MED15 function in SA signaling (Canet *et al.*, 2012). This conclusion is supported by the pull-down assay, which shows that the KIX domain is dispensable for MED15–RGA interaction (Fig. 1b). Therefore, different regions in MED15 appear to be important between GA and SA signaling although the KIX domain is considered as the major domain responsible for MED15–TF interactions (Thakur *et al.*, 2014; Kumar *et al.*, 2018; Dwivedi *et al.*, 2019). Our results indicate that there are multiple ways for MED15 to interact with target proteins in a context-dependent manner.

It still remains to be elucidated whether developmental and environmental cues affect the MED15–DELLA interaction. The DELLA protein level is finely tuned through developmental processes and responses to environmental changes. Given the ubiquitous expression of *MED15* (Kim *et al.*, 2016), modulation of MED15–DELLA interaction may serve as a general mechanism to regulate DELLA accumulation. In the case of DELLA–TF and DELLA–histone H2A interactions, post-translational modifications of DELLA play a key role in modulating these interactions (Blanco-Touriñán *et al.*, 2020b; Huang *et al.*, 2024). Therefore, it would be interesting to analyze the MED15's contribution to GA signaling under various conditions focusing on the DELLA post-translational modifications, which may modulates the extent of MED15's contribution from GA-biosynthesis activation and DELLA destabilization. A comparison between etiolated and greened seedlings would be a good starting point because MED15 negatively regulates RGA stability specifically after greening. A better understanding of the detailed molecular mechanisms underlying MED15 function in plant development could provide novel strategies for utilizing MED15 in molecular breeding to generate new crop cultivars with ideal body architecture.

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Competing interests

None declared.

Author contributions

NO and N-HC designed the experiments. NO, TLM, KC, NM, KB and DU executed the experiments. NO and N-HC wrote the manuscript.

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Data availability

The data that support the findings of this study are available in the main text and Figs S1–S17 and Tables S1, S2.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 MEDIATOR15 (*MED15*) expression levels in T-DNA insertion mutants and *MED15* overexpressors.

Fig. S2 Gibberellic acid-related phenotypes of *nrb4* and *MED15OE*.

Fig. S3 Gibberellic acid-*INSENSITIVE (GAI)* expression in *HS::gai-1D* lines.

Fig. S4 Images of adult *nrb4* and *MED15OE* plants.

Fig. S5 Phenotypes of *nrb4* and *MED15OE* grown under dark condition.

Fig. S6 Expression levels of MEDIATOR15 and *REPRESSOR OF ga1-3* in *XVE::MED15-myc*.

Fig. S7 GFP–RGA signal in etiolated seedlings.

Fig. S8 MEDIATOR15 protein level and subcellular localization are not affected by gibberellic acid in *Arabidopsis*.

Fig. S9 MEDIATOR15 induces expression of gibberellic acid-biosynthesis genes in *Arabidopsis*.

Fig. S10 MEDIATOR15 is not involved in the regulation of *GA20ox2* promoter via GAF1–RGA module in *Arabidopsis*.

Fig. S11 MEDIATOR15 destabilizes *REPRESSOR OF ga1-3* via gibberellic acid-dependent proteasomal degradation.

Fig. S12 MEDIATOR15 induction leads to *REPRESSOR OF ga1-3* degradation in the presence of endogenous gibberellic acid.

Fig. S13 Expression of DELLA degradation-related genes was not significantly affected in *nrb4* and *MED15OE*.

Fig. S14 Gibberellic acid does not affect the MED15–RGA interaction.

Fig. S15 MEDIATOR15 does not enhance RGA–GID1 and RGA–SLY1 interactions.

Fig. S16 MED15–YFP signal is not affected by dark and warm treatment in *Arabidopsis*.

Fig. S17 MED15 is a positive regulator of warm-induced petiole elongation in *Arabidopsis*.

Table S1 Primers used in this study.

Table S2 Destination vectors used in this study.

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