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Arabidopsis DELLA and JAZ Proteins Bind the WD-Repeat/bHLH/MYB Complex to Modulate Gibberellin and Jasmonate Signaling Synergy[C][W]

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Integration of diverse environmental and endogenous signals to coordinately regulate growth, development, and defense is essential for plants to survive in their natural habitat. The hormonal signals gibberellin (GA) and jasmonate (JA) antagonistically and synergistically regulate diverse aspects of plant growth, development, and defense. GA and JA synergistically induce initiation of trichomes, which assist seed dispersal and act as barriers to protect plants against insect attack, pathogen infection, excessive water loss, and UV irradiation. However, the molecular mechanism underlying such synergism between GA and JA signaling remains unclear. In this study, we revealed a mechanism for GA and JA signaling synergy and identified a signaling complex of the GA pathway in regulation of trichome initiation. Molecular, biochemical, and genetic evidence showed that the WD-repeat/bHLH/MYB complex acts as a direct target of DELLAs in the GA pathway and that both DELLAs and JAZs interacted with the WD-repeat/bHLH/MYB complex to mediate synergism between GA and JA signaling in regulating trichome development. GA and JA induce degradation of DELLAs and JASMONATE ZIM-domain proteins to coordinately activate the WD-repeat/bHLH/MYB complex and synergistically and mutually dependently induce trichome initiation. This study provides deep insights into the molecular mechanisms for integration of different hormonal signals to synergistically regulate plant development.

INTRODUCTION

To survive, plants must integrate diverse environmental and endogenous signals to regulate growth, development, and defense. The hormonal signals gibberellin (GA) and jasmonate (JA) regulate many aspects of plant growth, development, and defense. GA plays essential roles in promotion of plant growth and development (Ueguchi-Tanaka et al., 2007; Harberd et al., 2009; Sun, 2011; Hauvermale et al., 2012; Davière and Achard, 2013), including root growth (Fu and Harberd, 2003; Ubeda-Tomás et al., 2008), seed germination (Kahn et al., 1957; Piskurewicz et al., 2008), hypocotyl elongation (Silk and Jones, 1975), leaf expansion (Achard et al., 2009), stem elongation (Kato, 1956), flower development (Cheng et al., 2004), trichome initiation (Chien and Sussex, 1996; Gan et al., 2006; Gan et al., 2007), and in repression of plant defense (Navarro et al., 2008). The GA receptors GA-INSENSITIVE DWARF1a/b/c perceive GA signals (Griffiths et al., 2006; Murase et al., 2008) and recruit DELLA proteins, including RGA (Silverstone et al., 1997), GAI (Peng et al., 1997), RGL1 (Wen and Chang, 2002), RGL2 (Lee et al., 2002), and RGL3 (Wild et al., 2012), for ubiquitination and subsequent degradation (Willige et al., 2007; Ariizumi et al., 2008). Degradation of the DELLAs releases various DELLA-interacting transcription factors to activate their respective GA responses, including hypocotyl elongation, fruit patterning, and hook formation (Oh et al., 2007; de Lucas et al., 2008; Feng et al., 2008; Arnaud et al., 2010; Heo et al., 2011; Zhang et al., 2011; An et al., 2012; Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012; Park et al., 2013; Sarnowska et al., 2013).

JAs are a class of lipid-derived hormone molecules (Howe and Jander, 2008; Browse, 2009; Gfeller et al., 2010; Wasternack and Hause, 2013) that control diverse developmental processes, including stamen development (Feys et al., 1994; McConn and Browse, 1996; Huang et al., 2014), root growth (Staswick et al., 1992; Pauwels et al., 2010), trichome formation (Yoshida et al., 2009; Qi et al., 2011), and secondary metabolism (Hong et al., 2012; Pollier et al., 2013), and regulate various defense responses against pathogen infection (Thomma et al., 1998; Vijayan et al., 1998; Melotto et al., 2006; Yang et al., 2008; Rowe et al., 2010; Zheng et al., 2012) and insect attack (Howe et al., 1996; McConn et al., 1997; Moreno et al., 2009; Hu et al., 2013a; Mousavi et al., 2013; Schweizer et al., 2013; Song et al., 2014). The F-box protein CORONATINE INSENSITIVE1 (COI1) (Xie et al., 1998; Yan et al., 2013) perceives JA signals (Yan et al., 2009; Sheard et al., 2010) and recruits Jasmonate ZIM-domain (JAZ) proteins for ubiquitination and subsequent degradation through the 26S proteasomes (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007), leading to release of the downstream signaling cascades required for various JA responses (Cheng et al., 2011; Fernández-Calvo et al., 2011; Niu et al., 2011; Song et al., 2011; Zhu et al., 2011; Shan et al., 2012; Hu et al., 2013b; Nakata et al., 2013; Song et al., 2013b).

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GA and JA exhibit antagonistic actions in regulating hypocotyl elongation, root growth, flowering, and defense against necrotrophic pathogens and hemibiotrophic bacterial pathogens (Navarro et al., 2008; Hou et al., 2010, 2013; Wild et al., 2012; Yang et al., 2012). It was speculated that interaction between DELLAs and JAZs would attenuate their repression on their respective downstream transcription factors. GA signals would induce DELLAs degradation, abolish the DELLA-JAZ interactions, and release JAZs to bind and attenuate transcription factor MYC2, resulting in inhibition of JA-regulated root growth (Hou et al., 2010). Reciprocally, JA signals would promote JAZ degradation to release DELLAs, which further interact with and repress their downstream transcription factor PIF3, leading to inhibition of hypocotyl elongation (Yang et al., 2012).

In addition to antagonistic regulation, GA and JA also act synergistically to mediate many important developmental processes: Both GA and JA regulate development of stamens (Feys et al., 1994; McConn and Browse, 1996; Cheng et al., 2004; Mandaokar et al., 2006; Song et al., 2011) and induce initiation of trichomes (Chien and Sussex, 1996; Perazza et al., 1998; Traw and Bergelson, 2003; Li et al., 2004; Yoshida et al., 2009), which assist seed dispersal and protect plants against insect attack, pathogen infection, excessive water loss, and UV irradiation (Wagner et al., 2004; Ramsay and Glover, 2005; Ishida et al., 2008; Tissier, 2012). However, the molecular mechanism underlying the synergism between GA and JA signaling remains unclear. In this study, we reveal a mechanism for GA and JA signaling synergy and show that the WD-repeat/bHLH/MYB complex acts as a direct target of DELLAs in the GA pathway. We also show that DELLAs and JAZs interact with essential components of the WD-repeat/bHLH/MYB complex to modulate GA and JA signaling synergy in regulating trichome development. Identification of the WD-repeat/bHLH/MYB complex as a DELLA- and JAZ-interacting target, whose activation demands both GA and JA signaling, suggests a general mechanism for integration of different hormone signals to synergistically regulate plant development and growth.

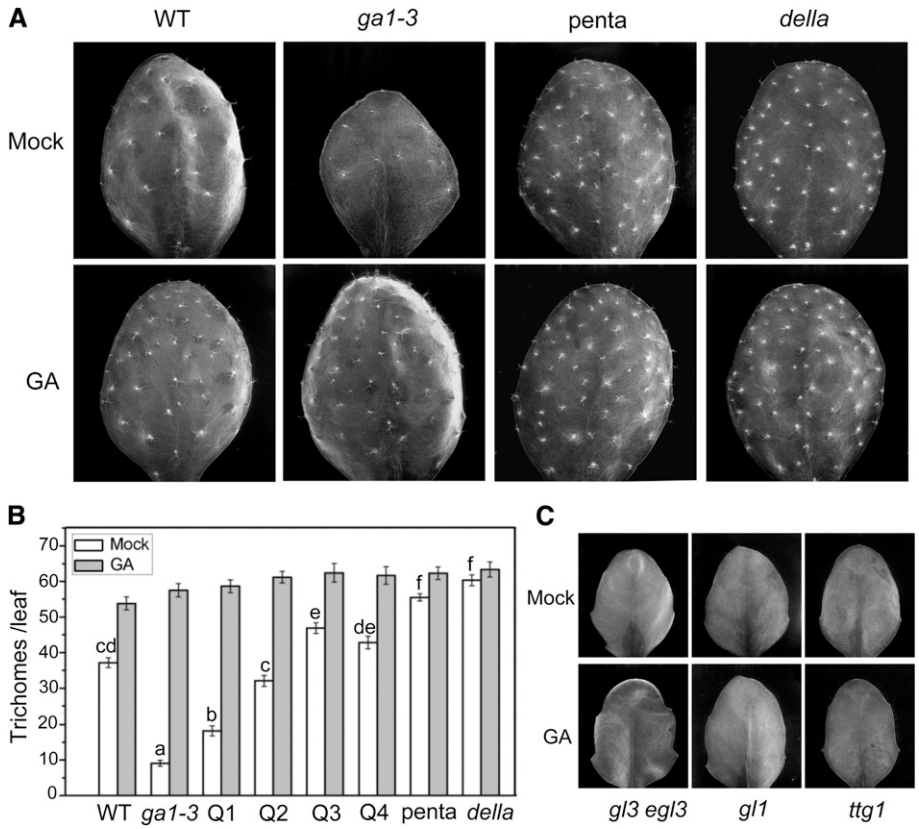

Figure 1. GA Promotes Trichome Formation in a WD-Repeat/bHLH/MYB Complex–Dependent Manner.

(A) Trichome images of wild-type (WT) Landsberg *erecta* (L*er*), *ga1-3*, penta (*ga1-3 rga gai rgl1 rgl2*), and *della* (*rga gai rgl1 rgl2 rgl3*) plants treated without (Mock) or with GA_3 (GA). The fifth true leaves were selected to count trichome numbers using an environmental scanning electron microscope.
(B) Statistical analysis of total trichome numbers in the fifth true leaves of L*er* (WT), *ga1-3*, Q1 (*ga1-3 gai rgl1 rgl2*), Q2 (*ga1-3 rga rgl1 rgl2*), Q3 (*ga1-3 rga gai rgl1*), Q4 (*ga1-3 rga gai rgl2*), penta, and *della* plants treated without (Mock) or with GA_3 (GA). Eight leaves for each genotype were used for trichome number measurement in each biological experiment. Data are means (±SE) of three biological replicates. Lowercase letters indicate significant differences by one-way ANOVA analysis with SAS software ($P < 0.05$).
(C) Trichome images of *Arabidopsis gl3 egl3*, *gl1*, and *ttg1* mutants treated without (Mock) or with GA_3 (GA). The fifth true leaves were selected to count trichome numbers. No trichome was observed in *gl3 egl3*, *gl1*, and *ttg1* mutants.

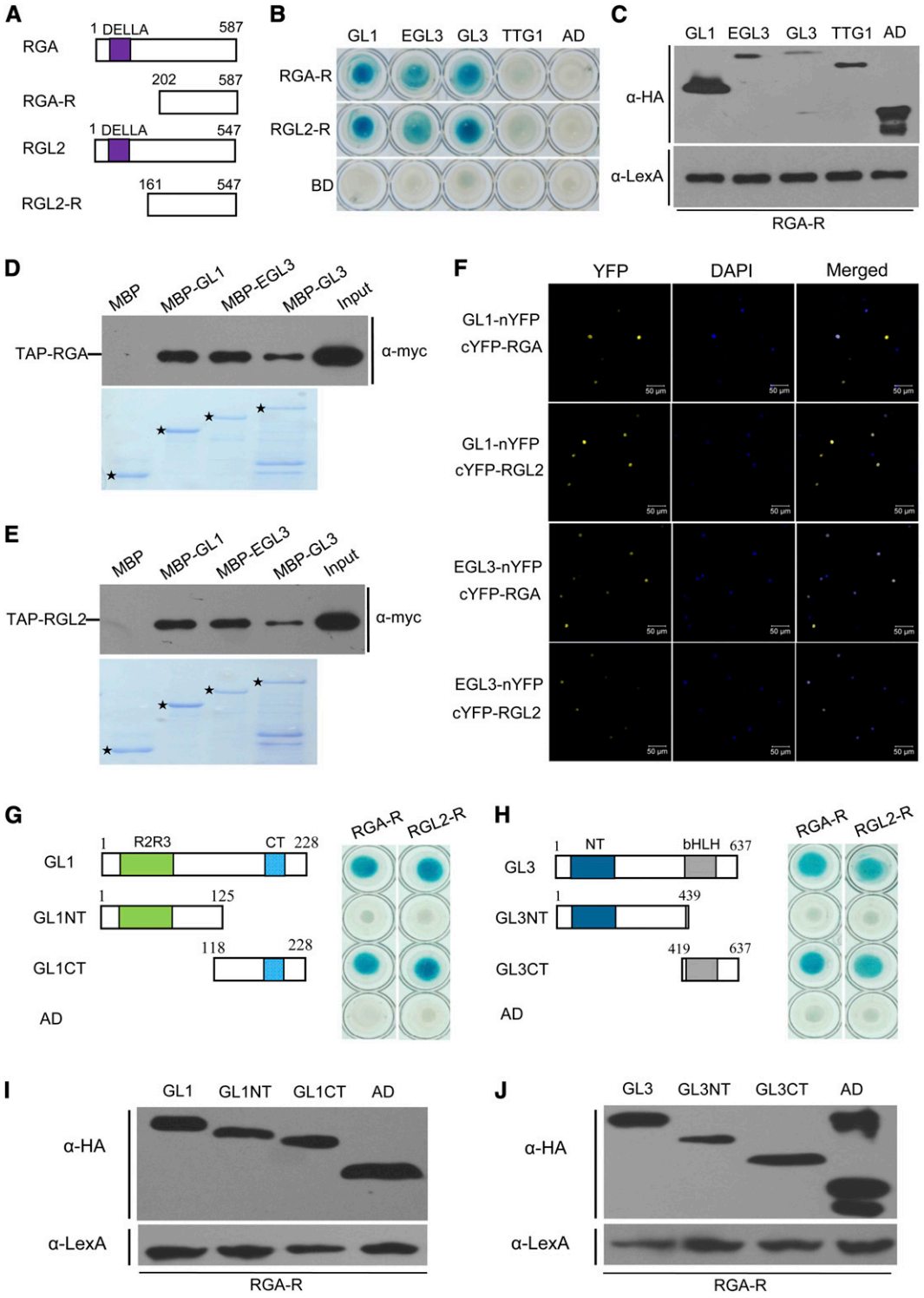

Figure 2. Interactions of DELLAs with the WD-Repeat/bHLH/MYB Complex.

(A) Schematic diagrams show domain constructs of RGA and RGL2. The diagrams display the conserved DELLA domain. The numbers indicate positions of the first and the last amino acid of the domain constructs.

(B) Y2H assays to test the interactions of DELLAs with GL1, EGL3, GL3, and TTG1. The RGA-R (202 to 587 amino acids) and RGL2-R (161 to 547 amino acids) were fused with the LexA DNA BD. GL1, EGL3, GL3, and TTG1 were fused with the activation domain (AD).

RESULTS

GA Promotes Trichome Initiation in a WD-Repeat/bHLH/MYB Complex-Dependent Manner

GA is required for trichome formation (Chien and Sussex, 1996; Perazza et al., 1998; Gan et al., 2007). Consistent with this, we also noticed that the GA biosynthetic mutant *ga1-3* exhibited a nearly trichome-less phenotype (Figures 1A and 1B) and that the penta mutant (*ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1*, wild-type for *RGL3*) rescued the trichome-less phenotype (Figures 1A and 1B). In addition, we found that the *della* mutant (*gai-t6 rga-t2 rgl1-1 rgl2-1 rgl3-1*) displayed maximum trichome density (Figures 1A and 1B), suggesting that the DELLA proteins repress trichome initiation.

Furthermore, we showed that exogenous application of GA induces maximum trichome density in the *ga1-3* mutant (wild-type for five *DELLA*s) and the mutants with various DELLA mutations, such as Q1 (*ga1-3 gai-t6 rgl1-1 rgl2-1*, wild-type for *RGA* and *RGL3*), Q2 (*ga1-3 rga-t2 rgl1-1 rgl2-1*, wild-type for *GAI* and *RGL3*), Q3 (*ga1-3 gai-t6 rgl1-1 rga-t2*, wild-type for *RGL2* and *RGL3*), Q4 (*ga1-3 gait6 rga-t2 rgl2-1*, wild-type for *RGL1* and *RGL3*), and penta (*ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1*, wild-type for *RGL3*) (Figures 1A and 1B). Quantitative analysis of trichome density demonstrated that, among these five DELLA proteins, RGA and GAI play the major roles in repressing trichome formation, RGL1 and RGL2 play moderate roles, and RGL3 plays a minor role (Figures 1A and 1B).

Previous studies have shown that the WD-repeat (TRANSPARENT TESTA GLABRA1 [TTG1])/bHLH (GLABRA3 [GL3] or ENHANCER OF GLABRA3 [EGL3])/MYB (GLABRA3 [GL1] or MYB23) complex mediates trichome development (Zhang et al., 2003; Ishida et al., 2008; Pesch and Hülskamp, 2009; Grebe, 2012). We further found that exogenous application of GA was unable to induce trichome initiation in *gl3 egl3*, *gl1*, and *ttg1*, the mutants of WD-repeat/bHLH/MYB complex (Figure 1C). As expected, the *gl3 egl3*, *gl1*, and *ttg1* mutants showed trichome-deficient phenotypes (Figure 1C) (Zhang et al., 2003). Taken together (Figure 1), these results indicated that GA induces the degradation of DELLAs to activate trichome formation in a WD-repeat/bHLH/MYB complex-dependent manner.

DELLA Proteins Interact with the bHLH Transcription Factors GL3/EGL3 and the MYB Factor GL1

Having shown that the WD-repeat/bHLH/MYB complex is required for GA-induced trichome initiation (Figure 1), we then investigated whether the DELLA repressors interact with the WD-repeat/bHLH/MYB complex to attenuate trichome initiation. The full-length DELLAs displayed strong autoactivation when fused with the LexA DNA binding domain (BD) in our yeast two-hybrid (Y2H) assay; therefore, the truncated C-terminal part of RGA (RGA-R), a representative DELLA protein with the strongest repression of trichome formation (Figure 1B), was fused with BD domain to test its interaction with TTG1, GL1, EGL3, and GL3, the major components of the WD-repeat/bHLH/MYB complex (Figures 2A and 2B).

We found that RGA-R interacts with GL1, EGL3, and GL3, but not with TTG1, in the Y2H system (Figures 2B and 2C). Similarly, the BD domain–fused C terminus of RGL2 (RGL2-R), another representative DELLA, also interacts with GL1, EGL3, and GL3, but not with TTG1 (Figure 2B). These results implied that DELLA proteins physically interact with the bHLH and MYB members of the WD-repeat/bHLH/MYB complex.

We next adopted a pull-down assay to verify the interaction of GL1, EGL3, and GL3 with DELLA proteins. The purified GL1, EGL3, or GL3 proteins, fused with the maltose binding protein (MBP), were incubated with total proteins from transgenic *Arabidopsis thaliana* plants expressing the DELLA proteins RGA or RGL2 fused with tandem affinity purification (TAP) tag (TAP-RGA or TAP-RGL2). Immunoblot analysis showed that all the MBP-fused GL1, EGL3, and GL3 could retain TAP-RGA, whereas MBP alone could not (Figure 2D). We observed a similar result for TAP-RGL2 when incubated with MBP-fused GL1, EGL3, and GL3 (Figure 2E). These results verified the physical interaction of DELLAs with GL1/EGL3/GL3 in vitro.

Figure 2. (continued).

(C) Expression of GL1, EGL3, GL3, TTG1, and RGA-R in the yeast strains shown in the top panel of **(B)**. GL1, EGL3, GL3, and TTG1 were detected with anti-HA antibody (top). RGA-R was detected with anti-LexA antibody (bottom).

(D) and **(E)** In vitro pull-down assay to verify the interactions of RGA **(D)** or RGL2 **(E)** with GL1, EGL3, and GL3. Purified MBP, MBP-GL1, MBP-EGL3, and MBP-GL3 fusion proteins were incubated with the total proteins extracted from the *Arabidopsis* seedlings transgenic for TAP-RGA **(D)** or TAP-RGL2 **(E)**. Bound proteins were washed, separated on SDS-PAGE, and immunoblotted with the anti-c-myc antibody (a-myc; top panel). The input lane shows the expression level of myc-RGA **(D)** or myc-RGL2 **(E)** in the transgenic plant. The positions of purified MBP, MBP-GL1, MBP-EGL3, and MBP-GL3 separated on SDS-PAGE are indicated with an asterisk (bottom panel; stained by Coomassie blue).

(F) BiFC assay to detect the interactions of RGA and RGL2 (fused with C-terminal fragment of YFP) with GL1 and EGL3 (fused with N-terminal fragment of YFP). Construct pairs indicated were coinfiltrated into leaves of *N. benthamiana*. YFP fluorescence was detected 50 h after infiltration. The nuclei are indicated by 4′,6-diamidino-2-phenylindole (DAPI) staining.

(G) and **(H)** Y2H assays to detect the interactions of RGA-R and RGL2-R with different domains of GL1 **(G)** and GL3 **(H)**. The schematic diagram showed the AD domain–fused GL1NT and GL1CT, the conserved R2R3 domain and CT domain of GL1 **(G)**, the AD domain–fused GL3NT and GL3CT, and the conserved NT domain and bHLH domain of GL3 **(H)**. The numbers indicate the positions of amino acids.

(I) Expression of GL1, GL1NT, GL1CT, and RGA-R in the yeast strains used in **(G)**. GL1, GL1NT, GL1CT, and AD were detected with anti-HA antibody (top). RGA-R was detected with anti-LexA antibody (bottom).

(J) Expression of GL3, GL3NT, GL3CT, and RGA-R in yeast strains used in **(H)**. GL3, GL3NT, GL3CT, and AD were detected with anti-HA antibody (top). RGA-R was detected with anti-LexA antibody (bottom).

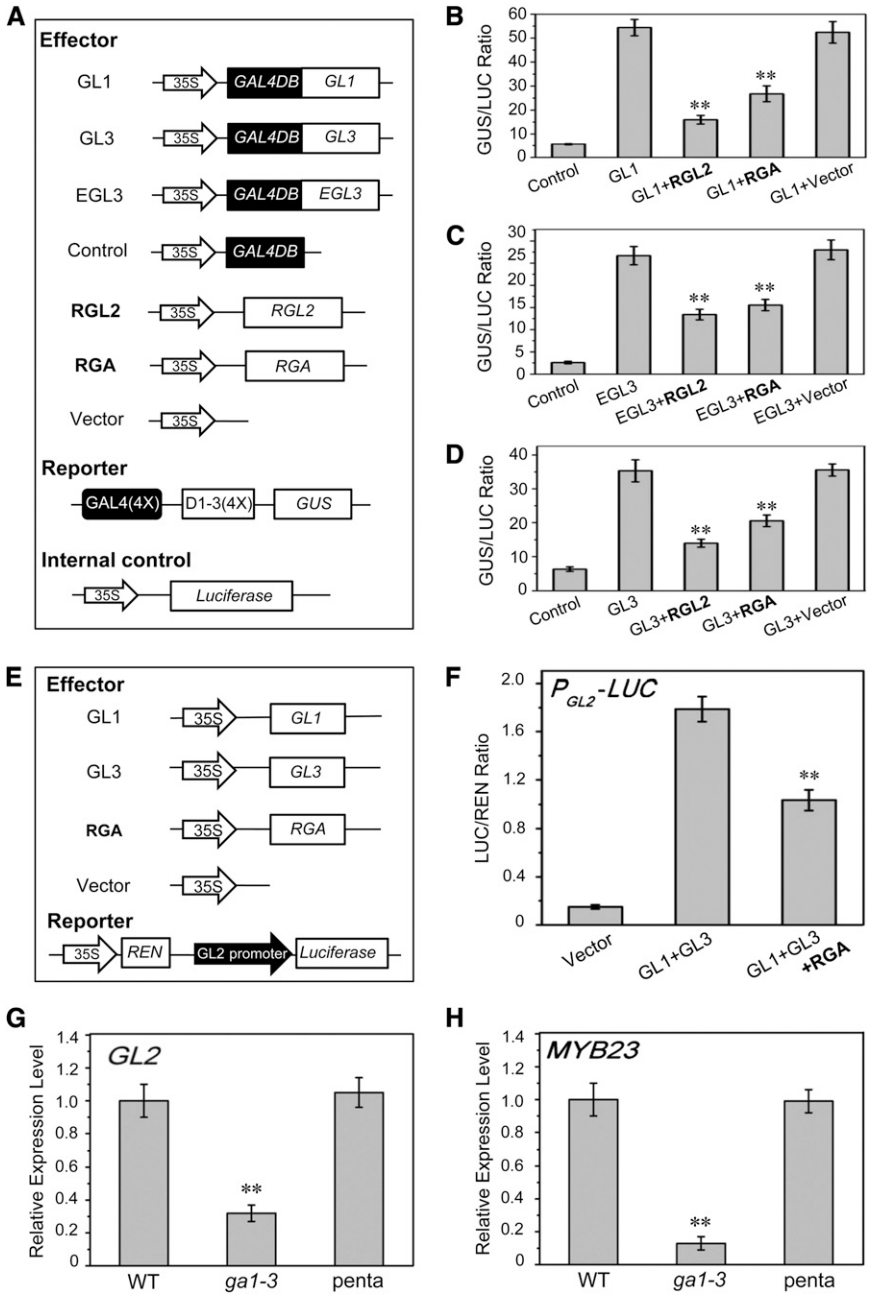

Figure 3. DELLA Proteins Inhibit Transcriptional Function of the WD-Repeat/bHLH/MYB Complex.

(A) The schematic diagram shows the constructs used in the transient expression assays of **(B)** to **(D)**.

(B) Transient expression assay shows that RGA and RGL2 inhibit transcriptional function of GL1. The *GUS* reporter and the internal control *LUC* were cotransformed with the indicated constructs. Data are means (±SE) of three biological replicates. Asterisks represent Student's *t* test significance compared with GL1 (**P < 0.01).

(C) Transient expression assay shows that RGA and RGL2 inhibit transcriptional function of EGL3. Data are means (±SE) of three biological replicates. Asterisks represent Student's *t* test significance compared with EGL3 (**P < 0.01).

(D) Transient expression assay shows that RGA and RGL2 inhibit transcriptional function of GL3. Data are means (±SE) of three biological replicates. Asterisks represent Student's *t* test significance compared with GL3 (**P < 0.01).

(E) The schematic diagram shows the constructs used in the transient expression assays of **(F)**.

(F) Transient expression assay shows that activation of *GL2* promoter by GL3/GL1 is repressed by RGA. The P_{GL2}-*LUC* reporter was cotransformed with the indicated constructs. Data are means (±SE) of three biological replicates. Asterisks represent Student's *t* test significance compared with GL1+GL3 (**P < 0.01).

(G) and **(H)** Real-time PCR analysis for *GL2* **(G)** and *MYB23* **(H)** in *Arabidopsis* L*er* wild-type (WT), *ga1-3*, and penta plants. *ACTIN8* was used as the internal control. Data are means (±SE) of three biological replicates. Asterisks indicate significant differences compared with the wild type by one-way ANOVA analysis with SAS software (**P < 0.01).

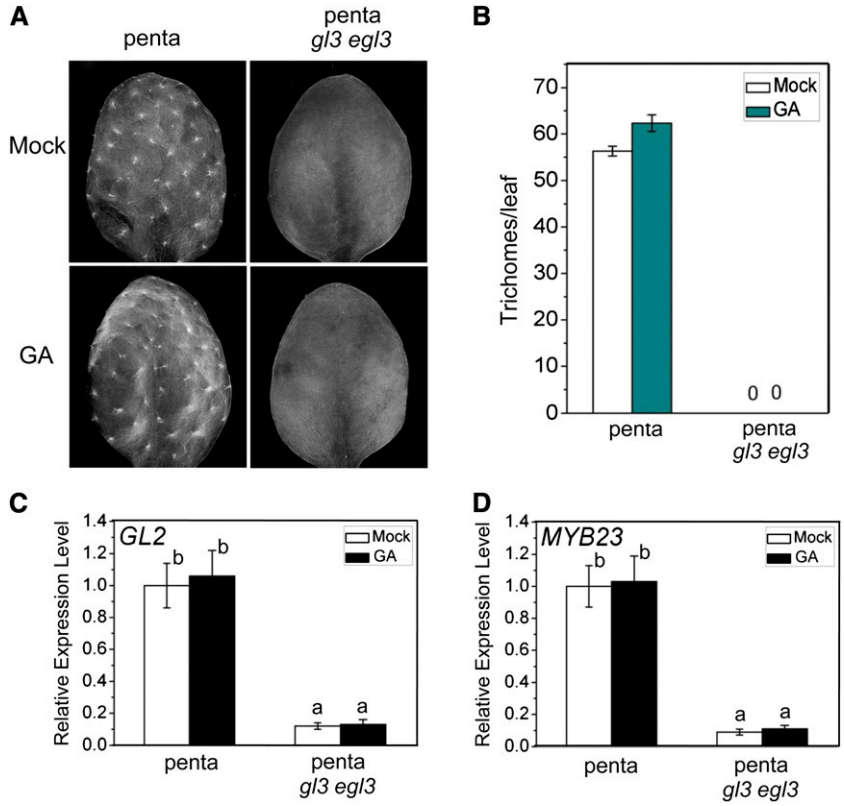

Figure 4. Double Mutations in *GL3* and *EGL3* Block Trichome Initiation in the *penta* Mutant.

(A) Images of the fifth true leaves of penta and penta *gl3 egl3* heptuple mutant treated without (Mock) or with GA_3 (GA).
(B) Statistical analysis of total trichome numbers per leaf indicated in **(A)**. Eight leaves for each genotype were used for trichome number measurement in each biological experiment. Data are means (±SE) of three biological replicates.
(C) and **(D)** Real-time PCR analysis for *GL2* **(C)** and *MYB23* **(D)** in penta and penta *gl3 egl3* mutants treated without (Mock) or with GA_3 (GA). *ACTIN8* was used as the internal control. Data are means (±SE) of three biological replicates. Lowercase letters indicate significant differences by one-way ANOVA analysis with SAS software ($P < 0.05$).
[See online article for color version of this figure.]

We further performed bimolecular fluorescence complementation (BiFC) assays to verify the interactions of DELLA with GL1 and EGL3. RGA and RGL2 were fused with the C-terminal part of yellow fluorescent protein (cYFP) individually to generate cYFP-RGA and cYFP-RGL2, while GL1 and EGL3 were ligated with the N-terminal fragment of YFP (nYFP) to generate GL1-nYFP and EGL3-nYFP, respectively. We found that coexpression of GL1-nYFP with cYFP-RGA or cYFP-RGL2 in *Nicotiana benthamiana* leaves produced strong YFP fluorescence in the nucleus (Figure 2F), whereas no YFP signal was observed in negative controls (Supplemental Figure 1). YFP fluorescence was also observed when EGL3-nYFP was coexpressed with cYFP-RGA or cYFP-RGL2 (Figure 2F). The BiFC results further demonstrated that the MYB (GL1) and bHLH factor (EGL3) interact with DELLAs in vivo.

To study which domains of the MYB and bHLH members are responsible for interaction with DELLA proteins, we further divided GL1 into N-terminal fragment (GL1NT) containing the R2R3 DNA BD and C-terminal fragment (GL1CT) (Figure 2G). GL3 was also divided into an N-terminal fragment (GL3NT) and a C-terminal fragment (GL3CT) containing the bHLH DNA BD (Figure 2H). As shown in Figures 2G to 2J, RGA-R and RGL2-R interact with both the C-terminal fragments of GL1 and GL3, but not with GL1NT and GL3NT, suggesting that C-terminal parts of MYB factor GL1 and bHLH factor GL3 are required for the interaction with DELLA proteins.

In summary, the Y2H, pull-down, and BiFC assays demonstrated that the DELLA repressors interact with the MYB (GL1) and bHLH (EGL3 and GL3) members of the WD-repeat/bHLH/MYB complex.

DELLAs Repress the Transcriptional Function of the WD-Repeat/bHLH/MYB Complex

To explore the significance of the interaction between DELLA proteins and these MYB/bHLH factors, we tested whether DELLAs can affect the transcriptional function of these MYB and bHLH factors. To do this, we used an *Arabidopsis* protoplast transient expression system based on the GAL4 DNA binding domain (GAL4DB) and its binding sites [GAL4(4X)-D1-3(4X)] (Tiwari et al., 2001).

The R2R3-MYB transcription factor GL1 and the two bHLH factors EGL3 and GL3 were fused with the 35S promoter–driven

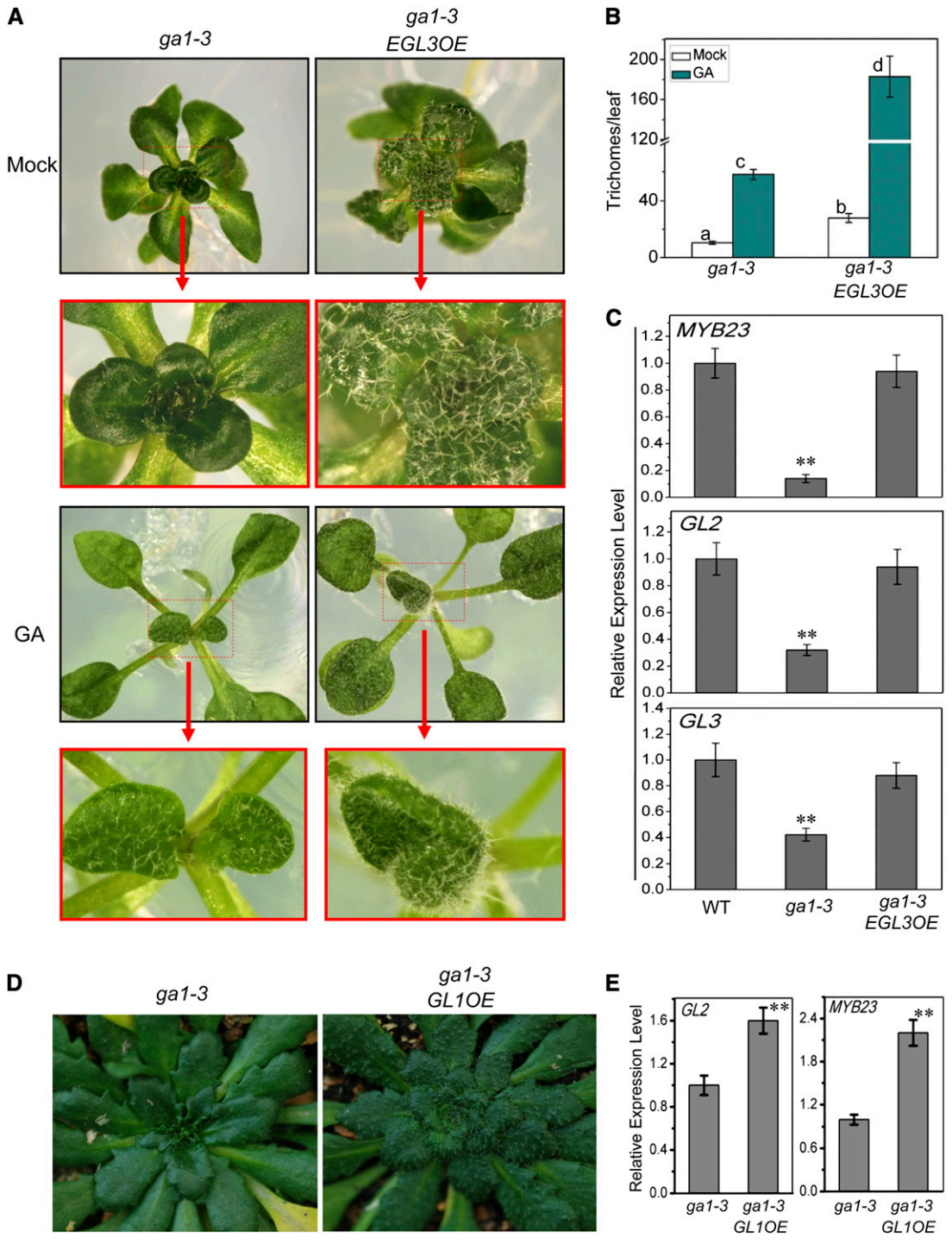

Figure 5. Overexpression of *EGL3* or *GL1* Rescues Trichome Initiation in *ga1-3* Mutant.

(A) Plant images of *ga1-3* and *EGL3* overexpression transgenic plant in *ga1-3* background (*ga1-3 EGL3OE*) supplied without (Mock) or with GA_3 (GA).
(B) Statistical analysis of total trichome numbers in the fifth true leaves of indicated plants in **(A)**. Eight leaves for each genotype were used for trichome number measurement in each biological experiment. Data are means (±SE) of three biological replicates. Asterisks indicate significant differences by one-way ANOVA analysis with SAS software (**P < 0.05).
(C) Real-time PCR analysis for *GL2*, *MYB23*, and *GL3* in *Arabidopsis* wild-type L*er* (WT), *ga1-3*, and *ga1-3 EGL3OE* plants plated on MS medium. *ACTIN8* was used as the internal control. Data are means (±SE) of three biological replicates. Asterisks indicate significant differences compared with *ga1-3 EGL3OE* by one-way ANOVA analysis with SAS software (**P < 0.01).
(D) Plant images of 3-month-old *ga1-3* and *ga1-3 GL1OE* transgenic plants. *GL1* overexpression obviously increased trichome formation in *ga1-3* background.
(E) Real-time PCR analysis for *GL2* and *MYB23* in *ga1-3* and *ga1-3 GL1OE* plants in **(D)**. *ACTIN8* was used as the internal control. Data are means (±SE) of three biological replicates. Asterisks indicate significant differences compared with *ga1-3* by one-way ANOVA analysis with SAS software (**P < 0.01).

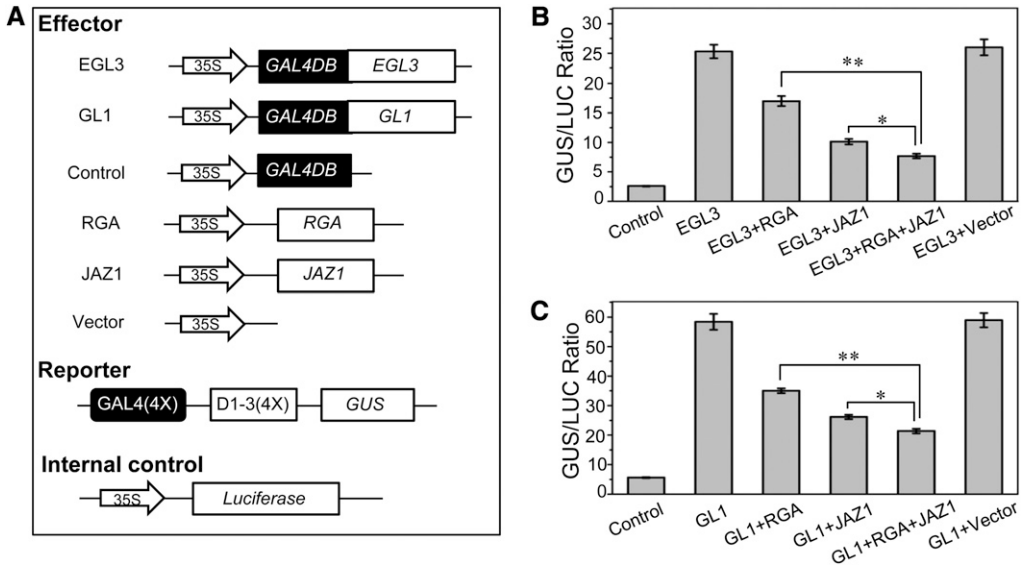

Figure 6. RGA and JAZ1 Coordinately Inhibit Transcriptional Function of EGL3 and GL1.

(A) Schematic diagram of the constructs used in transient expression assays in **(B)** and **(C)**.

(B) and **(C)** Transient expression assay showed that both RGA and JAZ1 proteins could significantly repress transcriptional function of EGL3 **(B)** and GL1 **(C)**. The GUS/LUC ratios were measured in *Arabidopsis* leaf protoplasts after transiently transfected with reporter, internal control, and indicated effectors. Data are means (±SE) of three biological replicates. Asterisks represent Student's *t* test significance between pairs indicated with brackets (*$P < 0.05$; **$P < 0.01$).

GAL4DB to serve as effectors. The *GUS* (β-glucuronidase) gene under control of four upstream GAL4 DNA binding sites [GAL4(4x)-D1-3(4x)] was used as a reporter, whereas the 35S promoter–driven *LUC* (luciferase) was the internal control (Figure 3A). The *DELLA* genes *RGA* and *RGL2* were inserted into pGreen62 vector under control of the 35S promoter (Figure 3A). As shown in Figure 3B, expression of GAL4DB-GL1 with the GUS reporter largely increased the GUS/LUC ratio, indicating that GL1 functions as a transcription activator. Coexpression of RGA or RGL2 with GAL4DB-GL1 significantly reduced the GUS/LUC ratio (Figure 3B), indicating that the GL1 transcription activity was inhibited by RGA or RGL2. Similar experiments also showed that RGA and RGL2 inhibit the transcription activity of EGL3 and GL3 (Figures 3A, 3C, and 3D).

As *GL2* is a direct target of GL1 and GL3 (Zhang et al., 2003; Morohashi et al., 2007), we used the *GL2* promoter–driven *LUC* reporter (P_{GL2}-*LUC*) system to further verify the inhibitory effect of DELLAs on transcriptional function of the WD-repeat/bHLH/MYB (Figure 3E). Consistent with a previous study (Wang and Chen, 2008), our results showed that expression of GL3 and GL1 induced P_{GL2}-*LUC* activity (Figure 3F). Furthermore, we observed that coexpression of RGA repressed GL3/GL1-induced P_{GL2}-*LUC* expression (Figure 3F).

Taken together (Figures 2 and 3), our results suggested that the DELLA proteins interact with GL1, EGL3, and GL3 to attenuate their transcriptional functions. The quantitative real-time PCR assay on GA-related mutants (*ga1-3* and penta) further demonstrated that endogenous DELLA proteins in planta attenuated the transcription function of the WD-repeat/bHLH/MYB complex and repressed expression of its downstream target genes, including *GL2* and *MYB23* (Zhang et al., 2003; Kang et al., 2009; Morohashi and Grotewold, 2009) (Figures 3G and 3H). Compared with the wild type, the GA-deficient mutant *ga1-3*, in which DELLA proteins accumulate (Silverstone et al., 2001), exhibited reduced expression of *GL2* and *MYB23* (Figures 3G and 3H), while the expression level of *GL2* and *MYB23* was fully rescued in the penta mutant (Figures 3G and 3H).

DELLAs Act through the WD-Repeat/bHLH/MYB Complex to Repress GA-Mediated Trichome Formation

To investigate whether the WD-repeat/bHLH/MYB complex acts downstream of DELLAs to regulate GA-induced trichome formation, we crossed the penta mutant with the *gl3 egl3* double mutant to produce the heptuple mutant (penta *gl3 egl3*). Comparison of trichome numbers in penta and penta *gl3 egl3* mutants showed that trichome initiation in the penta mutant was abolished by the *gl3 egl3* double mutations (Figure 4A). The penta mutant produced high density of trichomes (Figure 4B); however, the penta *gl3 egl3* heptuple mutant exhibited a no-trichome phenotype (Figures 4A and 4B), which is similar to the glabrous phenotype of the *gl3 egl3* mutant (Figure 1C). Consistent with this, the expression levels of *GL2* and *MYB23*, targets of the WD-repeat/bHLH/MYB complex, were significantly decreased in the penta *gl3 egl3* mutant (Figures 4C and 4D). These results demonstrated that the DELLAs act upstream of the WD-repeat/bHLH/MYB complex to regulate GA-mediated trichome formation.

To further verify that DELLAs act through the WD-repeat/bHLH/MYB complex to repress GA-mediated trichome formation, we

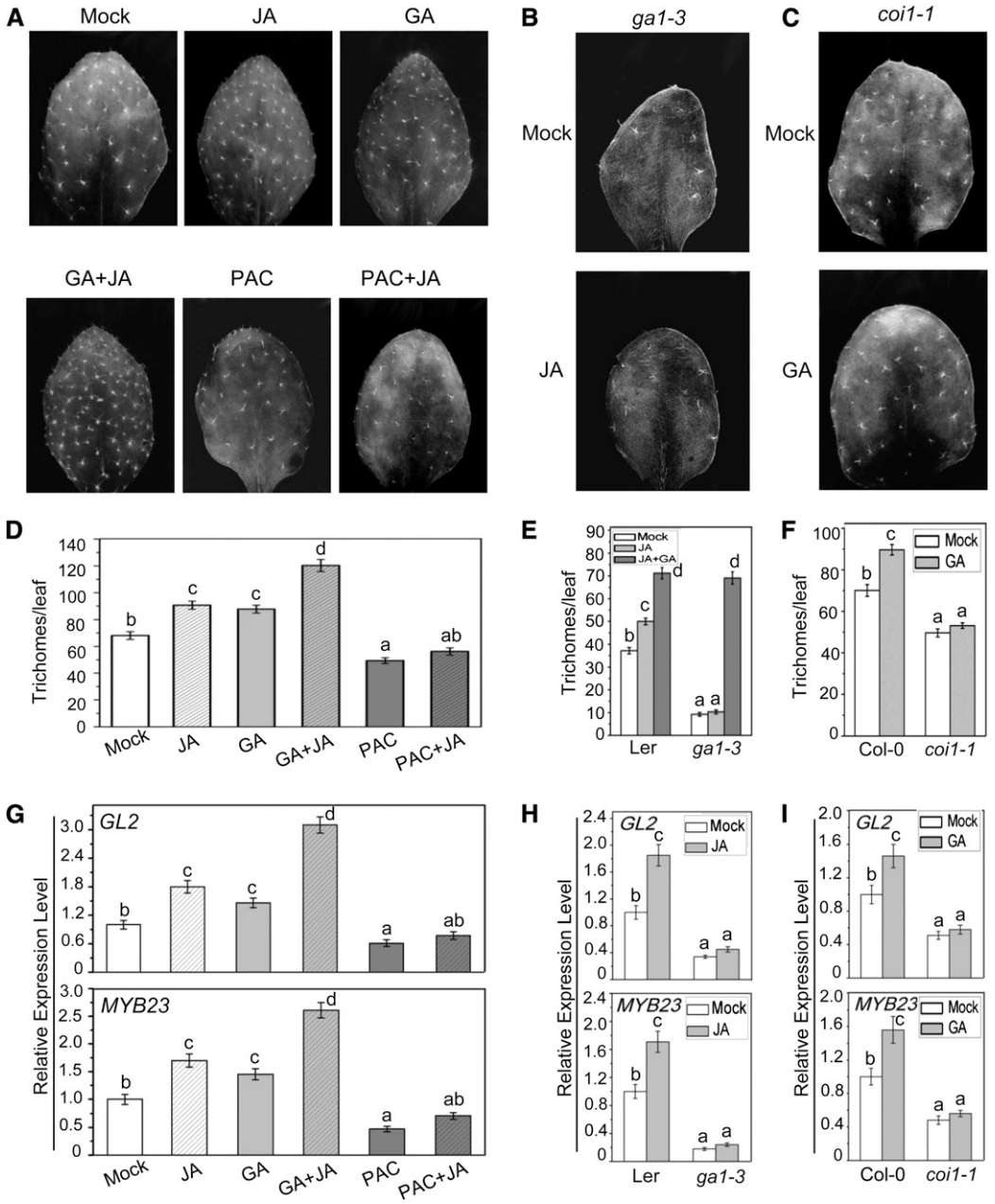

Figure 7. GA and JA Function Synergistically to Promote Trichome Initiation.

(A) Trichome images of the fifth true leaves from wild-type Columbia-0 (Col-0) treated without (Mock) or with MeJA (JA), GA_3 (GA), GA_3 plus MeJA (GA+JA), paclobutrazol (PAC), or paclobutrazol plus MeJA (PAC+JA).

(B) Trichome images of the fifth true leave from *ga1-3* treated without (Mock) or with MeJA (JA).

(C) Trichome images of the fifth true leave from *coi1-1* treated without (Mock) or with GA_3 (GA).

(D) Statistical analysis of total trichome numbers in the fifth true leaves of Col-0 with the indicated treatments in **(A)**. Eight leaves for each genotype were used for trichome number measurement in each biological experiment. Data are means (±SE) of three biological replicates. Lowercase letters indicate significant differences by one-way ANOVA analysis with SAS software ($P < 0.05$).

(E) and **(F)** Statistical analysis of total trichome numbers in the fifth true leaves of the indicated genotype with the indicated treatments. Eight leaves for each genotype were used for trichome number measurement in each biological experiment. Data are means (±SE) of three biological replicates. Lowercase letters indicate significant differences by one-way ANOVA analysis with SAS software ($P < 0.05$).

(G) Real-time PCR analysis for *GL2* and *MYB23* in Col-0 plants indicated in **(A)**. *ACTIN8* was used as the internal control. Data are means (±SE) of three biological replicates. Lowercase letters indicate significant differences by one-way ANOVA analysis with SAS software ($P < 0.05$).

examined whether overexpression of the bHLH factor EGL3 or MYB factor GL1 could escape the inhibition of DELLAs to rescue trichome formation in the GA-deficient mutant *ga1-3*. Trichome density analysis showed that the transgenic *ga1-3* plants overexpressing *EGL3* (*ga1-3 EGL3OE*) developed high density of trichomes (Figures 5A and 5B), suggesting that overexpression of *EGL3* could restore the trichome formation in *ga1-3*. Furthermore, we found that *EGL3* overexpression in *ga1-3* could rescue expression of *MYB23*, *GL2*, and *GL3* (target genes or component of the WD-repeat/bHLH/MYB complex) (Figure 5C).

In addition to overexpression of the bHLH factor *EGL3*, we also examined whether overexpression of MYB factor *GL1* restored trichome initiation in the *ga1-3* mutant. As shown in Figure 5D, overexpression of *GL1* in *ga1-3* increased trichome numbers. Consistent with this, overexpression of *GL1* in *ga1-3* enhanced the expression of its target genes *GL2* and *MYB23* (Figure 5E). Taken together, we demonstrated that overexpression of *EGL3* or *GL1* can restore trichome formation in *ga1-3* mutants, suggesting that the WD-repeat/bHLH/MYB complex acts downstream of DELLAs to regulate GA-induced trichome formation. These results collectively demonstrated that the WD-repeat/bHLH/MYB complex acts downstream of DELLAs to regulate GA-mediated trichome formation.

GA and JA Synergistically and Mutually Dependently Regulate Trichome Formation

Consistent with our previous studies (Qi et al., 2011), we further found that trichome initiation is induced by JA and attenuated by disruption of JA signaling (e.g., *coi1-1*) or biosynthesis (e.g., *aos*) (Supplemental Figures 2A and 2B) and that the WD-repeat/bHLH/MYB complex acts as a direct target of JAZ proteins to modulate trichome initiation (Qi et al., 2011) (Supplemental Figures 2 and 3).

As both DELLAs and JAZs interact with the WD-repeat/bHLH/MYB transcription complex to repress its transcription function (Figures 2 and 3; Supplemental Figure 2), we further investigated whether DELLAs and JAZs regulate the transcription function of the WD-repeat/bHLH/MYB complex synergistically. As expected, RGA or JAZ1 alone repressed the transcription activity of the bHLH factor EGL3 of the WD-repeat/bHLH/MYB complex (Figures 6A and 6B). We further found that coexpression of RGA and JAZ1 displayed a stronger repression of the transcriptional activity of EGL3 compared with RGA or JAZ1 alone in the transient expression assay (Figures 6A and 6B). We observed similar results for the repression of RGA and JAZ1 on the MYB factor GL1 (Figures 6A and 6C). These results demonstrated that DELLAs and JAZ proteins synergistically repressed the transcription activity of the WD-repeat/bHLH/MYB complex.

We further investigated whether GA and JA synergistically regulated trichome formation in planta. The wild-type plants were treated with GA, JA, or a combination of GA and JA and subjected for quantitative trichome analysis. As shown in Figures 7A and 7D, the wild-type plants displayed much more trichome numbers under treatment with both GA and JA, compared with treatment with GA or JA alone. Consistent with this, the expression of *GL2* and *MYB23*, the target genes of the WD-repeat/bHLH/MYB complex, was also highly induced by combination of GA and JA (Figure 7G). These results demonstrated that GA and JA synergistically induce trichome initiation in planta.

Interestingly, we found that paclobutrazol, a GA biosynthetic inhibitor that causes DELLAs accumulation in plant (Feng et al., 2008), repressed JA-induced trichome initiation (Figures 7A and 7D) and the expression of *GL2* and *MYB23* (Figure 7G). Furthermore, we observed that the GA biosynthesis mutant *ga1-3* (with high level of DELLAs) clearly suppressed the JA-induced trichome initiation and the expression of *GL2* and *MYB23* (Figures 7B, 7E, and 7H) and that the combination of GA and JA significantly induced trichome initiation in *ga1-3* (Figure 7E). These observations of DELLA suppression on JA-induced trichome formation suggested that GA signaling is required for JA-regulated trichome development.

Similarly, we found that the GA-induced trichome formation was attenuated in the JA signaling-deficient mutant *coi1-1* (with high level of JAZs) (Figures 7C and 7F). The GA-induced expression of *GL2* and *MYB23* in *coi1-1* was also inhibited (Figure 7I). These results suggested that GA-induced trichome formation requires JA signaling. Taken together, our results demonstrated that plant hormones GA and JA synergistically induce trichome initiation and that JA and GA signaling regulate trichome development in a mutually dependent fashion.

DISCUSSION

Plants integrate diverse environmental and endogenous signals to coordinately regulate growth, development, and defense to improve their survival in natural habitats. This study reveals a key molecular mechanism for integration of different hormonal signals to synergistically regulate plant development. Previous studies demonstrated that the GA-signaling repressors DELLAs interact with the JA-signaling repressors JAZs to modulate the antagonistic action between GA and JA in regulating root growth and hypocotyl elongation (Hou et al., 2010; Yang et al., 2012) (Figures 8B and 8C). The results reported here demonstrate that the WD-repeat/bHLH/MYB complex acts as a direct target of DELLAs and that both DELLAs and JAZs interact with the WD-repeat/bHLH/MYB complex to mediate the synergistic and mutually dependent action between GA and JA signaling in regulating plant trichome development. DELLAs and JAZs interact with bHLH factors (EGL3 and GL3) and MYB factor (GL1), essential components of the WD-repeat/bHLH/MYB complex, to repress their transcription activity, while GA and JA induce degradation of DELLAs and JAZs,

Figure 7. (continued).

(H) and **(I)** Real-time PCR analysis for *GL2* **(H)** and *MYB23* **(I)** in the indicated genotype with the indicated treatment in **(B)** and **(C)**, respectively. *ACTIN8* was used as the internal control. Data are means (±SE) of three biological replicates. Lowercase letters indicate significant differences by one-way ANOVA analysis with SAS software ($P < 0.05$).

respectively, to coordinately activate the WD-repeat/bHLH/MYB complex and synergistically and mutually dependently regulate trichome development (Figure 8A).

Consistent with this synergistic regulatory mechanism, in the mutants deficient in GA biosynthesis (e.g., *gal-3*), DELLA proteins accumulate to high levels to interact with and repress the WD-repeat/bHLH/MYB complex and inhibit trichome initiation. Similarly, in the mutants deficient in JA biosynthesis or signaling (e.g., *aos* and *coi1-1*), JAZ proteins accumulated to interact with and repress WD-repeat/bHLH/MYB and attenuate trichome initiation. GA and JA signals are mutually indispensable for activation of WD-repeat/bHLH/MYB, and disruption of either GA or JA signaling would lead to significant accumulation of either DELLAs or JAZs, which interact with and repress nearly all members of the WD-repeat/bHLH/MYB complex and attenuate trichome formation (Figures 1, 2, 3, 6, and 7; Supplemental Figure 2). Also, exogenous JA treatment of the GA-biosynthetic mutant *gal-3* did not rescue trichome formation (Figures 7B and 7E) as the high levels of DELLAs in *gal-3* are sufficient to repress WD-repeat/bHLH/MYB; reciprocally, GA treatment of the JA-related mutant *coi1-1*, which has high levels of JAZs, failed to restore trichome development (Figures 7C and 7F).

In response to developmental cues or environmental challenges, wild-type plants generate GA and/or JA signals to coordinately control levels of DELLAs and JAZs via SCF^{SLY1} and SCF^{COI1}-mediated 26S proteolysis. This maintains the appropriate transcriptional activity of WD-repeat/bHLH/MYB to activate expression of its downstream transcription factors (e.g., *GL2* and *MYB23*) and mediate proper trichome formation (Figures 7A, 7D, 7G, and 8A). Treatment of exogenous GA and/or JA further induces degradation of the remaining DELLAs and/or JAZs in the wild type to derepress WD-repeat/bHLH/MYB, leading to excessive trichome development in wild-type plants (Figures 1A, 1B, 7A, 7D, 7G, and 8A; Supplemental Figures 2A and 2B). The coordinated regulation of trichome development by both growth-related hormones (GA) and stress-triggered signals (JA) may benefit plant growth and survival in nature.

Our results and previous studies showed that the C-terminal part of DELLAs can bind to different transcriptional factors, including EGL3/GL3/GL1 (Figure 2), BZR1 (Bai et al., 2012), and EIN3/EIL1 (An et al., 2012). Similarly, the C-terminal Jas domain of JAZ proteins interact with various transcriptional factors, such as EGL3/GL3/TT8/GL1/MYB75 (Qi et al., 2011), MYC2/MYC3/MYC4 (Chini et al., 2009; Cheng et al., 2011), IIId bHLH factors (Song et al., 2013b), ICE1/ICE1 (Hu et al., 2013b), and MYB21/MYB24 (Song et al., 2011). As JAZs and DELLAs both interact with the bHLH and MYB components of the WD-repeat/bHLH/MYB complex (EGL3, GL3, and GL1) (Figure 2) (Qi et al., 2011), it will be interesting to investigate whether JAZ and DELLA proteins simultaneously bind to the WD-repeat/bHLH/MYB complex for synergistic repression.

In addition to trichome development, GA and JA also synergistically regulate sesquiterpene synthesis (Hong et al., 2012) and stamen development (Ma, 2005; Plackett et al., 2011; Song et al., 2013a). The R2R3 MYB transcription factors MYB21, MYB24, and MYB57 constitute a class of master regulators essential for stamen development (Mandaokar et al., 2006; Reeves et al., 2012). Our previous study demonstrated that JA modulates the interaction of JAZs with MYB21 and MYB24 to control stamen development (Song et al., 2011) and that GA promotes the expression of JA biosynthesis genes and induces JA accumulation in the flower to activate the COI1-JAZ-MYB21/MYB24 pathway

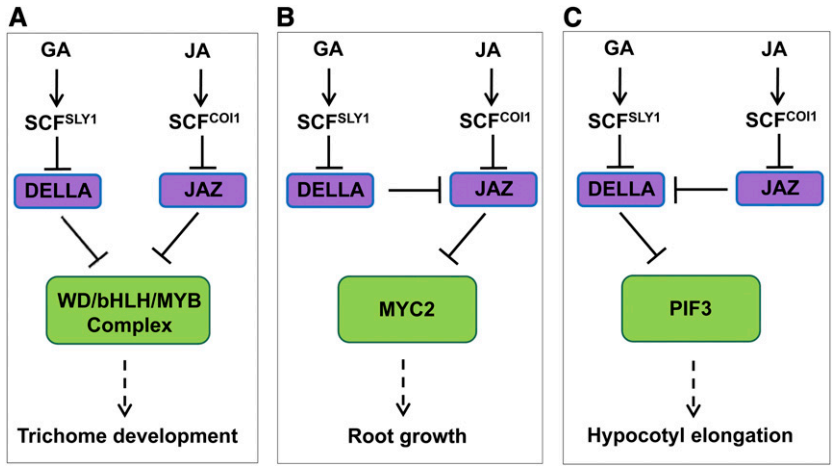

Figure 8. A Simplified Model for the Crosstalk between GA and JA.

(A) Both DELLAs and JAZs interact with and attenuate WD-repeat/bHLH/MYB complex to inactivate downstream genes and repress trichome formation. GA and JA signal induce degradation of DELLAs and JAZs, respectively, to derepress WD-repeat/bHLH/MYB complex and synergistically and mutually dependently modulate trichome development in *Arabidopsis*.

(B) A previous study showed that the *Arabidopsis* DELLAs interact with and repress JAZs to release MYC2 that mediates JA-regulated root growth (Hou et al., 2010).

(C) A previous study showed that JAZs interact with and repress DELLAs to release PIF3 that regulates GA-mediated hypocotyl elongation in *Arabidopsis* (Yang et al., 2012).

[See online article for color version of this figure.]

for stamen development (Cheng et al., 2009). It would be very interesting to investigate, in addition to the GA-regulated JA biosynthesis in flower, whether both DELLAs and JAZs also interact with MYB21/MYB24 to exert a synergistic and mutually indispensable effect in regulation of stamen development.

METHODS

Plant Materials and Growth Conditions

The *Arabidopsis thaliana* mutants *ga1-3*, penta, *della*, Q1, Q2, Q3, Q4, *gl3 egl3*, *gl1*, *ttg1*, *coi1-1*, and *aos* and the transgenic plants TAP-RGA and TAP-RGL2 were as described previously (Feys et al., 1994; Park et al., 2002; Feng et al., 2008; Cheng et al., 2009; Qi et al., 2011). The heptuple mutant penta *gl3 egl3* was generated by crossing penta with *gl3 egl3*.

After surface sterilization with 20% bleach for 10 min, *Arabidopsis* seeds were plated on Murashige and Skoog (MS) medium (Sigma-Aldrich) supplied with 3% Suc, chilled at 4°C in the dark for 3 d, and then transferred to growth house at 19 to 23°C with 16-h-light/8-h-dark photoperiod. Seeds with *ga1-3* background were immerged in 100 μM GA_3 at 4°C for 7 d before sowing. *Nicotiana benthamiana* was cultivated at 22 to 28°C under a 16-h-light/8-h-dark photoperiod.

Y2H Assays

The C-terminal fragments of RGA or RGL2 (RGA-R or RGL2-R) and coding regions of GL1, EGL3, GL3, TTG1, and their truncated forms were inserted into pLexA or pB42AD vectors. Y2H assays were performed following the manufacturer's instructions (Clontech) according to Qi et al. (2011). The EGY48 yeast cotransformed with the indicated constructs were selected on solid SD/-His/-Trp/-Ura medium. Surviving yeast transformants were then cultured with liquid SD/-His/-Trp/-Ura medium, harvested by centrifugation, and resuspended with 200 μL of distilled water, of which 5 μL were dropped onto a well of 96-well plates containing the induction medium (SD/Gal/Raf/X-gal/-Ura/-His/Trp/-Leu). Photos were taken after incubation at 30°C for 2 to 3 d. The expression of proteins fused with AD and BD domain were detected by immunoblots with anti-HA and anti-LexA antibodies, respectively. Primers for making these constructs are listed in Supplemental Table 1. The experiment was repeated for three independent biological replicates.

Trichome Measurement

For detecting the effect of GA on trichome formation, seeds were disinfected, chilled, and cultivated on MS medium containing 100 μM GA_3 for 6 d and then transferred to MS medium for another 2 weeks before trichome observation. The fifth true leaves were further used for trichome observation by environmental scanning electron microscopy (FEI Quanta 200). For exploring the interaction effect of GA and JA on trichome formation, seeds were disinfected, chilled, and plated on MS medium with or without 100 μM GA_3 for 6 d and then transferred to MS medium supplemented with indicated combinations of 0.2 μM paclobutrazol and 200 nM methyl jasmonate (MeJA) in a 15-cm-diameter plate. GA_3 and paclobutrazol were applied in the MS medium. For MeJA treatment, an Eppendorf tube cap with a piece of filter paper in it was placed in the center of plate. MeJA was dropped onto the filter paper for evaporation. After 2 weeks, the total trichome numbers of the fifth true leaves were calculated using environmental scanning electron microscopy.

BiFC Assay and Luciferase Complementation Imaging Assay

For BiFC assays, the full-length coding sequences of *RGA*, *RGL2*, *GL1*, and *EGL3* were first inserted into the pDONR207 vector and subsequently recombined into binary cYFP or nYFP vector through the Gateway reaction system (Invitrogen) (Qi et al., 2011). Primers used are presented in Supplemental Table 1. These vectors were subsequently transformed into *Agrobacterium tumefaciens* strain GV3101 and coexpressed in *N. benthamiana* leaves as described previously (Qi et al., 2011). YFP fluorescence signal was detected using confocal microscopy (LSM710; Zeiss) 50 h after infiltration. The nuclei were indicated by staining with 4′,6-diamidino-2-phenylindole. The experiment was repeated for three independent biological replicates.

For the firefly LUC complementation imaging assays, the full length coding sequences of *JAZ1* and *JAZ11* were fused with N-terminal fragment of luciferase (nLUC) to form JAZ1-nLUC and JAZ11-nLUC, respectively. The full-length coding sequence of EGL3 was ligated with C-terminal fragment of luciferase (cLUC) to form cLUC-EGL3. Primers used are presented in Supplemental Table 1. These vectors were subsequently transformed into *Agrobacterium* strain GV3101 and coexpressed in *N. benthamiana* leaves. The LUC complementation images were taken with a low-light cooled charge-coupled device imaging apparatus (Andor iXon) as described previously (Song et al., 2011). The experiment was repeated for three independent biological replicates.

Pull-Down Assay

The full-length coding sequences of *EGL3*, *GL1*, and *GL3* were inserted into the pMAL-c2x (NEB) vector to produce MBP-EGL3, MBP-GL1, and MBP-GL3, respectively. The MBP and MBP-fused EGL3, GL3, and GL1 were expressed in *Escherichia coli* and purified by amylose resin beads. Total proteins was extracted from 5 g of TAP-RGA and TAP-RGL2 transgenic plants using RB buffer (100 mM NaCl, 50 mM Tris-Cl, pH 7.8, 25 mM imidazole, 0.1% [v/v] Tween 20, 10% [v/v] glycerol, EDTA-free complete mini protease inhibitor cocktail, and 20 mM 2-mercaptoethanol), and subsequently concentrated to the volume of 400 μL. The procedure of pull-down assay was performed according to Qi et al. (2011). About 50 mg of purified MBP, MBP-EGL3, MBP-GL1, and MBP-GL3 proteins were incubated with 100 μL of amylose resin for 2 h at 4°C with gentle rocking and subsequently washed with 1 mL of RB buffer five times, then further incubated with 100 μL concentrated total proteins containing TAP-RGA and TAP-RGL2 for 2 h at 4°C. The proteins binding to amylose resin was further washed for five times with 1 mL RB buffer, resuspended in SDS loading buffer, and immunoblotted using anti-c-myc antibody (Roche). Coomassie Brilliant Blue was applied to determine protein levels. The experiment was repeated for three independent biological replicates.

Protoplast Transfection Assay

For transient expression assays using the GUS reporter, the coding sequences of *EGL3*, *GL3*, and *GL1* were fused with the GAL4DB under control of the *35S* promoter. The coding sequences of *RGA*, *RGL2*, and *JAZ1* were inserted into the pGreenII 62-SK under control of the 35S promoter (Hellens et al., 2005). Primers used for plasmid construction are listed in Supplemental Table 1. The GUS reporter contains four copies of upstream GAL4DB binding sites [GAL4(4x)-D1-3(4x)] followed by subsequent *GUS* gene (Tiwari et al., 2001). The internal control was a firefly *LUC* gene under control of 35S promoter. *Arabidopsis* mesophyll protoplasts preparation and subsequent transfection were performed according to the protocols reported previously (Yoo et al., 2007). The GUS:LUC ratio was presented. For Figures 3B to 3D, 10 μg for all the constructs were used in these experiments. For Figures 6B and 6C, 4 μg JAZ1 and 5 μg RGA were used, respectively, while for other reporters and effectors, 10 μg plasmid was individually used.

To perform transient transcription activity assay using the LUC reporter, a −2045-bp *GL2* promoter was amplified from genomic DNA and inserted into pGreenII 0800-LUC to generate the P_{GL2}-*LUC* reporter construct (Hellens et al., 2005). The coding sequences of *GL3* and *GL1*

were amplified and inserted into pGreenII 62-SK vector under control of the 35S promoter. All primers used for these constructs are listed in Supplemental Table 1. After protoplasts preparation and subsequent transfection, firefly LUC and renillia luciferase (REN) activities were measured using the dual-luciferase reporter assay system (Promega). The LUC:REN ratio was presented. For Figure 3F and Supplemental Figure 2F, 10 μg for all the constructs were used in these experiments.

Real-Time PCR Analysis

The indicated *Arabidopsis* seedlings were used for quantitative real-time PCR analysis of gene expression. Total RNAs were extracted using Trizol reagent (Invitrogen) and reverse transcribed using M-MLV (Takara). Real-time PCR was performed with the ABi7500 real-time PCR system using the SYBR Prime Script RT-PCR kit (Takara). The relative gene expression level was calculated by normalizing against the internal control *ACTIN8*. Primers used for real-time PCR analysis are listed in Supplemental Table 2.

Generation of *EGL3* and *GL1* Transgenic Plants

To generate *Arabidopsis EGL3* and *GL1* overexpression transgenic plants, the coding sequences of *EGL3* and *GL1* were amplified and inserted into the modified pCAMBIA1300 vector (Cambia) under control of 35S promoter. These vectors were transformed into *Agrobacterium* strain GV3101 and subsequently introduced into the *Arabidopsis* wild-type plants. *EGL3* overexpression transgenic plants were crossed with *ga1-3* and *coi1-1* to produce *ga1-3 EGL3OE* and *coi1-1 GL1OE* plants. *GL1* overexpression transgenic plants were crossed with *ga1-3* to generate *ga1-3 GL1OE*. Three individual transgenic lines for each gene (*EGL3* or *GL1*) were identified, and one transgenic line for *EGL3* (or *GL1*) was representatively displayed in the article.

Accession Numbers

The Arabidopsis Genome Initiative numbers for the genes mentioned in this article are as follows: *GAI* (AT4G02780), *RGA* (AT2G01570), *RGL1* (AT1G66350), *RGL2* (AT3G03450), *RGL3* (AT5G17490), *GA1* (AT4G02780), *COI1* (AT2G39940), *AOS* (AT5G42650), *JAZ1* (AT1G19180), *JAZ8* (AT1G30135), *JAZ10* (AT5G13220), *JAZ11* (AT3G43440), *GL3* (AT5G41315), *EGL3* (AT1G63650), *GL1* (AT3G27920), MYB23 (AT5G40330), *GL2* (AT1G79840), *TTG1* (AT5G24520), and *ACTIN8* (AT1G49240).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Negative Controls of BiFC Assay in *N. benthamiana* Leaves.

Supplemental Figure 2. JAZs Interact with the WD-Repeat/bHLH/MYB Complex to Modulate Jasmonate-Regulated Trichome Development.

Supplemental Figure 3. *EGL3* Overexpression Restores Trichome Formation in *coi1-1*.

Supplemental Table 1. Primers Used for Vector Construction.

Supplemental Table 2. Primers Used for Quantitative Real-Time PCR Analysis.

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AUTHOR CONTRIBUTIONS

T.Q., S.S., and D.X. designed the research. T.Q., H.H., D.W., and S.S. performed research. T.Q., J.Y., Y.Q., S.S., and D.X. analyzed data. T.Q., S.S., and D.X. wrote the article.

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