

ARTICLE ADDENDUM



## The C-terminal domains of *Arabidopsis* GL3/EGL3/TT8 interact with JAZ proteins and mediate dimeric interactions

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### ABSTRACT

The phytohormone jasmonates (JAs) regulate plant development, growth, secondary metabolism, and defense responses. JAs act through CORONATINE INSENSITIVE1 (COI1) to induce the degradation of JA ZIM-domain (JAZ) proteins, and activate JAZ-repressed transcription factors to regulate plant response. We previously showed that the basic helix-loop-helix (bHLH) and MYB members of the WD-repeat/bHLH/MYB complex interacted with JAZs and mediated JA-induced anthocyanin accumulation and trichome initiation. In this study, we showed that the C-terminal domain of the bHLH members (GLABRA3 [GL3], ENHANCER OF GLABRA3 [EGL3] and TRANSPARENT TESTA8 [TT8]) interacted with JAZs in yeast and plant, and mediated dimerizations between the bHLH members. Our study provides further understanding of the bHLH members of the WD-repeat/bHLH/MYB complex in JA pathway.

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Jasmonates (JAs), a class of cyclic fatty acid-derived phytohormones,<sup>1,2</sup> regulate plant developmental processes, including plant fertility, root growth, trichome formation and senescence,<sup>3-5</sup> control secondary metabolism,<sup>6,7</sup> and mediate plant defense responses against insects attack and pathogen invasion.<sup>8-10</sup> JA signals are perceived by the F-box protein CORONATINE INSENSITIVE1 (COI1), which recognizes and ubiquitinates JA ZIM-domain (JAZ) proteins for degradation, and it will release multiple JAZ-inhibited transcription factors to activate various JA response.<sup>1,11-16</sup>

In *Arabidopsis*, the WD-repeat/bHLH/MYB complex, consisting of the WD-repeat protein TRANSPARENT TESTA GLABRA1 (TTG1), the bHLH transcription factors (GLABRA3 [GL3], ENHANCER OF GLABRA3 [EGL3] and TRANSPARENT TESTA8 [TT8]), and the R2R3-MYB transcription factors (MYB75, MYB90, MYB113, MYB114, GLABRA1 [GL1]), activates expression of anthocyanin late biosynthetic genes and trichome developmental genes to promote anthocyanin accumulation and trichome initiation.<sup>17-19</sup> JAZs interact with bHLH, and MYB members of the WD-repeat/bHLH/MYB complex to inhibit their transcriptional activity, and JAs induce JAZs degradation to activate the WD-repeat/bHLH/MYB complex and enhance anthocyanin accumulation and trichome formation.<sup>20</sup>

In this study, we further mapped the domain responsible for the interactions between bHLH members (GL3/EGL3/TT8) and JAZs. EGL3, GL3 and TT8 contain a JAZ-interaction domain (JID) at N-terminus,<sup>21</sup> a bHLH domain<sup>22</sup> and a conserved C-terminal domain (CD) at C-terminus (Fig. 1A-C, Fig. S1). EGL3 and TT8 were respectively divided into EGL3NT and TT8NT containing the JID domain, and EGL3CT and TT8CT with bHLH and CD domains (Fig. 1A, B). Yeast-two hybrid (Y2H) assay showed that JAZ2 and JAZ11 interacted with EGL3CT and TT8CT, but not EGL3NT and TT8NT (Fig. 1D, E), implying that bHLH and/or CD domains are essential for interaction with JAZs, while JID is not. GL3 was truncated into GL3NT with JID and bHLH domains, and GL3CT containing bHLH and CD domains (Fig. 1C). Y2H analysis showed that JAZ2 and JAZ11 interacted with GL3CT, but not GL3NT (Fig. 1F), suggesting that CD domain is responsible for interaction with JAZs. Moreover, JAZ1 and JAZ8 also interacted with GL3CT, but not GL3NT (Fig. 1G).

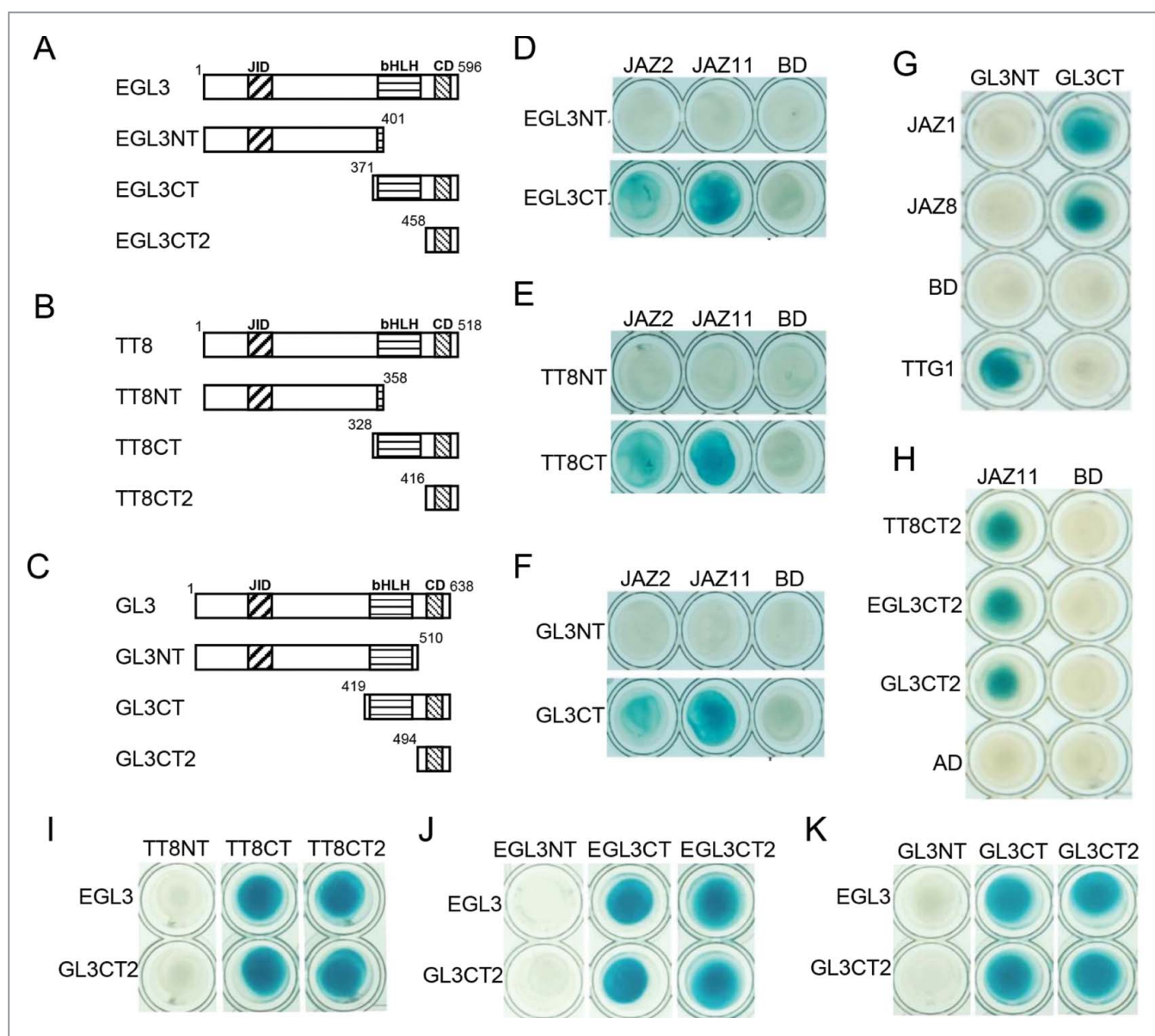
To further verify whether CD domain is responsible for interactions with JAZs. GL3, EGL3 and TT8 were further truncated into GL3CT2, EGL3CT2 and TT8CT2 harboring the CD domain (Fig. 1A-C). The results showed that all the GL3CT2, EGL3CT2 and TT8CT2 interacted with JAZ11 in yeast, suggesting that the CD domain at CT2 is responsible for interaction with JAZs (Fig. 1H).

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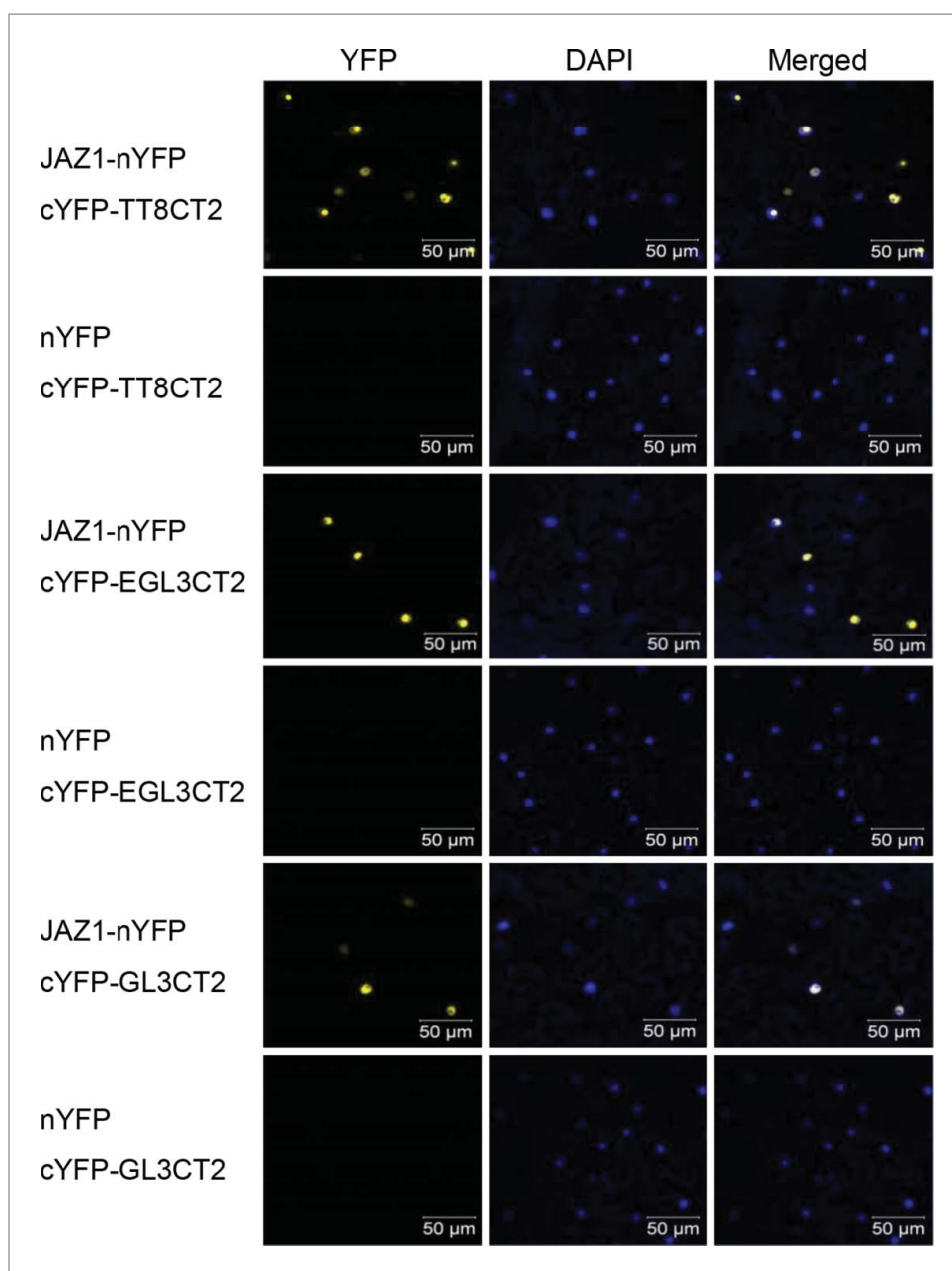
**Figure 1.** The CD domains of Egl3, Tt8 and Gl3 are responsible for interactions with JAZ proteins and dimeric interactions. (A) to (C) Schematic structures of Egl3, Tt8 and Gl3. JID, JAZ-interaction domain; CD, C-terminal domain. (D) to (H) Y2H assay to detect interactions of the truncated domains of Egl3, Tt8 and Gl3 with JAZ1, JAZ2, JAZ8 or JAZ11. JAZs, TTG1 and the domains of Gl3, Egl3 and Tt8 were fused with LexA DNA binding domain (BD) or activation domain (AD) respectively. (I) to (K) BD-fused Egl3 and Gl3CT2 interact with AD-fused CT and CT2 of Gl3, Egl3 and Tt8 in yeast. All the interactions were detected on SD/Gal/Raf/X-gal (-Ura/-His/-Trp/-Leu) medium.

We further employed a yellow fluorescence protein (YFP) based bimolecular fluorescence complementation (BiFC) assay<sup>23</sup> to verify the interactions of CD domain with JAZ1. As shown in Fig. 2, coexpression of cYFP-fused GL3CT2, EGL3CT2, or TT8CT2 with nYFP-fused JAZ1 produced YFP fluorescence in the nuclei, while the negative controls did not, suggesting that CD domain of GL3, EGL3 and TT8 interacts with JAZ1 in plant. Taken together, these data demonstrated that GL3, EGL3 and TT8 interacted with JAZs through the CD domain (Figs. 1 & 2).

GL3 was shown to form dimers.<sup>24</sup> We next examined the dimerization of derivatives of GL3, EGL3 and TT8 in yeast.

Both EGL3 and GL3CT2 exhibited interactions with TT8CT, TT8CT2, EGL3CT, EGL3CT2, GL3CT and GL3CT2, but not TT8NT, EGL3NT and GL3NT (Fig. 1I–K), demonstrating that CD domain, but not the bHLH domain, mediates the dimerization of GL3, EGL3 and TT8.

Previous studies showed that the JID domain of the IIIe bHLH factors (e.g. MYC2) mediated interactions with JAZs.<sup>16,21</sup> In this study, our results suggested that the CD domain at C-terminus of GL3, EGL3 and TT8 was essential for both interactions with JAZs and their dimerization, providing new perspectives on the interactions of JAZs with bHLH factors, and dimerization of bHLH factors.



**Figure 2.** The CT2 domains of GL3, EGL3 and TT8 interact with JAZ1 in plant. GL3CT2, EGL3CT2 and TT8CT2 were fused with C-terminal fragment of YFP (cYFP), and JAZ1 was fused with N-terminal fragment of YFP (nYFP). Agrobacterium strains containing indicated constructs pairs were co-infiltrated into the *N. benthamiana* leaves. The nuclei were stained by DAPI, and YFP fluorescence was tested 50h after co-infiltration.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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