Journal of Genetics and Genomics 45 (2018) 557–560

Contents lists available at ScienceDirect

Journal of Genetics and Genomics

Journal homepage: www.journals.elsevier.com/journal-of-genetics-and-genomics/

Letter to the editor

Control of flowering and inflorescence architecture in tomato by synergistic interactions between ALOG transcription factors

Inflorescences are flower-bearing shoots that originate from pools of stem cells in shoot apical meristems (SAM). Inflorescence architecture is determined by a process of meristem maturation, during which stem cell fate switches from a vegetative to a reproductive growth program. A major factor in plant reproductive success in nature and yield in agriculture is the number of branches and flowers on inflorescences (Kobayashi and Weigel, 2007; Lippman et al., 2008; Andrés and Coupland, 2012; Park et al., 2012, 2014; Lemmon et al., 2016). Diversity in inflorescence architecture is based on two major growth programs that influence meristem maturation in distinct ways (Park et al., 2014; Teo et al., 2014; Xu et al., 2016). Following a flowering transition, a meristem can either grow indefinitely (monopodial growth) or terminate into a flower (sympodial growth) (Park et al., 2012). In monopodial plants such as *Arabidopsis* and maize, the SAM matures into a persistent reproductive state, and the inflorescence meristems continuously generate floral meristems laterally, resulting in simple inflorescences (Hake, 2008). In contrast, in sympodial plants such as tomato and related Solanaceae (Knapp et al., 2004), the primary SAM terminates in a flower, and new growth continuously arises from specialized axillary (sympodial) meristems that also terminate into flowers, resulting in compound shoots with multiple flowers (Park et al., 2012, 2014; Xu et al., 2016).

Our past studies revealed a genetic pathway that promotes meristem maturation during reproductive development in tomato to control inflorescence architecture. Loss-of-function mutations in the homeobox transcription factor encoded by *COMPOUND INFLORESCENCE* (*S*, homolog of *WUSCHEL-RELATED HOMEOBOX 9*, *WOX9*) and a flower specification complex encoded by the F-box gene *ANANTHA* (*AN*, homolog of *UNUSUAL FLORAL ORGANS*, *UFO*) and its transcription factor partner *FALSIFLORA* (*FA*, homolog of *LEAFY*, *LFY*) result in highly branched inflorescences, caused by delayed meristem maturation in *s* mutant or blocked meristem maturation in *an* and *fa* mutants (Lippman et al., 2008; Park et al., 2012, 2014). Alongside the promotion of meristem maturation by S and AN-FA is a pathway that represses maturation to maintain a vegetative meristem state, defined by the tomato *TERMINATING FLOWER* (*TMF*) gene (MacAlister et al., 2012). *TMF* encodes a member of the ALOG (*Arabidopsis* LSH1 and *Oryza* G1) protein family, members of which contain a DNA-binding domain and have weak transcriptional activity (Iyer and Aravind, 2012; MacAlister et al., 2012; Yoshida et al., 2013). Loss of *TMF*, one of 12 ALOG genes in tomato, causes much earlier flowering and conversion of primary inflorescences into single flowers (MacAlister et al., 2012). The *tmf* single-flower phenotype is due to precocious adoption of floral identity, which hinders sympodial meristem development. Interestingly, *tmf* inflorescences from side shoots are unaffected, suggesting that *TMF* might function specifically to maintain a vegetative state during primary shoot meristem maturation, or that redundant factors or pathways work with *TMF* to maintain a vegetative state in axillary meristems (MacAlister et al., 2012). We recently showed that *TMF* acts together with three *Solanum lycopersicum BLADE-ON-PETIOLE* genes (*SlBOPs*, homologs of *Arabidopsis* BOP transcriptional cofactors) by forming a transcriptional complex. Elimination of all *SlBOP* function by CRISPR/Cas9 causes pleiotropic defects, including changes in leaf complexity and floral organ abscission. However, most notable is the simplification of all inflorescences into single flowers or two flowers, resembling single-flower primary inflorescences of *tmf* mutants (Xu et al., 2016).

Several ALOG genes in *Arabidopsis* and rice have been shown to have roles in light signaling and floral organ development (Zhao et al., 2004; Yoshida et al., 2009; Wang et al., 2010; Cho and Zambryski, 2011; Takeda et al., 2011; Sato et al., 2014). However, mutations in the closest homologs of *TMF* in *Arabidopsis* (*LIGHT-SENSITIVE HYPOCOTYL3* and *LIGHT-SENSITIVE HYPOCOTYL4*) have no effect on inflorescence architecture, and the closest homolog of *TMF* in rice (*TAWAWA1*) regulates panicle architecture (Yoshida et al., 2013).

Our previous study showed that *TFAM1* and *TFAM2*, two additional ALOG family members in tomato, are predominantly expressed in vegetative meristem stages and share similar expression dynamics with *TMF* (Fig. S1) (Xu et al., 2016). CRISPR/Cas9-generated null mutations in *TFAM1* and *TFAM2* cause modest changes in inflorescence complexity: *tfam1* mutants produce slightly fewer flowers in each inflorescence and inflorescences of *tfam2* mutants are weakly branched (Xu et al., 2016). Thus, both genes regulate inflorescence architecture; however, if and how these ALOG family members coordinate with TMF and possibly each other to regulate reproductive development remains unclear. Here, we have begun to dissect genetic and molecular interactions of TMF, TFAM1 and TFAM2 in the control of flowering, inflorescence architecture and flower production.

Plant ALOG family proteins have a DNA binding domain similar to retrotransposon integrases (Iyer and Aravind, 2012). We first examined the protein structures of the 12 tomato ALOG family members and found that all have a canonical ALOG domain, defined by the insertion of a zinc ribbon into the putative DNA-binding domain that is conserved with homologs in *Arabidopsis* and rice (Figs. 1A and S2) (Iyer and Aravind, 2012). We then tested the subcellular localization of TFAM1 and TFAM2 in tomato protoplasts. Like TMF, both TFAM1-GFP and TFAM2-GFP fusion proteins were exclusively localized to the nucleus (Fig. 1B), supporting their roles

https://doi.org/10.1016/j.jgg.2018.03.008

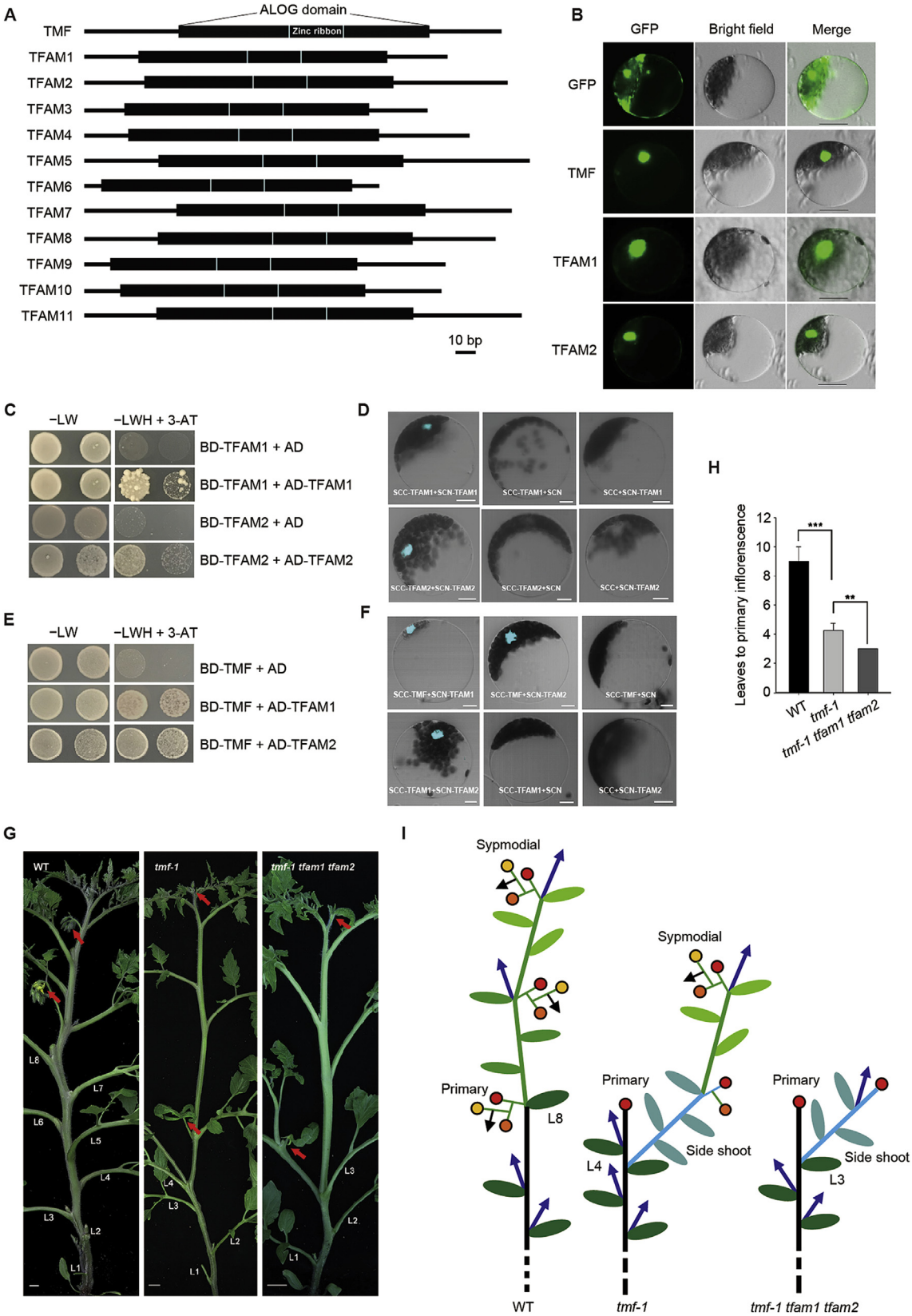

Fig. 1. TFAM1 and TFAM2 act synergistically with TMF to control inflorescence architecture and flower production in tomato. **A**: Cartoons showing structures of ALOG family proteins in tomato. The ALOG domain in each family member harbors a zinc ribbon motif. **B**: Transient expression of TFAM1-GFP and TFAM2-GFP fusion proteins in tomato protoplasts showing their nuclear localization. Bar = 10 μm. **C**: Yeast two-hybrid assays showing homodimerization of TFAM1 and TFAM2. **D**: BiFC assays in tomato protoplasts showing homodimerization of TFAM1 and TFAM2. Bar = 10 μm. **E**: Yeast two-hybrid assays showing the interactions of TMF with TFAM1 and TFAM2, respectively. **F**: BiFC assays in tomato protoplasts showing the interactions between TMF, TFAM1 and TFAM2. Bar = 10 μm. **G**: Representative shoots with two successive inflorescences of wild-type (WT), *tmf-1*, and *tmf-1 tfam1 tfam2* plants. L, leaf. The red arrow indicates an inflorescence. Bar = 1.5 cm. **H**: Quantification and comparison of flowering time measured by leaves to primary

as putative transcriptional regulators. We previously showed that TMF homodimerization is critical for its function (MacAlister et al., 2012; Xu et al., 2016). We therefore tested if TFAM1 and TFAM2 also form homodimers. Yeast two-hybrid assay using their full-length coding sequences showed that both TFAM1 and TFAM2 form homodimers (Fig. 1C). We further confirmed the homodimerization *in vivo* by bimolecular fluorescence complementation (BiFC) in tomato protoplasts, where all signals were localized to the nucleus (Fig. 1D). These results, along with similar expression dynamics during meristem maturation, indicated that TFAM1 and TFAM2 have similar molecular behavior to TMF, suggesting their redundant relationships.

Mutations in two or more related genes often produce a phenotype that is dramatically enhanced compared to each mutation's individual effects. A double mutant with a more extreme phenotype reflects a redundant, sometimes synergistic, interaction between the corresponding mutated genes. This phenomenon that defines synergistic interactions usually suggests the existence of compensatory pathways or protein complexes (Mani et al., 2008). Considering that all three proteins share similar molecular features, we tested if they interact with each other to form heterodimers. Using yeast two-hybrid assay, we found that both TFAM1 and TFAM2 directly interacted with TMF to form heterodimers (Fig. 1E). Further BiFC assays in tomato protoplasts showed that TMF, TFAM1 and TFAM2 interacted with each other to form heterodimers in the nucleus (Fig. 1F). The homo- and hetero-interactions between TMF, TFAM1 and TFAM2 suggested that they likely form one or more transcriptional complexes.

To test the functional relationships of these three ALOG genes on flowering and inflorescence development, we created triple mutants by crossing *tmf-1* mutant with our CRISPR/Cas9-generated *tfam1 tfam2* double mutant, and evaluated the F_3 progeny from F_2-derived triple mutants. Loss of all three genes dramatically enhanced flowering and inflorescence defects (Fig. 1G). Compared with *tmf-1* single mutants which flowered after four leaves, *tmf-1 tfam1 tfam2* triple mutants flowered after only two to three leaves, and the sepals of these flowers became even more leaf-like, suggesting that flower identity is adopted even earlier during meristem maturation (Fig. 1G–I). Most notably, inflorescences on side shoots in triple mutants produced fewer flowers than *tmf-1*, with some inflorescences developing single or two flowers with leaf-like sepals.. Thus, these three tomato ALOG family members function together in reproductive development.

In this study, we explored the molecular features of TFAM1 and TFAM2 (two tomato ALOG proteins) and revealed the molecular clues for how they coordinate activity with TMF to control flowering, inflorescence architecture and flower production in tomato. The homodimerization and heterodimerization of TMF, TFAM1 and TFAM2 in the nucleus strongly imply that one or more higher-order ALOG transcription complexes act in meristem maturation to control reproductive development, likely with SlBOP proteins and additional partners that have not yet been discovered. Our genetic analysis supported this by revealing a synergistic functional relationship of TFAM1 and TFAM2 with TMF to prevent precocious flowering and promote inflorescence complexity in both primary and lateral shoot systems. The physical interactions between these three ALOG transcription factors suggest that a large ALOG transcriptional complex might form in vegetative and early stage inflorescence meristems to repress the activity of downstream genes that promote meristem maturation and flower identity. Deeper dissection of the transcriptional activities of ALOG protein complexes and identification of their gene targets during meristem maturation will help expose the regulatory networks in which ALOG transcription factors function in plant reproductive development.

Acknowledgments

We thank Dr. Shuhua Yang for providing the yeast two-hybrid vectors, thank Dr. Xinping Yang for comments on this manuscript, and thank A. Krainer and S. Qiao for technical assistance. This research was supported by startup funding to C.X. from State Key Laboratory of Plant Genomics and Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, and a National Science Foundation grant (IOS-1556171) to Z.B.L.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jgg.2018.03.008.

References

Andrés, F., Coupland, G., 2012. The genetic basis of flowering responses to seasonal cues. Nat. Rev. Genet. 13, 627–639.
Cho, E., Zambryski, P.C., 2011. *ORGAN BOUNDARY1* defines a gene expressed at the junction between the shoot apical meristem and lateral organs. Proc. Natl. Acad. Sci. U. S. A. 108, 2154–2159.
Hake, S., 2008. Inflorescence architecture: the transition from branches to flowers. Curr. Biol. 18, R1106–R1108.
Iyer, L.M., Aravind, L., 2012. ALOG domains: provenance of plant homeotic and developmental regulators from the DNA-binding domain of a novel class of DIRS1-type retroposons. Biol. Direct 7, 39.
Knapp, S., Bohs, L., Nee, M., Spooner, D.M., 2004. Solanaceae - a model for linking genomics with biodiversity. Comp. Funct. Genomics 5, 285–291.
Kobayashi, Y., Weigel, D., 2007. Move on up, it's time for change mobile signals controlling photoperiod-dependent flowering. Genes Dev. 21, 2371–2384.
Lemmon, Z.H., Park, S.J., Jiang, K., Van Eck, J., Schatz, M.C., Lippman, Z.B., 2016. The evolution of inflorescence diversity in the nightshades and heterochrony during meristem maturation. Genome Res. 26, 1676–1686.
Lippman, Z.B., Cohen, O., Alvarez, J.P., Abu-Abied, M., Pekker, I., Paran, I., Eshed, Y., Zamir, D., 2008. The making of a compound inflorescence in tomato and related nightshades. PLoS Biol. 6, 2424–2435.
MacAlister, C.A., Park, S.J., Jiang, K., Marcel, F., Bendahmane, A., Izkovich, Y., Eshed, Y., Lippman, Z.B., 2012. Synchronization of the flowering transition by the tomato *TERMINATING FLOWER* gene. Nat. Genet. 44, 1393–1398.
Mani, R., StOnge, R.P., Hartman, J.L., Giaever, G., Roth, F.P., 2008. Defining genetic interaction. Proc. Natl. Acad. Sci. U. S. A. 105, 3461–3466.
Park, S.J., Eshed, Y., Lippman, Z.B., 2014. Meristem maturation and inflorescence architecture-lessons from the Solanaceae. Curr. Opin. Plant Biol. 17, 70–77.
Park, S.J., Jiang, K., Schatz, M.C., Lippman, Z.B., 2012. Rate of meristem maturation determines inflorescence architecture in tomato. Proc. Natl. Acad. Sci. U. S. A. 109, 639–644.
Sato, D.-S., Ohmori, Y., Nagashima, H., Toriba, T., Hirano, H.-Y., 2014. A role for *TRIANGULAR HULL1* in fine-tuning spikelet morphogenesis in rice. Genes Genet. Syst. 89, 61–69.
Takeda, S., Hanano, K., Kariya, A., Shimizu, S., Zhao, L., Matsui, M., Tasaka, M., Aida, M., 2011. CUP-SHAPED COTYLEDON1 transcription factor activates the expression of *LSH4* and *LSH3*, two members of the ALOG gene family, in shoot organ boundary cells. Plant J. 66, 1066–1077.
Teo, Z.W.N., Song, S., Wang, Y.-Q., Liu, J., Yu, H., 2014. New insights into the regulation of inflorescence architecture. Trends Plant Sci. 19, 158–165.
Wang, H. Sen, Yu, C., Tang, X.F., Wang, L.Y., Dong, X.C., Meng, Q.W., 2010. Antisense-mediated depletion of tomato endoplasmicr reticulum omega-3 fatty acid desaturase enhances thermal tolerance. J. Integr. Plant Biol. 52, 568–577.
Xu, C., Park, S.J., Van Eck, J., Lippman, Z.B., 2016. Control of inflorescence architecture in tomato by BTB/POZ transcriptional regulators. Genes Dev. 30, 2048–2061.
Yoshida, A., Sasao, M., Yasuno, N., Takagi, K., Daimon, Y., Chen, R., Yamazaki, R., Tokunaga, H., Kitaguchi, Y., Sato, Y., Nagamura, Y., Ushijima, T., Kumamaru, T.,

inflorescence. Data are represented by means ± SD ($n = 5$). A two-tailed, two-sample Student's *t*-test was performed, and significant differences are represented by black asterisks ($^{**}P < 0.01$ and $^{***}P < 0.001$). **I**: Schematic diagrams showing the compound vegetative and inflorescence shoot systems in wild-type plant, single- and early-flowered primary inflorescence and normal sympodial growth on side shoots in *tmf-1* mutant, and dramatically enhanced single- and early-flowered inflorescences in *tmf-1 tfam1 tfam2* triple mutant. L, Leaf.

Iida, S., Maekawa, M., Kyozuka, J., 2013. TAWAWA1, a regulator of rice inflorescence architecture, functions through the suppression of meristem phase transition. Proc. Natl. Acad. Sci. U. S. A. 110, 767–772.

Yoshida, A., Suzaki, T., Tanaka, W., Hirano, H.-Y., 2009. The homeotic gene *long sterile lemma* (*G1*) specifies sterile lemma identity in the rice spikelet. Proc. Natl. Acad. Sci. U. S. A. 106, 20103–20108.

Zhao, L., Nakazawa, M., Takase, T., Manabe, K., Kobayashi, M., Seki, M., Shinozaki, K., Matsui, M., 2004. Overexpression of *LSH1*, a member of an uncharacterised gene family, causes enhanced light regulation of seedling development. Plant J. 37, 694–706.

Xiaozhen Huang[a,b], Lingli Tang[a,c], Yuan Yu[a,c], Justin Dalrymple[d], Zachary B. Lippman[d,*], Cao Xu[a,b,c,*]

[a] *State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, and National Center for Plant Gene Research, Beijing 100101, China*

[b] *CAS-JIC Centre of Excellence for Plant and Microbial Science (CEPAMS), Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[c] *Graduate School of University of Chinese Academy of Sciences, Beijing 100049, China*

[d] *Cold Spring Harbor Laboratory, Cold Spring Harbor, 11724, USA*

[*] Corresponding authors.
E-mail addresses: lippman@cshl.edu (Z.B. Lippman), caoxu@genetics.ac.cn (C. Xu).

24 February 2018
Available online 25 May 2018