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A Role for APETALA1/FRUITFULL Transcription Factors in Tomato Leaf Development[C][W]

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Flexible maturation rates underlie part of the diversity of leaf shape, and tomato (*Solanum lycopersicum*) leaves are compound due to prolonged organogenic activity of the leaf margin. The *CINCINNATA -TEOSINTE BRANCHED1, CYCLOIDEA, PCF* (*CIN-TCP*) transcription factor LANCEOLATE (LA) restricts this organogenic activity and promotes maturation. Here, we show that tomato *APETALA1/FRUITFULL* (*AP1/FUL*) MADS box genes are involved in tomato leaf development and are repressed by LA. *AP1/FUL* expression is correlated negatively with LA activity and positively with the organogenic activity of the leaf margin. LA binds to the promoters of the *AP1/FUL* genes *MBP20* and *TM4*. Overexpression of *MBP20* suppressed the simple-leaf phenotype resulting from upregulation of LA activity or from downregulation of class I knotted like homeobox (KNOXI) activity. Overexpression of a dominant-negative form of *MBP20* led to leaf simplification and partly suppressed the increased leaf complexity of plants with reduced LA activity or increased KNOXI activity. Tomato plants overexpressing miR319, a negative regulator of several CIN-TCP genes including *LA*, flower with fewer leaves via an SFT-dependent pathway, suggesting that miR319-sensitive CIN-TCPs delay flowering in tomato. These results identify a role for *AP1/FUL* genes in vegetative development and show that leaf and plant maturation are regulated via partially independent mechanisms.

INTRODUCTION

Plant leaves are flat lateral organs that are produced repeatedly by the shoot apical meristem (SAM). While most leaf growth is determinate, young leaves feature transient indeterminate growth, during which they maintain organogenic activity in specific regions at their margins, termed marginal blastozones (MBs). The temporal and spatial extent of this organogenic activity underlies some of the morphological diversity of leaf form. An extended organogenic window enables the formation of elaborated leaf forms, such as compound leaves, which are composed of multiple leaflets, each resembling a simple leaf (Hagemann and Gleissberg, 1996; Dengler and Tsukaya, 2001; Kaplan, 2001; Efroni et al., 2010; Floyd and Bowman, 2010).

Studies on leaf development have identified several groups of transcription factors and hormonal cues that are involved in defining the organogenic window and its flexibility (Burko and Ori, 2013; Fambrini and Pugliesi, 2013). Class I knotted like homeobox (KNOXI) proteins play important roles in the maintenance of the organogenic activities of both the SAM and the MB (Hake et al., 2004; Hay and Tsiantis, 2009, 2010; Blein et al., 2010). Downregulation of KNOXI activity in *Cardamine hirsuta* and tomato (*Solanum lycopersicum*) resulted in accelerated leaf maturation and decreased leaf complexity (Hay and Tsiantis, 2006; Shani et al., 2009). Conversely, their overexpression in tomato mutants, such as *Mouse-ear* and *Curl*, or in transgenic lines in several species led to enhanced organogenic activity and delayed maturation (Hareven et al., 1996; Chen et al., 1997; Parnis et al., 1997; Janssen et al., 1998; Bharathan et al., 2002; Tsiantis et al., 2002; Müller et al., 2006; Kimura et al., 2008; Barth et al., 2009; Shani et al., 2009). The hormone cytokinin (CK) has been shown to promote the extended organogenic activity of tomato and lettuce (*Lactuca sativa*) leaves downstream of KNOXI proteins (Frugis et al., 2001; Shani et al., 2010).

CINCINNATA -TEOSINTE BRANCHED1, CYCLOIDEA, PCF (*CIN-TCP*) transcription factors, a subset of class II TCPs (Martín-Trillo and Cubas, 2010), restrain the organogenic activity of the MB by promoting leaf maturation. Their downregulation in *Antirrhinum majus* and *Arabidopsis thaliana* resulted in leaf rumpling due to delayed maturation of the leaf margin and in delayed senescence (Nath et al., 2003; Palatnik et al., 2003; Koyama et al., 2007; Efroni et al., 2008; Schommer et al., 2008), while their overexpression led to precocious maturation, smaller leaf size, and senescence (Palatnik et al., 2003; Efroni et al., 2008; Schommer et al., 2008; Sarvepalli and Nath, 2011). In tomato, the CIN-TCP protein LANCEOLATE (LA) promotes leaf maturation together with additional related CIN-TCPs (LA-like proteins). *LA-like* genes are regulated by miR319, and as a result, *LA* and miR319 show opposite expression dynamics during

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leaf development: miR319 expression is high in emerging leaf primordia and is downregulated at the P5 stage, when *LA* expression is upregulated (Shleizer-Burko et al., 2011). Gain-of-function *La* mutants show accelerated leaf maturation and differentiation, while downregulation of *LA-like* genes by overexpression of miR319 leads to indeterminate leaf growth, especially at the margin (Mathan and Jenkins, 1962; Stettler, 1964; Dengler, 1984; Ori et al., 2007; Shleizer-Burko et al., 2011). LA activity was recently shown to be partly mediated by gibberellin (GA; Yanai et al., 2011). Several potential CIN-TCP targets have been identified in *Arabidopsis*. These include jasmonate biosynthesis genes, miR167, genes involved in auxin signal transduction, miR164 and *ASYMMETRIC LEAVES1* (Kosugi and Ohashi, 2002; Schommer et al., 2008; Koyama et al., 2010; Danisman et al., 2012; Rubio-Somoza and Weigel, 2013). Recently, CIN-TCPs were shown to modify CK response in *Arabidopsis* leaves via an interaction with the chromatin remodeling ATPase BRAHMA (BRM) and activation of the CK response inhibitor *ARABIDOPSIS RESPONSE REGULATOR 16* (*ARR16*) (Efroni et al., 2013).

MADS box transcription factors are involved in many developmental processes in plants (Rounsley et al., 1995; Ng and Yanofsky, 2001; Becker and Theissen, 2003; Hileman et al., 2006; Dornelas et al., 2011; Smaczniak et al., 2012a). *APETALA1/FRUITFULL* (*AP1/FUL*) MADS box genes (also called SQUA) play conserved roles in the specification of floral meristem identity (Huijser et al., 1992; Bowman et al., 1993; Theissen et al., 1996, 2000; Ferrándiz et al., 2000; Litt and Irish, 2003; Parenicová et al., 2003; Smaczniak et al., 2012b) and are involved in the induction of flowering in many species (Immink et al., 1999; Yan et al., 2003; Ellul et al., 2004; Samach and Lotan, 2007; Ruokolainen et al., 2010; Kobayashi et al., 2012; Park et al., 2012). Their expression is induced by the flowering promoting factor FLOWERING LOCUS T (FT) upon induction to flowering (Mandel et al., 1992; Teper-Bamnolker and Samach, 2005; Berbel et al., 2012). In the core eudicots, this family is represented by three distinct clades, *euAPETALA1* (*euAP1*), *euFRUITFULL* (*euFUL*), and *AGAMOUS-like 79* (*AGL79*), thought to have evolved from a common eudicot through several duplication events (Litt and Irish, 2003; Shan et al., 2007). These clades and specific family members have acquired additional specific functions. For example, AP1 is involved in specification of organ identity (Irish and Sussex, 1990; Mandel et al., 1992; Bowman et al., 1993; Benlloch et al., 2006), and FUL functions in carpel development and promotes determinate growth. Loss-of-function mutations in the *Arabidopsis FUL* gene and suppression of *FUL-like* genes in poppy (*Papaver somniferum*) affect cauline leaf shape (Gu et al., 1998; Ferrándiz et al., 2000; Teper-Bamnolker and Samach, 2005; Pabón-Mora et al., 2012). The pea (*Pisum sativum*) *AGL79-like* gene *VEGETATIVE1* (*VEG1*) was found to be involved in specifying secondary inflorescence meristem identity (Berbel et al., 2012). Recently, *FUL-like* genes were shown to play a role in compound-leaf morphogenesis in *Aquilegia coerulea*, a basal eudicot that contains only *FUL-like* genes. Suppression of *A. coerulea FUL-like* genes resulted in reduced leaf complexity (Pabón-Mora et al., 2013).

The tomato genome contains five *AP1/FUL* MADS box genes, *MACROCALYX* (*MC*)*/AP1*, *MBP7* (*FUL*), *MBP20* (*AGL79*), *MBP10* (*AGL79L*), and *TM4* (*FULL*) (Busi et al., 2003; Butler, 1952; Pnueli et al., 1991; Vrebalov et al., 2002; Litt and Irish, 2003; Hileman et al., 2006; Leseberg et al., 2008; Park et al., 2012). MC was shown to be involved in sepal development and in fruit abscission and ripening (Vrebalov et al., 2002; Nakano et al., 2012). TM4 and MBP7 are involved in fruit ripening, likely via interaction with Ripening-Inhibitor (Bemer et al., 2012). The role of MBP20 and MBP10 in tomato development has not been described.

To explore the nature of the transient organogenic window in tomato leaf development, we compared gene expression between genotypes with different levels of LA-like activity. The expression of the *AP1/FUL* MADS box genes positively correlated with the extent of the organogenic window. Genetic analysis showed that MBP20 is involved in leaf development downstream of LA. LA binds to regulatory sequences upstream of *MBP20* and *TM4*. These findings reveal a role for *MBP20* and *TM4*, and possibly additional *AP1/FUL* genes, in the regulation of compound leaf development.

RESULTS

Expression of Tomato *AP1/FUL* MADS Box Genes Is Correlated with the Organogenic Activity of the Leaf Margin

Developing tomato leaves retain a window of organogenic activity that enables the elaboration of leaf shape (Burko and Ori, 2013). To identify genes that mediate this activity, we used microarray analysis to compare gene expression among tomato genotypes that vary in the extent of the organogenic window due to differences in the activity of the *LA* gene. These included the gain-of-function allele *La-2*, mutated in the miR319-recognition site, wild-type tomato (*S. lycopersicum* cv M82, *sp*), and transgenic plants overexpressing the *Arabidopsis* miR319 in leaves (Ori et al., 2007; Shleizer-Burko et al., 2011) under the control of the *FIL* promoter (Lifschitz et al., 2006; Shani et al., 2009). To minimize expression differences that are secondary to the developmental alterations, we collected shoot apices containing the SAM and the two youngest leaf primordia, the phenotype of which is very similar among these genotypes (Figure 1A).

The expression of four closely related MADS box genes from the *AP1/FUL* subfamily was positively correlated with the extent of the organogenic activity of the leaf margin and negatively correlated with the level of LA activity (Figure 1B). These included *MBP7* (*FUL2*), *MBP20* (*AGL79*), *TM4* (*FUL1*), and *MC*. These results were verified by quantitative RT-PCR (qRT-PCR) in apices containing the SAM and the four youngest leaf primordia (see Supplemental Figure 1A online). qRT-PCR also showed similar behavior of the fifth member of this gene family, *MBP10* (*AGL79L*), which was not present on the microarray, indicating that the expression of all members of this gene family correlates with the organogenic window (Figure 1C; see Supplemental Figures 1A and 2A online). Since in other species the expression of most *AP1/FUL* genes was reported mainly in reproductive organs, we examined the expression of *MBP20* and *TM4* in dissected P5 primordia. Their expression was decreased in *La-2* and increased in *FILpro>>miR319* primordia (Figures 1D and 1E). The leaf-specific effect of LA on the expression of these genes was further examined using plants

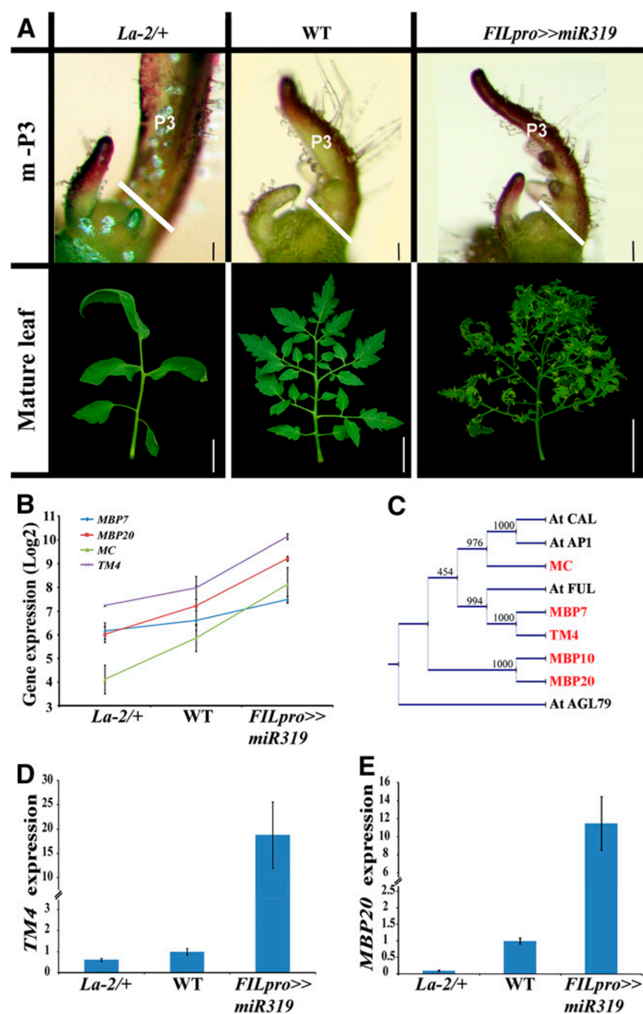

Figure 1. Expression of *AP1/FUL* Genes Is Positively Correlated with the Organogenic Activity of the Leaf Margin.

(A) Phenotypes of young leaf primordia and mature leaves of the genotypes used for the expression profiling. m-P3, SAM and three youngest leaf primordia. Genotypes are indicated at the top of each column. For microarray analysis, tissue was collected from the SAM and two youngest leaf primordia, as indicated by the white line in the top row. Bars = 0.1 mm (top row) and 5 cm (bottom row). WT, the wild type.

(B) Microarray expression data for four *AP1/FUL* genes, shown as an average of three biological repeats (±SE).

(C) Phylogenetic analysis of tomato and *Arabidopsis AP1/FUL* genes.

(D) and **(E)** Expression of *TM4* **(D)** and *MBP20* **(E)** in the fifth leaf at the P5 stage of the indicated genotypes. Expression was assayed by qRT-PCR relative to the reference gene *EXP* and is shown as an average of three biological repeats (±SE). *FILpro>>miR319* plants expresses miR319 under the control of the *FIL* promoter.

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expressing a mutated form of *LA* with reduced sensitivity to miR319 (LA^m) (Ori et al., 2007) or miR319 under the control of the *BLS* promoter, expressed specifically in leaves starting at the P4 stage (Shalit et al., 2009; Shani et al., 2009). Leaf primordia at the P4 stage were collected before flowering. At this stage, any observed expression changes are expected to be immediate effects of the changes in *LA* expression. The expression of *MBP20* and *TM4* was upregulated in *BLSpro>>miR319* and that of *MBP20* was also downregulated in *BLSpro>>*LA^m (see Supplemental Figure 1B online). In conclusion, LA-like proteins negatively regulate the expression of *AP1/FUL* genes.

miR319 Affects Leaf and Plant Maturation in an Opposite Manner and via Separate Pathways

Often a genetic alteration that affects the rate of leaf maturation similarly affects plant maturation, as manifested by flowering time, for example (Teper-Bamnolker and Samach, 2005; Lifschitz et al., 2006; Shalit et al., 2009). Maturation is accelerated in *La-2* leaves, while leaves overexpressing miR319 show delayed maturation and indeterminate growth (Ori et al., 2007; Shleizer-Burko et al., 2011). Interestingly, the effect of these genotypes on plant maturation was the opposite: *FILpro>>miR319* plants and *BLSpro>>miR319* plants flower with fewer leaves than the wild type, and the number of leaves produced by *La-2* mutants until flowering is slightly increased (Figure 2A). It should be noted that the effect of these genotypes on flowering is complex, as leaf production is faster in *La-2* and slower in *FILpro>>miR319* (Shleizer-Burko et al., 2011), resulting in *La-2* plants flowering after a shorter time and *FILpro>>miR319* after a longer time from planting relative to wild type.

SINGLE FLOWER TRUSS (SFT) is the tomato ortholog of the flowering promoting factor FT. *sft* mutants flower late and have more intercalary leaflets than the wild type, whereas *35S:SFT* shows early flowering and its leaves are simplified due to accelerated maturation (Molinero-Rosales et al., 2004; Lifschitz and Eshed, 2006; Lifschitz et al., 2006; Shalit et al., 2009). To examine whether the effect of *FILpro>>miR319* on flowering timing and *AP1/FUL* expression depends on SFT activity and whether it affects plant and leaf determination via common or separate pathways, we introduced the *FILpro>>miR319* transgene into the *sft* and *35S:SFT* backgrounds. *sft FILpro>>miR319* plants flowered after producing the same number of leaves as single *sft* mutants (Figure 2B), but their leaves resembled those of *FILpro>>miR319* (Figure 2C). *35S:SFT FILpro>>miR319* leaves showed indeterminate growth similar to *FILpro>>miR319* leaves and an intermediate phenotype with respect to leaf complexity (Figure 2D). Because of the very low fertility of *FILpro>>miR319* plants, we monitored flowering time in *BLSpro>>miR319 35S:SFT* plants. *BLSpro>>miR319 35S:SFT* plants flowered after three to four leaves, similar to *35S:SFT* plants (Figure 2H; see Supplemental Figures 3A to 3C online). Therefore, *sft* and *35S:SFT* were epistatic to *FILpro>>miR319* with respect to plant maturation, and *FILpro>>miR319* was epistatic to *35S:SFT* and *sft* with respect to leaf determination. This indicates that the effect of miR319 on flowering time is mediated by SFT and that miR319 and its target genes affect leaf development and flowering time via separate pathways.

The effect of miR319 overexpression on the expression of *TM4* and *MC* was milder in the *sft* background than in the wild type (Figures 2E and 2F), and the expression of *MBP20*, *MBP7*, and

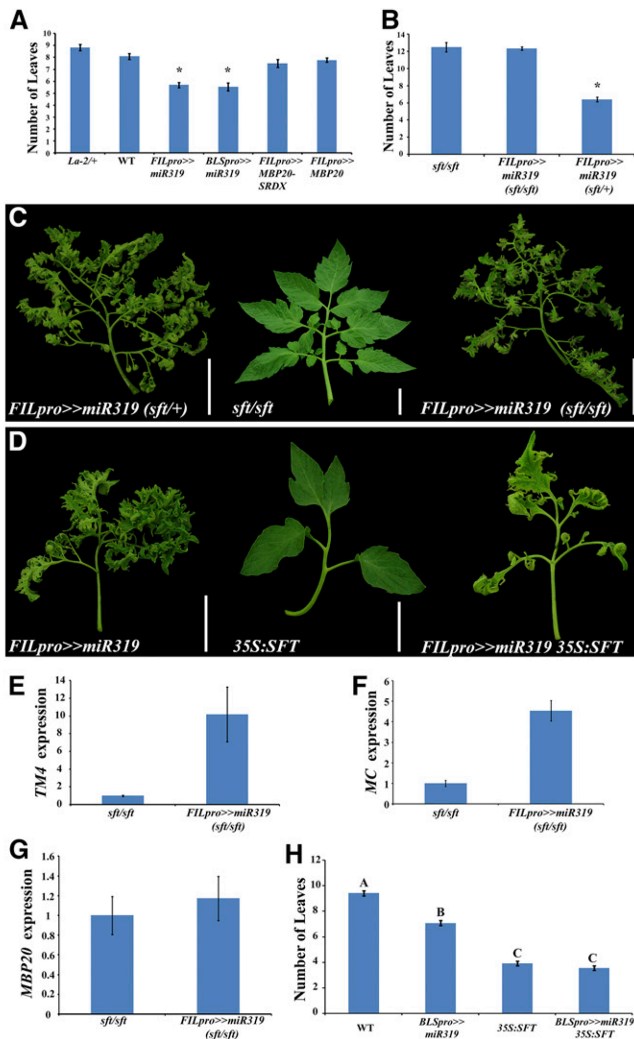

Figure 2. miR319 Affects Plant and Leaf Maturation via Separate Pathways.

(A) and **(B)** Number of leaves until the first visible flower. Shown are averages ± SE (for *FILpro>>MBP20-SRDX*, $n = 5$; for all other genotypes, $n = 10$). Asterisks indicate statistically significant differences from the wild type **(A)** or *sft* **(B)** at $P < 0.05$.

(C) and **(D)** Mature leaves of the indicated genotypes. *35S:SFT* plants express *SFT* under the control of the *35S* promoter by direct fusion; *FILpro>>miR319* plants express miR319 under the control of the *FIL* promoter using the transactivation system (Moore et al., 1998). Bars = 5 cm.

(E) to **(G)** Expression of *TM4* (E), *MC* (F), and *MBP20* (G) in apices containing the SAM and six youngest leaf primordia, in which the third leaf of the plant was at the P6 stage. Relative expression was assayed using qRT-PCR relative to the reference gene *EXP* and is shown as an average of four biological repeats ±SE.

(H) Number of leaves until the first visible flower. Shown are averages ±SE ($n > 7$). Letters indicate statistically significant differences at $P < 0.05$.

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MBP10 was similar between *sft* and *sft FILpro>>miR319* (Figure 2G; see Supplemental Figures 3D and 3E online). This suggests that the expression of these genes is positively regulated by SFT and negatively by LA-like proteins and that the effect of LA on their expression is at least partially dependent on functional SFT.

MBP20 Is Involved in Tomato Leaf Patterning

To further understand the role of *AP1/FUL* genes in leaf development, we examined the dynamics of their expression in the fifth leaf produced by the plant at successive developmental stages using qRT-PCR. Early stages were sampled with the SAM and younger primordia. In contrast with *AP1/FUL* genes from other species, all family members were expressed in tomato leaves (Figure 3A; see Supplemental Figure 2B online). *MBP20* mRNA was transiently and substantially upregulated in the P3 and P4 stage primordia. Its expression was then sharply downregulated at the P5 stage (Figure 3A, bars). Interestingly, *LA* expression is sharply upregulated at this stage (Figure 3A, dashed line; Shleizer-Burko et al., 2011). The closest homolog of *MBP20*, *MBP10*, showed similar but less profound expression dynamics (see Supplemental Figure 2B online). We chose to focus on *MBP20* for further investigation of its role downstream of LA in leaf patterning due to the negative correlation between its expression and *LA* expression and because we identified a putative LA binding site in its promoter (see below). In agreement with the mRNA expression, a 3209-bp *MBP20* promoter drove expression at early stages of leaf development, starting from the P1 stage and in the leaflet primordia, but not in the SAM (Figures 3B, 3D, and 3E). The expression was downregulated in the P2 and P3 primordia of *La-2* plants (Figures 3C, 3F, and 3G). Thus, expression of *MBP20* is negatively correlated with that of *LA*.

To understand the role of MBP20 in leaf development, we overexpressed in leaves wild-type *MBP20* or a fusion of *MBP20* with the *SRDX* repression motif (Hiratsu et al., 2003), using the *trans*-activation system (Moore et al., 1998) and the *FIL* promoter (Shani et al., 2009). We reasoned that as LA activity similarly affects the entire tomato *AP1/FUL* clade, genes in this clade may act redundantly during leaf development. The *MBP20-SRDX* fusion was expected to downregulate their redundant targets, affecting the downstream developmental pathways. Overexpression was verified using qRT-PCR. Overexpression of *MBP20* in leaves had a mild effect on leaf shape in four out of eight independent lines (Figures 3H to 3J; see Supplemental Figures 4A, 4C, and 4D online). Leaves overexpressing *MBP20-SRDX* were simplified, with fewer primary leaflets, a lack of higher order leaflets, and smooth leaflet margins (Figures 3H to 3J; see Supplemental Figures 4A, 4B, and 4D online), in agreement with the observation that downregulation of *FUL-like* genes in *A. coerulea* results in simplified leaves (Pabón-Mora et al., 2013). A similar phenotype was observed in crosses of eight independent transgenic lines to the *FIL* promoter, with three lines showing a strong phenotype, two lines showing an intermediate phenotype, and three lines showing a weak phenotype. One of the strong lines was used in further analyses. To verify the relevance of this phenotype to MBP20 function, we coexpressed *MBP20* and *MBP20-SRDX* in leaves. While *MBP20* overexpression had a very mild effect on leaf shape, it substantially suppressed the *FILpro>>MBP20-SRDX* phenotype (see Supplemental Figure 4D online), implying that the *FILpro>>MBP20-SRDX* phenotype results at least in part from impaired MBP20 function. *FILpro>>MBP20* and *FILpro>>MBP20-SRDX* plants flowered after producing a similar number of leaves as wild-type plants (Figure 2A). It should be noted that the *MBP20-SRDX* transgene could possibly affect other genes in addition to

the natural targets of the *AP1/FUL* family, as they may share binding sites with additional MADS box transcription factors (Riechmann et al., 1996; Melzer et al., 2006) and may be engaged in unnatural interactions. *35Spro>>MBP20-SRDX* plants showed severe developmental alterations, including enlarged sepals (see Supplemental Figure 4E online), suggesting that the activity of MC is compromised by this transgene, as predicted. Together, these results suggest that *MBP20*, and possibly additional *AP1/FUL* genes, are involved in compound-leaf development.

LA Binds to Sequences in the *MBP20* and *TM4* Regulatory Regions

xamination of the *MBP20* and *TM4* promoters revealed several putative TCP binding sites (Kosugi and Ohashi, 2002; Schommer et al., 2008; Aggarwal et al., 2010; Viola et al., 2012), which were designated sites I-IV and sites I-II, respectively (Figures 4A and 4B). We tested whether LA binds to these sequences using electrophoretic mobility shift assay (EMSA) and a recombinant, bacterially expressed LA protein, fused to maltose binding protein (MBP-LA). MBP-LA bound to a 60-bp-long probe that contained *MBP20* site IV but not to a probe that contained a mutation in the putative binding site (Figure 4A). A nonlabeled probe competed with the binding, indicating that the binding of LA to site IV is specific. Furthermore, inclusion of anti-MBP antibodies in the reaction resulted in a supershift (Figure 4A). No binding to sites I-III could be detected (see Supplemental Figure 5A online). Recombinant MBP-LA also bound to site II in the *TM4* promoter, and competition and supershift assays confirmed the specificity of this binding (Figure 4B). We verified the binding of LA to site IV in the *MBP20* promoter by a yeast one-hybrid assay (Li and Herskowitz, 1993; Pruneda-Paz et al., 2009), in which a fusion of LA to the GAL4 activation domain (prey) showed binding to a wild-type 500-bp fragment (bait) surrounding site IV but not to a fragment with two point mutations in site IV (see Supplemental Figure 5B online).

We further verified in vivo binding of LA to the identified sites in the promoters of *MBP20* and *TM4* using chromatin immunoprecipitation (ChIP) assays. We used transgenic plants expressing a fusion of *LA^m* and green fluorescent protein (GFP) under the control of the *LA* promoter (*LApro>>LA^m-GFP*) and anti-GFP antibodies. ChIP samples were tested using PCR and quantitative PCR with primer pairs spanning the *MBP20-IV* and *TM4-II* binding sites and the *TUBULIN* gene as a negative control. An enrichment of the *MBP20-IV* and *TM4-II* sites was detected in the *LApro>> LA^m-GFP* immunoprecipitation sample compared with the input sample and the wild-type samples (Figures 4C to 4E). In conclusion, LA binds specifically to the *MBP20* and the *TM4* promoters.

MBP20 Genetically Mediates LA Activity

To test whether *MBP20* mediates LA-like activity, we introduced *LApro>>MBP20* and *LApro>>MBP20-SRDX* into genotypes with altered LA activity by crosses. Expression of *LA^m* under the control of the *LA* promoter (*LApro>>LA^m*) led to simple, reduced leaves with fused primary leaflets and no secondary leaflets (Figures 5A and 5D; see Supplemental Figure 6A online). Coexpression of *MBP20* via the same promoter did not affect the phenotype of the first few leaves, but substantially suppressed the *LApro>>LA^m* phenotype of later leaves, reinstating the formation of separated primary, secondary, and intercalary leaflets (Figures 5A to 5D; see Supplemental Figure 6A online). Analysis of *LA* expression confirmed that this suppression did not result from silencing of the *LA* transgene (see Supplemental Figure 6B online). Curiously, *LApro>>MBP20* did not suppress the phenotype of the *La-2* gain-of-function allele, which could result from inaccurate expression of the *LA* promoter. Coexpression of miR319 and *MBP20-SRDX* under the control of the *LA* promoter resulted in an intermediate phenotype between the *LApro>>miR319* and *LApro>>MBP20-SRDX* phenotype (Figures 5E to 5G). Together, these results suggest that MBP20 partly mediates the effects of LA and miR319 on leaf shape but that additional factors are also involved, likely including the closely related gene *MBP10* and additional tomato *AP1/FUL* genes.

MBP20 Modulates the Effects of KNOXI and CK in Tomato Leaf Development

To further understand the context of MBP20 activity in leaf development, we tested its genetic interaction with the tomato *KNOXI* gene *Tkn2* and the hormone CK, both of which promote the organogenic activity of the tomato leaf margin. Leaves overexpressing *Tkn2-SRDX* are simple (Figure 6D; Shani et al., 2009). Notably, coexpression of *MBP20* suppressed this phenotype, restoring the formation and separation of primary and secondary leaflets (Figures 6B, 6D, and 6G), and coexpression of *MBP20-SRDX* enhanced the *Tkn2-SRDX* phenotype (see Supplemental Figure 6C online). Whereas overexpression of *Tkn2* resulted in filamentous leaves, leaf expansion was restored by coexpression of *MBP20-SRDX* (Figures 6C, 6E, and 6H). *MBP20-SRDX* also partly suppressed the increased leaf complexity phenotype of the *Me*/+ mutant (Figures 6C, 6F, and 6I). *FILpro>>CKX3* leaves, overexpressing the CK deactivation gene *CYTOKININ OXIDASE3* (*CKX3*) (Werner and Schmülling, 2009; Shani et al., 2010), are simplified with only primary leaflets and smooth margins. Coexpression of *MBP20* partly suppressed this phenotype, whereas coexpression of *MBP20-SRDX* enhanced it (Figures 6A to 6C and 6J to 6L). Cumulatively, these results suggest that MBP20, possibly along with additional family members, play a role downstream of *LA* and *KNOXI* genes in maintaining the organogenic activity of the leaf margin.

DISCUSSION

This study shows that some of the tomato *AP1/FUL* genes promote indeterminate growth in tomato leaves and are negatively regulated by LA-like proteins.

Role of AP1/FUL in Tomato Leaf Development

Our results identify a role for AP1/FUL transcription factors during vegetative development, in modulating the extent of the indeterminate growth of developing tomato leaves. Overexpression of several MADS box genes affected leaf shape and fate in *Arabidopsis* (Goodrich et al., 1997; Honma and Goto, 2001). *AP1/FUL* genes in core eudicots have been divided into

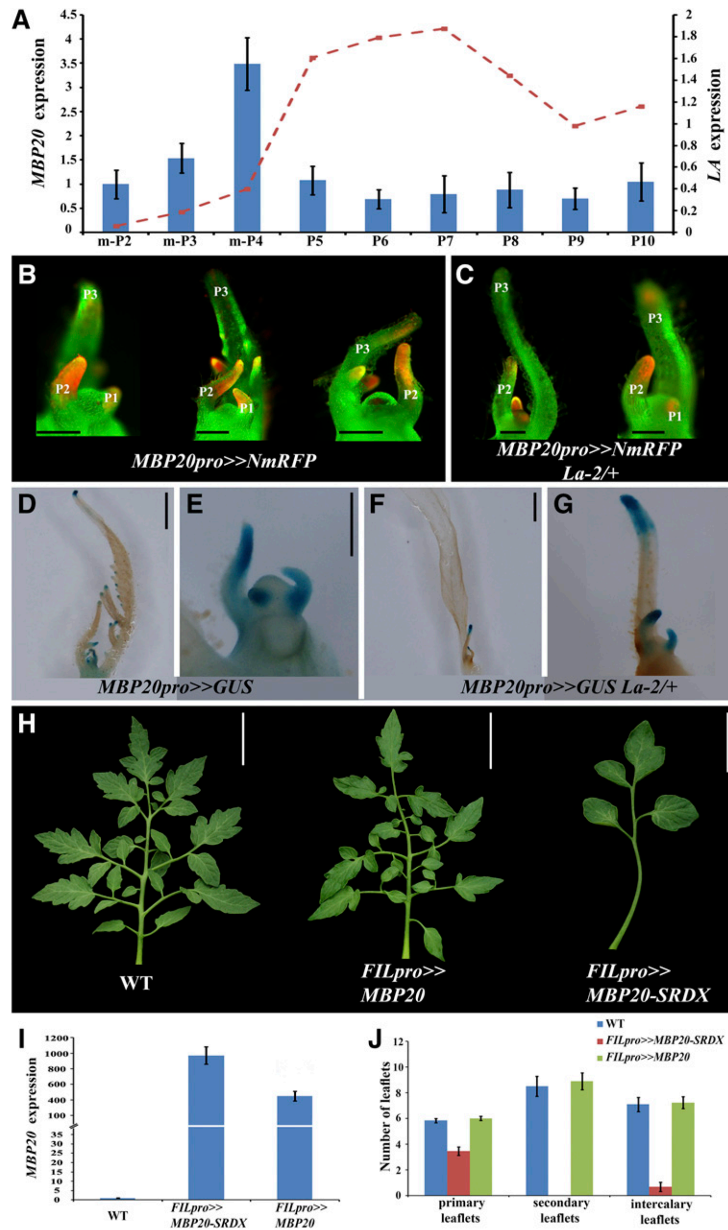

Figure 3. Expression and Function of *MBP20* in Tomato Leaf Development.

(A) Expression dynamics of *MBP20* (columns, left axis) and *LA* (red dashed line, right axis) along the development of the fifth leaf of wild-type tomato plants, assayed by qRT-PCR relative to the reference gene *EXP*. Shown are averages ± SE (*n* = three to six biological repeats). m-P2, m-P3, and m-P4 represent SAM and two, three, or four youngest leaf primordia, respectively. The *LA* expression data is illustrated according to Shleizer-Burko et al. (2011).

(B) and **(C)** Fluorescence of the mRFP protein expressed under the control of the *MBP20* promoter (red) at stages P1 to P3 in the wild type **(B)** and *La-2/+* **(C)** viewed with a stereomicroscope using a Nuance camera and software (CRi).

(D) to **(G)** Histochemical staining of β-glucuronidase (GUS) activity (blue). β-Glucuronidase was expressed under the control of the *MBP20* promoter. Shown are SAM and five youngest leaf primordia (**[D]** and **[F]**) and magnifications of the SAM and three youngest primordia (**[E]** and **[G]**) of the wild type (**[D]** and **[E]**) and *La-2/+* (**[F]** and **[G]**).

(H) Fifth leaves of the indicated genotypes. WT, the wild type.

(I) *MBP20*-SRDX and *MBP20* expression in apices containing the SAM and five youngest leaf primordia of *FILpro>>MBP20* and *FILpro>>MBP20-SRDX* transgenic plants compared with the wild type. Expression was assayed by qRT-PCR relative to the reference gene *EXP* and is shown as an average of four biological repeats (±SE).

(J) Number of primary, secondary, and intercalary leaflets on the fifth leaves of the indicated genotypes, shown as an average of seven to nine leaves from different plants (±SE).

Bars = 250 μm **(B)**, **(C)**, **(E)**, and **(G)**, 1 mm **(D)** and **(F)**, and 5 cm in **(H)**.

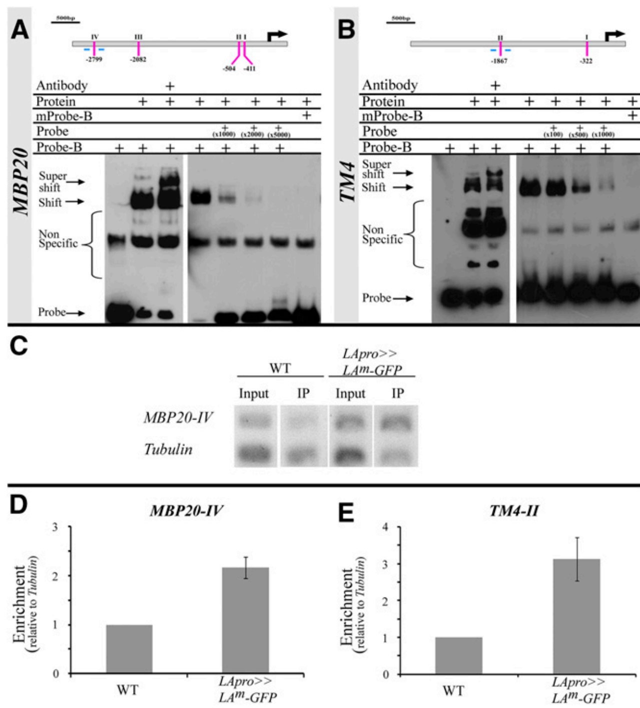

Figure 4. LA Binds to the *MBP20* and *TM4* Promoters.

(A) and **(B)** Top: Schematic diagrams of the *MBP20* **(A)** and *TM4* **(B)** promoters and potential core TCP binding sites (GGNCC, indicated with lines and roman numerals). Black arrows indicate the translation start sites. Bottom: EMSA performed with biotin-labeled probes (Probe-B) and recombinant LA protein fused to MBP (Protein). The components included in each reaction are indicated above each lane. Probe, unlabeled probe (folds of the amount of labeled probe indicated); mProbe and mProbe-B, unlabeled and labeled mutated probe (…GGNaCt…), respectively; Antibody, antibodies against MBP.

(C) to **(E)** PCR and quantitative PCR analyses of a ChIP assay, performed with wild-type plants (WT) or plants expressing a LA^m-*GFP* fusion under the control of the *LA* promoter (*LApro>>*LA^m-*GFP*) and anti-GFP antibodies.

(C) PCRs were performed with specific primers for *MBP20-IV* (lines below the gene diagrams in **[A]**) and *Tubulin*. Input, nonimmunoprecipitated samples; IP, samples after ChIP.

(D) and **(E)** Quantitative PCR reactions were performed with specific primers for *MBP20-IV* **(D)** or *TM4-II* **(E)** (lines below the gene diagrams in **[A]** and **[B]**, respectively) or *Tubulin*. Shown are averages (±SE) of fold enrichment, compared with the wild type (n = two technical and three biological repeats in **[D]** and three biological repeats in **[E]**).

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

three distinct lineages, *euFUL*, *euAP1*, and *AGL79*, that evolved following two duplication events (Litt and Irish, 2003; Shan et al., 2007). Whereas *AP1-like* genes are expressed and function mainly during reproductive development, *FUL* is also expressed in leaves. *ful* mutants have wider cauline leaves (Gu et al., 1998; Teper-Bamnolker and Samach, 2005), and *ful soc* double mutants show variable phenotypes related to indeterminate growth, including reversion of inflorescence meristems to vegetative growth (Melzer et al., 2008). The role of the AGL79-like clade is less characterized, but recently the pea *AGL79-like* gene *VEG1* was shown to be involved in secondary inflorescence meristem identity (Berbel et al., 2012). Basal eudicots contain only one clade of *FUL-like* genes, most closely related to the FUL lineage of core eudicots. These *FUL-like* genes are expressed more broadly, similar to *FUL-like* genes of core eudicots. Interestingly, all three classes of *AP1*/*FUL* genes from tomato are expressed in leaves (Hileman et al., 2006; Shalit et al., 2009; this study), suggesting that leaf expression of *AP1-like* and *AGL79-like* genes has either been maintained or reacquired in tomato and possibly other species with compound leaves. Recently, *FUL-like* genes have been shown to be expressed in leaves and involved in compound-leaf morphogenesis in *A. coerulea* but not in other basal eudicot species (Pabón-Mora et al., 2013). Pabón-Mora et al. suggested that this unique role of *FUL-like* gene may have been acquired specifically in the lineage leading to *A. coerulea*. The role shown here for the *ALG79-like* gene *MBP20*

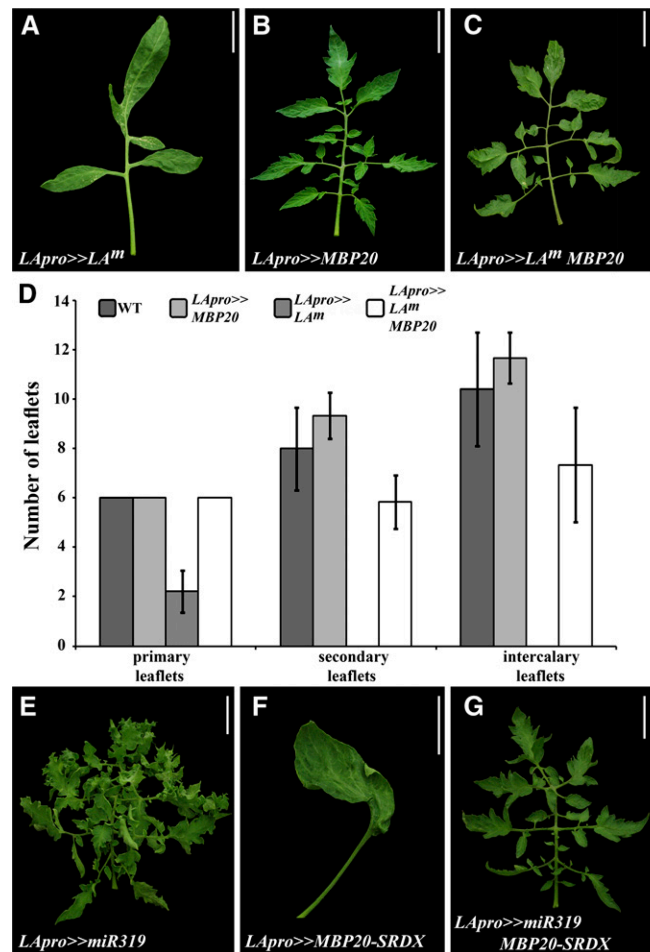

Figure 5. MBP20 Genetically Mediates LA Activity.

(A) to **(C)** and **(E)** to **(G)** Mature leaves of the indicated genotypes. All the transgenes were expressed using the transactivation system. All leaves shown are from plants heterozygous for all transgenes, which include the promoter and the expressed gene. Bars = 5 cm.

(D) Number of primary, secondary, and intercalary leaflets on mature leaves of the indicated genotypes, shown as an average of four to five leaves from different plants (±SE). WT, the wild type.

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

and likely additional *AP1/FUL* genes in tomato compound-leaf development could be explained by two alternative scenarios: (1) The role in compound-leaf development has evolved independently at least twice. (2) This role evolved early but has been lost or is redundant with other genes in most of the species, which may be supported by the leaf expression of *FUL-like* genes in basal eudicots and the *FUL* clade in core eudicots. Analysis of the role of these genes in additional species with compound leaves may help distinguish between these possibilities.

MADS box transcription factors have been implicated in the regulation of growth and differentiation in *Arabidopsis* floral organs, being expressed both during the initiation of floral organs and at later developmental stages (Dornelas et al., 2011; Johnson and Lenhard, 2011). The effect of MC on sepal size in tomato is in agreement with this proposed role. Floral organs are leaf derivatives, and many common factors are involved in the growth and development of these organs (Johnson and Lenhard, 2011), including class II *TCPs* and MADS box genes (Wellmer et al., 2006; Nag et al., 2009). Therefore, *AP1/FUL* genes may have evolved diverse functions in the regulation of leaf and flower organ growth, which differ among species and organs.

Interestingly, to date, *AP1/FUL* genes have been found to promote determinate growth and to be upregulated as the plant matured. These results suggest that during tomato leaf development they act to promote indeterminate growth and are upregulated transiently during early leaf development. This suggests that this gene family has adopted distinct roles in specific developmental contexts. Tomato AP1/FUL proteins show both conserved and unique protein–protein interaction profiles compared with *Arabidopsis* (Leseberg et al., 2008). These unique interactions may be involved in the species- and process-specific roles of these proteins.

AP1/FUL Genes Mediate LA-Like Activity during Leaf Development

Our results identify *MBP20* and *TM4* as targets of LA-like proteins. Whereas *MBP20* was dramatically upregulated in *FILpro>>miR319* plants relative to the wild type, leaf overexpression of *MBP20* in an otherwise wild-type background only slightly affected leaf shape. This suggests that its activity is not a limiting factor in the wild-type leaf and that the indeterminate growth phenotype of *FILpro>>miR319* results from altered expression of additional genes. These may include other *AP1/FUL* genes that were also upregulated in *FILpro>>miR319*, as well as additional pathways. Recently, LA was shown to act in part by positive regulation of GA homeostasis (Yanai et al., 2011). In *Arabidopsis*, several genes that are positively regulated by miR319-sensitive CIN-TCPs have been identified (Schommer et al., 2008; Koyama et al., 2010; Danisman et al., 2012; Rubio-Somoza and Weigel, 2013). Interestingly, the class I TCP TCP15 binds to the regulatory sequences of a partially overlapping set of genes (Uberti-Manassero et al., 2012). This suggests that class I and class II TCPs may have common targets, which is also supported by the finding that they bind similar DNA sequences (Kosugi and Ohashi, 2002; Danisman et al., 2012; Viola et al., 2012). It has been previously suggested that class I and class II TCPs affect growth in an antagonistic manner (Li et al., 2005; Hervé et al., 2009; Koyama et al., 2010; Martín-Trillo and Cubas, 2010). Class I TCPs were recently shown to promote CK responses

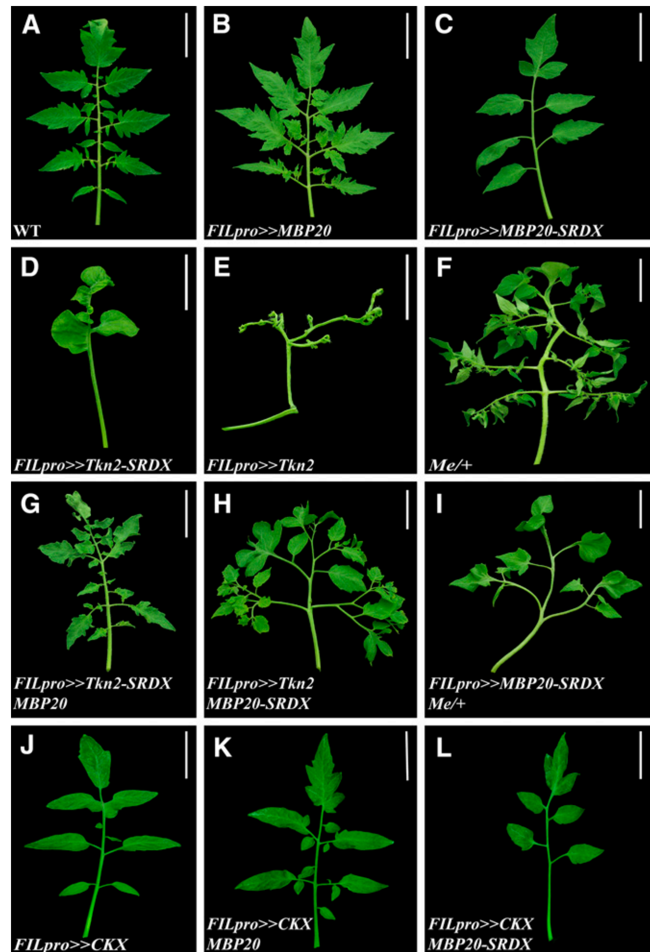

Figure 6. MBP20 and MBP20-SRDX Modify the Leaf Phenotypes Caused by Altered KNOXI and CK Activity.

Mature leaves of the indicated genotypes. All transgenes were expressed using the transactivation system. WT, the wild type. Bars = 5 cm. [See online article for color version of this figure.]

in both *Arabidopsis* and tomato (Steiner et al., 2012a, 2012b), and *Arabidopsis* CIN-TCP were shown to dampen CK response by activating *ARR16* via an interaction with the chromatin-modifying complex BRM (Efroni et al., 2013). Moreover, BRM was recently shown to affect GA biosynthesis and response (Archacki et al., 2013). As CK promotes and GA restricts the window of indeterminate growth in the tomato leaf margin, TCPs possibly regulate the extent of this window by modulating the balance between CK and GA, and class I and class II TCPs may affect this balance antagonistically. Therefore, LA-like proteins likely promote leaf maturation via the regulation of an array of genes, such that the alteration of the activity of only one of these targets is not expected to mimic the *FILpro>>miR319* phenotype.

TCP genes are involved in floral organ development, and class II *TCPs* and *FUL* show opposite expression dynamics during flower development (Wellmer et al., 2006; Nag et al., 2009; Rubio-Somoza and Weigel, 2013). Therefore, the interaction between TCPs and MADS box genes may be conserved in leaves

and floral organs. *TCPs* were recently identified as potential SEP3 and AP1 targets in *Arabidopsis* (Kaufmann et al., 2009) Thus, TCPs and MADS box transcription factors may be involved in a regulatory feedback loop, as has been suggested for additional AP1 regulators (Kaufmann et al., 2009, 2010).

The finding that coexpression of *MBP20-SRDX* modifies both the *Tkn2* and miR319 overexpression phenotypes suggests that *MBP20*, or the pathway it regulates, may be an antagonistic target of LA-like and KNOXI proteins. As *KNOXI* genes have been shown to affect the balance between GA and CK (Hay et al., 2002, 2004; Jasinski et al., 2005; Yanai et al., 2005; Shani et al., 2010), this balance is a possible additional common target of KNOXI and LA-like proteins. Alternatively, the suppression of the *Tkn2* overexpression phenotype by *MBP20-SRDX* could result from a requirement for both increased KNOXI activity and decreased LA-like activity to promote organogenesis.

MBP20 and *TM4* are shown here to be repressed by LA. Other genes have been reported to be activated by CIN-TCPs (Schommer et al., 2008; Koyama et al., 2010; Martín-Trillo and Cubas, 2010; Danisman et al., 2012). Thus, it appears that CIN-TCPs can act as either activators or repressors, depending on the species, the specific target, and the developmental contexts.

Independent Programs Regulate Leaf and Plant Determination

Downregulation of miR319-sensitive *CIN-TCPs* results in indeterminate leaf growth (Ori et al., 2007; Shleizer-Burko et al., 2011) and in reduced numbers of leaves produced before flowering (Figure 2). These results indicate that in tomato, organ and plant determination are regulated via independent pathways that can be separated. Interestingly, in *Arabidopsis*, TCP proteins promote determination of both the leaf and the plant. Downregulation of *Arabidopsis* CIN-TCP activity caused late flowering (Palatnik et al., 2003; Schommer et al., 2008; Koyama et al., 2010), and expression of an activated form of TCP4 from its endogenous promoter induced early flowering (Sarvepalli and Nath, 2011). Therefore, while the effect of TCPs on leaf maturation is conserved between these species, their effect on flowering varies between tomato and *Arabidopsis*.

Unlike TCPs, the ratio between the activities of SFT and SP similarly affects all aspects of determinate growth in tomato (Lifschitz et al., 2006; Shalit et al., 2009; Efroni et al., 2010). High SFT/SP ratio promotes flowering and accelerates leaf maturation, leading to simpler leaves. In this research, we show that overexpression of miR319 is epistatic to both reduced and increased SFT activity with respect to leaf shape, but *SFT* is epistatic to miR319 overexpression with respect to flowering time. This further supports the notion that LA-like proteins influence leaf and plant maturation via separate pathways. The effect on leaf maturation is either downstream of or partly independent of SFT, while the effect on flowering requires intact SFT.

METHODS

Plant Materials and Genetics

Tomato (*Solanum lycopersicum* cv M82, *sp*) plants were grown as described previously (Shleizer-Burko et al., 2011). Except for the *35S*:*SFT* plants (Lifschitz et al., 2006), all the described transgenic plants were produced using the LhG4 transactivation system (Moore et al., 1998; Shani et al., 2009). The following tomato driver and responder lines have been previously described: *FILpro*:*LhG4*, *BLSpro*:*LhG4*, *OP*:*NLS-mRFP* (Shalit et al., 2009; Shani et al., 2009), *OP*:*GUS* (Lifschitz et al., 2006; Efroni et al., 2008), *OP*:*miR319*, *OP*:*LA^m* (Ori et al., 2007), *LApro*:*LhG4* (Shleizer-Burko et al., 2011), *OP*:*Tkn2*, *OP*:*Tkn2-SRDX* (Shani et al., 2009), and *OP*:*CKX* (Shani et al., 2010). *35Spro*:*LhG4* is a gift from Yuval Eshed (Weizmann Institute). The tomato lines *OP*:*MBP20*, *OP*:*MBP20-SRDX*, *OP*:*LA^m-GFP*, and *MBP20pro*:*LhG4* were generated during this research as described below.

Plasmids and cDNA Clones

To generate *OP*:*MBP20-SRDX*, assembly PCR was used to introduce the 36-nucleotide-long SRDX motif CTCGATCTGGATCTAGAACTCCGTTTGGGTTTCGCT + TAA stop codon (Hiratsu et al., 2003) into the C terminus of the MBP20 protein. *MBP20-SRDX*, *LA^m-GFP*, and *MBP20* were cloned downstream to an OP array (Moore et al., 1998) and subsequently cloned into the pART27 binary vector (Gleave, 1992). The *MBP20* promoter (3209 bp) was amplified from the BAC clone CO2Le0092m23 (Tomato Functional Genomics Database) and cloned into pART27, upstream to the LhG4 array.

For the yeast one-hybrid assay, the *MBP20* promoter fragment IV (504 bp), DNA Bait, was cloned into the pLacZi plasmid and introduced into the yeast strain YM-4271. LA, protein prey, was cloned into the pDEST22 plasmid to produce translational fusions with the GAL4 activation domain (GAL4-AD) and introduced into the yeast strain YU-187 (Pruneda-Paz et al., 2009). *MBP20*, *MBP20-IV*, *MBP20m-IV*, and *LA* were cloned using the gateway homologous recombination system (Hartley et al., 2000) starting with the pENTR/D-TOPO cloning kit (Invitrogen). MBP20m-IV was generated by a two-step PCR using the following primer combinations: 1, pMBP20-IV-f + pMBP20m-IV-r and pMBP20-IV-r + pMBP20m-IV-f; 2, pMBP20-IV-f + pMBP20-IV-r. Primers used for cloning are listed in Supplemental Table 1 online.

Analysis of Transgenic Lines

Phenotypic analyses were performed with progeny of crosses between a driver (promoter:LhG4) and a responder (OP:GENE) line. For the initial characterization of the transgenic lines, at least seven independent driver or responder lines were crossed to *OP*:*NLS-mRFP* or *FILpro*:*LhG4*, and a representative line was selected for further analysis. Leaflet number was counted on fully expanded leaves from four to 10 different plants.

Tomato and Yeast Transformation

Tomato cotyledon transformation was performed according to McCormick (1991). Yeast was transformed by the lithium-acetate method (Daniel Gietz et al., 2002). When *pLacZi* plasmids were used, they were linearized at the *ApaI* site prior to transformation.

β-Galactosidase Activity Assay

β-Galactosidase activity was determined as described (Pruneda-Paz et al., 2009). Briefly, transformed yeast cells were grown for 24 h at 30°C in 400 μL SD medium lacking Trp and uracil. Then, 100 μL of the culture was combined with 400 μL YPD and grown for 6.5 h at 30°C. A total of 150 μL of this culture was used to determine the OD_{600}, and 300 μL was centrifuged and washed with Z buffer (10 mM KCl and 1 mM $MgSO_4$ in phosphate buffer, pH 7.0), resuspended in 30 μL of the same buffer, and lysed by four freeze/thaw cycles. Enzymatic reaction was initiated by addition of 170 μL Z buffer + BME (0.27 mL/100 mL), with 120 μg 2-nitrophenyl-β-D-galactopyranoside (Sigma-Aldrich), followed by a 19 h incubation at 30°C. The enzymatic reaction was stopped by addition of 80 μL 1 M Na_2CO_3,

followed by centrifugation. A total of 150 μL of the supernatant was used to determine the OD_{420} and to calculate the β-galactosidase activities.

Phylogenetic Analysis

Amino acid sequences were aligned using a progressive alignment algorithm (see Supplemental Data Set 1 online). A maximum likelihood tree was constructed using a distance-based method and a neighbor-joining algorithm with 1000 bootstrap replicates, using the CLC Main Workbench 5.6.1 program (www.clcbio.com).

Tissue Collection, RNA Analysis, and Statistical Analysis

P1 designates the youngest leaf primordium; it becomes P2 when a new primordium initiates, etc. For leaf primordia at the P1 to P3 stages, the leaf at the respective developmental stage was collected with younger leaf primordia and the SAM. At least three biological repeats, each consisting of at least five plants, were used for RNA expression analysis. RNA was extracted using the RNeasy Micro Kit (Qiagen) according to the manufacturer's instructions, except that samples were incubated for 30 min at room temperature after addition of the lysis buffer. cDNA synthesis was performed using the Verso cDNA kit (Thermo Scientific) with 1 μg RNA. Quantitative PCR and qRT-PCR analysis was performed using a Corbett Rotor-Gene 6000 real-time PCR machine, with TaqMan probes (PrimerDesign) and Premix Ex Taq (TaKaRa) for *MBP20* and *LA* or SYBR Premix Ex Taq II (TaKaRa) for all other genes. Levels of mRNA were calculated relative to the *EXPRESSED* (*EXP*) gene as an internal control as follows: In each biological repeat, the levels of the analyzed gene (*GENE*) and *EXP* were separately calculated relative to a standard curve obtained by a dilution series of a reference sample. GENE/EXP ratio was calculated for each sample, and the average expression values of all repeats are presented as "relative expression." Primers are listed in Supplemental Table 1 online.

Microarray Analysis

Microarray expression analysis was performed with total RNA from 12-d-old apices that contained the SAM and the two youngest leaf primordia. Labeled RNA was hybridized to an Affymetrix GeneChip Tomato Genome Array (900738). Three biological repeats were analyzed for the wild-type and *La-2*/+ genotypes and two for the *FILpro>>miR319* genotype. Affymetrix Microarray Suite version 5.0 was used to simultaneously normalize data from all groups (Hubbell et al., 2002). One-way analysis of variance was applied to compare the three genotypes ($P < 0.05$). Each pair of genotypes was compared based on *t* tests and false discovery rates to identify differentially expressed genes.

Imaging, Microscopy, and GUS Staining

Fluorescence imaging was performed using a SMZ1500 fluorescence stereomicroscope (Nikon) equipped with a Nuance camera (CRi) as described previously (Shleizer-Burko et al., 2011). Scanning electron microscopy was performed using a JEOL 5410 LV microscope as described previously (Brand et al., 2007). β-Glucuronidase staining was performed as described previously (Ori et al., 2000).

Expression and Purification of the MBP-Tagged LA Protein

The *LA* coding region was amplified from cDNA (primers listed in Supplemental Table 1 online) and cloned into the pMal plasmid (New England Biolabs) at the *Xho*I and *Eco*RI sites to generate a fusion with MBP (MBP-LA) and transformed into the *Escherichia coli* strain Rosetta (EMD-Novagen). Sixteen milliliters of overnight-grown culture starter was introduced into 1600 mL of fresh Luria-Bertani and grown at 37°C until ~0.6 OD_{600}. Recombinant protein expression was induced with 0.4 mM isopropyl β-D-1 thiogalactoside. Cells were harvested by centrifugation and incubated for 30 min on ice with 160 mL lysis buffer (20 mM Tris-HCl, pH 7.4, 200 mM NaCl, 1 mM EDTA, and 0.2 mg/mL Lysozim), and then disrupted by three freeze/thaw cycles in liquid nitrogen and 37°C, respectively, and by sonication. The lysate was centrifuged, and the protein purified from the supernatant using amylose/agarose beads (NEB E8021S). Elution of bound protein was performed with 20 mM Tris-HCl, pH 7.4, 200 mM NaCl, 1 mM EDTA, and 10 mM maltose.

EMSA

DNA probe was generated by end labeling of a 60-base single-stranded oligonucleotide using the DNA 3′ End Biotinylation Kit (Pierce 89818) and hybridization to complementary synthetic oligonucleotides (see Supplemental Table 1 online). EMSAs were performed using the LightShift chemiluminescent EMSA kit (Pierce 20148). Briefly, 10 μL of purified recombinant MBP-LA fusion protein was incubated at room temperature in 1× binding buffer, 50 ng/μL poly(dI/dC), 2.5% glycerol, 0.05% Nonidet P-40, 50 fmol biotin-labeled probe, and 3.75 μg BSA for 30 to 40 min. For the Supershift, after 30 to 40 min of incubation, 3 μg of antibody against MBP was added and the reaction incubated for an additional 20 to 30 min. The samples were resolved on 6% nondenaturing polyacrylamide gels, electrotransferred onto 0.45 μm Biodyne B nylon membrane (Pierce 7701), and cross-linked to the membrane. The migration of the biotin-labeled probe was detected on x-ray film (5-h exposure) using streptavidin–horseradish peroxidase conjugates and chemiluminescent substrate according to the manufacturer's protocol.

ChIP

ChIP was performed on dissected shoots (containing the SAM and young leaves) of 21-d-old plants grown on nitch medium as described (Ricardi et al., 2010). Briefly, 1.5 g plant tissue was cross-linked by 30 min of vacuum infiltration in 1% formaldehyde. Cross-linking was stopped with Gly and vacuum infiltration for an additional 5 min. Nuclei were isolated and chromatin was fragmented by sonication, calibrated to reach an average fragment size of 0.4 kb. ChIP reactions were performed using anti-GFP antibody (Abcam ab290) prebound to protein A-agarose beads (Santa Cruz Biotechnology sc-2001) and salmon sperm (Sigma-Aldrich B1626), followed by DNA elution, cross-linking reversal with Proteinase K (Roche 03115879), and DNA recovery. Enrichment for LA-bound sequences was assayed by PCR or quantitative PCR on the immunoprecipitated DNA. Quantitative PCR enrichment was calculated by normalizing to *TUBULIN* and to the total input of each sample. Two technical repeats and three biological replicates per genotype were analyzed for the enrichment of *MBP20-IV* and three biological replicates per genotype were analyzed for the enrichment of *TM4-II*.

Accession Numbers

Sequence data for genes used in this study can be found in the Arabidopsis Genome Initiative, Sol Genomics Network, or the National Center for Biotechnology Information under the following accession numbers: *MBP20/AGL79* (Solyc02g089210, BT013126.1), *TM4/TDR4/FULL/FUL1* (Solyc06g069430, AY098732.1), *MBP7/FUL/FUL2* (Solyc03g114830, AY306156.1), *MBP10/AGL79*L (Solyc02g065730), *MC/AP1* (Solyc05g012020, AF448521.1), *LA* (Solyc07g062680, EF091571), *Tkn2* (Solyc02g081120, U76407.1), *SFT* (Solyc03g063100, AY186735.1), *Tubulin* (Solyc04g077020), *EXP* (Solyc07g025390), *FUL* (AT5G60910), *AP1* (AT1G69120), *CAL* (AT1G26310), and *AGL79* (AT3G30260).

Supplemental Data

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

Supplemental Figure 1. Expression of *AP1/FUL* Genes Is Negatively Correlated with *LA* Expression.

Supplemental Figure 2. Sequence Comparison and Dynamic Expression of *AP1/FUL* Genes.

Supplemental Figure 3. miR319 Affects Leaf Maturation and Flowering Time via Separate Pathways.

Supplemental Figure 4. Overexpression of *MBP20-SRDX* Leads to Reduced Formation of Secondary and Intercalary Leaflets.

Supplemental Figure 5. LA Binds the *MBP20* and *TM4* Promoters.

Supplemental Figure 6. Genetic Interaction of MBP20 with LA and Tkn2-SRDX.

Supplemental Table 1. Primers Used in This Work.

Supplemental Data Set 1. Text File of the Alignment Used for the Phylogenetic Analysis in Figure 1C.

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AUTHOR CONTRIBUTIONS

Y.B., S.S.-B., O.Y., L.-E.W., and N.O. designed the research. Y.B., S.S.-B., I.S., I.D.Z., and O.Y. performed research and analyzed the data. O.Y., J.J.-H., and I.K. performed microarray and did the statistical analysis. Y.B., S.S.-B., and N.O. wrote the article.

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