ASYMMETRIC LEAVES2 and FASCIATA2 cooperatively regulate the formation of leaf adaxial-abaxial polarity in Arabidopsis thaliana

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Abstract  The establishment of adaxial–abaxial polarity in the early stage of leaf development is important for the expansion of lamina. In Arabidopsis thaliana, asymmetric leaves2 (as2) and as1 mutations cause defects in the leaf adaxial–abaxial polarity, which are exhibited as abaxialized filamentous leaves in the various modifier mutant backgrounds. Several modifier single mutants generate pointed leaves in common. Mutations in FASCIATA2 (FAS2) also cause pointed leaves. The FAS2 gene encodes a component of Chromatin Assembly Factor-1 (CAF-1), a histone chaperone, which affects mRNA levels of various genes through the regulation of chromatin states. In the present study, we demonstrate that as2 fas2 double mutants frequently generate abaxialized filamentous leaves and show increased mRNA levels of genes for leaf abaxialization, KANADI1 (KAN1), KAN2, YABBY5 (YAB5), ETTIN/AUXIN RESPONSE FACTOR3 (ETT/ARF3) and ARF4. In addition, the transcript levels of all four class 1 KNOTTED1-like homeobox genes that are involved in the maintenance of shoot apical meristem and the Kip-related Protein2 (KRP2) and KRP5 genes that are involved in the cell cycle progression are elevated in the as2 fas2 double mutants. The mRNA levels of all genes other than YAB5, whose transcript levels rise in as2 fas2, were increased in the fas2 single mutants. Our data suggest that FAS2 participates in the establishment of leaf adaxial–abaxial polarity through the repression of the transcript levels of these genes on the as2 background.

Key words: AS2, FAS2, leaf development, adaxial–abaxial polarity, Arabidopsis thaliana.

In seed plants, aerial organs, such as leaves and floral organs, are initiated from the peripheral regions of the shoot apical meristem (SAM) composed of undifferentiated cells. Leaf primordia that are developed from the SAM grow up along the proximal-distal, mediolateral, and adaxial–abaxial axes through repeated cell divisions and cell differentiations. The establishment of adaxial–abaxial polarity at the initial stage of leaf development is crucial for the acquisition of leaf flatness (Steeves and Sussex 1989; Waites and Hudson 1995). ASYMMETRIC LEAVES2 (AS2) and AS1 genes of Arabidopsis thaliana function in the establishment of appropriately expanded flat symmetric leaves, with generation of the three structural axes (Byrne et al. 2000, 2001, 2002; Iwakawa et al. 2002; Lin et al. 2003; Ori et al. 2000; Rédei and Hirono 1964; Semiarti et al. 2001; Tsukaya and Uchimiya 1997; Xu et al. 2003). To date, various modifier mutations that generate filamentous leaves surrounded by abaxialized epidermis in the as2 or as1 mutant backgrounds have been identified [for example, enhancer of asymmetric leaves2 and asymmetric leaves1-1 (eal-1) and elongator3-27 (elo3-27), and so on], and it has been reported that the development of the adaxial domain of leaves is severely defective in these double mutants (Garcia et al. 2006; Horiguchi et al. 2011 a, b; Huang et al. 2006; Inagaki et al. 2009; Ishibashi et al. 2012; Kojima et al. 2011; Li et al. 2005; Pinon et al. 2008; Szakonyi and Byrne 2011; Ueno et al. 2007; Wang et al. 2011; Xu et al. 2012; Yang et al. 2006; Yao et al. 2008; Yuan et al. 2010). On the base of the extreme synthetic phenotype of the double mutants, it has been proposed that AS2 (and AS1) and modifier genes are involved in adaxial development in independent and parallel fashions during the leaf formation. Interestingly, most of the single modifier mutants show the common phenotype, formation of slightly narrow leaves with pointed tip (pointed leaves). (Horiguchi et al. 2011; Ishibashi et al. 2012; Moschopoulos et al. 2012; Pinon et al. 2008). Mutations in the FASCIATA2 (FAS2) gene, which encodes a component of Chromatin Assembly Factor-1 (CAF-1) complex, a histone chaperone, also generate such a leaf phenotype (Kay et al. 2001). In the present paper, we examined whether the fas2 mutation might also affect the establishment of leaf

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adaxial–abaxial polarity in as2 mutant backgrounds and performed gene expression analyses in the as2 fas2 double mutant. Xu et al. (2012) reported that mutation and performed gene expression analyses in the as2 mutant backgrounds. The genetic interaction between as2 and fas2 has not been investigated.

In this study, we used fas2-2 loss-of-function mutants, which was originally isolated from ecotype Nossen. The fas2-2 mutation causes shaded leaves, abnormal leaf phyllotaxy, narrow leaves and short roots (Kaya et al. 2001). As shown in Figure 1A–D and Table 1, although the wild-type Col-0, as1-1, as2-1, and fas2-2 three-times-backcrossed with Col-0 plants did not have filamentous leaves, 25% and 95% of the as1-1 fas2-2 and as2-1 fas2-2 double mutants showed filamentous leaves, respectively. These results show that the fas2-2 mutation influences the establishment of leaf adaxial–abaxial polarity in the as1-1 and as2-1 mutant backgrounds. The as2-1 fas2-2 double mutant generated filamentous leaves with higher efficiency.

We investigated patterns of expression of cDNA for green fluorescent protein (GFP) under the control of the FIL promoter (FILp:GFP), which is expressed only in abaxial cells of leaf primordia (Ishibashi et al. 2012; Watanabe and Okada 2003). We observed signals due to GFP from FILp:GFP by using a confocal laser scanning microscope (LSM510 META; Carl Zeiss, Inc., Oberkochen, Germany). In the wild type, as2-1, and fas2-2 plants, we detected GFP signals only in cells of the abaxial domain of expanded leaves (Figure 2A–C). In the filamentous leaves of as2-1 fas2-2 double mutants, however, we detected GFP signals all over the epidermal cells (Figure 2D). These results suggest that the filamentous leaves in the as2-1 fas2-2 double mutants are abaxialized.

By quantitative reverse transcriptional-PCR (qRT-PCR) using RNA from the shoot apices of wild-type, as2-1, fas2-2, and as2-1 fas2-2 plants, we investigated the transcript levels of the genes that are involved in the establishment of leaf adaxial–abaxial polarity (Figure 3). We quantified transcripts of genes in the HD-ZIP III family [PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV)], which specify the adaxial cell fate; genes in the KANADI (KAN) family [KAN1, KAN2]; genes in the YABBY (YAB) family [FILAMENTOUS FLOWER (FIL), YAB2, YAB5]; and genes in the AUXIN RESPONSE FACTOR (ARF) family [ETTIN (ETT)/ ARF3, ARF4], which specify the abaxial cell fate, as described by Kojima et al. (2011). There were no significant differences among the mRNA levels of PHB, PHV, and REV in the wild-type, as2-1, fas2-2, and as2-1 fas2-2 plants (Figure 3A). In contrast, the mRNA levels of YAB5 and ETT/ARF3 were increased in the as2-1 mutant compared with those in the wild-type plants, consistent with our previous report (Figure 3B, Iwakawa et al. 2007). The mRNA levels of KAN1, KAN2, ETT/ARF3, and ARF4 genes were increased in the fas2-2 mutant compared with those in the wild-type plants (Figure 3B). In the as2-1 fas2-2 double mutants, the mRNA levels of the genes, whose mRNA levels were up-regulated...
in either as2-1 or fas2-2, increased, as compared with those in the wild type (Figure 3B). The mRNA level of ETT/ARF3 in the as2-1 fas2-2 double mutant was higher than that in both the as2-1 and fas2-2 single mutants, and the level of ARF4 transcripts was higher than that in the as2-1 single mutant (Figure 3B). These results are consistent with a conclusion that the filamentous leaves in the as2-1 fas2-2 double mutants were abaxialized. To date, it has been reported that the formation of filamentous leaves in several double mutants combined with the as2-1 mutant are, at least partially, due to increased mRNA levels of ETT and ARF4 (Ishibashi et al. 2012; Takahashi et al. 2013). The increased levels of ETT and ARF4 transcripts in the as2-1 fas2-2 double mutant might be responsible for the formation of the filamentous leaves.

In the double mutants combined with the as1 or as2 mutation exhibiting the formation of filamentous leaves, the mRNA levels of class 1 KNOTTED1-like homeobox (KNOX) genes, BREVIPEDECELLUS (BP), KNAT2, KNAT6, and SHOOT MERISTEMLESS (STM), significantly increased, as compared with those of the wild type (Horiguchi et al. 2011a; Ishibashi et al. 2012; Kojima et al. 2011; Yang et al. 2006). We quantified the mRNA levels of these four class 1 KNOX genes in the wild-type, as2-1, fas2-2, and as2-1 fas2-2 mutant plants (Figure 3C). Consistent with the previous reports, the mRNA levels of BP were raised in as2-1 (Iwakawa et al. 2007; Semiarti et al. 2001). In fas2-2, the mRNA levels of all four class 1 KNOX genes were markedly increased over those levels in the wild type. In the as2-1 fas2-2 double mutants, the mRNA levels of KNAT2 and KNAT6 were similar to those in the fas2-2 single mutants, while the mRNA level of STM was higher than that of wild type, but lower than that of fas2-2. The level of BP transcripts accumulated in as2-1 fas2-2 was further increased over those in the fas2-2 mutant (Figure 3C). Ikezaki et al. (2010) have reported that the increased mRNA levels of BP, KNAT2, and KNAT6 affect the leaf expansion along the proximal-distal direction, but they have no effect on the establishment of the leaf adaxial-abaxial polarity. However, the effects of elevated levels of STM mRNA on polarity have not been examined. A question remains whether STM might be involved in the establishment of the adaxial-abaxial polarity. The high efficiency of filamentous leaf formation that we observed might be due to the combined effects of the increased expression of all of KAN1, KAN2, YAB5, ETT/ARF3, ARF4, and all four class 1 KNOX genes.

In this report, we show a novel mutant with pointed leaves generated filamentous leaves in the as1 and as2 mutant backgrounds (Figure 1, Table 1). Takahashi et al. (2013) show that the mRNA levels of genes for the CDK inhibitor, Kip-related Proteins (KRP5), are increased, in common, in the as2 eal and as2 elo3 double mutants exhibiting abaxialized filamentous leaves, and that the
increased levels of KRP transcripts are due to the up-regulation of ETT/ARF3 in the as2 eal double mutant. We investigated the mRNA levels of KRP genes in the as2-1 fas2-2 double mutants. In the fas2-2 single mutant, the mRNA levels of KRP2 and KRP5 genes were increased by 1.9-fold and 2.2-fold, respectively, as compared to those of wild type (Figure 3D). The mRNA level of KRP2 in as2-1 fas2-2 was similar to that of fas2-2, that is, increased 2.0-fold over that of wild type. While, the mRNA level of KRP5 in as2-1 fas2-2 was increased by 3.1-fold over that of wild type, and was higher than that of fas2-2 (Figure 3D). In Arabidopsis thaliana, the overexpression of both KRP2 and KRP5 stimulate the transition from the mitotic cell cycle to the endoreduplication cycle (Jégou et al. 2013; Verkest et al. 2005; Wen et al. 2013), suggesting that these KRP genes have a negative effect on cell cycle progression. The formation of filamentous leaves in these double mutants might be caused by inhibition of cell division for the lateral expansion of leaves due to the misexpression of KRP genes. In the wild type, precise modulation of KRP expression might be critical for the development of flat leaves. Adachi et al. (2009) reported that the CDKA;1 promoter region contains a regulatory region that controls abaxial side-biased expression. Our microarray analyses, however, showed that levels of CDKA;1 transcripts were not significantly increased in shoot apices of mutant plants forming abaxialized filamentous leaves (our unpublished data). The abaxial region-biased expression of CDKA;1 may reflect differential cell division activity on each side of leaves; thus, KRP misexpression in double mutants might inhibit CDK activity more on the adaxial region.

The class 1 KNOXs, ETT, ARF4, KAN1, KAN2, KRP2, and KRP5 were all increased in the fas2-2 mutant, suggesting that FAS2 represses the levels of these gene transcripts, although it remains unclear whether the effects of FAS2 is direct or indirect. The AS1–AS2 complex (Guo et al. 2008; Yang et al. 2008) directly repressed transcript levels of BP, KNAT2, and ETT/ARF3 (Guo et al. 2008; Iwasaki et al. 2013). FAS2 is an Arabidopsis ortholog of a p60 subunit of the CAF-1 complex, which has activity of histone chaperone and affects the mRNA levels of various genes including those involved in the cell cycle progression (Kay et al. 2001; Ono et al. 2006; Schönrock et al. 2006). It should be intriguing to examine whether the genes whose transcript levels are increased in fas2-2, as described above, are responsible for the morphological phenotypes.

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