The complex of ASYMMETRIC LEAVES (AS) proteins plays a central role in antagonistic interactions of genes for leaf polarity specification in Arabidopsis

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Leaf primordia are born around meristem-containing stem cells at shoot apices, grow along three axes (proximal–distal, adaxial–abaxial, medial–lateral), and develop into flat symmetric leaves with adaxial–abaxial polarity. Axis development and polarity specification of Arabidopsis leaves require a network of genes for transcription factor-like proteins and small RNAs. Here, we summarize present understandings of adaxial-specific genes, ASYMMETRIC LEAVES1 (AS1) and AS2. Their complex (AS1–AS2) functions in the regulation of the proximal–distal leaf length by directly repressing class 1 KNOX homeobox genes (BP, KNAT2) that are expressed in the meristem periphery below leaf primordia. Adaxial–abaxial polarity specification involves antagonistic interaction of adaxial and abaxial genes including AS1 and AS2 for the development of two respective domains. AS1–AS2 directly represses the abaxial gene ETTIN/AUXIN RESPONSE FACTOR3 (ETT/ARF3) and indirectly represses ETT/ARF3 and ARF4 through tasiR-ARF. Modifier mutations have been identified that abolish adaxialization and enhance the defect in the proximal–distal patterning in as1 and as2. AS1–AS2 and its modifiers synergistically repress both ARFs and class 1 KNOXs. Repression of ARFs is critical for establishing adaxial–abaxial polarity. On the other hand, abaxial factors KANADI1 (KAN1) and KAN2 directly repress AS2 expression. These data delineate a molecular framework for antagonistic gene interactions among adaxial factors, AS1, AS2, and their modifiers, and the abaxial factors ARFs as key regulators in the establishment of adaxial–abaxial polarity. Possible AS1–AS2 epigenetic repression and activities downstream of ARFs are discussed. © 2015 The Authors. WIREs Developmental Biology published by Wiley Periodicals, Inc.

INTRODUCTION

Leaves develop as lateral organs from the peripheral zone of a shoot apical meristem (SAM) along three structural axes. A group of cells is initially patterned along the proximal–distal axis and then along the adaxial–abaxial axis. Subsequent cell proliferation along the medial–lateral axis results in flat and mediolateral symmetric leaves1–9 (Figure 1).
The process of leaf differentiation is a good model to study organ development from stem cells. The SAM consists of stem cells in a central zone (CZ), which divide slowly and replenish a peripheral zone (PZ) of more rapidly dividing cells, in which leaf initiation occurs. Leaf primordia are detected as transcriptionally distinct groups of leaf founder cells before they become morphologically distinct from the SAM. This process was first clearly demonstrated as the disappearance of class-1 KNOTTED-like homeobox (class 1 KNOX) gene transcripts from the leaf primordia. In dicotyledonous plants, a leaf primordium 0 (p0) is initially contained entirely within the SAM (see Figure 1), and then begins to grow outward. It has been speculated that the primordium acquires adaxial-abaxial polarity in the radial dimension, soon after it becomes visible, which is between the p1 and p2 stages of development. Based on the observation that if adaxial-abaxial polarity is perturbed, filamentous shaped leaves are formed, Waites and Hudson proposed that cell proliferation might be induced at the boundary between the adaxial and abaxial domains, and result in the expansion of leaf lamina in the medial-lateral direction. Genetic and molecular studies of leaf development in dicotyledonous plants support their concept. The molecular mechanisms underlying the cell proliferation induced by the juxtaposition of these two domains, however, are not well understood.

As summarized in Figure 1, genes involved in the adaxial-abaxial partitioning of leaves have been isolated and characterized in Arabidopsis thaliana. Analyses of these genes have shown that networks of several families of transcription factor-like proteins and small RNAs must play critical roles in the specification of leaf polarity. The PHABULOSA (PHB), PHAVOLUTA (PHV), and REVOLUTA (REV) genes, which encode class III homeodomain-leucine zipper (HD-ZIPIII) proteins, determine adaxial cell fate. The accumulation of their transcripts in the adaxial domain is a consequence of their degradation in the abaxial domain by microRNA165 (miR165) and miR166 (miR165/166). The ASYMMETRIC LEAVES1 (AS1) and AS2 genes, which encode nuclear proteins with the MYB (SANT) domain and the plant-specific AS2/LOB domain (https://www.arabidopsis.org/browse/genefamily/AS2.jsp), respectively, were initially identified as factors involved in the formation of symmetric flat lamina of leaves. It has recently been shown, however, that they are related to the formation of proper morphology along three leaf axes including the adaxial-abaxial axis, which will be mainly discussed in this article.
The idea that leaf polarity is specified by antagonistic interactions between adaxial and abaxial genes was proposed on the basis of genetic and expression analyses of these genes. Such expression patterns change during leaf development. During this process, both adaxial and abaxial promoting genes are initially expressed throughout the primordium (see p0–p1 in Figure 1), and subsequently their expression patterns are restricted to their respective complementary domains (p2). The patterning of expression of the polarity genes is generated by the mutually exclusive actions of their protein products. For example, expression of the abaxial gene FIL/YAB is abolished by the ectopic expression of PHB (HD-ZIPIII adaxial gene). Although leaf regions expressing PHB and KAN messenger RNAs (mRNAs) are mutually exclusive, and these gene families act genetically in an antagonistic manner during embryo patterning, it has not been clearly demonstrated that KAN regulates HD-ZIPIII expression directly.

Recently, Nakata et al. showed that PRESSED FLOWER/WUSCHEL-RELATED HOMEOBOX3 (WOX3) and WOX1, which are expressed in the middle domain between the adaxial and abaxial domains, function redundantly in lateral-specific lamina outgrowth and leaf margin-specific cell fate and, furthermore, that expression patterns of the two WOX genes are negatively and positively regulated by the KAN and YAB genes, respectively. They also propose a three-domain model, in which these WOX genes would coordinate adaxial/abaxial patterning in cooperation with adaxial- and abaxial-specific regulators, including the ASYMMETRIC LEAVES2 (AS2) and YAB3. YUCCA genes, responsible for auxin biosynthesis, are expressed in response to the juxtaposition of adaxial and abaxial domains, which is responsible at least in part for leaf margin expansion.

AS1 and AS2, both of which are referred to as adaxial genes and exhibit similar laminar abnormalities, repress the expression of abaxial genes, such as KAN2, YAB5, ETT/ARF3, and ARF4, but do not affect the HD-ZIPIII family genes, suggesting that they are involved in the antagonistic interactions between genes that specify adaxial–abaxial polarity. The direct repression of AS2 by KAN was first reported by Wu et al. Our group has recently reported the direct repression of ETT/ARF3 by transcriptional gene silencing (TGS) through AS1–AS2 and indirect repression of both ARF3 and ARF4, a redundant member of the ARF gene family, by post TGS (PTGS) through AS1–AS2 functions. These results provide a molecular framework for the antagonistic interaction of genes involved in adaxial–abaxial specification. In this article, we will...
overview the recent results on molecular mechanisms for the opposing interplay of polarity-related genes by AS1 and AS2 and discuss prospects for a novel epigenetic system of gene repression to guarantee the polarity specification necessary for leaf development.

CHARACTERIZATION OF ADAXIAL-SPECIFIC AS1 AND AS2 GENES

The PHANTASTICA (PHAN) gene of Antirrhinum majus is involved in the growth and adaxial–abaxial determination of lateral organs and its expression is required early in the establishment of the proximal–distal axis. Because plants with a mutation in PHAN are known to generate abaxialized filamentous leaves only when grown at 15–17°C or in the handlebars (hb) mutation background, it is proposed that a cold-sensitive pathway and some other gene might be redundantly involved in the adaxial–abaxial determination of leaves together with PHAN (see the later section of ‘Modifier mutations’ of Arabidopsis). In the as1 mutant in A. thaliana, the PHAN MYB ortholog is disrupted 17

Both as1 and as2 mutants exhibit pleiotropic phenotypes, (Figure 2(b)). These mutants produce petioles and leaf blades that are much shorter than those of the wild type, in addition to asymmetrically lobed and downwardly curled leaf blades, which are bilaterally asymmetric. Furthermore, as1 and as2 also often generate leaflet-like structures from petioles in asymmetric positions and fail to produce a thick and distinct midvein; and as2 often generates leaflet-like structures from petioles in asymmetric positions. Higher-ordered veins are asymmetric and simplified. The observation that the leaf laminas of as1 and as2 are often plump and swelled at their base implies that adaxial development in the leaves of these mutants is slightly diminished. Thus, the AS1 and AS2 genes are involved not only in the symmetric development along the mediolateral axis, but also in development along the adaxial–abaxial axis and the proximal–distal axis. In addition, expression levels of many genes, including both class-1 KNOX genes (BP, KNAT2, KNAT6) and abaxial-determinant genes, such as ETT/ARF3, KAN2 and YAB3, are elevated.

Both AS1 and AS2 transcripts accumulate in the early stage of above-ground organ primordia, and AS1 and AS2 expression sites become restricted to middle/inner regions and the adaxial epidermis of cotyledonary and leaf primordia. AS2 transcripts are also accumulated in the columnella root cap. After leaf maturation, the expression levels of these genes are reduced. AS2-fused YFP (AS2-YFP) proteins are localized to subnuclear bodies adjacent to the nucleoli in leaf cells, called AS2 bodies, and some are also dispersed in the nucleoplasm. GFP-fused AS1 proteins are located as speckles in the nucleoplasm and are also concentrated in the AS2 bodies by an AS2-dependent process. AS1 and AS2 form the AS1–AS2 complex, which represses the expression of two class 1 KNOX genes, BP and KNAT2, by binding to their respective promoter regions, showing that these KNOX genes are direct targets of AS1–AS2. In addition, AS1–AS2 directly represses ETT/ARF3 by binding to its promoter region.

Although genes that are predicted to encode AS1 orthologs and members of the AS2/LOB protein family are detected in genome databases of many plant species, genes that might encode amino acid sequences entirely homologous to the AS2 sequence are not detected in rice genome databases, and even complementary DNA (cDNAs) encoding AS2 orthologs have yet to be reported from monocotyledonous plants. Although AS2 homologues have been predicted to be present in various dicotyledonous plants, their roles in plants other than Arabidopsis are not yet intensively studied.

PROXIMAL–DISTAL POLARITY DEVELOPMENT OF LEAVES BY AS1–AS2

Genes involved in the formation of proximal–distal polarity were first identified in maize. While leaves of dicotyledonous plants are composed of stipule, petiole, and leaf blade along with proximal–distal axis, monocotyledonous plants such as maize and rice develop other distinct leaf features: the sheath in the proximal region of the leaf and the blade in its distal region. The sheath and blade are separated at their boundary by the auricle and ligule, which are not present in leaves of dicotyledonous plants (http://www.fsl.orst.edu/forages/projects/regrowth/print-section.cfm?title=Grass Structures). Recessive mutants of the rough sheath2 (rs2) gene of maize, an ortholog of PHAN and AS1, exhibit a disruption of the blade-sheath boundary owing to disorganized cell growth and acropetal ligule displacement, and the semi-bladeless phenotype of leaves. In rs2 mutants, class 1 KNOX genes are ectopically expressed, a condition that is also observed in some dominant mutants exhibiting phenotypes similar to those of rs2. Thus, rs2 is involved in the proximal–distal patterning of maize leaves through repression of class 1 KNOX genes.
AS1 and AS2 of *A. thaliana* are also involved in regulation of the proximal–distal development of leaves through repression of class 1 KNOX genes (Figure 3). Although petiole and leaf lengths are markedly reduced both in *as1* and *as2*, the extent of the reduction is more severe in *as1*.

To investigate effects of the elevated expression of class 1 KNOX genes on the phenotypes of *as1* and *as2* mutants, a number of studies have been performed using over- and ectopic-expression systems of class 1 KNOXs under the control of the 35S promoter of Cauliflower mosaic virus. Overexpression of KNOX genes in tobacco and *A. thaliana* plants repressed transcription of the gibberellin-synthetic (GA-synthetic) genes that encode GA-20 oxidase, and application of GA partially suppressed the abnormal phenotypic features of *PHANTASTICA*-antisense transgenic tobacco plants. Analyses of multiple loss-of-function mutants of KNOX genes (*bp knat2 knat6*) in *as1* and *as2* backgrounds show that the formation of shorter petioles and leaf blades is due to repression of GA-synthetic genes by the upregulation of *BP KNAT1, KNAT2, and KNAT6* (Figure 3). Thus, elevated expression of KNOXs is responsible for limited numbers of *as1* and *as2* phenotypes including petiole and lamina sizes, the less prominent midvein, and the lower potential of root regeneration from leaf sections in *in vitro* culture. The formation of asymmetric leaf lobes, leaf curling, leaflet-like structures from petioles, and the increased potential of shoot regeneration are, however, not due to upregulation of class 1 KNOXs.

*PHAN* in *Antirrhinum* is also involved in elaboration of the proximal–distal axis as well as the adaxial–abaxial polarity in leaves. *NSPHAN* of *Nicotiana sylvestris* is also proposed to be involved in proximal–distal development. Taken together, the KNOX-repressive systems mediated by *AS1* orthologs (*PHAN* and *RS2*), which appear to be involved in the proximal–distal polarity patterning, might be conserved at least in the plants mentioned in this section. Nevertheless, roles of *AS2* orthologs in such patterning have not been determined in these plants other than *Arabidopsis*.

![Figure 3](image_url) Roles of the AS1–AS2 complex in the regulation of class 1 KNOX, *ETT/ARF3* and *ARF4* genes in early stages of leaf primordia in *Arabidopsis thaliana*. The introduction of *bp knat2 knat6* triple mutations into *as1-1* or *as2-1* efficiently suppressed the phenotypes of short petiole and leaf blade seen in Figure 2(b).

**ADAXIAL–ABAXIAL POLARITY SPECIFICATION OF LEAVES BY AS1–AS2**

**Molecular Roles of AS1–AS2: Repression of Abaxial Genes**

Gene expression analyses of *as1* and *as2* show that transcript levels of several abaxial side-specific genes (*ETT/ARF3, KNAN2, YAB5*) are significantly increased, whereas those of *HD-ZIPIII* do not change. These results suggest that *AS1* and *AS2* directly or indirectly repress expression of the abaxial-specific genes (Figure 3). In addition, systematic molecular and genetic analyses have identified a target gene, *ETT/ARF3*, which encodes an abaxial factor acting downstream of the AS1–AS2 complex. As schematically summarized in Figure 4, the AS1–AS2 complex represses *ETT/ARF3* by the direct binding of AS1 to the *ETT/ARF3* promoter and also indirectly induces accumulation of miR390 and *tasiR-ARF*, which negatively regulate the expression of both *ETT/ARF3* and *ARF4*. Thus, the complex dually represses the expression of *ETT/ARF3*. Several abnormalities of *as2* plants are slightly suppressed by the introduction of an *ett* or *arf4* single mutation into *as2* plants. The introduction of *ett arf4* double mutations into *as2* efficiently suppresses these asymmetric leaf phenotypes (Figure 5(a)). These results are consistent with the observation that overexpression of a *tasiR-ARF*-insensitive *ETT/ARF3* cDNA yields *as2*-like phenotypes. Similarly, some lamina phenotypes of *as1* were also rescued by the introduction of *ett arf4* (Figure 5(a)). The phenotype of wrinkled lamina with patches of abaxialized cells on the adaxial surface, which indicates a slight
FIGURE 4 | Dual regulation of ETT/ARF3 gene expression, including by the possibly epigenetic system of AS1–AS2. The AS1–AS2 complex represses ETT/ARF3 directly, and ETT/ARF3 and ARF4 indirectly, via stimulating the miR390 and tasiR-ARF pathway. In addition, AS1 and AS2 maintain gene-body DNA methylation of the ETT/ARF3 gene. Solid lines indicate direct regulation and dashed black lines indicate indirect regulation.

FIGURE 5 | (a) The ett and arf4 mutations suppressed major leaf phenotypes of as1-1 and as2-1. Representative gross morphology of 40-day plants and magnified views of their leaves. Gross morphology of Col-0 (wild type), as1-1, as1-1 ett-13 arf4-1, as2-1, and as2-1 ett-13 arf4-1 plants is shown. The genotype of each plant is indicated. Red arrowheads indicate leaf lobes and the arrow indicates a leaflet-like structure. The introduction of ett arf4 double mutations into as1-1 or as2-1 efficiently suppressed the phenotypes of asymmetrically curled leaf blades, asymmetric lobes, and plump and swollen leaf lamina in both mutants in Figure 2(b). Scale bars: 5 mm (upper) and 2 mm (lower). (b) Gross morphology of typical double mutants (as2-1 elo3-27 and as2-1 eal-1 bob1). Introduction of ett and arf4 mutations into the double mutants efficiently suppressed the abaxialized leaf phenotypes to form flat symmetric leaves. See details of modifier mutations in Table 1. Scale bars: 5 mm. Plants were photographed at 28 days after sowing. White arrowheads indicate filamentous leaves. Scale bars: 5 mm. Higher magnification views of filamentous leaves are shown. Scale bars: 1 mm in higher magnification views. Photographs (a) and (b) are reproduced and modified from Ref 34 (Development 2013, 140:1958–1969) and Ref 69 (Plant Cell Physiol 2013, 54:418–431), respectively.
increase in abaxialization,\textsuperscript{14,53} was also rescued in both \textit{as1} and \textit{as2} by the introduction of \textit{ett arf4}.\textsuperscript{34} These results suggest that several leaf abnormalities, including those related to adaxial–abaxial polarity defects in \textit{as1} and \textit{as2} plants, result from elevated expression of the abaxial genes \textit{ETT} and \textit{ARF4} (Figure 4). Analyses of modifier mutations of \textit{as1} and \textit{as2} have further confirmed that repression of these \textit{ARFs} by \textit{AS1}–\textit{AS2} is important for the adaxialization of leaves. Although expression levels of \textit{KAN2} and \textit{YAB5} are increased in \textit{as1} and \textit{as2}, they are indirectly regulated by \textit{AS1}–\textit{AS2}.\textsuperscript{34}

Although the systems whereby tasiR-\textit{ARFs} regulate \textit{ARF3} expression are conserved in both \textit{Arabidopsis} and maize plants, the contribution of tasiR-\textit{ARFs} to adaxial–abaxial patterning in \textit{Arabidopsis} seems to be different from that in maize; the extents of adaxial defects in mutations in tasiR-ARF biogenesis components of \textit{Arabidopsis} are subtle as compared with those of maize.\textsuperscript{13} This might be due to difference in the involvement of \textit{AS1} in repressing \textit{ARF3} expression in \textit{Arabidopsis} from that of \textit{RS2} in repressing \textit{ARF3} in maize. Recently, loss-of-function mutants of small RNA biogenesis components (\textit{RDR6, SGS3, AGO7, and DCL4}) in tomato (\textit{Solanum lycopersicum}) have been isolated. In severe cases, they generate shoestring leaves that lack leaf blade expansion (\textit{wiry leaves}).\textsuperscript{54} In the tomato mutants, levels of tasiR-\textit{ARFs} are reduced and \textit{ARF3} and \textit{ARF4} are upregulated, suggesting that the repressive system of \textit{ARF3} and \textit{ARF4} regulation by tasiR-\textit{ARFs} is also conserved in the pathway for adaxial–abaxial specification in leaves of tomato; increased activity of either of \textit{ARFs} phenocopies results in \textit{wiry} leaves. Interestingly, overexpression of these \textit{ARFs} in \textit{Arabidopsis}, tobacco (\textit{Nicotiana tabacum}), and potato (\textit{Solanum tuberosum}), however, fails to produce \textit{wiry} leaves, suggesting that such a response in tomato is distinct from those in other dicotyledonous plants. The tomato system of adaxial–abaxial specification by tasiR-\textit{ARFs} appears to be somewhat similar to the developmental control of adaxial–abaxial patterning by the tasiR-\textit{ARFs} in maize.

### Modifier Mutations Enhancing Leaf Polarity Defects of \textit{as} Mutants

Many mutations that enhance leaf polarity defects of \textit{as1} and \textit{as2} have been isolated and characterized, which is reminiscent of the presence of the cold-sensitive pathway in the original \textit{phan} mutant of \textit{Antirrhinum} and \textit{handlebars} as the enhancer mutation of \textit{phan}.\textsuperscript{2,35} The causative genes are designated as modifiers of \textit{AS1} and \textit{AS2}\textsuperscript{34,69} and, as listed in Table 1, \textsuperscript{24,30,34,39,58,59,62–64,69–72,74–79,81,82,84,88–90,93,95} they include those for biogenesis of tasiR-\textit{ARF}, biogenesis of ribosomes, chromatin modification and nucleosome assembly proteasome-mediated protein degradation, genomic stability, and cell proliferation. Prominent phenotypes in almost all double mutants with \textit{as2} and a modifier mutation involve the generation of filamentous leaf-like organs (Figure 5(b)), which are surrounded by an abaxialized epidermis and possess no or markedly premature vascular tissues. Double mutations in \textit{PRESSED FLOWER/WUSCHEL-RELATED HOMEOBOX3 (WOX3)} and \textit{WOX1} also cause the formation of severely abaxialized filamentous leaves in the \textit{as2} background.\textsuperscript{30,89}

In the double mutants that have been examined, transcript levels of several abaxial-specific genes (\textit{KAN2, YAB5, ETT/ARF3, ARF4}) as well as class 1 \textit{KNOX} genes are markedly increased; these genes are upregulated in the \textit{as2} single mutant and slightly upregulated in some of the modifier single mutants. When the double mutations of \textit{ETT/ARF3} and \textit{ARF4} (see Figure 4), are introduced into double mutants with \textit{as2} and one of the modifier mutations, such as \textit{elo3} and \textit{bob1usal-1}, the phenotype of abaxialized filamentous leaves is restored to flat and expanded shapes\textsuperscript{34,69} (Figure 5(b)). These results show that the upregulation of these \textit{ARF} genes in the double mutants is responsible for the disappearance of adaxial specification of the mutants, which suggests that repression of these \textit{ARF} genes by the synergistic action of \textit{AS1}–\textit{AS2} and modifier proteins is critical for proper development of the adaxial domain.

How can the modifiers and \textit{AS1}–\textit{AS2} synergistically repress expression of \textit{ARFs} in a wild type plant? As mentioned in the previous section, \textit{ETT/ARF3} expression is regulated dually by \textit{AS1}–\textit{AS2}-dependent TGS and tasiR-ARF-mediated PTGS through \textit{AS1}–\textit{AS2}, and \textit{ARF4} is regulated by the PTGS. Therefore, the synergistic repression of these \textit{ARFs} is achieved by the two independent pathways, \textit{AS1}–\textit{AS2} and factors involved in small RNA biogenesis such as \textit{RDR6, AGO7}, and \textit{DCL4}, as illustrated in Figure 4. Molecular mechanisms for the prominent repression by other modifiers and \textit{AS1}–\textit{AS2} have yet to be elucidated, but they might be involved in such repression through independent pathways\textsuperscript{65,70,72,90} (Figure 6). It might be worthwhile, however, to stress that modifier mutations so far identified are weak alleles of genes that are essential for cell viability or, conversely, strong alleles of one of the functionally redundant members of such essential gene families. In addition, it is also interesting to note that most of the proteins encoded by such modifier genes are localized in the nucleus or nucleus-related structures or compartments, such
| Gene (Mutation) | AGI Code | Protein | Cellular Process and Status | Subcellular Localization | References |
|----------------|----------|---------|-----------------------------|--------------------------|------------|
| **I. Biogenesis of small RNA** | | | | | |
| RNA-DEPENDENT RNA POLYMERASE6 (rdr6/sde1/sgs2) | AT3G49500 | RNA-dependent RNA polymerase | Duplication of TAS3 mRNAs; biogenesis of ta-siRNA | Cytoplasm, nucleus | 24, 58 |
| ARGONAUTE7 (ago7/zip) | AT1G69440 | ARGONAUTE family protein: RNA slicer | Biogenesis of miR390 for ta-siRNA production | Cytoplasm | 24, 59 |
| SUPPRESSOR OF GENE SILENCING3 (sgs3) | AT5G23570 | Unknown | Biogenesis of siRNA, stabilization of ta-siRNA | Cytoplasm | 24, 59 |
| DICER-LIKE4 (dc4) | AT5G20320 | DICER-LIKE protein: RNase III-like enzyme | Processing of ta-siRNA intermediates | Nucleus | 24, 59 |
| ARGONAUTE1 (ago1) | AT1G48410 | ARGONAUTE family protein: RNA slicer | Recruit of miRNA and siRNA to mRNAs to be degraded | Nucleus (D-body) and cytoplasm | 59, 62, 63 |
| **II. Chromatin modification and remodeling** | | | | | |
| PICKLE (pkl/gymnos) | AT2G25170 | Chromodomain helicase DNA-binding (CHD3) family protein | Component of chromatin remodeling complex SWI/SNF | | 64 |
| SERRATE (se) | AT2G27100 | C2-H2-type zinc finger protein | miRNA-mediated gene expression | Nucleus | 65 |
| HDT1 (hdt1/hd2al/hda3) | AT3G44750 | Histone deacetylase (plant-specific class) | Deacetylation of nucleosomal histone H3, transcription of rDNAs | Nucleolus | 39 |
| HDT2 (hdt2/hd2b) | AT5G22650 | Histone deacetylase (plant-specific class) | Deacetylation of nucleosomal histone H3, transcription of rDNAs | Nucleolus | 39 |
| ELONGATA3 (elo3/east1); ELO2 (elo2/elp1/abo1); ELONGATOR PROTEIN2 (elp2) | AT5G50320; AT5G13680; AT1G49540 | Histone acetyltransferase; scaffold proteins | Core subcomplex of holo-elongator; stimulation of transcriptional elongation; DNA replication | Nucleus (predominant) and cytosol (lesser extent) | 69–71 |
| ELON1 (elo1/elp4); ELP5 (elp5); ELP6 (elp6) | AT3G11220; AT2G18410; AT4G10090 | | Accessory subcomplex of holo-elongator; stimulation of transcriptional elongation; DNA replication | | 70 |
| ELONGATA4/DRL1 (elo4/drl1) | AT1G13870 | Associated protein of elongator complex | Stimulation of transcriptional elongation; DNA replication | | 70, 71 |
| FASCIATA1 (fas1); FAS2 (fas2) | AT1G65470; AT5G64630 | H3 and H4 histone chaperone | p150 subunit of chromatin assembly factor-1 (CAF1); p60 subunit of CAF1; chromatin replication | | 71, 72 |
### TABLE 1

Continued

| 1. Gene (Mutation) | 2. AGI Code | 3. Protein | 4. Cellular Process and Status | 5. Subcellular Localization | 6. References |
|--------------------|-------------|------------|-------------------------------|-----------------------------|--------------|
| **III. Ribosomal protein (or biogenesis of ribosomes)** | | | | | |
| RPL10a (rp10a/pgy1); RPL9c (rp9c/pgy2); RPL5a (pgy3/a6/ol5); RPL28a (ae5); RPL24b (rp24b/stv1); RPL5b (rp5b/oli7) | AT2G27530; AT1G33140; AT3G25520; AT2G19730; AT3G53020; AT5G39740 | L10a; L9; L5; L28e; L24b; L5b | Subunits of ribosome; components of pre-rRNA-protein complex | Cytoplasm, nucleus, and nucleolus | 74–77 |
| RPL4d (rp4d); RPL7b (rp7b); RPL18c (rp18c); RPL38b (rp38b); RPL39c (rp39c); RPS6a (rps6a); RPS21b (rps21b); RPS24b (rps24b); RPS28b (rps28b); RPS15ab (rps15ab); APICULATA2/RPL36AB (api2); RPL36aA (rpl36aa) | AT5G02870; AT2G01250; AT5G27850; AT3G59540; AT4G31985; AT4G31700; AT3G53890; AT5G28060; AT5G03850; AT2G19720; AT1G70600; AT4G14320; AT3G23390 | L4d (L1) family; L30/L7 family (translational regulation); L18e (L15) superfamily; L38e family; L39 family; S6; S21e; S24e; S28; S15AB; L18e/L15 superfamily; L44e | Subunits of ribosome; components of pre-rRNA-protein complex | Cytoplasm, nucleus, and nucleolus | 76–79 |
| APUM23 (apum23) | AT1G72320 | Pumilio protein containing PUF domain | Pre-rRNA processing and rRNA maturation | Nucleolus | 80 |
| **IV. DNA replication and repair** | | | | | |
| TEBICHI (tebichi) | AT4G32700 | A homologue of Drosophila MUS308 and mammalian DNA polymerase | Repair at damaged DNA | | 82 |
| ABA OVERLY SENSITIVE4 (abo4) | AT1G08260 | POL2A, DNA polymerase epsilon catalytic subunit | Interaction with PCNA; DNA-directed DNA polymerase | | 71 |
| ASYMMETRIC LEAVES1/2 ENHANCER7 (ae7/duf59) | AT1G68310 | | | Nucleus and cytoplasm | 84 |
| **V. Proteasome** | | | | | |
| RPN8a (asymmetric leaves enhancer3/rpn8a); RPT2a (hir-2/rpt2a); PBE1 (pbe1); RPT5a (rpt5a); RPT1a (rpt1a); RPN9a (rpn9a); RPT4a (rpt4a); PAD1 (pad1) | AT5G05780; AT4G29040; AT1G13060; AT3G05530; AT1G53750; AT5G45620; AT5G43010; AT3G51260 | 26S proteasome subunit; 20S β subunit; one of the six AAA-ATPases of the proteasome; proteasome component domain; 20S proteasome α subunit | Component of 26S or 20S proteasome | Endoplasmic reticulum and golgi (RPT2a), cytoplasm and nucleus | 88 |
### TABLE 1

| 1. Gene (Mutation) | 2. AGI Code | 3. Protein | 4. Cellular Process and Status | 5. Subcellular Localization | 6. References |
|-------------------|-------------|------------|-----------------------------|----------------------------|---------------|
| VI. Pressed Flower | AT2G28810 | AT3G10810 | Transcription | Nucleus | 30, 89 |
| VII. Others | AT5G03400 | AT5G06400 | Growth-regulating factor 1 | Cytoplasm | 69, 90 |
| VII. Others | AT5G28640 | AT5G24120 | Glycine-RNA ligase | Nucleus | 93 |
| VII. Plastid genes | AT3G48110 | AT2G24120 | DNA-directed RNA polymerase | Nucleus | 95 |

**TABLE 1 (Continued)**

| 1. Gene (Mutation) | 2. AGI Code | 3. Protein | 4. Cellular Process and Status | 5. Subcellular Localization | 6. References |
|-------------------|-------------|------------|-----------------------------|----------------------------|---------------|
| VII. Others | AT5G28640 | AT5G24120 | DNA-directed RNA polymerase | Nucleus | 95 |

**FIGURE 6** Model for repression of ETT/ARF3 and ARF4 by the AS1–AS2 complex and modifiers in the early stage of leaf primordia in Arabidopsis thaliana. Such repression events are crucial for the establishment of adaxial–abaxial polarity and then cell division and growth along the medial–lateral axis. Class 1 KNOX genes are similarly repressed by AS1–AS2 together with modifier genes, although that is not depicted in this figure.

It should be interesting to solve the question of how AS1–AS2, which is localized to nuclear bodies adjacent to the nucleolus, might repress coordinate ETT/ARF3, ARF4, and class 1 KNOX genes with modifier proteins, after they might complete roles in polarity development of leaves. As many modifiers are localized to the nucleus or nuclear compartments, they function in certain nuclear processes to repress directly or indirectly the expression of KNOXs and ARFs. In cases where modifiers might be involved in unidentified nuclear processes, including such known processes as chromatin assembly and ribosome biogenesis mediated by some modifiers, any gene-repressive functions of AS1–AS2 must be associated with such unidentified processes.

**Genes Downstream of the AS-Abaxial Factor Pathway**

Transcript levels of Kip-related protein 2 (KRP2), KRP5, and Isopentenyltransferase 3 (IPT3) increase on the as2 and modifier (eal and elo3) backgrounds, and such upregulation events are canceled by the introduction of an ett arf4 double mutation into as2.69 These results suggest that expression of KRP2, KRP5, and IPT3 genes was negatively controlled by AS1–AS2...
through repression of ARF3/ETT and ARF4 functions in the wild type plant. KRP2 and KRP5 of A. thaliana encode cyclin-dependent kinase inhibitors (CKIs), which interact with CDKs to inhibit their kinase activities and act as key regulators of cell cycle progression. It is possible that cell proliferation required for leaf formation might be achieved by the proper repressive control of KRP2 and KRP5 expression by AS1–AS2.

The IPT genes encode adenylate isopentenyltransferase, a cytokinin biosynthesis enzyme, in A. thaliana. Among members of the IPT family, IPT3 is expressed throughout a plant including the shoot apex and leaves. These data predict that the endogenous level of cytokinin might increase around the SAM in as1 and as2 mutants, which might affect developmental states of cells in the leaf primordia of these mutants.

These results suggest that the AS1-AS2-ETT pathway plays a critical role in controlling the cell cycle progression and the cytokinin level at the shoot apex for leaf growth and development. The relationship between the control of these downstream genes and adaxial development of leaves by AS1–AS2, however, has been unknown.

**POTENTIAL EPIGENETIC REGULATION BY AS1–AS2**

The AS1–AS2 complex targets the cis elements in the promoters of BP and KNAT2. AS1 and maize RS2, an AS1 ortholog, interact with the plant HIRA proteins which is predicted to be a histone chaperone that maintains KNOX gene silencing. Polycomb-repressive complexes (PRCs) ensure the correct spatiotemporal expression of numerous key developmental regulators. Recently, it was shown that the AS1–AS2 complex physically interacts with PRC2 and recruits this complex to the homeobox genes BP and KNAT2 to stably silence in differentiating leaf cells. This recruitment mechanism resembles the Polycomb response element-based recruitment of PRC2 originally defined in flies and provides the first such example in plants. These findings reveal a conserved paradigm to epigenetically regulate homeobox gene expression during development.

It has also been shown that levels of DNA methylation in exon 6 of ETT were depressed in both as1 and as2 mutants. It was reported that over one third of expressed genes in A. thaliana contain DNA methylation within their transcribed regions, and loss of such methylation results in enhanced levels of transcription. Recently, it has been verified that DNA demethylation increases ETT expression in a mutant for MET1. Levels of ETT transcripts increase in shoot apices of met1, implying the involvement of AS1–AS2 in epigenetic control through DNA methylation.

**CONCLUSION**

In Arabidopsis and some other dicotyledonous plants, development of the adaxial domain requires two types of genes: genes for the HD-ZIPIII protein family and those for the AS1–AS2 complex. In the present review, we have summarized two characteristic features of the AS1–AS2-mediated adaxialization (Figures 6 and 7): (1) This complex dually represses expression of

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**FIGURE 7** | AS1–AS2 plays a central role in the antagonistic interaction of genes for adaxial–abaxial polarity specification. Solid lines indicate direct regulation and dashed lines indicate indirect regulation or unconfirmed interactions. Faded names of genes indicate those to be repressed.
the abaxial-specific ARF gene family, ETT/ARF3 and ARF4. These repression systems are experimentally demonstrated to be critical for development of the adaxial domain in Arabidopsis leaves. (2) The repression is further achieved by at least one other molecular system controlled by a modifier gene independently from the AS1–AS2 system. Although molecular mechanisms of the synergistic repression by AS1–AS2 and the modifiers have not been elucidated, their concerted actions should play a critical role in adaxial development. Events that repress the expression of these abaxial genes might occur in the presumptive adaxial domain of the leaf anlagen at its early developmental stages (p0–p1: Figure 7). Unlike the situation in maize, the contribution of tasiR-ARFs to adaxialization is not apparent in Arabidopsis. In the presumptive abaxial domain at such early stages, AS2 is also directly repressed by KAN1 and KAN2 to induce abaxial specification.33 Considered collectively, AS1–AS2 is a central player in the antagonistic interactions of genes expressed in the process of adaxial–abaxial polarity specification.

Recently, Qi et al.108 have reported the existence of a transient low auxin zone in the adaxial domain of leaf primordia from p1 to p9, and suggested that auxin flow from leaf primordia to the SAM is responsible for the adaxial low auxin domain and, thus, acts as a signal influencing formation or maintenance of the leaf adaxial domain. The relationship between the auxin flow and the AS1-AS2-ETT pathway remains to be elucidated.

Antagonistic interaction has been proposed between KAN and YAB families and the HD-ZIPIII family in leaf polarity development. Recently, many phytohormone-related genes have been identified as candidates downstream of the respective KAN1 and REVOLUTA, a member of the HD-ZIPIII family,109–111 suggesting the involvement of genes for phytohormone biosynthesis, response, and transport in the antagonistic interaction. Molecular networks of the interaction are, however, still not clear. Loss-of-function as2 mutations and double mutations of AS2 and its modifier genes do not significantly affect expression of HD-ZIPIII genes,32,70,90 suggesting that the adaxialization mediated by HD-ZIPIII is independent from that by AS1–AS2.

AS1–AS2 and various modifiers synergistically repress these developmentally important genes through certain nuclear processes. Taken together with these observations, it is likely that the repression system mediated by AS1–AS2 and modifier proteins might be, at least in part, involved in epigenetic processes. It will be intriguing to elucidate how coordinate actions of these molecules might determine epigenetic states of repression of KNOX and ARF family genes and how the MET1-dependent ETT methylation might be involved in establishment of the epigenetic state during leaf polarity specification.

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