Antagonistic actions of FPA and IBM2 regulate transcript processing from genes containing heterochromatin

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Summary sentence:
Intronic heterochromatic marks, associated with alternative polyadenylation sites, are decoded by RNA-binding proteins like FPA and IBM2, to tune the expression of key regulator genes such as IBM1 or RPP7.

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AD, CLR, YI, CB, AA, IG and NB performed the research; AD, CLR, YI and NB analyzed the data; NB designed the research; JP and NB wrote the paper.
ABSTRACT

Repressive epigenetic marks, such as DNA and histone methylation, are sometimes located within introns. In Arabidopsis (*Arabidopsis thaliana*), INCREASE IN BONSAI METHYLATION2 (IBM2), an RNA-binding protein containing a BAH domain, is required to process functional transcript isoforms of genes carrying intronic heterochromatin. In a genetic screen for suppressors of the *ibm2* mutation, we identified FPA, an RNA-binding protein which promotes use of proximal polyadenylation sites in genes targeted by IBM2, including *IBM1* encoding an essential H3K9 histone demethylase and the disease resistance gene *RECOGNITION OF PERONOSPORA PARASITICA7 (RPP7)*. Both IBM2 and FPA are involved in the processing of their common mRNA targets: transcription of IBM2 target genes is restored when FPA is mutated in *ibm2* and impaired in transgenic plants over-expressing FPA. By contrast, transposons targeted by IBM2 and localised outside introns are not under this antagonistic control. The DNA methylation patterns of some genes and transposons are modified in *fpa* plants, including the large intron of *IBM1*, but these changes are rather limited and reversed when the mutant is complemented, indicating that FPA has a restricted role in mediating silencing. These data reveal a complex regulation by IBM2 and FPA pathways in processing mRNAs of genes bearing heterochromatic marks.

Keywords

Arabidopsis;
FPA;
INCREASE IN BONSAI METHYLATION (IBM1 & IBM2);
mRNA processing;
RECOGNITION OF PERONOSPORA PARASITICA7 (RPP7).
INTRODUCTION

DNA and histone methylations are epigenetic marks found in plants and animals that influence chromatin structure and have a direct impact on gene function and transposon mobilization. Chromatin can be modified and remodeled in several ways. In plants, the Jumonji C (JmjC) domain-containing protein INCREASE IN BONSAI METHYLATION1 (IBM1) is a histone demethylase which removes methylation on lysine 9 of histone H3 (H3K9me). IBM1 function is essential in plants because it prevents deposition of these heterochromatic silencing marks at transcribed genes (Saze et al., 2008; Miura et al., 2009; Inagaki et al., 2010). H3K9me and CHG DNA methylation (where H = A, T or C) are tightly correlated. Indeed, CHG methylation is controlled by the DNA methyltransferase CHROMOMETHYLASE3 (CMT3) recruited to regions enriched in H3K9me, which it directly binds (Du et al., 2012; Du et al., 2014). In a reciprocal manner, H3K9me is catalysed by three histone methyltransferases, SU(VAR)3-9 HOMOLOG4/KRYPTONITE (SUVH4/KYP), SUVH5, and SUVH6 (Ebbs and Bender, 2006). KYP binds CHG-methylated cytosines through its SRA domain (Johnson et al., 2007). Thus, CMT3 and KYP participate in a self-reinforcing loop between DNA and histone methylation, which is needed for silencing transposons and repeat sequences but is deleterious to genes when IBM1 is absent. Consequently, ibm1 mutants accumulate both H3K9me and CHG in coding regions with drastic consequences for development (Saze et al., 2008; Miura et al., 2009).

Two other categories of Arabidopsis (Arabidopsis thaliana) mutants share the ibm1 developmental and molecular phenotype: mutants of the IBM2 / ANTI-SILENCING1 / SHOOT GROWTH1 gene, hereafter called IBM2 (Saze et al., 2013; Wang et al., 2013; Coustham et al., 2014) and mutants of ENHANCED DOWNY MILDEW2 (EDM2) (Tsuchiya and Eulgem, 2013). The IBM1 gene encodes two different transcripts of which only the longest encodes a functional protein (Rigal et al., 2012), and its production is controlled by both IBM2 and EDM2 in a yet unclear manner. IBM2 is a protein of unknown function containing a Bromo-Adjacent Homology (BAH) domain and an RNA-Recognition Motif (RRM) (Saze et al., 2013; Wang et al., 2013; Coustham et al., 2014). EDM2 contains several zinc-finger domains and a region similar to the active domains of certain methyltransferases (Tsuchiya and Eulgem, 2013). Both EDM2 and IBM2 are found in the same protein complex, bridged by the ASI1-IMMUNOPRECIPITATED PROTEIN1 (AIPP1) (Duan et al., 2017). In addition to the IBM1 gene, EDM2, AIPP1 and IBM2 share another target, the disease resistance gene RECOGNITION OF PERONOSPORA PARASITICA7 (RPP7) (Saze et al., 2013; Tsuchiya and
Like IBM1, RPP7 contains a heterochromatic domain within a long (>2 kb) intron associated with H3K9me and DNA methylated in all cytosine contexts. The IBM2 complex associates with these methylated intronic regions (Saze et al., 2013; Tsuchiya and Eulgem, 2013; Wang et al., 2013) to produce the full-length functional transcript by an unknown molecular mechanism. One hypothesis is that EDM2/AIPP1/IBM2 function by enhancing the use of distal polyadenylation sites over proximal sites located in large introns.

Polyadenylation is one key mRNA processing step, and the choice between alternative polyadenylation sites impacts the regulation of gene expression. FPA is an RNA-binding protein with three RRMs involved in polyadenylation site choice and plays a major role in repressing floral transition by favoring the proximal polyadenylation site of an antisense of the FLOWERING LOCUS C (FLC) transcript (Hornyik et al., 2010; Liu et al., 2010) and more broadly in regulating the 3′-end site choice of diverse mRNAs (Sonmez et al., 2011; Duc et al., 2013), including its own transcript (Macknight et al., 2002; Hornyik et al., 2010). So far, FPA has not been identified as a member of any splicing or polyadenylation complexes, and the precise function of FPA and its mode of action are still unclear. fpa mutants have also been identified in a genetic screen aimed at finding components required for RNA-mediated chromatin silencing (Bäurle et al., 2007), but the role played by FPA in silencing has not been explored. In addition, FPA is involved in plant defense responses (Lyons et al., 2013), pointing toward a more general role in addition to flowering.

Here, we identify fpa as a suppressor of the ibm2 phenotype. The transcription of both IBM1 and RPP7 is restored in a double fpa ibm2 mutant and impaired when FPA is over-expressed. We show that fpa mutants are depleted in CHG methylated cytosine within the largest introns of IBM1, providing evidence that mutating FPA has an effect on chromatin structure that nevertheless seems to be limited to specific regions. We demonstrate that RNA-binding proteins, like FPA and IBM2, are involved in an intricate crosstalk between chromatin and RNA processing to regulate the production of key genes such as IBM1 and RPP7. We further show that transposons controlled by IBM2 localised outside introns are unaffected by this mechanism.

RESULTS

fpa is a genetic suppressor of ibm2
To uncover new genes impacting the function of IBM2, we performed a forward genetic
screen to isolate suppressors of ibm2-4, an allele previously called sg1-1 (Coustham et al.,
2014). Approximately 7,000 ibm2-4 seeds were treated with EMS (see Materials & Methods).
The genetic screen was performed in two steps on 88,000 M3 seedlings. Because the ibm2
phenotype is related to a deficiency in production of the long functional IBM1 mRNA (Saze et
al., 2013; Wang et al., 2013; Coustham et al., 2014), we screened for mutants showing a wild-
type phenotype, aiming to select suppressors in which the function of IBM1 was restored. We
postulated that this pool of plants contained suppressors of ibm2, but also suppressors restoring
the effects of a non-functional IBM1. Mutations like cmt3 or kyp, for instance, suppress ibm1
by preventing the accumulation of heterochromatic marks on a large range of IBM1 targets
(Saze et al., 2008). These mutations did not restore the transcription of IBM2 targets such as
AT3G05410 or RPP7 (Supplemental Figure S1). Next, to isolate genetic suppressors of ibm2
more specifically from the first screen, we determined the level of transcription of AT3G05410
(Saze et al., 2013; Wang et al., 2013), a target of IBM2 which is not targeted by IBM1. We
isolated three M3 plants (from the same M2 pool) which resembled wild-type plants but had
more serrated leaves (Figure 1A) and were late flowering (Supplemental Figure S2). In these
plants, mRNA levels of the known IBM2 target AT3G05410 were intermediate between ibm2-
4 and wild type (Supplemental Figure S3). Therefore, the three mutants are likely progeny from
the same M2 plant. By sequencing and comparing the genomes of these plants and the original
ibm2-4 mutant, we identified mutations that were homozygous and common to the three plants
but not the original mutant. Sequencing revealed that the suppressor of ibm2-4 carries a
nucleotide change (C-to-T) in the fifth exon of FPA (AT2G43410) at position 586, creating a
premature stop codon. The ibm2-4 suppressor was therefore designated a new fpa allele (fpa-
11). These results show that fpa is epistatic over ibm2.

We then extracted genomic DNA from leaves of fpa-11 ibm2-4 mutants to perform
whole-genome bisulfite single-base resolution sequencing (WGBS; Supplemental Table S1)
and determine the patterns of methylation for genes. On average, both CG and CHH
methylation levels were similar in genes of ibm2-4 and fpa-11 ibm2-4 mutants (Supplemental
Figure S4), however, the CHG hypermethylation accumulating in ibm2 genes (Saze et al., 2013;
Wang et al., 2013; Coustham et al., 2014) was lost in genes of fpa-11 ibm2-4 (Figure 1B). We
confirmed the results by identifying the Differentially-Methylated Regions (DMRs) in ibm2-4
or fpa-11 ibm2-4 compared to wild type (Figure 1C). Indeed, the number of ibm2 CHG
hyperDMRs (n=4722) was reduced by 35-fold in fpa-11 ibm2-4, implying that mutating FPA
in ibm2 suppresses the CHG hypermethylation in genes.
Next, we quantified mRNA levels of known IBM2 targets (Supplemental Figure S5) in $fpa$ and $fpa$ $ibm2$ mutants grown in vitro for 21 days. RT-qPCR analyses revealed that the IBM1-$L$ transcripts were more abundant compared to wild type in all $fpa$ allelic backgrounds tested, including $fpa$-3, which is a previously described allele (Hornyik et al., 2010), $fpa$-$11$, $fpa$-$3$ $ibm2$-$4$, and $fpa$-$11$ $ibm2$-$4$ (Figure 2; IBM1-$L$). The opposite trend was observed for IBM1-$S$ (Figure 2; IBM1-$S$). We also detected a general increase of IBM1 transcripts in $fpa$ mutants using a set of primers amplifying all IBM1 transcript isoforms (Figure 2; IBM1-total). Together, the expression data show that mutating $FPA$ in both $ibm2$ and wild-type plants increased IBM1 transcripts by ~1.8-fold. Furthermore, in $fpa$ backgrounds, the production of the long IBM1 transcript is favored over the shortest one. Levels of two other IBM2 targets ($AT3G05410$ and $AT1G11270$) were restored to 40% of wild type in the double $fpa$ $ibm2$ mutants (Figure 2; AT3G05410-$L$ and AT1G11270-$L$). Finally, RPP7 ($AT1G58602$) mRNA levels were restored to 80% of wild type in the suppressor $fpa$ $ibm2$ backgrounds (Figure 2; RPP7-$L$). Altogether, the genetic screen for $ibm2$ suppressors, the methylome sequencing of $fpa$ $ibm2$ mutants, and the RT-qPCR analyses of IBM2 targets demonstrate that a mutation in $fpa$ counterbalances the absence of IBM2 by restoring the production of its target transcripts.

FPA contributes to processing of IBM2 target genes containing intronic heterochromatin

To understand better the links between FPA and the processing of IBM2 targets, the levels of their transcripts were monitored when FPA was over-expressed. Compared to wild type, the production of IBM1-$L$, RPP7-$L$, AT1G11270-$L$, and AT3G05410-$L$ mRNAs was reduced by 29%, 60%, 47%, and 54%, respectively, in plants expressing $35S$:FPA-YFP constructs in a $fpa$-$8$ background (Figure 3 and Supplemental Figure S6). Therefore, the long RPP7, IBM1, AT1G11270, and AT3G05410 transcripts are produced incorrectly in these transgenic plants, confirming the role played by FPA in their processing.

Next, we assessed the function of the Col-0 RPP7 gene in race-specific disease resistance against the biotrophic oomycete Hyaloperonospora parasitica isolate Hiks1 (Hpa Hiks1) (Slusarenko and Schlaich, 2003). The triple $svuh456$ mutant, which has lost the RPP7 intragenic methylation, and two $ibm2$ alleles ($ibm2$-$1$ and $ibm2$-$4$) displayed reduced RPP7 resistance, indicated by increased growth of Hpa Hiks1 in leaves (Figure 4), whereas $fpa$-$3$ and $fpa$-$11$ mutants were as resistant as Col-0 (Figure 4). In agreement with the partial restoration of RPP7-$L$ transcript levels in $fpa$-$11$ $ibm2$-$4$ (Figure 2), this double mutant also exhibited partially restored RPP7-mediated resistance (Figure 4). Therefore, FPA controls the resistance function of RPP7.
Direct RNA sequencing (DRS) helps to define polyadenylation sites by direct sequencing of RNAs in the absence of reverse transcription and is therefore a method of choice to localise regions where FPA promotes polyadenylation. We used the DRS data published previously for fpa mutants to identify polyadenylated 3' ends in fpa-7 and Col-0 (Duc et al., 2013). We found that distant polyadenylation sites of AT3G05410, IBM1, and RPP7 were more frequently used in fpa mutants at the expense of proximal polyadenylation sites (Figure 5). Indeed, the number of normalized DRS reads corresponding to distal polyadenylated sites increased in the fpa-7 background by 2.4, 1.9, and 1.7 fold for IBM1, AT3G05410, and RPP7, respectively. By contrast, the number of DRS reads corresponding to proximal sites decreased by 28.8 and 3.4 fold for IBM1 and AT3G05410, respectively. Hence, polyadenylation of IBM2 targets is mediated by FPA. The data indicate that FPA and IBM2 pathways are interconnected in processing of their common targets, including IBM1 and RPP7 transcripts.

IBM2 promotes the transcription of the methylated Copia element AT4G16870 (Duan et al., 2017), which is a non-intronic transposon localized upstream of the RPP4 resistant gene (Figure 6A). A chimeric RPP4-AT4G16870 mRNA consisting of both RPP4 and this Copia element (Wang and Warren, 2010) was detected in the wild type but not in ibm2-4, aipp1-1, edm2-4, or suvh456 (Figure 6B and Supplemental Figure S7A), confirming that the transcription of RPP4-AT4G16870 is promoted by an EDM2/AIPP1/IBM2 complex and relies on the presence of heterochromatic marks controlled by SUVH proteins. Similarly, RPP4-AT4G16870 mRNAs were not detected in fpa-11 ibm2-4 mutants but were expressed in fpa-11 (Figure 6B). Thus, the loss of FPA does not restore the transcription of RPP4-AT4G16870 mRNAs in ibm2. We verified that the RPP4-mediated resistance to Hpa isolate EMWA1 was not compromised in ibm2, edm2, fpa ibm2, or fpa mutants (Supplemental Figure S8). To identify additional non-intronic IBM2 targets, transposons differently expressed in ibm2, edm2 and aipp1, compared to wild-type plants, were listed using published RNA-seq data (Duan et al., 2017). We found a total of 18 transposons that were significantly (FDR threshold ≤ 0.05) downregulated (log2FC(ibm2 and edm2 and aipp1/WT) < -2) in ibm2, edm2, and aipp1 (Supplemental Table S2). Six transposons corresponded to known IBM2 targets like the intronic RPP7 transposons or the Copia element AT4G16870. In addition, we found that AT4TE21110 is another non-intronic IBM2 target expressed in Col-0 and fpa mutants but not in ibm2 or fpa ibm2 mutants (Figure 6C and Supplemental Figure S7B), as observed for RPP4-AT4G16870. Altogether, our data show that FPA and IBM2 pathways are antagonistic at genes containing heterochromatin within their introns, like IBM1 or RPP7, but not at non-intronic IBM2 targets such as the Copia element AT4G16870 or AT4TE21110.
Intronic DNA methylation of *IBM1* decreases in *fpa*

The genes targeted by IBM2 contain introns carrying heterochromatic marks which regulate their transcription. Since FPA was previously identified in a mutant screen for genes required for the silencing of an inverted repeat (Bäurle et al., 2007), we tested whether the DNA methylation patterns of IBM2 targets were modified in an *fpa* background. For this, we monitored methylation levels of the large *IBM1* intron in the *fpa* mutants. After bisulfite conversion, we sequenced the corresponding *IBM1* region in *ibm2-4, fpa-11 ibm2-4*, and *fpa-3*. Compared to wild type or to *ibm2* controls, CHG methylation was reduced by almost half in the *fpa-11 ibm2-4* and the *fpa-3* mutants (Figure 7A and Supplemental Figure S9). We examined the methylation patterns of the same *IBM1* region in mutants for which the whole methylomes were sequenced (Stroud et al., 2013), and we found reduced CHG methylation in both *fpa-7* and *fca-9 fpa-7* plants (Supplemental Figure S10). Therefore, the methylation of *IBM1* is modified in *fpa* backgrounds.

To explain the reduction of CHG methylation observed at *IBM1* in *fpa*, we hypothesized that IBM1 could control the production of its own mRNA by removing intronic epigenetic marks contained within the largest intron of the *IBM1* gene. If this hypothesis was correct, increased levels of *IBM1-L* transcript – as observed in *fpa* – would result in the demethylation of the *IBM1* intron. To test whether such a feedback loop exists, we monitored patterns of DNA methylation in the intron of *IBM1* when *IBM1-L* was over-expressed ectopically (Supplemental Figure S6). Indeed, IBM1 controls methylation of H3K9 which cross-regulates the levels of mCHG (Johnson et al., 2007; Du et al., 2012; Du et al., 2014). Our data show that cytosine methylation levels of the *IBM1* intron were comparable between wild type and *ibm1* mutants that over-express the *IBM1-L* cDNA (Figure 7A and Supplemental Figure S9). As the methylation patterns of the *IBM1* intron remained unchanged when *IBM1-L* was more abundant, we concluded that the hypomethylation of *IBM1* in *fpa* is likely not associated with the increased production of *IBM1-L* transcripts observed in this mutant background.

**The absence of FPA induces transient methylation changes**

To understand whether the decrease of methylation we observed for *IBM1* was widespread or limited to specific regions of the genome, we sequenced the whole methylome of *fpa* mutants. Levels of methylation per cytosine confirmed that biological replicates were closely correlated (Pairwise Pearson correlation values between biological replicates 0.97 for CGs, 0.98 for CHG and 0.94 for CHH). When the average methylation levels were calculated...
in 100-bp windows partitioning the genome, we observed no broad changes between wild-type plants and fpa-3 (Figure 8A), confirming results obtained with the fpa-7 T-DNA allele (Stroud et al., 2013). Next, we identified the DMRs in fpa-3 and compared them to the wild type and to the transgenic fpa-8 line complemented by a 35S:FPA-YFP construct (Bäurle et al., 2007) that was also sequenced. The spontaneous DMRs naturally occurring within the Arabidopsis Col-0 accession were filtered (Zhang et al., 2018). We identified 61 CG hypoDMRs, 73 CG hyperDMRs, 7 CHG hypoDMRs, 7 CHG hyperDMRs, 2 CHH hypoDMRs, and 6 CHH hyperDMRs arising in fpa-3 and returning to wild-type methylation patterns when the function of FPA was restored (Supplemental Table S3). Most of the CG hyperDMRs were found in genes (Figure 8B) and were de novo methylated in fpa (Figure 8C; CGhyper), while CG hypoDMRs overlapped with transposons (Figure 8B) that were demethylated in fpa (Figure 8C; CGhypo). The IBM1 intronic region carrying the heterochromatic marks (Figure 7B) was identified among the 7 CHG hypoDMRs, confirming the results obtained by targeted bisulfite sequencing (Figure 7A and Supplemental Figure S9). A limited number of other regions remained differentially methylated in both fpa-3 and the fpa complemented line compared to their respective wild-type controls: 12 CG hypoDMRs, 9 CG hyperDMRs, 2 CHG hypoDMRs, 2 CHG hyperDMRs and 1 CHH hypoDMR. Therefore, most of the changes of methylation patterns in fpa, including those at IBM1, are reversible when the mutant is complemented by a construct overexpressing FPA.

Next, we examined the methylation profiles of transposable elements that are derepressed in fpa. Previous studies have revealed that transposons like AtSN1, which is a SINE retroelement, AtMu1, which is a DNA transposon, and the helitron AT1TE93275 are expressed in the fpa backgrounds in contrast to wild type (Bäurle et al., 2007; Sonmez et al., 2011). No changes in DNA methylation were observed for AtMu1 in fpa-3 or for AtSN1 (Supplemental Figure S11). We confirmed that AT1TE93275 is demethylated in all cytosine contexts in fpa-3 (Supplemental Figure S11), matching one of the 4 CHG DMRs that remained hypomethylated when fpa was complemented. These results indicate that some transposons, which are up-regulated in fpa, are associated with differences in DNA methylation patterns, but their number is low because we found no widespread changes of transposon methylation patterns in fpa.

**DISCUSSION**

Here we identify fpa as a genetic suppressor of the ibm2 mutation. The IBM2 protein complex interacts with heterochromatic marks localized within the large introns of IBM2 target genes (Deremetz et al., 2018).
genes to promote production of their long transcripts. FPA, by promoting polyadenylation of shorter transcripts, antagonizes the function of IBM2. In addition, FPA affects methylation of the largest intron of IBM1 and a limited number of other regions.

Crosstalk between FPA and IBM2 in polyadenylation site choice

Our forward genetic screen to isolate suppressors of IBM2 resulted in the isolation of FPA. Levels of AT1G11270 and AT3G05410 long transcripts were restored to 40% of wild-type levels in a double fpa-11 ibm2-4 mutant, and levels of RPP7 long mRNAs were restored to 80% (Figure 2; RPP7-L). In addition, IBM1 long mRNAs were ~1.8-fold more abundant in fpa compared to wild type (Figure 2; IBM1-L). Consequently, we found that the most distant polyadenylation sites of IBM2 target genes are favored in an fpa background (Figure 5). At the same time, the large intron of IBM1 contained less methylation in fpa mutants (Figure 7, Supplemental Figure S9 and Table S3), although both CHG and H3K9 methylation within introns appear to be crucial for processing IBM2 target transcripts. First, these transcripts are incorrectly processed when intronic transposons are depleted of CHG methylation (Le et al., 2015). Indeed, RT-qPCR analyses revealed that levels of long RPP7 and AT3G05410 transcripts are reduced in cmt3 (Le et al., 2015), confirming results obtained for IBM1 long transcripts (Rigal et al., 2012). Second, compromising the functions of H3K9 histone methyltransferases has similar consequences. Northern blot analyses show that kyp/suvh4 mutants produce lower levels of IBM1 long transcripts compared to wild type (Rigal et al., 2012). If methylation marks are necessary to correctly transcribe IBM2 target genes, how then can IBM2 targets be transcribed in an fpa background in which intragenic heterochromatic marks are reduced? Because fpa is epistatic to ibm2, it is likely that IBM2 is important for the production of its target transcripts only when FPA is functional. A possible explanation is that FPA promotes the recruitment of the polyadenylation complex at proximal sites, while IBM2 antagonizes this binding. We suggest that IBM2 prevents the polyadenylation of short transcripts only when FPA is active. Whether IBM2 and/or its partners interact directly with proteins of the polyadenylation complex remains to be determined. This mechanism might apply to other plant species. In oil palm (Elaeis guineensis), for instance, the transcription of an essential homeotic gene is regulated by methylation of an intronic LINE retroelement (Ong-Abdullah et al., 2015). Levels of transcription also correlate with the size of heterochromatic regions. Expression of genes with long methylated introns, such as RPP7, AT3G05410, or AT1G11270, is decreased by more than 70% in ibm2 and partially rescued in fpa ibm2 (Figure 2). However, the transcription of IBM1, which contains a shorter methylated intronic region, is
decreased by only 50% in ibm2 and restored in fpa ibm2 (Figure 2) in which IBM1 is fully functional (Figure 1B and 1C). By inserting into introns, transposons introduce alternative polyadenylation sites, making the targeted gene regulated by both the IBM2 and FPA pathways. By contrast, FPA does not antagonize IBM2 for IBM2 transposon targets localized outside genic regions (Figure 6).

Links between RNA processing and methylation changes

The role played by FPA in controlling silencing remains controversial. The fpa mutants were first retrieved from a forward genetic screen to identify genes involved in RNA silencing (Bäurle et al., 2007). Even if some transposons are strongly reactivated in fpa (Bäurle et al., 2007; Sonmez et al., 2011), the analysis of DRS data showed no widespread differences of expression for transposons between fpa-7 and the wild type (Duc et al., 2013). In addition, fpa-7 methylome analyses revealed no major differences in methylation patterns (Stroud et al., 2013). fpa-7 is a T-DNA allele, and recent studies have demonstrated that fpa mutations can rescue T-DNA insert mutants (Zhang et al., 2016), possibly explaining the phenotypic discrepancies existing between T-DNA and point mutation fpa alleles (Duc et al., 2013). By sequencing the methylome of the point mutation fpa-3 allele, we confirmed that no major changes of DNA methylation patterns were observed genome-wide (Figure 8A), but we found that CG methylation was gained in some genes and lost in some transposons of fpa (Figure 8B and 8C). Most of these changes revert to wild-type patterns when the function of FPA is restored, indicating that the changes of methylation are rather limited and transient.

Mutating FPA more specifically disturbs DNA methylation of heterochromatic regions localised within the largest intron of IBM1 in contrast to other IBM2 targets. By sequencing the methylome of fpa-3, the largest intron of IBM1 was identified as a CHG hypomethylated DMR (out of 7 in total) (Supplemental Table S3), confirming results obtained by targeted bisulfite sequencing in fpa-11 ibm2-4 (Figure 7A). Likewise, the CHG methylation, localized at the endogenous phytoene desaturase (PDS) locus silenced by an inverse repeat introduced transgenically in trans, is compromised in the fpa-8 background (Bäurle et al., 2007).

Hypomethylation of IBM1 observed in fpa is probably not coupled to an increase of IBM1-L production, since transgenic ibm1 plants overexpressing IBM1-L ectopically show no alteration of methylation at IBM1 (Figure 7A and Supplemental Figure S9). Moreover, the limited number of CHG DMRs found in an fpa background (Supplemental Table S3) argue against FPA directly controlling the activity of CMTs at transposons localized near or within genes. Therefore, the loss of methylation at IBM1 in fpa is probably independent of IBM1 activity. Other factors...
might account for this hypomethylation, such as those associated with the changes of polyadenylation site in fpa mutants and the subsequent effects on IBM1 mRNA processing. Previous studies have demonstrated that transcription initiation and/or the Pol II elongation rates are influenced by choice of polyadenylation sites at FLC (Wu et al., 2016). Similarly, Pol II occupancy is increased near the proximal polyadenylation sites of RPP7 in edm2, aipp1 and suvh456 plants, indicating that the enzyme is probably pausing in this region (Lai et al., 2018). Therefore, changes of polyadenylation sites in fpa also very likely modify the rates of transcription for many genes, including IBM1 and transposons, which might impact their methylation patterns. Furthermore, the recent discovery that human RBM15 proteins, related to FPA, direct methylation to specific non-coding RNAs (Patil et al., 2016), is consistent with a role for FPA in mRNA methylation, implying that a previously unrecognized interplay exists between epigenetic silencing marks and methylation of IBM2 target RNAs.

**Conclusion**

We show that FPA and IBM2 pathways are crucial for controlling the transcription of their common targets and provide evidence that they act antagonistically when transposons are inserted in introns. The tight regulatory control of RPP7 mRNA levels is likely critical to limit the accumulation of a long functional RPP7 transcript in pathogen-unchallenged conditions and to prevent autoimmunity. In the presence of Hpa Hiks1, the fine regulation of RPP7 transcripts mediated by both IBM2 and FPA favors accumulation of a long transcript to induce a rapid and specific immune response (Tsuchiya and Eulgem, 2013). Similarly, the importance of H3K9 methylation in resistance to viruses has been described (Sun et al., 2015), and the virulence of geminiviruses requires a viral protein that inhibits expression of the main plant H3K9 methyltransferase, KYP. To reinforce the action of KYP, rapid modulation of IBM1 gene expression, mediated by both FPA and IBM2, is likely essential. Our data suggest that intronic heterochromatic marks associated with alternative polyadenylation sites can be decoded by RNA-binding proteins like FPA and IBM2 to tune the expression of key regulator genes such as IBM1 or RPP7.

**MATERIALS AND METHODS**

**Plant materials and growth conditions**

Arabidopsis (*Arabidopsis thaliana*) accession Ksk-1 was described previously (Slusarenko and Schlaich, 2003). All other plants were in the Arabidopsis Col-0 background.
The ibm2-4 point mutation was previously named sg1-1, and ibm2-5 (SAIL_310B06) was sg1-2 (Coustham et al., 2014). The following mutants were previously described: edm2-4 SALK_142563 (Eulgem et al., 2007), fpa-3 (Hornyik et al., 2010), fpa-7 (Michaels and Amasino, 2001; Veley and Michaels, 2008), fpa-8 (Bäurle et al., 2007), ibm1-1 (Saze et al., 2008), ibm2-1 (Saze et al., 2013), the triple suvh456 mutant (Ebbs and Bender, 2006), cmt3-11 ibm2-5 (Coustham et al., 2014), and kyp ibm2-5 (Coustham et al., 2014). The following lines were previously described: the transgenic fpa-8 line (Col-0 background) carrying a 35S:FPA-YFP construct (Bäurle et al., 2007) and the ibm1-3 complemented lines expressing a 35S:YFP-IBM1-L cDNA (Fan et al., 2012).

For ethylmethane sulfonate (EMS) mutagenesis, ~7,000 ibm2-4 seeds were incubated in water containing 0.1% (v/v) EMS for 15 hours at room temperature and washed several times with water. Plants were then grown in pools of 16 (440 M1 pools in total) in greenhouses in long-day conditions at 20°C. The next generation was obtained by growing 200 M2 plants per pool that were selfed to obtain the M3 generation. The genetic screen was performed on M3s.

Seeds of plants grown in vitro were first surface sterilized and then sown on Gamborg B5 medium containing 1% (w/v) Sucrose. Plants were cultivated in growth chambers at 21°C in long day conditions.

Gene expression analyses

Total RNA was isolated from the aerial parts of 21-day-old seedlings grown in vitro using the RNeasy Plant Mini kit (Qiagen) followed by a DNAse treatment (Fermentas). RT-PCR was performed on 500 ng (except for RPP7 amplifications where 1 µg was used) of total RNAs with the M-MLV reverse transcriptase (Fermentas), and cDNAs were diluted 10 times. Five µl was used for RT-qPCR using a CFX96 real-time PCR machine (BioRad) with a SYBR solution (Eurogentec) and primers listed in Supplemental Table S4. Expression levels were normalized against the Arabidopsis UBC21 gene (AT5G25760).

Pathogen infection assays

To test RPP7 disease resistance function, plants were inoculated with Hyaloperonospora arabidopsidis (Hpa) isolate Hiks1, which is an oomycete pathogen specifically recognized by RPP7 in Arabidopsis accession Col-0 and virulent on accession Ksk-1 (Slusarenko and Schlaich, 2003). The function of another Hpa resistance gene, RPP4, was assessed by inoculating the plants with Hpa isolate EMWA1, which is specifically recognized by RPP4 in Arabidopsis accession Col-0 and virulent on the Col-0 eds1-2 mutant (García et al., 2012).
Briefly, 14-day-old plants were sprayed with water containing $4 \times 10^4$ Hpa Hiks1 spores per ml. Plant cell necrosis and Hiks1 or EMWA1 hyphal development were monitored by staining leaves with lactophenol trypan blue as described (Koch and Slusarenko, 1990). Stained leaves were viewed under a binocular light microscope. Infection assays were repeated independently at least three times with similar results.

**Whole-genome sequencing and bioinformatic analyses**

The genomes of *fpa-11 ibm2-4* and *ibm2-4* were sequenced using HiSeq technology (*Illumina*). Mutations were identified using the *MutDetect* pipeline described previously (Girard et al., 2014). For *fpa-3, fpa-8 35S:FPA-YFP*, and *fpa-11 ibm2-4* methylome sequencings, bisulfite treatment, library preparation, and whole-genome sequencing (final depth of 20X) were performed by the BGI (China) using HiSeq technology (*Illumina*) producing 100 bp paired-end reads (Supplemental Table S1). Reads were trimmed with *Trim_Galore* (*Babraham Bioinformatics*) and aligned to the Col-0 Arabidopsis TAIR10 reference genome with *Bismark* version 0.14.5 (*Babraham Bioinformatics*) using standard options (*Bowtie2*; 1 mismatch allowed). Identical pairs were collapsed. Subsequent analyses were done using the following R packages: *bsseq* version 1.7.7 (Hansen et al., 2012) and *DSS* version 2.11.3 (Wu et al., 2015) to call Differentially-Methylated Regions (DMRs) as previously described (Corem et al., 2018). The hcDMR pipeline was used as indicated (Zhang et al., 2018) to filter spontaneous DMRs occurring in Arabidopsis Col-0 bisulfite sequencings. DMRs arising in *fpa* and restored to wild-type patterns in *fpa* complemented lines corresponded to DMRs found between *fpa-3* and the corresponding Col-0 controls that overlapped with DMRs found between *fpa-3* and the *fpa* complemented line but not with DMRs found between the *fpa* complemented line and its Col-0 control. DMRs that remained differentially methylated when the FPA function was restored corresponded to DMRs found between *fpa-3* and the corresponding Col-0 controls that overlapped with DMRs found between the *fpa* complemented line and its Col-0 control.

For DRS analyses, we retrieved the data corresponding to the study PRJEB3993 deposited at the ENA (Duc et al., 2013). Raw DRS reads were aligned using *TopHat2* (Kim et al., 2013), allowing a maximum of two mismatches and no gaps.

For transposon expression analyses, we used *ibm2* and *aipp1* RNA-seq data described previously (Stroud et al., 2012; Saze et al., 2013; Wang et al., 2013; Duan et al., 2017). Reads were trimmed with *Trim_Galore* (*Babraham Bioinformatics*) and aligned to the Col-0 Arabidopsis TAIR10 reference genome with *HISAT2* version 2.1.0 (Kim et al., 2015) using...
standard options. Differential expression analyses were done with DESeq2 version 1.20.0 (Love et al., 2014) in R version 3.5.1. To define transposon transcripts differently expressed, we used a significance cut-off of 0.05 and a 2-fold change relative to wild type. RNA-seq read coverage files were produced and normalized with deepTools2 (Ramírez et al., 2018).

Targeted bisulfite sequencing
For each sample, 1 to 2 µg of genomic DNA was extracted from leaves corresponding to bulks of 10 to 15 plants, using the NucleoSpin Plant II kit (Macherey-Nagel). DNA was treated with bisulfite using the EpiTect Bisulfite Kit (Qiagen). Treated DNA was amplified using primers listed in Supplemental Table S4. PCR fragments were then cloned in pTOPO (Life Technologies) and sequenced individually. Results were analyzed with the Kismeth tool (Gruntman et al., 2008).

ACCESSION NUMBERS
WGBS data described in this study are available from the ENA database under the accession number PRJEB28432.

SUPPLEMENTAL DATA
Supplemental Figure S1. Expression analysis of two IBM2 targets in cmt3 ibm2 and kyp ibm2
Supplemental Figure S2. Late flowering phenotype of fpa-11 ibm2-4 and fpa-3 ibm2-4 mutants
Supplemental Figure S3. ibm2-4 suppressor screen
Supplemental Figure S4. CG and CHH methylation levels of genes in ibm2 and fpa ibm2 mutants.
Supplemental Figure S5. Schematic representation of the IBM2 target genes and localization of the regions amplified by qPCR
Supplemental Figure S6. Characterization of plants overexpressing FPA or IBM1
Supplemental Figure S7. Expression of IBM2 non-intronic target transposons
Supplemental Figure S8. Host responses and Hpa EMWA1 growth in Arabidopsis mutant lines
Supplemental Figure S9. Methylation patterns of the IBM1 intron in ibm2 and fpa mutants
Supplemental Figure S10. Methylation of IBM1 intron in fpa determined by whole-genome sequencing after bisulfite conversion
Supplemental Figure S11. Methylation profiles of transposons derepressed in fpa
Supplemental Table S1. Bisulfite sequencing statistics

Supplemental Table S2. List of transposons differentially expressed in *ibm2*, *edm2*, and *aipp1*

Supplemental Table S3. DMRs identified between *fpa-3* and the wild type in CG, CHG, and CHH contexts

Supplemental Table S4. List of primers

ACKNOWLEDGMENTS

The authors wish to thank Eric Kemen (MPIPZ) for providing Ksk-1 seeds, Thomas Eulgem (UC Riverside) for *Hpa* isolate Hiks1, Caroline Dean for the *35S:FPA-YFP* line, and Ligeng Ma for the *35S:YFP-IBM1* line. We acknowledge funding from the Agence Nationale de la Recherche (Project 11-JSV7-0013) to NB, the Max-Planck Society and a H2020-Marie Skłodowska-Curie Actions Individual Fellowship (705631-CHERI) to CLR and JEP. The Institut Jean-Pierre Bourgin benefits from the support of the LabEx Saclay Plant Sciences-SPS (Project 10-LABX-0040-SPS). We are grateful to the Genotoul bioinformatics platform Toulouse Midi-Pyrénées for providing help and computing resources.
LEGENDS OF FIGURES

Figure 1. Phenotype of the ibm2 suppressor
(A) Wild type (Col-0), ibm2-4, and the ibm2 suppressor (fpa-11 ibm2-4) plants were grown in the greenhouse and pictured after 25 days. Scale bar = 1 cm.
(B) CHG methylation levels of genes in ibm2-4 and fpa-11 ibm2-4 mutants. The average methylation levels of genes were determined by dividing the genes into 100 bp bins. Regions located 1 kb upstream and 1 kb downstream are shown.
(C) Total number of DMRs found in the three methylation contexts (mCG, mCHG, and mCHH). Hypo- and hypermethylated DMRs are shown.

Figure 2. Expression analyses of IBM2 target genes in fpa mutants
The expression of IBM2 targets were determined by RT-qPCR in ibm2-4, fpa-3, and fpa-11 back-crossed twice to Col-0 and the double ibm2-4 fpa-11 and ibm2-4 fpa-3 mutants. Results were normalized to Col-0 (expression fixed at 1 for each experiment). The PCR fragments amplified are shown in Supplemental Figure S5. Error bars represent SD (n=9). The asterisks indicate a significant difference between the sample and the corresponding Col-0 control determined by Student’s t-test (* p<0.05; ** p<0.01; *** p<0.001).

Figure 3. Reduced expression of IBM2 target genes in plants over-expressing FPA
Expression analysis of RPP7, IBM1, AT1G11270, and AT3G05410 transcript isoforms in an fpa-8 mutant complemented by a 35S:YFP-FPA construct resulting in the over-expression of FPA (Supplemental Figure S6). The expression was determined by RT-qPCR using primers (Supplemental Table S4) specific of IBM2 targets as shown in Supplemental Figure S5. Error bars represent SD (n=9). The asterisks indicate a significant difference between the sample and the corresponding Col-0 control determined by Student’s t-test (*** p<0.001).

Figure 4. Host RPP7 resistance and Hpa Hiks1 growth in Arabidopsis mutant lines
Two-week-old seedlings of the indicated genotypes were inoculated with Hpa Hiks1 (see Materials and Methods). At 4 days after inoculation, the two first true leaves of >10 plants per genotype were stained with lactophenol trypan blue to reveal necrotic plant cells and pathogen structures. Hpa Hiks1 is recognized by resistance gene RPP7. Col-0 expressing RPP7 is resistant and Ksk-1 lacking RPP7 is susceptible to Hpa Hiks1 infection. Hpa Hiks1 hyphal growth is restricted at HR sites in Col-0 whereas hyphae ramify through Ksk-1 leaves. Col-0
IBM2 or IBM2-4 and Col-0 SUVH456 display reduced RPP7 resistance to Hpa Hiks1. EDM2 is regulating RPP7 transcript levels and Col-0 edm2-4 mutants are therefore susceptible to Hpa Hiks1 (Eulgem et al., 2007). fpa-11 ibm2-4 double mutant exhibits intermediate RPP7 resistance. Col-0 fpa-3 or fpa-11 mutants and Col-0 ibm1-1 are not affected in RPP7 resistance. Plants shown were grown and inoculated at the same time. Similar results were obtained in three independent experiments. HR, host hypersensitive response; h, Hpa Hiks1 hyphae; Sp, Hpa Hiks1 sporangiophore. Scale bars = 1 mm.

Figure 5. Polyadenylation of IBM2 intronic targets

Reads corresponding to the direct RNA sequencing (DRS) of fpa-7 and the Col-0 wild type (Duc et al., 2013) were aligned to the sequence of Col-0 (TAIR10 version).

(A) DRS reads for IBM1, AT3G05410, and RPP7 loci were visualized using the Integrated Genome Browser (IGB). Proximal (Prox.) and distal (Dist.) polyadenylation sites are indicated. Each biological repeat is presented individually (rep#1 to #3). The scale is identical for all repeats presented for a given gene. The gene model is shown with exons represented by black boxes.

(B) DRS reads mapping the proximal or distal polyadenylation site regions were counted and normalized in reads per million mapped reads (RPM). Error bars represent SD (n=3). The asterisks indicate a significant difference between the sample and the Col-0 control determined by Student’s t-test (* p<0.05; ** p<0.01).

Figure 6. Expression analysis of IBM2 non-intronic target transposons

(A) Schematic representation of the Copia (AT4G16870) - RPP4 (AT4G16860) locus targeted by IBM2. The exons of RPP4 are in blue, and the Copia element is in green.

(B) Expression analysis of RPP4, AT4G16870, and the chimeric RPP4-AT4G16870 transcripts in Col-0 and different mutant backgrounds. cDNAs were amplified using primers indicated in (A) and described previously (Wang and Warren, 2010). ATEF cDNA amplifications served as controls.

(C) Expression analysis of AT4TE21110 in Col-0 wild type and different mutant backgrounds. AT4TE21110 is localized in the pericentromeric region of chromosome 4. RNAs were extracted from bulks (#1 and #2) of 20 plants grown in vitro for 15 days and cDNAs were amplified using primers described in Supplemental Table S4. ACTIN cDNA amplifications served as controls.

Figure 7. Methylation of IBM1 intron in fpa mutants and an IBM1 overexpressing line
(A) The methylation levels within the large intron of *IBM1*, in the region containing the heterochromatic marks (chromosome 3, position 2,430,285 to 2,430,595), are indicated. Data were obtained by amplifying the region after bisulfite conversion and correspond to the average methylation ratio determined between the repeats (Supplemental Figure S9).

(B) Methylation on top (positive values) and bottom (negative values) strands across the coding sequence of *IBM1* in *fpa-3*. The *IBM1* gene model is shown according to TAIR10. Mean methylation levels per cytosine are plotted on a 0 to 100% scale for each strand. Data correspond to the combination of two biological repeats for each genotype. CG methylation is in red, CHG in blue, and CHH in green. The CHG hypoDMR identified between *fpa-3* and Col-0 is represented by a blue rectangle.

**Figure 8. Patterns of methylation in *fpa***

(A) Pairwise comparison of methylation in wild type and *fpa-3* mutants. Each dot represents a 100 bp-window, and their methylation levels were determined as follows. The Arabidopsis genome (TAIR10 release) was partitioned in 100 bp-tiles and methylation levels correspond to the ratios of methylated cytosines over the total number of cytosines. Only cytosines covered by at least five reads were considered. The average methylation levels were determined by combining the two biological replicates for each genotype. The color scale measures the density of points (red being very dense). The Pearson correlation coefficients between the samples are 0.97 for mCG, 0.98 for mCHG and 0.94 for mCHH.

(B) Nature of CG hypo- and hyperDMRs identified in *fpa-3*. ‘gene+TE’ corresponds to DMRs overlapping with both genes and transposons, ‘gene’ corresponds to DMRs overlapping with genes, and ‘TE’ corresponds to DMRs overlapping with transposons. All other DMRs were classified as ‘Intergenic’.

(C) Methylation levels of CG hypo- and hyperDMRs. The average methylation levels of the DMRs were determined by dividing the DMR into 100 bp bins. Regions located 1 kb upstream and 1 kb downstream are shown.
LEGENDS OF SUPPLEMENTARY MATERIALS

Supplemental Figure S1. Expression analysis of two IBM2 targets in cmt3 ibm2 and kyp ibm2
RT-qPCR analyses of RPP7-L and AT3G05410-L expression in Col-0, ibm2-4, cmt3-11 ibm2-5 (Coustham et al., 2014) and kyp ibm2-5 (Coustham et al., 2014). Results were normalized to Col-0 (expression fixed at 1 for each experiment). Error bars represent SD (n=4).

Supplemental Figure S2. Late flowering phenotype of fpa-11 ibm2-4 and fpa-3 ibm2-4 mutants

Supplemental Figure S3. ibm2-4 suppressor screen
Expression analyses of an IBM2 target in 9 M3 plants (#1 to #9) retrieved from the ibm2-4 suppressor screen. RNAs of plants were extracted and cDNAs were amplified using primers corresponding to AT3G05410-L (Supplemental Figure S5). ATEF amplifications served as controls. The GeneRuler DNA Ladder Mix (Ref SM0331, Thermo) is the DNA ladder used.

Supplemental Figure S4. CG and CHH methylation levels of genes in ibm2 and fpa ibm2 mutants
The average methylation levels of genes were determined by dividing the genes into 100 bp bins. Regions located 1 kb upstream and 1 kb downstream are shown.

Supplemental Figure S5. Schematic representation of the IBM2 target genes and localization of the regions amplified by RT-qPCR
Schematic representation of the IBM2 target gene drawn to scale. The region (chromosome 3, position 2,430,285 to 2,430,595) containing intronic heterochromatic marks in IBM1 is represented by a blue bar.

Supplemental Figure S6. Characterization of plants overexpressing FPA or IBM1
RNAs were extracted from bulks of plants carrying the 35S:FPA-YFP or 35S:YFP-IBM1 constructs. Both FPA (A) or IBM1-L (B) cDNAs were amplified using specific primers (Supplemental Table S4). ATEF or ACTIN cDNA amplifications served as controls.

Supplemental Figure S7. Expression of IBM2 non-intronic target transposons
RNA-seq data were retrieved from the following GEO projects: GSE38286 for *suvh456* and the three corresponding wild-type repeats (Stroud et al., 2012), GSE98655 for *aipp1-1, edm2-4, ibm2/asi1-2* and the two wild-type repeats (Duan et al., 2017), GSE48026 for *asi1-1* and the corresponding wild type (Wang et al., 2013) and PRJDB2180 for *ibm1-4, ibm2-2* and the wild type (Saze et al., 2013). The methylation levels for mCG, mCHG and mCHH are shown for the wild type and *fpa-3* plants. The screenshots were obtained with Integrative Genome Browser (IGB). The scales indicated on the left are identical for all RNA-seq or methylation tracks, respectively. *RPKM*: Reads Per Kilobase per Million mapped reads. (A) *RPP4* locus, (B) *AT4TE21110* locus.

**Supplemental Figure S8. Host responses and *Hpa* EMWA1 growth in Arabidopsis mutant lines**

Two-week-old seedlings of the indicated genotypes were inoculated with *Hpa* EMWA1 (see Materials & Methods). At 4 days after inoculation, the two first true leaves of >10 plants per genotype were stained with lactophenol trypan blue to reveal necrotic plant cells and pathogen structures. *Hpa* EMWA1 is specifically recognized by *RPP4*. Col-0 expressing *RPP4* is resistant and *eds1-2* mutants (García et al., 2010) are susceptible to *Hpa* EMWA1 infection. *Hpa* EMWA1 hyphal growth is restricted at HR sites in Col-0 whereas hyphae ramify through *eds1-2* leaves. None of the mutants (in the Col-0 background) are affected in *RPP4* resistance. Plants were grown and inoculated at the same time. Similar results were obtained in three independent experiments.

**Supplemental Figure S9. Methylation patterns of the *IBM1* intron in *ibm2* and *fpa* mutants**

After bisulfite conversion of DNAs, the region indicated in Supplemental Figure S5 (*IBM1*, blue bar) was amplified in the large intron of *IBM1* using primers described in Supplemental Table S4. Samples are corresponding to leaves from 10 to 15 plants bulked together. The number of clones sequenced (n) is indicated. Sequences were aligned and the methylation quantified using the Kismeth tool (Gruntman et al., 2008). Cytosines are represented by circles (red for CGs, blue for CHGs, green for CHHs; solid circles: methylated cytosines).

**Supplemental Figure S10. Methylation of *IBM1* intron in *fpa* determined by whole-genome sequencing after bisulfite conversion**
The methylation levels within the large intron of *IBM1*, in the region containing heterochromatic marks (chromosome 3, position 2,430,285 to 2,430,595), are indicated. Data for three wild-type (Col-0) repetitions, *fpa*-7, *fca*-9 *fpa*-7 and *suvh4* were obtained from raw data publicly available (Stroud et al., 2013). Data for *ibm2*-4 and the corresponding wild type (Col-0) were obtained from our previous methylome sequencing data (Coustham et al., 2014).

Supplemental Figure S11. Methylation profiles of transposons derepressed in *fpa*

Mean methylation levels for mCG, mCHG and mCHH were obtained by combining the two biological replicates for the wild type or *fpa*-3, respectively. The screenshots were obtained with Integrative Genome Browser (IGB). HypoDMRs between the mutant and the wild type are contained in the boxed area. The scale is identical for all tracks.

Supplemental Table S1. Bisulfite sequencing statistics

<table>
<thead>
<tr>
<th>Col #1, biological replicate #1</th>
<th>Col-0 methylome sequencing; Col #2, biological replicate #2</th>
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| Col-0 methylome sequencing; *fpa*-3 #1, biological replicate #1 *fpa*-3 mutant methylome sequencing; *fpa*-3 #2, biological replicate #1 *fpa*-3 mutant methylome sequencing; 35S:*FPA*-YFP, 35S:*FPA*-YFP methylome sequencing; Col-0 (35S:*FPA*-YFP control), Col-0 methylome sequencing; *fpa*-11 *ibm2*-4, *fpa* *ibm2* methylome sequencing. To determine the bisulfite conversion rates, reads were aligned to the TAIR10 Arabidopsis chloroplast sequence.

Supplemental Table S2. Transposons differentially expressed in *ibm2*, *edm2* and *aipp1* compared to wild type

The IBM2 targets previously identified are in red.

Supplemental Table S3. DMRs identified between *fpa*-3 and the wild type in CG, CHG and CHH contexts

DMRs were defined using two biological repeats per genotype with the bsseq and DSS R packages (see Materials & Methods).

Supplemental Table S4. List of primers
REFERENCES


Figure 1. Phenotype of the *ibm2* suppressor

(A) Wild type (Col-0), *ibm2*-4, and the *ibm2* suppressor (*fpa-11 ibm2*-4) plants were grown in the greenhouse and pictured after 25 days. Scale bar = 1 cm.

(B) CHG methylation levels of genes in *ibm2*-4 and *fpa-11 ibm2*-4 mutants. The average methylation levels of genes were determined by dividing the genes into 100 bp bins. Regions located 1 kb upstream and 1 kb downstream are shown.

(C) Total number of DMRs found in the three methylation contexts (mCG, mCHG, and mCHH). Hypo- and hypermethylated DMRs are shown.
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The expression of IBM2 targets were determined by RT-qPCR in *ibm2-4, fpa-3,* and *fpa-11* back-crossed twice to Col-0 and the double *ibm2-4 fpa-11* and *ibm2-4 fpa-3* mutants. Results were normalized to Col-0 (expression fixed at 1 for each experiment). The PCR fragments amplified are shown in Supplemental Figure S5. Error bars represent SD (n=9). The asterisks indicate a significant difference between the sample and the corresponding Col-0 control determined by Student’s *t*-test (* *p*<0.05; ** *p*<0.01; *** *p*<0.001).
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Expression analysis of RPP7, IBM1, AT1G11270, and AT3G05410 transcript isoforms in an fpa-8 mutant complemented by a 35S::YFP-FPA construct resulting in the over-expression of FPA (Supplemental Figure S6). The expression was determined by RT-qPCR using primers (Supplemental Table S4) specific of IBM2 targets as shown in Supplemental Figure S5. Error bars represent SD (n=9). The asterisks indicate a significant difference between the sample and the corresponding Col-0 control determined by Student’s t-test (*** p<0.001).
Figure 4. Host RPP7 resistance and Hpa Hiks1 growth in Arabidopsis mutant lines.

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Figure 5. Polyadenylation of IBM2 intronic targets

Reads corresponding to the direct RNA sequencing (DRS) of fpa-7 and the Col-0 wild type (Duc et al., 2013) were aligned to the sequence of Col-0 (TAIR10 version).

(A) DRS reads for IBM1, AT3G05410, and RPP7 loci were visualized using the Integrated Genome Browser (IGB). Proximal (Prox.) and distal (Dist.) polyadenylation sites are indicated. Each biological repeat is presented individually (rep#1 to #3). The scale is identical for all repeats presented for a given gene. The gene model is shown with exons represented by black boxes.

(B) DRS reads mapping the proximal or distal polyadenylation site regions were counted and normalized in reads per million mapped reads (RPM). Error bars represent SD (n=3). The asterisks indicate a significant difference between the sample and the Col-0 control determined by Student’s t-test (* p<0.05; ** p<0.01).
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(A) Schematic representation of the Copia (AT4G16870) - RPP4 (AT4G16860) locus targeted by IBM2. The exons of RPP4 are in blue, and the Copia element is in green.

(B) Expression analysis of RPP4, AT4G16870, and the chimeric RPP4-AT4G16870 transcripts in Col-0 and different mutant backgrounds. cDNAs were amplified using primers indicated in (A) and described previously (Wang and Warren, 2010). ATEF cDNA amplifications served as controls.

(C) Expression analysis of AT4TE21110 in Col-0 wild type and different mutant backgrounds. AT4TE21110 is localized in the pericentromeric region of chromosome 4. RNAs were extracted from bulks (#1 and #2) of 20 plants grown in vitro for 15 days and cDNAs were amplified using primers described in Supplemental Table S4. ACTIN cDNA amplifications served as controls.
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(A) The methylation levels within the large intron of \textit{IBM1}, in the region containing the heterochromatic marks (chromosome 3, position 2,430,285 to 2,430,595), are indicated. Data were obtained by amplifying the region after bisulfite conversion and correspond to the average methylation ratio determined between the repeats (Supplemental Figure S9).

(B) Methylation on top (positive values) and bottom (negative values) strands across the coding sequence of \textit{IBM1} in \textit{fpa}-3. The \textit{IBM1} gene model is shown according to TAIR10. Mean methylation levels per cytosine are plotted on a 0 to 100% scale for each strand. Data correspond to the combination of two biological repeats for each genotype. CG methylation is in red, CHG in blue, and CHH in green. The CHG hypoDMR identified between \textit{fpa}-3 and Col-0 is represented by a blue rectangle.
Figure 8. Patterns of methylation in *fpa*

(A) Pairwise comparison of methylation in wild type and *fpa*-3 mutants. Each dot represents a 100 bp-window, and their methylation levels were determined as follows. The Arabidopsis genome (TAIR10 release) was partitioned in 100 bp-tiles and methylation levels correspond to the ratios of methylated cytosines over the total number of cytosines. Only cytosines covered by at least five reads were considered. The average methylation levels were determined by combining the two biological replicates for each genotype. The color scale measures the density of points (red being very dense). The Pearson correlation coefficients between the samples are 0.97 for mCG, 0.98 for mCHG and 0.94 for mCHH.

(B) Nature of CG hypo- and hyperDMRs identified in *fpa*-3. ‘gene+TE’ corresponds to DMRs overlapping with both genes and transposons, ‘gene’ corresponds to DMRs overlapping with genes, and ‘TE’ corresponds to DMRs overlapping with transposons. All other DMRs were classified as ‘Intergenic’.

(C) Methylation levels of CG hypo- and hyperDMR. The average methylation levels of the DMRs were determined by dividing the DMR into 100 bp bins. Regions located 1 kb upstream and 1 kb downstream are shown.


regulates plant DNA methylation by controlling mRNA processing at the intronic heterochromatin-containing gene IBM1. Proc Natl Acad Sci U S A 110: 15467-15472


