

Role of alternative polyadenylation in epigenetic silencing and antisilencing

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Epigenetic marks such as DNA methylation and histone modifications are widely involved in regulating different aspects of developmental and environmental responses (1). Meanwhile, DNA methylation and histone modification are also used constitutively to silence transposable elements and repeat elements (TREs) (2). Such TRE-mediated silencing should necessarily be limited to the intended targets only and not spread to adjacent genes and their regulatory elements. Higher eukaryotic organisms have evolved antisilencing mechanisms to keep the balance between silencing and antisilencing that is required for precise gene expression regulation. Earlier work revealed that *repressor of silencing 1* (*ROS1*) is one such antisilencing gene (3). Recently, a group of unique antisilencing genes has been discovered, including *enhanced downy mildew 2* (*EDM2*) reported both by Lei et al. in PNAS (4) and earlier by Tsuchiya and

Eulgem (5), *anti-silencing 1* (*ASI1*) by Wang et al. (6), and *increase in bonsai methylation 1* (*IBM1*) by Saze et al. (7). What distinguishes the latter group of genes from *ROS1* is that they seem to be involved in posttranscriptional regulation through alternative polyadenylation (APA).

Polyadenylation is an essential mRNA processing step for almost all genes in eukaryotes (8, 9). The poly(A) site defines the end of the transcript and thus its 3'-UTR. However, an alternate site of processing and poly(A) addition would create a different transcript by an inclusion or exclusion of certain RNA sequences such as the miRNA target site, a recognition site of an mRNA stability factor, or a translational repressor. In recent years, APA has been shown to change the fate of a cell or a developmental process and to alter cellular responses to the environment, including tumorigenesis, flowering time control, and

oxidative responses (10–12). Genomewide analyses by deep sequencing have revealed that 70–80% of human and plant genes are subject to APA (13–15). The role of such abundant APAs in cellular functions, however, remains largely unknown.

It was previously suggested that histone modification might influence poly(A) choice through correlation analysis (16). The significance of the work on *EDM2* and *ASI1* is that they provide direct genetic evidence demonstrating an involvement of epigenetic marks in the selection of APA sites. *EDM2*, identified as an enhancer of fungal disease resistance (17), specifically recognizes intronic heterochromatin in its target genes presumably through its composite plant homeodomain (PHD) domain (4). This binding of *EDM2* somehow blocks the poly(A) site choice of the transcript in the vicinity of the binding location. This is deduced from the fact that in the *edm2* mutant, use of intronic poly(A) sites [so called proximal poly(A) sites, in contrast to the full-length distal poly(A) sites] results in the generation of shortened transcripts that encode no or nonfunctional proteins (4, 5). In other words, the normal function of *EDM2* is to reduce the production of nonfunctional transcripts and to promote the generation of full-length mRNA ending at the distal poly(A) site. The net result is the avoidance of reduced gene expression caused by silencing due to TREs in the intron (hence antisilencing).

The antisilencing phenomena of *EDM2* were clearly demonstrated in the APA of a group of genes with large TRE-containing genes such as the fungal disease resistance gene *resistance to Peronospora parasitica* (*RPP7*) (5) and *IBM1* (4). Interestingly, another gene (*ASI1*) was also found to have very similar function of generating APA of *IBM1* transcripts (6). Adding an additional layer of complexity to this story is the fact that *IBM1* itself is a histone H3K9 demethylase that prevents plant-specific CHG methylation (7).

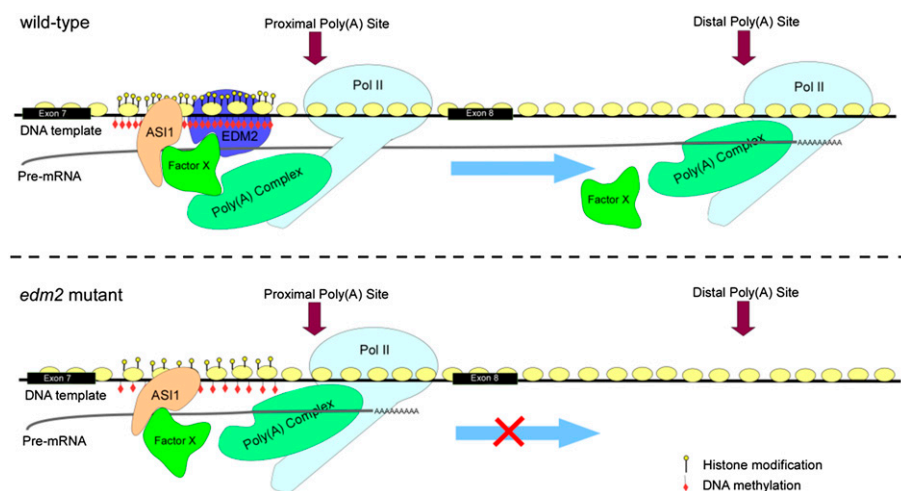


Fig. 1. Hypothetic model for antisilencing regulation of alternative polyadenylation. In WT *Arabidopsis*, *EDM2* and *ASI1* bind to the intronic heterochromatin region by recognizing enriched epigenetic marks. With the assistance of an unknown factor X, the three proteins form a complex that potently masks the poly(A) signals from being recognized by the polyadenylation apparatus riding on the C-terminal domain of RNA polymerase II. Consequently, the distal poly(A) site is used. In the *edm2* or *asi1* mutant, absence of either *EDM2* or *ASI1* reduces the masking effect on the proximal poly(A) site. The result is an extensive use of the proximal site, producing shortened transcripts encoding nonfunctional proteins, leading to a silencing effect of the target gene.

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Moreover, genomewide epigenetic modification profiles of all three mutants (*asi1*, *edm2*, and *ibm1*) largely overlap, which indicates that they work together through a similar antisilencing pathway. In fact, it was suggested that both ASI1 and EDM2 function mostly through IBM1 by modulating APA of *IBM1* transcripts (4, 5).

The question is how are these epigenomic marks sensed by the polyadenylation machinery so that a different poly(A) site is chosen? Polyadenylation is a cotranscriptional event where numerous factors affecting transcription would impact polyadenylation (18). The polyadenylation machinery is composed of 20–25 different proteins in plants that recognize a set of poly(A) signals on the pre-mRNA (9). The use of one poly(A) site over the other is likely owing to the relative strength of the polyadenylation signal being perceived by poly(A) machinery, as well as the availability of the site. It is also possible that associated protein(s) may recruit (or repel) the polyadenylation apparatus close to a particular site. It is equally possible that a protein could inhibit the function of the complex. One possibility in the EDM2 or ASI1 cases is that, while binding to epigenetic marks, these proteins also interact with the poly(A) signals, thus blocking the recognition of the proximal poly(A) site. Indeed, ASI1 was found to have an RNA recognition motif and a bromo-adjacent homology domain involved in preventing the genomewide CHG methylation (6). EDM2, on the other hand, possesses an N6-adenine methyltransferase domain (4), providing a potential to methylate adenine residues in RNA. Methylated adenine was shown to affect 3'-end formation (19). Binding of EDM2 to chromatin through its PHD domain could bring the methyltransferase domain to close proximity to the nascent RNA. However, there is no evidence to show a direct interaction between ASI1 and EDM2. Thus, a conceivable model would be that both ASI1 and EDM2 bind to target DNA and to associated nascent RNA, forming a complex through an unidentified factor X. The unknown factor may also mediate the interaction with the polyadenylation complex that is associated to the C-terminal domain of RNA polymerase II (Fig. 1).

The analysis of both *edm2* and *asi1* mutants also implied that the transcription rates of *IBM1* and *RPP7* do not change in the mutants, because there were no differences in the distribution of pol II along the genes (4–6). Clearly this is a posttranscriptional regulation event. Particularly in *RPP7*, the degree

of disease resistance conferred is tightly associated with its transcript level (5). Could the APA just be a byproduct of other modes of regulation? This possibility is very unlikely. There exist other examples showing that APA is a predominant way to attenuate the expression of

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genes. One involves the *flower control locus A (FCA)* gene, a regulator of *flower locus C (FLC)* expression, and of flowering time in *Arabidopsis* (12). Another is the *oxidative tolerant 6 (OXT6)* gene in *Arabidopsis* that encodes two proteins: a smaller one produced through use of an intronic poly(A) site and a larger one produced using a distal poly(A) site (11). Both *FCA* and *OXT6* genes have large introns where the proximal APA occurs. However, it seems that these APA events use a different mechanism because these introns do not contain TREs (4). Recent work by Duc et al. indicated that a spen family protein FPA might be involved in the selection of some intronic polyadenylation (20). Nonetheless, recent genome-level profiling discovered a significant amount

of intronic poly(A) sites in *Arabidopsis* and rice (14, 15). Further analysis of these intronic sites would broaden our view on the interrelationship of RNA processing and epigenetic regulation.

The connection between gene silencing and polyadenylation was demonstrated by Herr et al. (21) where mutations of poly(A) protein factors led to enhanced silencing of some genes. This work is indicative that normal functions of these polyadenylation factors prevent silencing of the tested genes, an equivalent to an antisilencing effect. While there was not clear demonstration of the involvement of TREs, this is further evidence that motivates investigations to elucidate a potential direct engagement of poly(A) factors in antisilencing.

Meanwhile, many interesting questions remain. What are the specific functions of ASI1 and EDM2 during this process? If any, what are other factors involved? Is binding of ASI1 or EDM2 to intronic heterochromatin region needed to influence poly(A) site choice? Is RNA modification (e.g., methylation) or RNA secondary structure involved? What is the role of the splicing of the large intron in all this? Addressing these questions would ensure a fine-grained understanding of posttranscriptional regulation of genes in this ever-increasing complex world of the epigenome.

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