Splicing and dicing with a *SERRATE*d edge

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The maturation and quality control of mRNA in eukaryotes is a tightly regulated, multistep process that begins on nascent transcripts. A 7-methyl guanosine (^{7}MeG) cap structure is added to the 5′ end of pre-mRNA as it emerges from the exit channel of RNA Polymerase II. The multifunctional nuclear cap-binding complex (CBC), consisting of two protein subunits (CBP80 and CBP20), assembles at the pre-mRNA cap early during transcript formation and helps recruit the splicesome machinery to the cap-proximal intron (1, 2). Termination of transcription involves cleavage and polyadenylation at the 3′ end, and the mature mRNA is retained in the nucleus or exported to the cytoplasm. In either case, the mRNA undergoes a pioneer round of translation and surveillance by the nonsense-mediated mRNA decay (NMD) pathway to eliminate defective or misspliced transcripts. Although the CBC localizes primarily to the nucleus, it remains associated with mRNAs during export to the cytoplasm and during the pioneer round of translation and mRNA surveillance. After the first round of translation, the CBC is replaced by the eukaryotic initiation complex eIF4F, and the mRNA steady-state translation initiation complex is formed (3). But not all RNA Pol II transcripts are predestined for translation. Primary transcripts for microRNA (pri-miRNA) are retained in the nucleus, where they are processed into ≈21- to 22-nt miRNA that generally function as posttranscriptional regulators of mRNA expression. Although it is known that pri-miRNA transcripts form by RNA Pol II transcription, the extent to which pri-miRNA and pre-mRNA transcripts share common processing components is far from settled. In this issue of PNAS, Laubinger *et al.* (4) identify an important relationship between mRNA maturation and miRNA primary transcript processing in plants.

The CBC was initially identified in human cells through its role in splicing and has since been shown to be important for multiple mRNA functions (1, 5). The plant homolog of the large subunit of the CBC, CBP80, was first isolated in a genetic screen for *Arabidopsis thaliana* mutants with hypersensitivity to the plant hormone abscisic acid (ABA) and thus was designated *ABA HYPERSENSITIVE1* (*ABH1*). ABH1/CBP80 was shown to interact with an *Arabidopsis* homolog of the smaller subunit of the CBC, CBP20, and to form a complex that binds the ^{7}MeG cap of mRNA, confirming that the CBC shares biochemical functions in plants, humans, and yeast (6). Although ABH1/CBP80 and CBP20 are not essential for plant viability under controlled or luxurious growth conditions, plants lacking or deficient in these factors display pleiotropic abnormalities, including jagged-edge leaf morphology defects and increased drought tolerance (6–8).

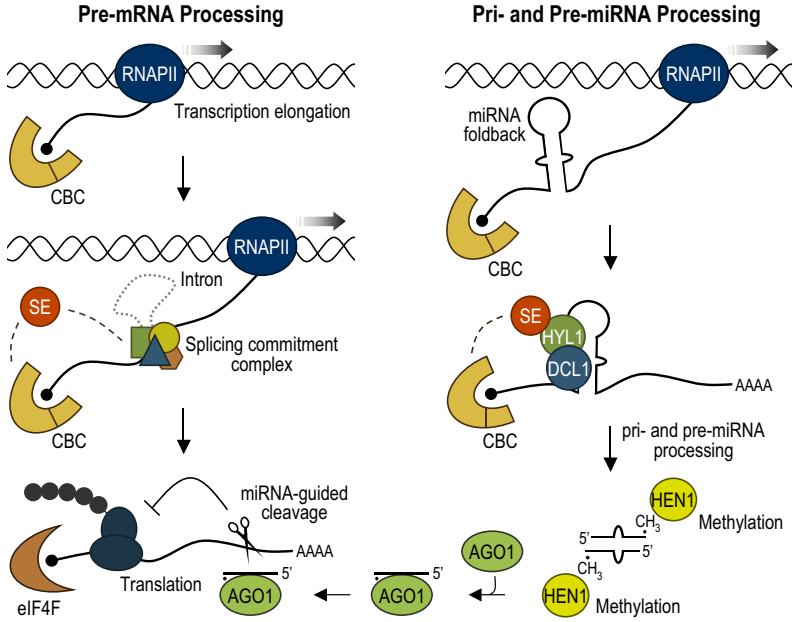

Fig. 1. Pre-mRNA and pri-miRNA processing in plants. The CBC consists of two subunits: CBP20 and CBP80. The eIF4F complex consists of eIF4E, eIF4A, and eIF4G. The AGO-miRNA complex suppresses target mRNA by cleavage or through translational repression. SE, SERRATE.

Laubinger *et al.* (4) recognized, as did others (7, 9), that the serrated leaf phenotype of *abh1*/*cbp80* and *cbp20* mutant plants was reminiscent of a phenotype observed nearly 40 years earlier in the mutant *serrate* (10). Convergence of this specific leaf phenotype could mean that the affected genes function in the same biochemical or developmental pathway. Whereas *abh1*/*cbp80* and *cbp20* mutants have relatively mild phenotypes, hypomorphic *serrate* alleles have a variety of severe developmental defects relating to developmental timing, phylotaxy, meristem function, and patterning in leaves and flowers (11–13). *serrate*-null mutants are embryonic-lethal (14), and, similar to *abh1*/*cbp80* mutants, *serrate* mutants are hypersensitive to ABA (7). *SERRATE* encodes a zinc-finger protein (12). Although *SERRATE* had not been directly implicated in mRNA metabolism, Bezerra *et al.* (7) presciently suggested that *SERRATE* and the CBC might function through a common mechanism involving RNA metabolism. More recently, *SERRATE* was shown to be a critical component of the pri-miRNA processing machinery and to interact and colocalize with other miRNA biogenesis factors (14–17). Until now, the link between *SERRATE* and the CBC remained elusive.

SERRATE is one of several factors involved in miRNA processing. Similar to other RNA Pol II products, pri-mRNA transcripts contain a ^{7}MeG cap structure at the 5′ terminus and a 3′ poly(A) tail, but they are distinguished from mRNA by their lack of a functional coding sequence and possession of a self-complementary foldback re-

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gion. In addition to SERRATE, miRNA processing in plants requires DICER-LIKE1 (DCL1), an RNase III-type enzyme, and HYPONASTIC LEAVES1 (HYL1), a double-stranded RNA-binding protein. Hypomorphic mutations in any of the critical components of the miRNA processing machinery cause severe developmental defects. Similar to *SERRATE*, null alleles of *DCL1* are embryonic-lethal. Null alleles of *HYL1* are viable, possibly because of redundancy with closely related factors. After processing, the mature miRNA is loaded as a guide RNA into a complex containing an ARGONAUTE (AGO) protein, the effector component. The AGO–miRNA complex functions to suppress transcripts by either irreversible cleavage or translational repression (Fig. 1) (18).

Laubinger *et al.* (4) describe an elegant series of experiments using whole-genome tiling array technology, as well as traditional RNA blot and PCR assays, to examine the roles of the CBC and SERRATE in both mRNA splicing and miRNA processing. The authors first tested whether the CBC was required for normal accumulation of miRNA by using RNA blot assays. Strikingly, several miRNA tested by blot assays were reduced in *abh1*/*cbp80* and *cbp20* mutants, relative to wild-type plants, indicating a previously unknown role for the CBC in the miRNA pathway.

The reduction in miRNA levels observed in *abh1*/*cbp80* and *cbp20* mutants could be due to reduced accumulation of the pri-miRNA, defective processing of pri-miRNA transcripts into mature miRNA, or destabilization of the mature miRNA. To distinguish among these possibilities, the authors analyzed pri-miRNA transcript levels at great depth in *abh1*/*cbp80*, *cbp20*, and *serrate* mutants. More than 160 pri-miRNA transcripts were analyzed by using a tiling array platform containing 3.2 million probe pairs tiled across most of the non-repetitive component of the *Arabidopsis* genome. A subset of the pri-miRNAs analyzed had significantly elevated levels in *abh1*/*cbp80*, *cbp20*, and *serrate* mutants relative to wild-type plants. Although *serrate* mutants had the broadest effect on pri-miRNA processing, considerable overlap was seen in the pri-miRNA affected in *serrate*, *abh1*/*cbp80*, and *cbp20* mutants. This indicates a novel role for the CBC in pri-miRNA processing and provides a comprehensive analysis of SERRATE-dependent miRNA loci. Another recent study, using a similar tiling array-based approach, also identified a role for ABH1/CBP80 in pri-miRNA processing (9).

Although the CBC was previously shown to be an important factor in recruiting splicing machinery to pre-mRNA in yeast (2), there was only suggestive evidence that it was involved in splicing in *Arabidopsis* as well (19). Armed with whole-genome tiling array data, Laubinger *et al.* (4) set out to exhaustively analyze the role of the CBC, as well as the role of SERRATE, in splicing across the *Arabidopsis* genome. The authors identified a significant number of introns that had elevated hybridization signals in the *abh1*/*cbp80* and *cbp20* mutants, indicating intron retention resulting from decreased splicing efficiency. Surprisingly, *serrate* mutants also had elevated hybridization signals for a partially overlapping, although somewhat smaller, set of introns. In each CBC mutant, and *serrate*, typically only a single intron from a gene was affected, most commonly the 5′ cap proximal intron, consistent with the role of the CBC in yeast (1, 2). Importantly, the authors observed no difference in intron retention in *dcl1* and *hyl1* mutants relative to wild-type plants, indicating that the splicing defect observed in *serrate* mutants was not an indirect consequence of disruption of the miRNA pathway. These findings indicate a novel role for SERRATE in mRNA splicing that is independent of its role in pri-miRNA transcript processing and also point to independent roles for the CBC in pri-miRNA processing and mRNA splicing.

From this and previous studies, a picture emerges of SERRATE as a mediator between the CBC and both the splicing commitment complex and the pri-miRNA processing machinery. One possibility is that SERRATE functions as a physical link between the CBC and distinct RNA processing machineries for pre-mRNA splicing and pri-miRNA processing (Fig. 1). SERRATE was previously shown to interact with HYL1 and DCL1 in specialized nuclear dicing bodies (16, 17). It will be important to learn whether SERRATE also interacts with components of the CBC and splicing commitment complex. Unlike SERRATE, the CBC is not essential for survival, indicating that SERRATE might interact with other factors bound to the 5′ cap, such as the eIF4F complex, to mediate loading of splicing and pri-miRNA processing machinery. Does a similar mediator function in mRNA and miRNA metabolism in other eukaryotes? There are several potential homologs of *SERRATE* in nonplant species, including the mammalian gene *ASR2*, as well as genes in *Drosophila*, *Caenorhabditis elegans*, and *Schizosaccharomyces pombe* (12). The CBC, which is conserved across eukaryotes, may function in pri-miRNA processing in other eukaryotes as well. In support of this idea, a mutant for one component of the cap-binding complex was identified in a screen for RNAi-deficient mutants in *C. elegans* (20). Finally, given its role in splicing and dicing, one wonders whether additional roles are yet to be discovered for *SERRATE* in RNA metabolism.

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