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Review

SERRATE: a key factor in coordinated RNA processing in plants

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The SERRATE (SE) protein is involved in the processing of RNA polymerase II (RNAPII) transcripts. It is associated with different complexes engaged in different aspects of plant RNA metabolism, including assemblies involved in transcription, splicing, polyadenylation, miRNA biogenesis, and RNA degradation. SE stability and interactome properties can be influenced by phosphorylation. SE exhibits an intriguing liquid–liquid phase separation property that may be important in the assembly of different RNA-processing bodies. Therefore, we propose that SE seems to participate in the coordination of different RNA-processing steps and can direct the fate of transcripts, targeting them for processing or degradation when they cannot be properly processed or are synthesized in excess.

Highlights

SERRATE (SE) is a key factor for processing all RNA polymerase II transcripts.

To date, the identity of least 50 proteins that directly interact with SE has been validated, which implies that SE is a master regulator of RNA metabolism.

The phosphorylation status of SE influences its stability and interactome properties.

Liquid–liquid phase separation is triggered by SE.

SE: an important factor in RNA metabolism

Protein coding genes are transcribed in plants, as in all other eukaryotes, by **RNAPII** (see Glossary). The path from gene transcription to protein production is long and, during this journey, the primary transcripts synthesized through RNAPII are extensively modified. Shortly after RNAPII initiates transcription, a cap structure (N7-methylated guanosine) is added to the first transcribed nucleotide in all RNAPII primary transcripts [1]. This cap structure is immediately recognized and bound by the nuclear **Cap-binding protein complex (CBC)**, which in plants interacts with the conserved multifunctional scaffold protein **SE** [the mammalian homolog of SE is Arsenite resistance protein 2 (ARS2)] and is part of the protein complex that binds to the cap [2–4].

SE is essential for cell viability and a null mutant, *se-4*, is embryonic lethal, similar to mammalian ARS2-null embryos that die at the approximate time of implantation [5,6]. However, over the years, several arabidopsis (*Arabidopsis thaliana*) hypomorphic mutants of *SE* have been described: *se-1*, *se-2*, and *se-3* [6–8]. Studies carried out on viable SE mutants showed that a small deletion in the 3′ end of the *SE* gene (*se-1*) or a T-DNA insertion in the tenth exon (*se-2*) or the first exon (*se-3*) of the SE-encoding gene causes a wide range of developmental disorders, including aberrant phyllotaxy and altered meristem tissue functions, and leads to characteristic serrated leaves (*se-1*). In *se-2* mutants, the leaves are smaller in size and exhibit hyponastic growth; reduced fertility has been observed in *se-3* mutants [6–9]. Moreover, *se-3* plants are extremely small and embryonic lethal under certain environmental conditions [6,8]. All *se* mutants show a profound reduction in **miRNA** levels accompanied by an accumulation of miRNA primary precursors (pri-miRNAs) [6,8,10,11]. Because of these important miRNA biogenesis-related defects, SE was first described as an important miRNA biogenesis factor [6,8,12]. Further studies revealed that SE, together with the endonuclease **Dicer-like 1 (DCL1)** and the double-stranded RNA-binding protein **Hyponastic leaves 1 (HYL1)**, form the core of the **Microprocessor** protein complex, which is involved in miRNA-related biogenesis in plants [13,14]. Reports have shown, however, that SE exerts a global effect on RNA metabolism by regulating transcription [15,16], splicing [2,17,18], and RNA degradation [11,19]. Recently, a comprehensive *in vivo*

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SE interactome has been described [11] and this analysis revealed that miRNA biogenesis factors are not the main SE partners. Moreover, the interactome data have shown that SE is mainly associated with two complexes: the **Nuclear exosome targeting (NEXT) complex** and the Transcription and export (TREX) complex [11].

In parallel to functional studies, the structure of SE has also been investigated. In 2011, Machida and coworkers described the SE core crystal structure as 'walking man-like', consisting of N-terminal, middle, and C-terminal noncanonical zinc finger (ZnF) domains. In addition to these highly structured fragments, SE also includes unstructured N- and C-terminal regions [20]. Interestingly, SE has recently been shown to contain three intrinsically disordered regions (IDRs), of which one (IDR1) that is located its N-terminal region enables SE induction of phase separation [21]. This finding suggests a potential role played by SE in the formation of various protein complexes via liquid–liquid phase separation. New data on SE, which have been supported by studies on the human homolog ARS2, depict SE as a scaffolding protein that can determine the fate of RNAPII transcripts. In this review, we summarize the knowledge of SE functions to date, discuss new findings, and provide novel ideas as well as exciting directions for future studies.

The role played by SE in transcription

Several reports linked SE with proteins/complexes that influence the transcription of miRNA genes (*MIR*s) (Figure 1A, Key figure). SE associates with RNAPII [15] and interacts with both subunits of CBC: Cap-binding protein 20 (CBP20) and Cap-binding protein 80 (CBP80) [2,3]. Recent results have shown that the association between RNAPII and SE is mediated by the pre-mRNA-processing protein 40a/b (PRP40a/b) [22]. Moreover, SE interacts with the **Elongator** complex, a multisubunit complex with histone acetyltransferase activity that is associated with hyperphosphorylated RNAPII [23]. Elongator interacts not only with SE but also with DCL1 and HYL1, two other proteins in the Microprocessor core. Published results clearly show that the Elongator complex enhances *MIR* transcription and promotes cotranscriptional miRNA processing by bringing Microprocessor close to chromatin [23]. Another protein that brings Microprocessor and RNAPII together is the Negative on TATA less 2 proteins (NOT2a/b). NOT2 transcription factors interact directly with the C-terminal domain (**CTD**) of RNAPII and key Microprocessor factors DCL1 and SE. The same study showed the influence of NOT2a/b on the transcription of both miRNA and protein-coding genes [24]. The **Mediator** complex that influences transcription of RNAPII genes, including *MIR* genes, interacts also with Microprocessor. Interestingly, this interaction is stabilized by the Hasty protein (HST) [25,26].

The cell division cycle 5 (CDC5) protein is a conserved DNA-binding protein of the **MOS4-associated complex (MAC)**. MAC is the highly conserved **spliceosome**-associated complex that in plants plays a role in development and immunity without fully understanding the molecular mechanism behind it. CDC5 interacts with promoters of *MIR*s, SE, DCL1, and RNAPII. Moreover, CDC5 plays a dual role in the biogenesis of miRNAs: first, it positively influences *MIR* transcription, and second, via interactions with Microprocessor components, it influences the processing of miRNA precursors [27]. Another important factor interacting with both RNAPII and SE is CTD phosphatase-like 1 protein (CPL1) (Figure 1A). CPL1 is critical for the dephosphorylation of serine 5 in the CTD of RNAPII and the dephosphorylation of HYL1. Interestingly, CPL1, together with proteins in the CBC and NEXT and TREX complexes, belongs to a group of proteins that have been identified in SE coimmunoprecipitation (co-IP) experiments [11].

Through chromatin immunoprecipitation followed by high-throughput sequencing, two recent studies showed that SE is associated with chromatin in intronless gene regions as well as

Glossary

Cap-binding protein complex (CBC): a complex consisting of Cap-binding protein 20 (CBP20) and Cap-binding protein 80 (CBP80) that together binds the 5′ cap of nascent RNA polymerase II (RNAPII) transcripts. CBC is involved in various steps of RNA processing, including constitutive and alternative mRNA splicing and export of RNA from the nucleus to the cytoplasm.
CTD: the C-terminal domain of the largest RNAPII subunit, NRPB1, the CTD includes the tandemly repeated heptad Tyr-Ser-Pro-Thr-Ser-Pro-Ser, which undergoes cycles of phosphorylation and dephosphorylation. The phosphorylation of different residues in each heptad is characteristic of particular stages of transcription and controls the RNAPII transcriptional cycle.
Dicer-like 1 (DCL1): an RNase III-type enzyme that is critical for endonucleolytic cleavages of pri- and pre-miRNAs.
Dicing bodies (D-bodies): nuclear bodies consisting of core Microprocessor proteins.
Elongator: a multisubunit complex consisting of six component proteins (Elp1–6) associated with active RNAPII transcription. It facilitates transcription through histone acetylation.
Exosome: a conserved 3′–5′ RNA degradation machinery involved in turnover of every class of RNA in both cytoplasmic and nuclear compartments.
Hyponastic leaves 1 (HYL1): a double-stranded RNA-binding protein that facilitates DCL1 processing that converts pri-miRNA to mature miRNA.
Mediator: a highly conserved multisubunit transcriptional coactivator that regulates gene expression by enabling communication between transcription factors and RNAPII. The Mediator complex is involved in plant development, flowering, hormone signaling, and biotic stress responses.
Microprocessor: a miRNA biogenesis machinery. In plants, the core components of Microprocessor are endoribonuclease DCL1, HYL1, and SE.
miRNA: a short, noncoding, single-stranded RNA (~21 nt) that together with Argonaute1 (AGO1) forms an RNA-induced silencing complex (RISC) that regulates gene expression at the post-transcriptional level.
MOS4-associated complex (MAC): a complex involved in plant immunity. It consists of a core protein MOS4 and

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Key figure

SERRATE (SE) coordinates miRNA biogenesis and pri-miRNA degradation

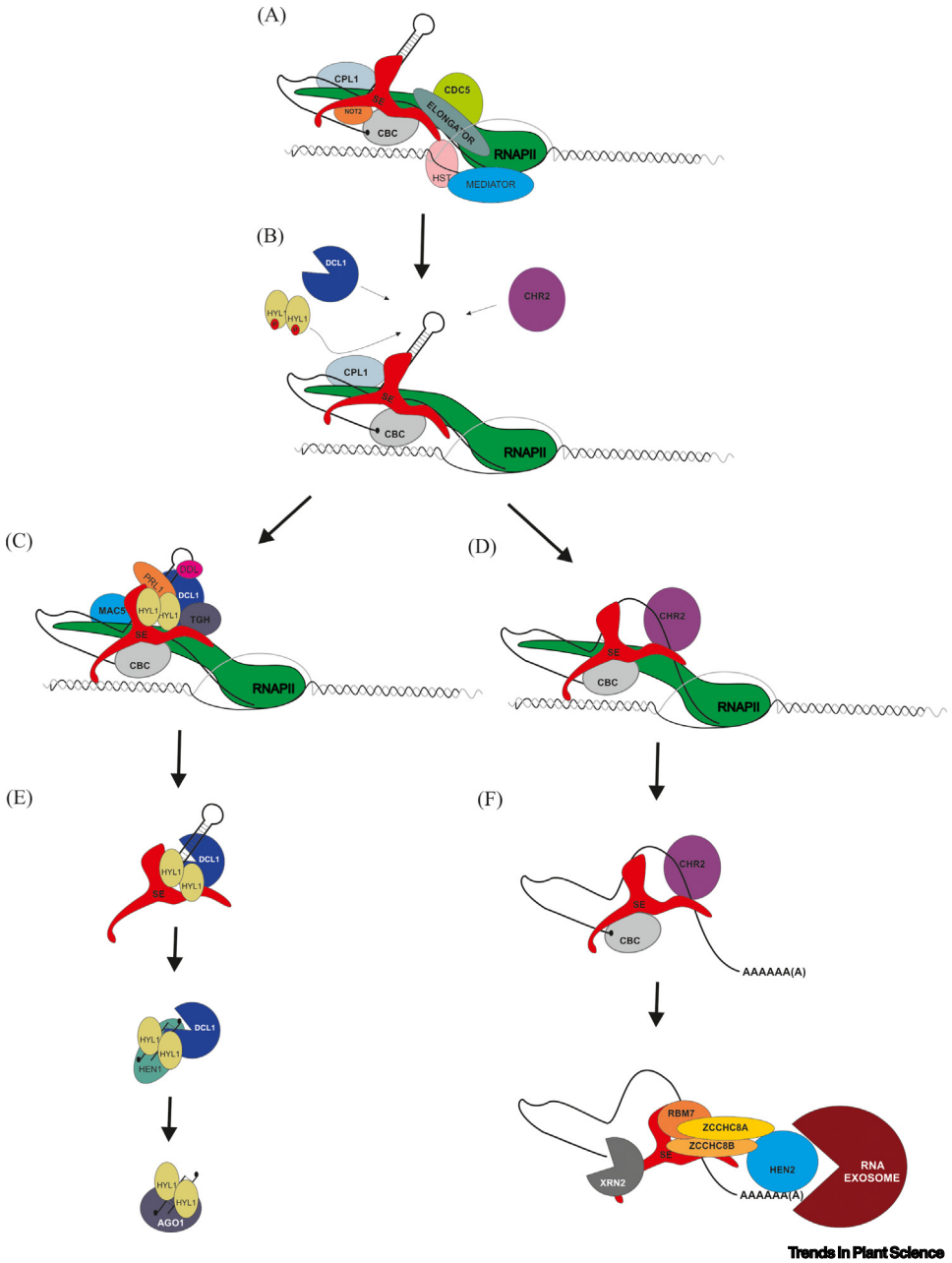

Figure 1. The figure shows miRNA biogenesis with SE as a key factor that coordinates all steps of the process. (A) During miRNA gene transcription, SE connects RNA polymerase II (RNAPII) with many transcription factors: Elongator complex, Mediator complex, CTD phosphatase-like 1 protein (CPL1), Negative on TATA less 2 protein (NOT2), Cell division cycle 5 (CDC5), Hasty (HST), and nuclear Cap-binding protein complex (CBC). (B) SE is recruited to miRNA precursor

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accompanying CDC5 and PRL1, as well as MAC3A/B and MAC5A/B/C.

Nuclear exosome targeting (NEXT) complex: plant NEXT consists of RNA helicase HEN2, RBM7, and two homologs of ZCCHC8: ZCCHC8A and ZCCHC8B. It facilitates RNA exosome-induced degradation of imprecisely processed RNAs.

RNA polymerase II (RNAPII): a DNA-dependent RNA polymerase critical for the transcription of protein-coding genes and certain noncoding RNAs [most small nuclear RNAs (snRNAs) and miRNAs]. RNAPII is a multicomponent complex in which the largest subunit NRPB1 includes a CTD domain.

SERRATE (SE): a zinc finger domain-containing protein involved in RNA metabolism.

Spliceosome: a ribonucleoprotein (RNP) complex that consists of small nuclear RNAs (snRNAs) and numerous proteins. It removes intron sequences from RNAPII transcripts.

transposable elements (TEs) [15,16]. Notably, approximately 1000 chromatin regions are bound to SEs and, of these loci, almost one-half belong to the same group of intronless genes. Moreover, the expression levels of 216 intronless genes have been shown to be downregulated in the *se-1* mutant. It has also been shown that stress responsive genes containing no or low numbers of introns are usually lowly expressed, however, this is not the case for genes whose expression is controlled by SE. Considering this observation, the authors concluded that SE positively regulates the transcription of intronless genes that can be important for the rapid response to stresses [15]. In another report, more than 5400 SE-bound chromatin regions have been identified. Approximately 1000 of these regions include TEs. Hence, the authors showed that SE directly interacts with Arabidopsis trithorax-related proteins 5 and 6 (ATXR5/6), which deposit a methyl group on lysine 27 of histone 3 (H3K27me1), a repressive chromatin mark. This study showed that 61% of the bound TE regions in SE were monomethylated at H3K27. All these findings showed that the interaction between SE and ATXR5/6 is important for the fine-tuning of transposon expression [16]. Notably, in the two aforementioned published studies, only 500 genes were found to overlap, which may suggest that the interaction of SE with chromatin is broad but transient and varies under different environmental and developmental conditions.

Taken together, these data indicate a functional connection between SE and RNAPII and this interplay appears to be important for the transcription of *MIR*s as well as that of other mRNAs by RNAPII.

SE: a crucial factor in miRNA biogenesis

The first report showing that SE is involved in miRNA biogenesis was published by Grigg and coworkers in 2005 [8]; these authors showed that altered expression of *MIR165* and *MIR166* in *se-2* and *se-3* mutants resulted in dysregulation of shoot apical meristem and leaf development [8]. Further analyses revealed a global effect of SE on the processing of pri-miRNAs. The levels of pri-miRNAs in *se-1* were increased, while the levels of mature miRNAs were reduced compared with those of wild-type plants. These and other results have clearly shown that SE is involved in the processing of pri-miRNAs catalyzed by DCL1, influencing the cleavage efficiency of the DCL1 endonuclease [7,12,13]. The *in vitro* experiments performed by Dong and colleagues have shown that SE increased both DCL1-mediated pri- and pre-miRNA cleavage rate and accuracy. In addition, the double-stranded RNA-binding protein HYL1 further enhanced the cleavage rate and accuracy of the reaction catalyzed by DCL1, suggesting synergistic functions of both HYL1 and SE in pri-miRNA processing [13]. DCL1 cleavage accuracy was further promoted by Tough (TGH), which interacted with all three Microprocessor proteins: DCL1, SE, and HYL1 [28]. Experiments performed with truncated versions of SE (SE variants lacking the N-terminal, C-terminal, or ZnF domain) showed that SE stimulated DCL1 activity without direct interaction with RNA and that formation of the DCL1/pri-miRNA complex stimulated the binding of SE to DCL1 [14].

Interestingly, the core Microprocessor components DCL1, SE, and HYL1 localized in the nucleus were either dispersed within the nucleoplasm or concentrated in nuclear structures called **Dicing**

Microprocessor components, such as Dicer-like 1 protein (DCL1) and Hyponastic leaves 1 (HYL1), during miRNA biogenesis, or Chromatin-remodeling factor 2 (CHR2) to prevent miRNA biogenesis. (C) DCL1 and HYL1 bind to the stem–loop structure facilitated by other proteins that are known to stabilize the miRNA precursor hairpin: Tough (TGH), Protein pleiotropic regulatory locus 1 (PRL1), and MOS4-associated complex protein (MAC5), Dawdle protein (DDL). (D) CHR2 remodels the RNA structure of the miRNA precursor, which interrupts HYL1 and DCL1 binding to the miRNA precursor. (E) The miRNA precursor is then processed into a miRNA/miRNA* duplex, which is methylated by Hua enhancer 1 (HEN1) and loaded into Argonaute 1 (AGO1). (F) SE is recruited to the misfolded polyadenylated miRNA precursor Nuclear exosome-targeting complex (NEXT) [including Hua enhancer protein 2 (HEN2), ZCCHC8A, ZCCHC8B, RNA-binding protein 7 (RBM7)] and Exoribonuclease 2 (XRN2), which leads to pri-miRNA degradation.

bodies (D-bodies) [29]. Since they contain all the key proteins important for pri-miRNA processing, D-bodies have been suggested to be sites of miRNA biogenesis. Moreover, bimolecular fluorescence complementation (BiFC) assays showed that SE, HYL1, and DCL1 interacted with each other in D-bodies and that pri-miR173 was directed to these specialized nuclear structures. The data presented by Fang and Spector suggest direct involvement of D-bodies in pri-miRNA processing, but the authors did not rule out the possibility that D-bodies are storage/assembly sites for Microprocessor factors [29]. This proposed function seems to be a very likely possibility since the inhibition of RNAPII activity increased the number of cells with D-bodies [30] (see Outstanding questions). The important role played by D-bodies in miRNA biogenesis has been recently supported by results showing that D-body formation depended on liquid–liquid phase separation mediated by SE [21]. Using SE tagged with a fluorescent protein, Xie and coworkers showed that SE formed dynamic droplets independently and that these droplets were enriched with DCL1, HYL1, and pre-miRNA/miRNA [21]. Moreover, the results indicated that the SE IDR spanning the first 134 amino acids (IDR1) was critical to this process. Interestingly, IDR1 induced liquid–liquid phase separation, as indicated by droplets recovered after photobleaching. All these data have demonstrated that SE is a crucial factor for D-body formation [21].

DEAD-box RNA helicases involved in D-body formation and miRNA biogenesis

The assembly of D-bodies seems to be a complex process. In 2021, Li and coworkers suggested the engagement of three DEAD-box RNA helicases, RH6, RH8, and RH12, in controlling D-body formation [31]. All three helicases colocalize with known D-body components SE, HYL1, and DCL1. In addition, direct interactions of all three helicases with SE and HYL1 have been confirmed by co-IP assays. When the expression of all three helicases was knocked down in plants, the percentage of nuclei with D-bodies was decreased in inflorescence cells compared with the levels in wild-type plants; moreover, in root tip elongation cells, D-bodies were completely eliminated. In addition, *in vitro* studies showed that the RH12 helicase promoted the formation of SE phase separation droplets [31]. The levels of several examined pri-miRNAs were decreased in the absence of RH6, RH8, and RH12 helicase expression. Moreover, the association of RNAPII with *MIRs* was diminished in plants lacking the helicases studied [31]. Recent reports describing SE protein partners pointed to certain other DEAD-box RNA helicases, SMALL1 (SMA1) and RH27, as two novel players in miRNA biogenesis [32,33]. The lack of RH27 or SMA1 expression led to embryo lethality, and partial loss-of-function mutations of the gene encoding either RH27 or SMA1 reduced the levels of pri-miRNAs. Both the RH27 and SMA1 helicases have been associated with other components of the Microprocessor complex; that is, SMA1 interacts with DCL1 and RH27 interacts with HYL1 and DCL1 [32,33].

Post-translational modifications of SE and its protein partners

The miRNA biogenesis pathway can be affected by post-translational modifications of core Microprocessor proteins. By interacting with CPL1 phosphatase, SE acts as a platform for HYL1 dephosphorylation by CPL1. The activity of HYL1 in miRNA biogenesis is phosphorylation-dependent and only dephosphorylated HYL1 is functional [10] (Figure 1B). Whether SE acts as a similar platform for modifications of other proteins dephosphorylated by CPL1 is unknown. Moreover, global studies of the arabidopsis phosphoproteome revealed 16 phosphorylation sites in SE [34]. SNF1-related protein kinase 2 (SnRK2) and the phosphatase CPL1 have been predicted to be involved in the phosphorylation and dephosphorylation of SE, respectively [10,11,35,36]. Recently, Wang and coworkers showed that pre-mRNA processing 4 kinase A (PRP4KA) interacted with and phosphorylated at least five SE residues. These modifications changed both SE activity and stability. Hyperphosphorylated SE exhibited a lower binding affinity for HYL1. Moreover, hyperphosphorylated SE was less resistant to 20S proteosome activity [37]. These results clearly showed that post-translational modifications of SE are important for

controlling SE activity and stability. Notably, ten of the 16 described phosphorylated sites were located within the unstructured N-terminal sequence in SE that is critical for liquid–liquid phase separation [21,37,38]. This structural discovery raises an interesting question: are these post-translational modifications important to the regulatory function of SE?

The SE interactome

As mentioned earlier, SE interacts with a plethora of different proteins involved in various biological processes [2,6,10–12,20,22,28,39–41]. These interactions may enable proteins to access other molecules involved in processes in which SE is involved or regulate these processes at different levels. One of the SE protein partners, Chromatin-remodeling factor 2 (CHR2), is an ATPase subunit in the switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex. CHR2 plays two roles in miRNA biogenesis. It is related to the stimulation of the transcription of *MIR*s. However, through the interaction of CHR2 with SE, this ATPase remodels SE-bound pri-miRNAs, making these RNAs inaccessible to Microprocessor and thus inhibits pri-miRNA processing (Figure 1D). In an arabidopsis transgenic line expressing CHR2 with mutation that disrupted its molecular contact with SE, the miRNA levels were only partially restored. This observation suggested that the CHR2-SE interaction is essential for the post-transcriptional regulatory role played by CHR2 in miRNA biogenesis. However, the expression level of the GUS reporter driven by the *MIR159A* promoter in plants expressing CHR2 with the mutated site that abrogated the interaction with SE is the same as that in wild-type plants. This finding indicated that the transcriptional and post-transcriptional roles played by CHR2 are uncoupled and that CHR2 must interact with SE for its post-transcriptional activity [42].

Recent data have shown that the subunits of the TREX complex are also coimmunopurified with SE [11]. TREX is a multicomponent complex that affects various maturation stages of RNAPII transcripts: transcription, splicing, maturation of RNA 3′ ends and export of RNA from the nucleus to the cytoplasm [43]. In addition to the canonical role played by TREX, several reports connected TREX with miRNA biogenesis [44,45]. Mutations in genes encoding two main TREX subunits, THO1 and THO2, led to reduced levels of miRNAs. Interestingly, plants with mutant *THO1* acquired a phenotype that was very similar to that of the *se-1* mutant [44]. THO2 interactions with SE were required for loading miRNA precursors into Microprocessor and/or for stabilizing miRNA precursors [45]. However, the exact role played by TREX in miRNA biogenesis remains an open question (see Outstanding questions).

The role played by SE in splicing

Laubinger and coworkers observed the accumulation of pre-mRNA transcripts with retained introns in *se-1* mutant plants, which strongly supported a role for SE in splicing regulation [17]. Additionally, a report indicated the involvement of SE in alternative splicing (AS), showing changes in the ratios of AS isoforms in *se-1* mutant plants [2]. Both of the described splicing aberrations involved the first intron in the respective RNAPII transcripts [2,17]. Moreover, a significant number of *se* mutant transcripts with irregular splicing were changed when both CBC subunits, *cbp20* and *cbp80*, were mutated. These overlapping changes in splicing indicated marked cooperation between CBC and SE during splicing regulation and interactions between SE and CBC subunits have also been described [2]. Interestingly, SE interacted with certain U1 small nuclear ribonucleoprotein (U1 snRNP) auxiliary proteins: Pre-mRNA-processing protein 39b (PRP39b), Pre-mRNA-processing protein 40a/b (PRP40a/b), and Lethal unless CBC 7 RL (LUC7rl) [41]. Disruption of the PRP40a/b and SE interaction caused a decrease in the levels of certain miRNAs and reduced the efficiency of intron-containing *MIR* transcript splicing. The interaction between SE and PRP40a/b has been shown to stimulate Microprocessor assembly and activity when the miRNA-containing stem–loop structure was located upstream of the 5′ splice site (5′SS) but

inhibited its formation and activity when the miRNA-containing stem–loop was downstream of the 5′SS [22,41] (Figure 2). Interestingly, SE has been copurified with certain polyadenylation factors, such as Flowering time control protein (FPA) [46], suggesting that SE may be involved in the selection of polyadenylation sites. This supposition has been supported by results showing that AS in the absence of SE may have led to the activation and selection of new polyadenylation sites [47]. Furthermore, SE has been found in nuclear speckles in association with Regulator of CBF gene expression 3 (RCF3) and two serine-/arginine-enriched splicing factors: RS40 and RS41. RCF3, RS40, and RS41 are involved in both pre-mRNA splicing and miRNA biogenesis. These observations suggested a link between splicing and efficient miRNA processing [48].

The involvement of SE in RNA degradation

To date, only a few reports linking SE with RNA degradation have been published [11,19,49]. In plants, two main RNA degradation machines are involved in the nucleus: 5′ exoribonucleases and the RNA **exosome**. Exoribonuclease 2 (XRN2) and exoribonuclease 3 (XRN3) degrade uncapped RNA from the 5′ end of an RNA molecule [50]. However, the RNA exosome is involved in RNA degradation from its 3′ end [51]. Recently, SE has been shown to interact with proteins involved in both RNA degradation pathways. For example, SE has been shown to interact with XRN2 [19]. Moreover, the crosstalk between SE, XRN2, and MAC5, a subunit of the MAC complex, has been shown to be important for pri-miRNA protection from XRN2/3 activity, which is SE-dependent. MAC5 interacted with SE and the stem–loop fragment of pri-miRNAs and these interactions stabilized the precursors and probably protected them from the 5′ RNA decay machinery [19] (Figure 1C). MAC5 and XRN2 might interact with the same portion of SE, but to confirm this possibility, additional studies are needed. Additionally, other subunits in the MAC complex, namely, PRL1 and MAC3, interacted with SE and enhanced the stability of pri-miRNAs [52,53]. The stability of pri-miRNAs was additionally enhanced by the Dawdle (DDL) protein, which interacts with DCL1 [54] (Figure 1C).

SE has also been connected with the main cofactor of nuclear RNA exosome, namely, the NEXT complex. The plant NEXT complex consists of an RNA helicase, Hua enhancer 2 (HEN2); two ZnF

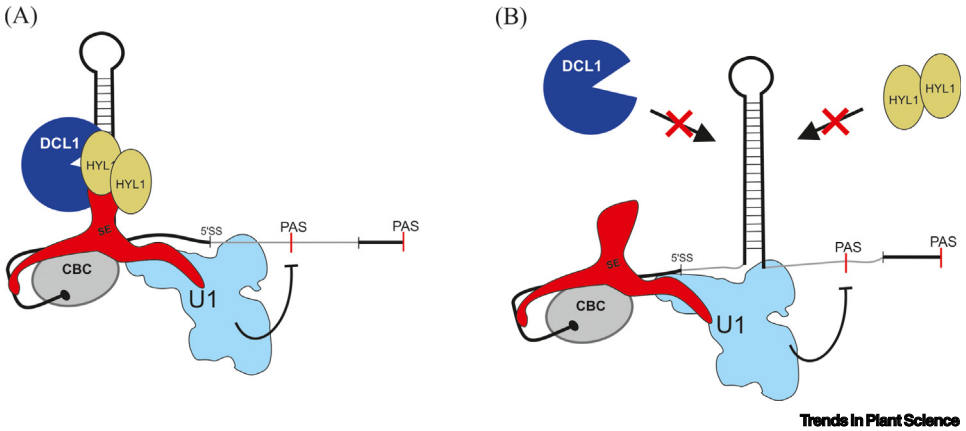

Figure 2. Proposed model of SERRATE (SE) action in the coordination of miRNA biogenesis, splicing, and polyadenylation. (A) SE recruits Dicer-like 1 protein (DCL1) and Hyponastic leaves 1 (HYL1) to the stem–loop structure of the miRNA primary precursor. When a precursor consists of an intron, the 5′ splice site (5′SS) is recognized by U1 small nuclear ribonucleoprotein (U1 snRNP), which leads to more efficient splicing, blocking of proximal polyadenylation, and more efficient miRNA biogenesis. (B) In the case of a miRNA encoded within introns, SE recruits U1 snRNP, which leads to more efficient splicing, inhibition of proximal polyadenylation, and inhibition of miRNA biogenesis. Abbreviations: CBC, Cap-binding protein complex; PAS, proximal and distal polyadenylation site.

proteins, ZCCHC8A and ZCCHC8B; and RNA-binding protein 7 (RBM7). NEXT targets RNA molecules for degradation, which is performed by the nuclear RNA exosome [55]. Recent data have shown that SE was copurified with all NEXT subunits and interacted directly with three of them: ZCCHC8A, ZCCHC8B, and RBM7 [11]. Combining these results with the fact that NEXT, as well as the RNA exosome, are involved in the degradation of pri-miRNAs as well as in the degradation of pri-miRNA process byproducts, it has been suggested that SE recruits NEXT/exosome to pri-miRNAs (Figure 1F). In the *se-2* mutant, the accumulation of known exosome targets, such as small nuclear RNA (snRNA), aberrant mRNAs, and long noncoding RNAs (lncRNAs), has been observed [11]. Moreover, in plants with mutations in the *SE* and *HEN2* genes, the sequential accumulation of many exosome targets has been described [11]. These findings suggested that the crosstalk between SE and NEXT/RNA exosome is not limited to miRNA precursors but may involve other RNAPII transcripts [11]. However, whether the molecular contact between SE and XRN2 is important for the degradation of different RNA classes, in addition to pri-miRNAs, remains to be determined.

SE-mediated coordination of different RNA processing pathways

The SE size (81 kDa) and complex architecture (three highly structured domains and unstructured C- and N-terminal regions) suggest its potential molecular interactions with various partners. Indeed, an analysis of SE interactome has shown that SE is associated with several different complexes known to be engaged in RNA metabolism [11]. To date, more than 50 proteins capable of interacting directly with SE have been identified (Table 1). Among these candidates, many proteins are involved in transcription, splicing, polyadenylation, miRNA biogenesis, and other RNA maturation processes, suggesting that SE may coordinate the synthesis and processing of various RNAPII transcripts. However, the list of identified SE protein interactors consists of components of both aforementioned RNA degradation machines: 5′ exonucleases XRNs and subunits of the NEXT complex. Thus, SE may define the fate of primary RNAPII transcripts, targeting them for processing or degradation. An increasing number of studies support a role for SE in RNA metabolism [11,19,42]. For example, the competition between HYL1/DCL1 and CHR2 for interaction with SE seems to be a key step in determining whether a single primary transcript is a miRNA precursor that is cleaved by DCL1 or is targeted for degradation when the precursor structure is altered by CHR2 [42] (Figure 1C,D).

SE coordination of different RNA-processing events is particularly clear when the miRNA biogenesis pathway is analyzed. Approximately one-half of known arabidopsis *MIRs* contain introns and 29 miRNAs are encoded within the introns of host genes [9]. Moreover, alternative transcription start sites, alternative SSs and polyadenylation sites have been identified within pri-miRNAs and this variety is essential for the production and fine-tuning of miRNA levels [9,56]. Thus, the post-transcriptional processing of intron-containing pri-miRNAs, as well as the pre-mRNAs encoding miRNA within introns, and Microprocessor activity, require other RNA-processing machinery activity, including spliceosome and polyadenylation protein complex functions. Interestingly, to a large extent, this coordination is based on direct communication between SE, which is an important component in Microprocessor, and certain U1 snRNP auxiliary proteins. As previously described, the location of the U1 snRNP-binding site (5′SS) relative to a miRNA-encoded stem–loop structure within a pri-miRNA is critical to its influence on miRNA biogenesis. Moreover, the binding of U1 snRNP to the 5′SS protects synthetized RNAPII transcripts from premature termination and polyadenylation [41]. Interestingly, in the list of proteins coimmunoprecipitated with polyadenylation factors, SE has been identified, suggesting that SE may be involved in polyadenylation site selection in plants [46]. To confirm this possibility, however, further investigation is needed because no experimental data supporting this functional connection in plants have been provided to date (see Outstanding questions). Thus, on the one hand,

Outstanding questions

What is the order of cotranscriptional Microprocessor assembly in plants? Does SERRATE coordinate the sequential inclusion of miRNA biogenesis factors into the Microprocessor complex?

What is the molecular mechanism underlying the involvement of SERRATE in intron recognition and excision?

Is SERRATE involved in the regulation of the polyadenylation of RNA polymerase II transcripts?

What is the role played by the interaction of SERRATE with the TREX complex? Is SERRATE engaged in RNA export from the nucleus?

Is the liquid–liquid phase separation induced by SERRATE required for the formation of SERRATE-containing complexes in addition to D-bodies? Is this feature of SERRATE necessary for cotranscriptional Microprocessor assembly?

If D-bodies are not the major sites for pri-miRNA processing, what role do they play in miRNA biogenesis?

What is the function of DEAD-box RNA helicases that interact with SERRATE? Do they stimulate binding of other RNA-binding proteins?

Table 1. List of proteins interacting with the SERRATE protein

Protein	Locus	Complex	Process	Interaction site in SE	Confirmed by method[a]	Refs
CBP20	At5g44200	CBC	Transcription	Core domain	BiFC, pull down, co-IP	[2,24]
CBP80	At2g13540	CBC	Transcription	Core domain	BiFC, pull down, co-IP	[2,24]
NOT2B	At5g59710		Transcription		BiFC, Y2H, pull down	[24]
ELP2	At1g49540	Elongator	Transcription		Y2H, BiFC	[23]
ELP4	At3g11220	Elongator	Transcription		BiFC	[23]
ELP5	At2g18410	Elongator	Transcription		Y2H, BiFC	[23]
ATXR5	At5g09790		Transcription	642–695	Y2H, co-IP, pull down	[16]
ATXR6	At5g24330		Transcription		Y2H, co-IP, pull down	[16]
CHR2	At2g46020	SWI2/SNF2	Transcription, miRNA biogenesis	642–694	LCi, Y2H, pull down, co-IP	[42]
CDC5	At1g09770	MAC	Transcription, miRNA biogenesis		BiFC, co-IP	[27]
SMA1	At2g33730		Transcription, miRNA biogenesis		BiFC, co-IP	[32]
HST	At3g05040		Transcription, miRNA biogenesis		BiFC	[26]
NTR1	At1g17070		Transcription, miRNA biogenesis		BiFC, co-IP	[57]
ILP1	At5g08550		Transcription, miRNA biogenesis		BiFC, co-IP	[57]
DCL1	At1g01040	Microprocessor	miRNA biogenesis	469–543	co-IP, BiFC, pull down	[20,40,42]
HYL1	At1g09700	Microprocessor	miRNA biogenesis	Core domain	Y2H, pull down, BiFC	[6,10,20,24,58,59]
TGH	At5g23080		miRNA biogenesis		Pull down, BiFC	[28]
CPL1	At4g21670		miRNA biogenesis		co-IP, BiFC, Y2H	[10]
MAC5A	At1g07360	MAC	miRNA biogenesis		BiFC, co-IP	[19]
MAC3A	At1g04510	MAC	miRNA biogenesis		BiFC, co-IP	[52]
MAC3B	At2g33340	MAC	miRNA biogenesis		BiFC	[52]
PRL1	At4g15900	MAC	miRNA biogenesis		BiFC, co-IP	[53]
RACK1A	At1g18080		miRNA biogenesis		Y2H, BiFC, co-IP	[60]
RACK1B	At1g48630		miRNA biogenesis		Y2H	[60]
RACK1C	At3g18130		miRNA biogenesis		Y2H	[60]
RH6	At2g45810		miRNA biogenesis		BiFC, co-IP	[31]
RH8	At4g00660		miRNA biogenesis		BiFC, co-IP	[31]
RH12	At3g61240		miRNA biogenesis		BiFC, co-IP, pull down	[31]
RH27	At5g65900		miRNA biogenesis		Pull down, LCi, BiFC, co-IP	[33]
PAG1	At2g27020	20S proteasome	miRNA biogenesis	469–720	co-IP, Y2H, BiFC	[38]
PAB1	At1g16470	20S proteasome	miRNA biogenesis		Y2H, BiFC	[38]
PAE1	At1g53850	20S proteasome	miRNA biogenesis		Y2H	[38]
PBA1	At4g31300	20S proteasome	miRNA biogenesis		Y2H, BiFC	[38]
PBE1	At1g13060	20S proteasome	miRNA biogenesis		Y2H, BiFC	[38]
PBE2	At3g26340	20S proteasome	miRNA biogenesis		Y2H, BiFC	[38]
HIGLE	At2g30350		miRNA biogenesis	1–541	Pull down, co-IP	[61]
SEAP1	At4g24270		miRNA biogenesis		BiFC, co-IP	[62]

(continued on next page)

Table 1. (continued)

Protein	Locus	Complex	Process	Interaction site in SE	Confirmed by method[a]	Refs
THP1	At2g19560	TREX-2	miRNA biogenesis		Y2H, co-IP	[63]
SAC3A	At2g39340	TREX-2	miRNA biogenesis		Y2H, BiFC	[63]
SnRK2.2	At3g50500		miRNA biogenesis		Y2H, LCi	[35]
SnRK2.3	At5g66880		miRNA biogenesis		Y2H, LCi	[35]
SnRK2.6	At4g33950		miRNA biogenesis		Y2H, LCi	[35]
PRP4KA	At3g25840		miRNA biogenesis	469–720	LCi, co-IP, BiFC, Y2H	[37]
PRP4KB	At1g13350		miRNA biogenesis		LCi, co-IP, BiFC, Y2H	[37]
FHA2	At3g07220		miRNA biogenesis		Pull down, co-IP, BiFC	[64]
PRP40A	At1g44910	U1 snRNP	miRNA biogenesis, splicing	681–701	Y2H, pull down, FRET-FLIM	[41]
PRP40B	At3g19670	U1 snRNP	miRNA biogenesis, splicing	681–701	Y2H, pull down, FRET-FLIM, PLA	[22,41]
PRP39B	At5g46400	U1 snRNP	miRNA biogenesis, splicing		Y2H, pull down, FRET-FLIM	[41]
LUC7rl	At5g51410	U1 snRNP	miRNA biogenesis, splicing	681–701	Y2H, pull down, FRET-FLIM	[41]
HOS5	At5g53060		miRNA biogenesis, splicing		BiFC	[48]
RS40	At4g25500		miRNA biogenesis, splicing		BiFC	[48]
RS41	At5g52040		miRNA biogenesis, splicing		BiFC	[48]
ZCCHC8A	At5g38600	NEXT	Degradation		Y2H, pull down, FRET-FLIM	[11]
ZCCHC8B	At1g67210	NEXT	Degradation		Y2H, pull down, FRET-FLIM	[11]
RBM7	At4g10110	NEXT	Degradation		Y2H, FRET-FLIM	[11]
XRN2	At5g42540		Degradation		co-IP	[19]

[a]Abbreviations: FRET-FLIM, Förster resonance energy transfer-fluorescence lifetime imaging; LCi, luciferase complementation assay; PLA, proximity ligation assay; Y2H, yeast two-hybrid assay.

binding of U1 snRNP to a 5′SS influences Microprocessor assembly and polyadenylation site selection, but on the other hand, SE affects 5′SS selection, as has been demonstrated by the role it plays in AS [2]. This complicated SE-mediated crosstalk between several machines is important for a proper miRNA-connected response to biotic and abiotic stresses, ensuring plant survival in a changing environment [41]. It is very likely that SE also coordinates the processing of other primary RNAPII transcripts.

Interestingly, the first 134 amino acids of the unstructured N-terminal region of SE have been identified as IDR1, which is critical for the liquid–liquid phase separation of SE [21]. This unique property is necessary to form SE-containing nuclear foci. These foci can also contain other proteins and/or RNA molecules. SE phase separation has previously been shown to be crucial for the formation of D-bodies, where DCL1 and HYL1 proteins accumulate [21,29]. It is well known that there are only a few D-bodies per nucleus; however, there is a plethora of SE-containing nuclear foci in each cell [29]. It is very likely that SE forms droplets that include different SE partners; therefore, these SE condensates can be platforms attracting other factors involved in different processes such as miRNA biogenesis (Microprocessor), RNA degradation (NEXT/RNA exosome), and splicing (snRNPs) (Figure 3). Moreover, SE liquid–liquid phase separation and the ability of SE to interact with different proteins that are involved in RNA metabolism make SE a perfect candidate for a coordinator of different RNA-processing pathways (Figure 3). Furthermore, SE liquid–liquid phase separation ability can be promoted by interaction with other proteins (e.g., DEAD-box helicases: RH6, RH8, RH12) [31] that leads to the suggestion that appropriate interactors of SE can force formation of special nuclear bodies.

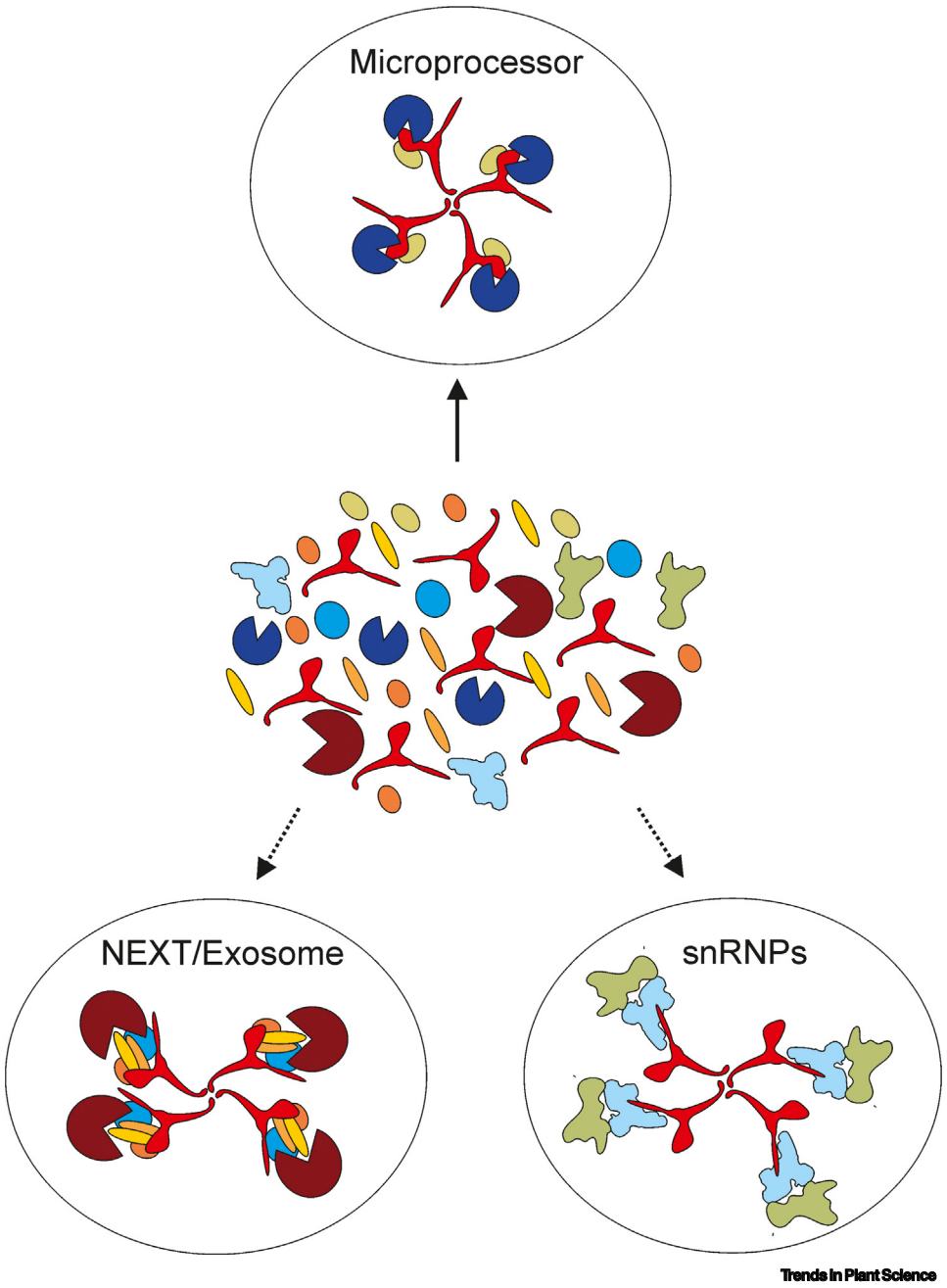

Figure 3. SERRATE (SE) is a driving force of nuclear speckle formation by liquid–liquid phase separation. SE forms droplets (ultimately, nuclear bodies) that include different SE partners and therefore are platforms for different processes such as miRNA biogenesis (Microprocessor), RNA degradation [Nuclear exosome targeting (NEXT)/RNA exosome], and splicing [small nuclear ribonucleoproteins (snRNPs)].

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Declaration of interests

No interests are declared.

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