Minireview Article

Regulatory role of nucleus in miRNA processing at early developmental stages in plants

ABSTRACT

Subcellular droplets of the Dicing complex component, which is essential in microRNA synthesis, were identified in plants called nuclear dicing bodies. Liquid–liquid stage segregated condensates containing inherently disordered sections of component of the SERRATE Dicing are now known to drive microRNA processing and phase separation in tandem.

Keywords: MicroRNA; retrotransposons; dicing complexes; nuclear D-bodies; microRNA biogenesis

1. INTRODUCTION

During plant and animal development, microRNAs (miRNAs) play a crucial regulatory function by controlling expression of genes and regulating retrotransposons [1,2]. Various species use various synthetic methods to produce microRNA, and the steps involved in this process are complicated and vary from one species to the next. An enzyme called as DCL1 fused with a green fluorescent protein (GFP) was used in plants to see subcellular chambers called nuclear dicing bodies (GFP) [3,4]. When these D-bodies were first discovered, it was not clear whether they represented the region where processing of microRNA was really occurring or were just a storage mechanism. Dicing complexes, that are made up of HYL1 and DCL1 and a zinc-finger protein, SERRATE, have been shown to play a major role in the synthesis of microRNAs in plants (SE) [5] (Figure 1). MicroRNA synthesis occurs in the D-bodies of cells, which are liquid–liquid stage segregated condensates of SE protein with inherently disordered areas [3,5,6]. Despite the fact that this work reveals insights into the mechanisms into microRNA synthesis in plants, similarities with mammalian cells may also be made.

Observation

After the initial cleavage, microRNA loci are transcribed by RNA polymerase II to form pri-microRNA, which is then clipped to a stem-loop pre-microRNA by RNA polymerase III [1,7]. In both plants and animals, the pre-microRNA hairpin structure is a common trait [5,7]. In the presence of dicer proteins, RNase III action further cleaves the pre-microRNA, resulting in a pair of microRNA/microRNA of 21 nucleotides (nt) in length. Pre-microRNA is converted by Dicer in the cytoplasm of mammals into the microRNA-microRNA duplex [7]. DCL1 is a nuclear enzyme that cleaves pre-microRNA in plants [1,5]. Dcl1 null mutations cause embryonic death, and even minor mutations cause developmental abnormalities because of the lack of the microRNA [5]. The nuclear D-bodies are formed by the interaction of SE and HYL1 with DCL1 [1,5]. Se and hyl1 mutants had decreased aggregation of

microRNAs, indicating that these both proteins are necessary for microRNA synthesis [8]. The link between D-bodies and microRNA synthesis has remained uncertain, despite the fact that HYL1, DCL1, and SE have already been identified as significant contributors to microRNA synthesis.

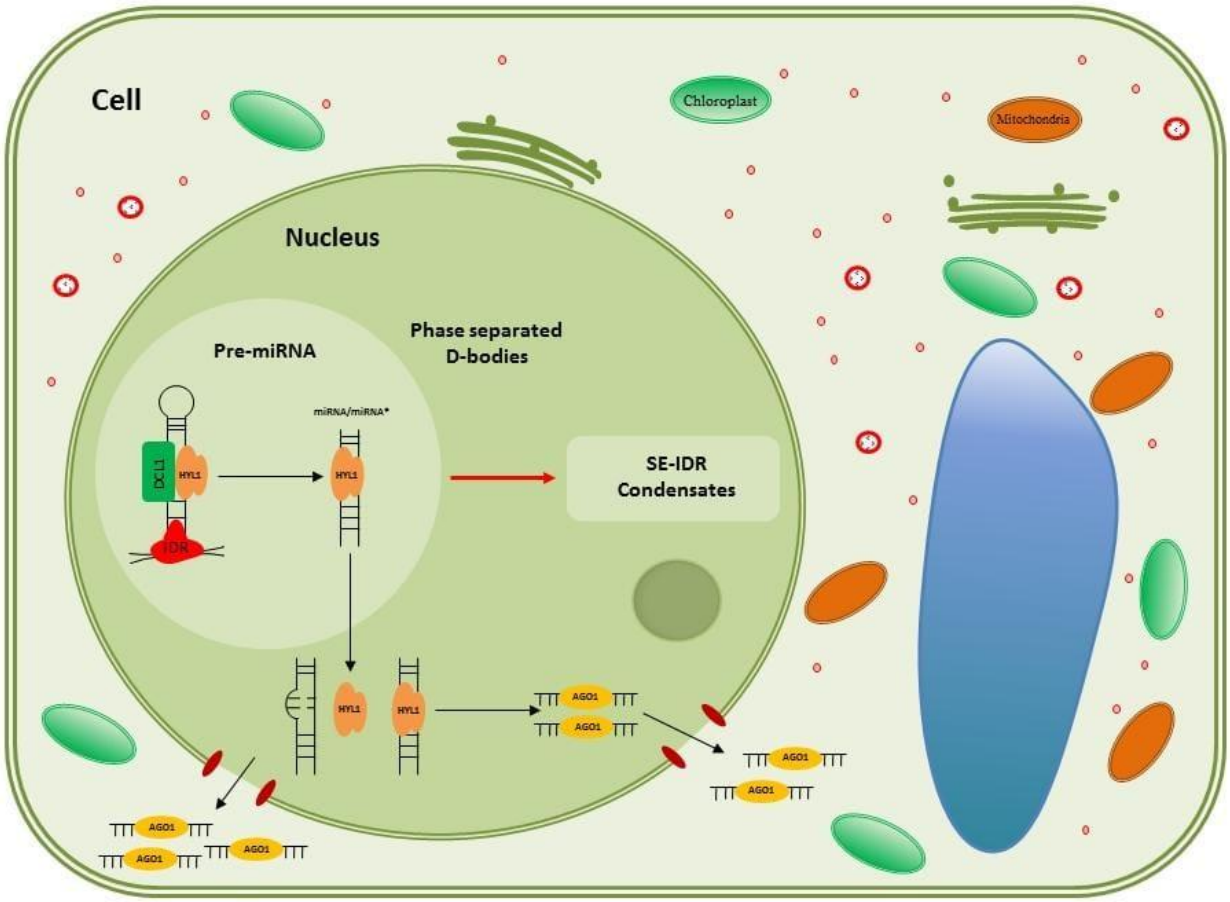

Fig. 1. Plant miRNA processing occurs in liquid-liquid phase-separated condensates

According to the present study, Xie, Chen, and Niu investigated whether dicing bodies may possess phase separation features and discovered intrinsically disordered regions (IDRs) in SE but not in HYL1 or DCL1. Subcellular chambers without membranes are formed through the liquid–liquid phase separation (LLPS) of proteins carrying IDRs [9]. A technique known as fluorescence recovery after photobleaching was used by the researchers to conduct LLPS tests on proteins that had been labelled with fluorescent proteins (FRAP). Whereas HYL1 and DCL1 could not produce droplets in vitro, SERRATE was adequate. For example, in vitro SERRATE condensates included microRNA, HYL1 and DCL1, which suggests that the microRNA processing machinery has been recruited by SERRATE into these condensates.

On the next page, the authors discuss how they used in vitro biochemical experiments to determine if SE is required for microRNA processing. Processing of 21-nt microRNA/microRNA* duplexes was reliant on

SERRATE protein content and the protein-protein interactions between DCL1 and SERRATE were preserved after microRNA processing. When microRNA was cleaved by DCL1, microRNA/microRNA* duplexes linked with HYL1 were identified in the supernatant, whereas DCL1 and SERRATE remained in the condensates. There is strong evidence to show that DCL1 and SERRATE have a high affinity for pre-microRNA, whereas HYL1 seems to be critical for microRNA/microRNA* export. When HYL1 was removed, most of the microRNA/microRNA* duplexes remained in the condensates, supporting this result.

As a consequence of this research, it was possible to conclude that the LLPS of Dicing-bodies is essential for the synthesis of microRNAs. According to these findings, full-length SERRATE but not an IDR deletion mutant was able to restore virtually all Dicing-bodies in SERRATE mutant nuclei. There was a correlation between microRNA levels and Dicing-bodies in se mutants restored with wild-type SERRATE or IDR-deficient SERRATE. Furthermore, The Fused in Sarcoma (FUS) oncoprotein (LCD) was fused to the IDR-deletion mutant of SERRATE and the phenotypes of the se mutant were restored, throwing out the hypothesis that microRNA processing was defective rather than relying on phase separation.

There are several fascinating issues raised by the study of Xie, Chen, Niu et al. It is still a mystery why condensates process microRNAs more effectively than supernatants or soluble fractions do. SE has a greater affinity for pri-microRNA, HYL1 and DCL1 than the IDR-deleted protein. SE may be able to entice the other members of the Dicing complex to move closer together in this way. The presence of D-bodies may also concentrate microRNA processing components, as shown by the higher enzyme reaction rates found in the phase-separated condensed solutions [9]. However, it is possible that the metabolic and physiological circumstances in the SERRATE condensates are more suited to the microRNA processing machinery.

In addition, it is important to understand why there are so few D-bodies in each nucleus, given that earlier research have discovered just one or two such structures in *Arabidopsis* leaf cell nuclei [3,4]. In *Nicotiana benthamiana*, more D-bodies have been discovered, and it will be vital to determine how widespread they are employed for processing the dozens of microRNAs that have been discovered in various species [10]. It has been shown that SE and DCL1 are both zinc-finger chromatin proteins [11]. According to the researchers, a lesser percentage of D-bodies may contain numerous microRNA loci. This might be due to the fact that only a few microRNA loci create D-bodies that are big enough to be seen under the microscope. Future research will need to investigate the relationship between co-transcriptional microRNA processing and the LLPS of Dicing-bodies. Environmental and developmental factors may influence the expression of various microRNAs in plants [1]. For example, in *Arabidopsis* pollen with a highly differentiated vegetative nucleus, microRNA 845b creates epigenetically activated small interfering RNA (easiRNA) [2]. In order to determine if SE-dependent subcellular entities always work in the same manner for various types of microRNAs, it will be necessary to do more research. With the help of nuclear exosome proteins, SERRATE may be able to downregulate the synthesis of microRNAs [12].

microRNA biogenesis

When it comes to microRNA biogenesis, there are some differences between animal and plant systems. MicroRNA-induced silencing occurs in plants when the microRNA/microRNA* duplex is synthesized in the nucleus and transferred to the effector protein ARGONAUTE 1 (AGO1) in both the nucleus and cytoplasm [13] (Figure 1). An enzyme called DGCR8 cleaves pri-microRNA in *Drosophila* nuclear microprocessor (Drosha) [7]. The stem-loop hairpin pre- microRNA is then cleaved by Dicer-1 into a 22-nt microRNA in the cytoplasm. SiRNA loading onto Ago2 rather than microRNA processing seems to be the

role of R2D2, a closely related homolog of HYL1 [14]. It would be interesting to study if phase separation is essential for the function of the nuclear protein Ars2, which is conserved in *Drosophila* and mammals and interacts with both the microprocessor and Dicer-2 and is required for microRNA processing. Ars2 is the metazoan orthologue of SE and interacts with both the microprocessor and Dicer-2 and is required for microRNA processing [15]. It is not yet apparent whether nuclear or cytoplasmic condensates are also involved in the processing of short RNAs in animals.

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