The spliceosome factor and RNA helicase Brr2a moonlights in miRNA production

The secondary structure of pri-miRNAs determines the efficiency and accuracy of miRNA production. RNA helicase Brr2a, a key component of spliceosomes in *Arabidopsis*, can interact with the pri-miRNA processing machinery component HYL1 and fine-tune the structures of pri-miRNAs to enhance miRNA production, whether the pri-miRNAs contain introns or not.

This is a summary of:
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The problem

RNA molecules have various shapes that can be altered by different RNA helicases (RHs, enzymes that unwind RNA helices) to meet biological needs[1]. MicroRNAs (miRNAs), a group of small noncoding RNAs, regulate numerous biological processes by cleaving their target mRNAs or reducing their translation. These miRNAs are produced from primary miRNA precursors (pri-miRNAs) in a processing centre that, in plants, mainly comprises DICER-LIKE 1 (DCL1, an enzyme that cuts RNA strands) and the regulatory proteins SERRATE (SE) and HYPONASTIC LEAVES 1 (HYL1). Previous studies have shown that the efficiency and precision of miRNA production are influenced by the hairpin structures of pri-miRNAs that result from base-pairing[2,3]. Intriguingly, the miRNA production machinery is frequently visited by RHs, but the purpose of these visits remains largely unclear. We speculate that some RHs might directly change the structures of pri-miRNAs to control miRNA yield. Although the RH chromatin remodelling factor 2 (CHR2) disrupts pri-miRNA structure to inhibit miRNA production[4], it is intriguing to consider whether certain RHs might fine-tune pri-miRNA structures to enhance miRNA production.

The discovery

To search for RHs that might enhance miRNA production, we mined *Arabidopsis* proteomic datasets for key components of the pri-miRNA processing machinery (HYL1 and SE), and we identified a few RH candidates that interacted with HYL1 or SE. We then searched candidate RHs that are co-expressed with DCL1. This effort pinpointed one RH, Brr2a. The physical interaction between Brr2a and HYL1 was confirmed by co-immunoprecipitation and other experiments. Furthermore, small-RNA-sequencing and RNA-sequencing analyses suggested that Brr2a and HYL1 might share certain genetic functions and miRNA pathways. These results implicated Brr2a as a new partner in the miRNA production machinery.

Brr2a has been best known as an essential and conserved component in spliceosomes[5] (enzyme complexes that remove introns (non-coding regions) from precursor messenger RNAs). Because a portion of pri-miRNAs contain introns, improper splicing of these pri-miRNAs reduced miRNA production in transgenic plants carrying a loss-of-function mutant *brr2a* gene (called *brr2a-3* mutants). Surprisingly, most pri-miRNAs lack introns, but their miRNAs were also decreased in *brr2a-3* plants. Moreover, the reduced accumulation of miRNAs was not caused by defective transcription of miRNA-encoding genes or by altered splicing of transcripts that encode key components of the miRNA production machinery, indicating that Brr2a directly participates in miRNA production via a splicing-independent route.

We found that Brr2a binds to pri-miRNAs in vivo and in vitro. Furthermore, its binding can be strengthened by HYL1. Notably, pri-miRNAs substantially stimulate the RH activity of Brr2a; consequently, Brr2a unwinds nicked pri-miRNAs in vitro. Finally, we decoded the shapes of pri-miRNAs in different plants. Interestingly, most detected pri-miRNA structures become unsuitable for miRNA processing in *brr2a-3* compared to wild-type plants, indicating that Brr2a adjusts pri-miRNA structures for their processing. We conclude that Brr2a has a dual role in promoting miRNA production: it participates in splicing of intron-containing pri-miRNAs to generate correct pri-miRNA sequences, and it effectively modulates the pri-miRNA structures to improve their fit with the DCL1–HYL1–SE complex, regardless of intron presence (Fig. 1).

The implications

In addition to RNA sequence, RNA shape also contains information. Here, Brr2a reshapes pri-miRNA structures, indicating that it can re-wire the structure-associated information to regulate miRNA production. Understanding how Brr2a recognizes and latches onto pri-miRNAs with heterogenous structures is a critical mechanistic question. Deciphering the atomic structures of the Brr2a and pri-miRNA complex will hold the key to understand how Brr2a functions in miRNA production.

Whereas CHR2 is a negative regulator, Brr2a is a positive one. One immediate question is to identify the physiological conditions and/or environmental responses that determine the activities of Brr2a and CHR2. The next question would be whether these two enzymes compete or coordinate with each other to adjust the pri-miRNA structures, thereby balancing the miRNA yield for biological processes. Notably, Brr2a was initially identified owing to the sensitivity of *brr2a* mutants to refrigeration. Assessment of miRNA production under cold stress is another goal. Beside CHR2 and Brr2a, several other RHs also exist in the miRNA production factory. Their helicase activity and potential pri-miRNA reshaping roles warrant further investigation.

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EXPERT OPINION

As a spliceosome component, Brr2a can regulate alternative splicing of intron-containing pri-miRNAs to promote miRNA biogenesis. However, the production of miRNAs from properly spliced pri-miRNAs is affected in *brr2a* mutants. The authors investigated the splicing-independent role of Brr2a in miRNA biogenesis and provided biochemical, structural and genetic evidence that Brr2a can remodel secondary structures of pri-miRNAs, thereby promoting miRNA biogenesis. The study uncovers a non-canonical role of Brr2a and the involvement of Brr2a-mediated RNA remodelling — a novel structural mechanism — in miRNA biogenesis. **Yijun Qi, Tsinghua University, Beijing, China.**

FIGURE

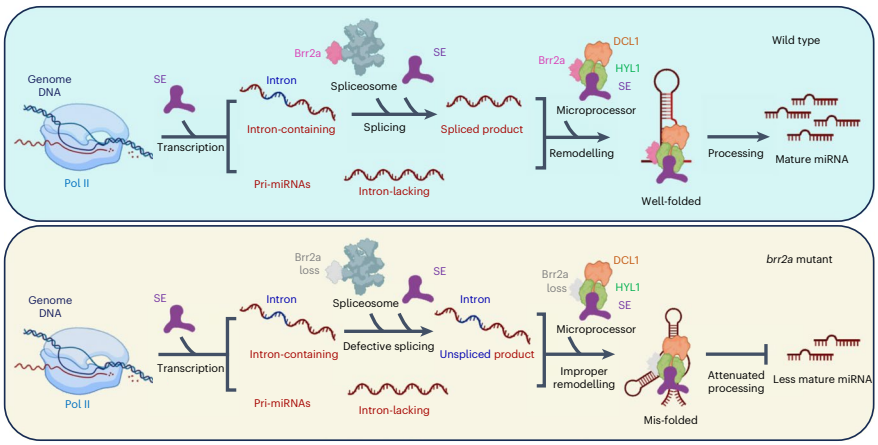

Fig. 1 | A proposed model for Brr2a function in miRNA production. Under wild-type conditions (top), Brr2a plays a dual part in miRNA production. It promotes splicing of intron-containing pri-miRNAs to generate correct pri-miRNA sequences, thereby facilitating miRNA production. Meanwhile, it exerts its RNA helicase activity to adjust the secondary structures of pri-miRNAs, consequently enhancing their processing. By contrast, in the loss-of-function *brr2a* mutant (bottom), intron-containing pri-miRNAs fail to have their introns removed and cannot be processed further. Also, pri-miRNAs tend to have structures unsuitable for processing. These defects ultimately result in reduced miRNA production. Pol II, polymerase II.

BEHIND THE PAPER

X.L. was a graduate student of Hongliang Zhu, who pioneered research on the secondary structures of pri-miRNAs and miRNA production in X.Z.'s laboratory back in 2013. Thus, two 'generations' of scientists have been studying this complicated but exciting scientific question.

Brr2a is a large (250 kDa) protein with two helicase cassettes. We have unsuccessfully tried to purify the full-length protein from bacteria and an insect–baculovirus expression system for years. Eventually, we had to use recombinant truncated cassettes for some biochemical assays. We are now exploring yeast and mammalian systems for full-length protein production for further studies.

We have also used AlphaFold to simulate a Brr2a–pri-miRNA structure, comparing how Brr2a binds these diverse structures with how it binds RNA as a spliceosome component. Simulations match our biochemical data but lack atomic-level detail. We will revisit this issue after obtaining the cryo-electron microscopy structure of Brr2a–pri-miRNAs. **X.L. & X.Z.**

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FROM THE EDITOR

The RNA helicase Brr2a has a known function in pre-mRNA splicing. This study stands out because it reveals a new function of Brr2a in modulating the RNA secondary structure of pri-miRNAs to regulate miRNA production, advancing our knowledge of how RNA secondary structure is modulated during miRNA biogenesis. **Jun Lyu, Senior Editor, *Nature Plants***