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Forkhead-associated domain 2 links light signal to miRNA biogenesis

MicroRNAs (miRNAs) are one of the most important regulators of gene expression. Their biogenesis starts with the transcription of primary miRNA transcripts (pri-miRNAs). In plants, the RNase III enzyme DICER-LIKER 1 (DCL1), together with its partner, the double-stranded RNA-binding protein HYPONASTIC LEAVES 1 (HYL1), and the zinc-finger protein SERRATE (SE), process the imperfect miRNA-residing stem loop of pri-miRNAs to release the miRNA/miRNA* duplexes in the nucleus, which are then methylated by HUA1 ENHANCER 1 (Rogers and Chen, 2013; Song et al., 2019). Then, most miRNAs are sorted into AGONAUTE 1 to mediate mRNA-cleavage or translation inhibition based on sequence complementarity (Rogers and Chen, 2013; Song et al., 2019). Studies have shown that miRNA biogenesis is controlled through modulating pri-miRNA transcription, stability and processing, which is often coupled with various developmental and environmental signals.

In a recent study, Park et al. (2021) found that forkhead-associated domain 2 (FHA2) negatively regulates miRNA biogenesis. It interacts with DCL1 to repress pri-miRNA processing. Remarkably, FHA2 is stabilized by light and modulates light-triggered changes in pri-miRNA processing during de-etiolation, suggesting that FHA2 links light signal to miRNA biogenesis (Park et al., 2021).

MULTIFACETED ROLES OF FHA DOMAIN-CONTAINING PROTEINS IN miRNA BIOGENESIS

The FHA domain spanning ~80-100 amino acid (aa) residues is a conserved motif that specifically recognizes phosphothreonine-containing motifs (Chevalier et al., 2009). The FHA domain exists in diversified proteins that act in various cellular processes including signal transduction, DNA transport, protein degradation and others. The FHA domain-containing DAWDLE (DDL) protein from Arabidopsis is a positive regulator of miRNA biogenesis. It interacts with DCL1 through its C-terminal FHA domain, which recognizes the helicase and RNase III domains of DCL1 and promotes DCL1 activity (Figure 1A) (Yu et al., 2008; Machida and Yuan, 2013; Zhang et al., 2018). These two protein domains of DCL1 contain potential phosphothreonine motifs recognized by the FHA domain (Machida and Yuan, 2013), indicating that phosphorylation may control the DDL-DCL1 interaction and modulate miRNA biogenesis. Notably, DDL also binds and stabilizes pri-miRNAs.

FHA2 is another FHA domain-containing protein sharing very low sequence similarity with DDL. It harbors an N-terminal FHA domain and an acidic aa-enriched C-terminal region (Figure 1A). The loss-of-function *fha2* mutants display male sterility and slightly enlarged leaves with mild serration (Park et al., 2021). Most miRNAs are increased in abundance in *fha2* relative to wild-type plants, suggesting that FHA2 negatively regulates miRNA biogenesis (Park et al., 2021). FHA2 directly interacts with HYL1 and DCL1, but not SE. In addition, FHA2 co-elutes with HYL1, DCL1, and SE in a size-exclusion chromatography analysis, demonstrating that FHA2 is associated with the DCL1 complex (Park et al., 2021). Moreover, FHA2 recognizes the PAZ domain and RBD of DCL (Figure 1A) (Park et al., 2021). However, it is unknown if the FHA domain mediates the DCL1-FHA2 interaction. Notably, the DCL1 complex from *fha2* displays increased pri-miRNA processing activity relative to wild type, which can be repressed by the addition of the recombinant FHA2 protein, demonstrating that FHA2 inhibits the DCL1 activity (Figure 1B) (Park et al., 2021). Supporting this notion, *fha2* reduces pri-miRNA accumulation.

To investigate how FHA2 represses the DCL1 activity, Park et al. (2021) examined the impact of FHA2 on the interaction of the recombinant DCL1-PRR (PAZ-RNase III-RBD) with pri-miRNAs. The results show that FHA2 represses the pri-miRNA-DCL1-PRR interaction, showing that FHA may inhibit DCL1 activity through preventing its access to pri-miRNAs (Park et al., 2021). Intriguingly, FHA2 also stimulates the HYL1-pri-miRNA interaction (Figure 2B), which seems to be contradictory to its negative role in miRNA biogenesis (Park et al., 2021). Perhaps, FHA2 has at least two non-mutually exclusive roles in miRNA biogenesis. On the one hand, it may facilitate HYL1 to compete with other double-stranded RNA-binding proteins for pri-miRNAbinding, and thereby ensure HYL1 function. On the other hand, it represses DCL1 activity, which is a rate limiting step for miRNA biogenesis. The balance of these two roles of FHA2 may ensure proper miRNA biogenesis.

FHA2 negatively modulates DCL1 activity whereas DDL has an opposite effect, revealing that FHA-containing proteins have a multifaceted role in miRNA biogenesis. Their different impacts on pri-miRNA processing are likely due to that they recognize different protein domains of DCL1. Arabidopsis encodes 19 FHA-containing proteins. It will be interesting to test the function of other FHA domain-containing proteins in miRNA pathway.

FHA2 LINKS LIGHT SIGNAL TO miRNA BIOGENESIS

Several studies suggest that light modulates miRNA biogenesis by affecting the stability of proteins essential for pri-miRNA

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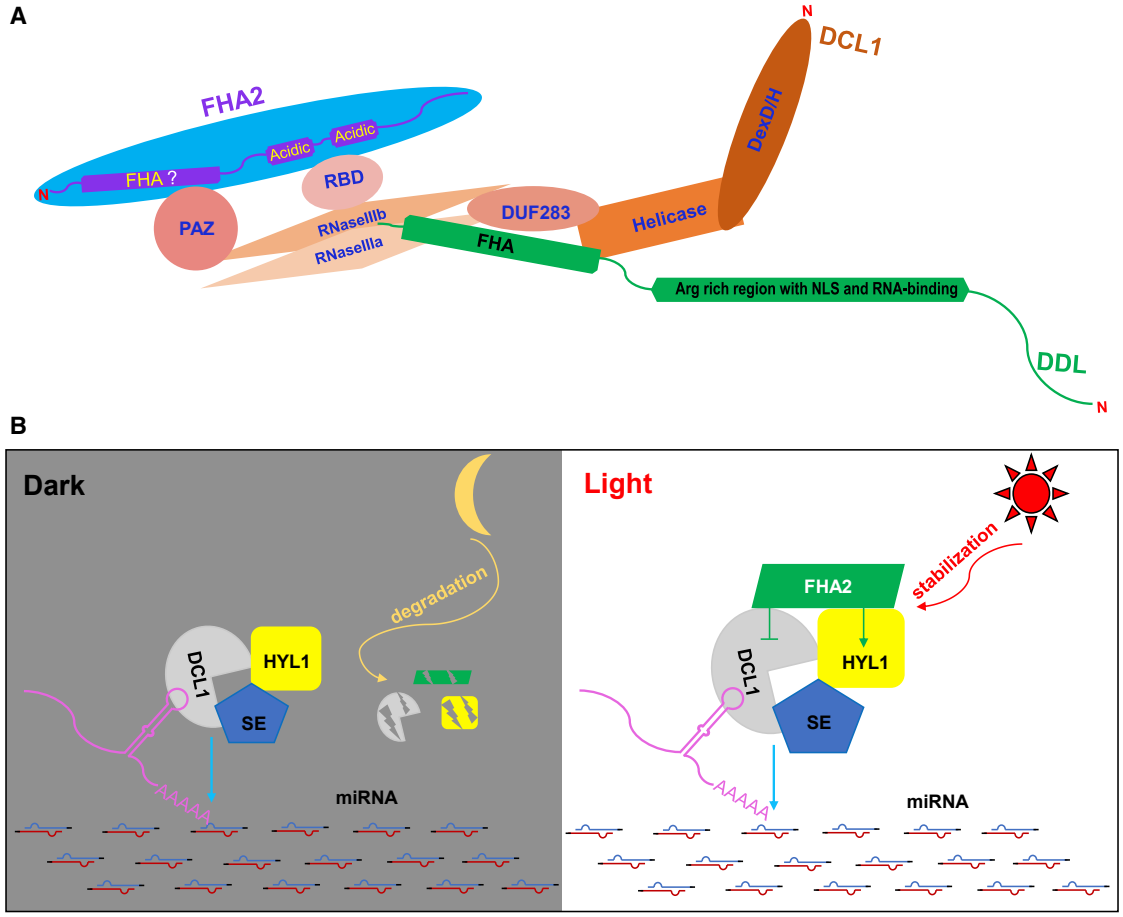

Figure 1. FHA2 links light signal to miRNA biogenesis.

(A) Diagram of the DDL-DCL1 and FHA2-DCL1 interactions. N, N-terminal; "?" indicates that it is unknown if the FHA domain mediates the FHA2-DCL1 interaction.

(B) Plants balance miRNA biogenesis under dark and light through orchestrating the amounts of the DCL1 and FHA2 proteins. FHA2 interacts with DCL1 to limit its access to pri-miRNAs, suppressing miRNA production. FHA2 and DCL1 are stabilized by light during de-etiolation, resulting in increased protein abundance (right) and vice versa under dark (left). The coordinated regulation of the DCL1 and FHA2 abundance equalizes the production of miRNAs under light and dark. FHA2 interacts with HYL1, which is also stabilized by light, to increase its affinity to pri-miRNAs. However, the role of the FHA2-HYL1 interaction in miRNA biogenesis remains to be determined.

processing (Song et al., 2019). For instance, the stability of HYL1 under light depends on CONSTITUTIVE PHOTOMORPHOGENIC 1 E3 ligase, a crucial regulator of the light signaling pathway (Cho et al., 2014). Consistent with this observation, prolonged darkness causes degradation of HYL1. Notably, during prolonged darkness, a small portion of HYL1 is phosphorylated, retained and stabilized in the nucleus as an inactive form (Achkar et al., 2018). Upon expose to light, phosphorylated HYL1 is quickly dephosphorylated and reactivated (Achkar et al., 2018).

Interestingly, miRNA biogenesis also subjects to regulation during de-etiolation (Choi et al., 2020). During de-etiolation, the protein levels of DCL1 and HYL1 are elevated caused by light-dependent stabilization (Choi et al., 2020). However, most miRNAs do not show changes in abundance in de-etiolated plants relative to etiolated ones, accompanied with increased levels of pri-miRNAs (Figure 1B) (Choi et al., 2020). This result suggests that light may also inhibit pri-miRNA processing (Figure 1B). Indeed, the DCL1 complex from light-exposed plants displays a reduced activity relative to that from plant in the dark (Choi et al., 2020). The reduced DCL1 activity can be restored by overexpression of the PAZ domain of DCL1 (Choi et al., 2020). These results show that light may trigger a negative regulator of DCL1, which can be decoyed by the PAZ domain of DCL1 (Choi et al., 2020), and raise the need to look for the light-triggered suppressor of DCL1.

Park et al. (2021) found that the protein levels of FHA2 are increased in light without changes in transcript levels. In addition, FHA2 protein levels are increased in dark under the presence of MG132, a proteasome inhibitor, suggesting that FHA2 is stabilized by light (Park et al., 2021). These results together with facts that FHA2 interacts with the PAZ domain and inhibits DCL1 activity demonstrate that FHA2 is the light-triggered suppressor of DCL1 (Figure 1B) (Park et al., 2021). Further supporting this notion, miRNA levels in *fha2* are similar

to those in the transgenic plants overexpressing the PAZ domain of DCL1 (Park et al., 2021).

In summary, the study of FHA2 not only reveals multiple roles of the FHA domain-containing proteins in miRNA biogenesis, but also explains how miRNA biogenesis is balanced between dark and light during de-etiolation. In the light, the levels of both DCL1 and its suppressor FHA2 are elevated due to increased protein stability under light (Figure 1B). In turn, miRNAs are not produced according to the amounts DCL1. In contrast, both DCL1 and FHA2 are reduced in abundance in dark so that considerable amounts of DCL1 activity still exist (Figure 1B). Thus, through synchronizing the stability of DCL1 and FHA2 under light or dark, plants balance the production of miRNAs. Clearly, how light and dark modulate the stability of DCL1 and FHA2 requires further investigation.

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