Utilizing Illumina high-throughput sequencing technology to gain insights into small RNA biogenesis and function

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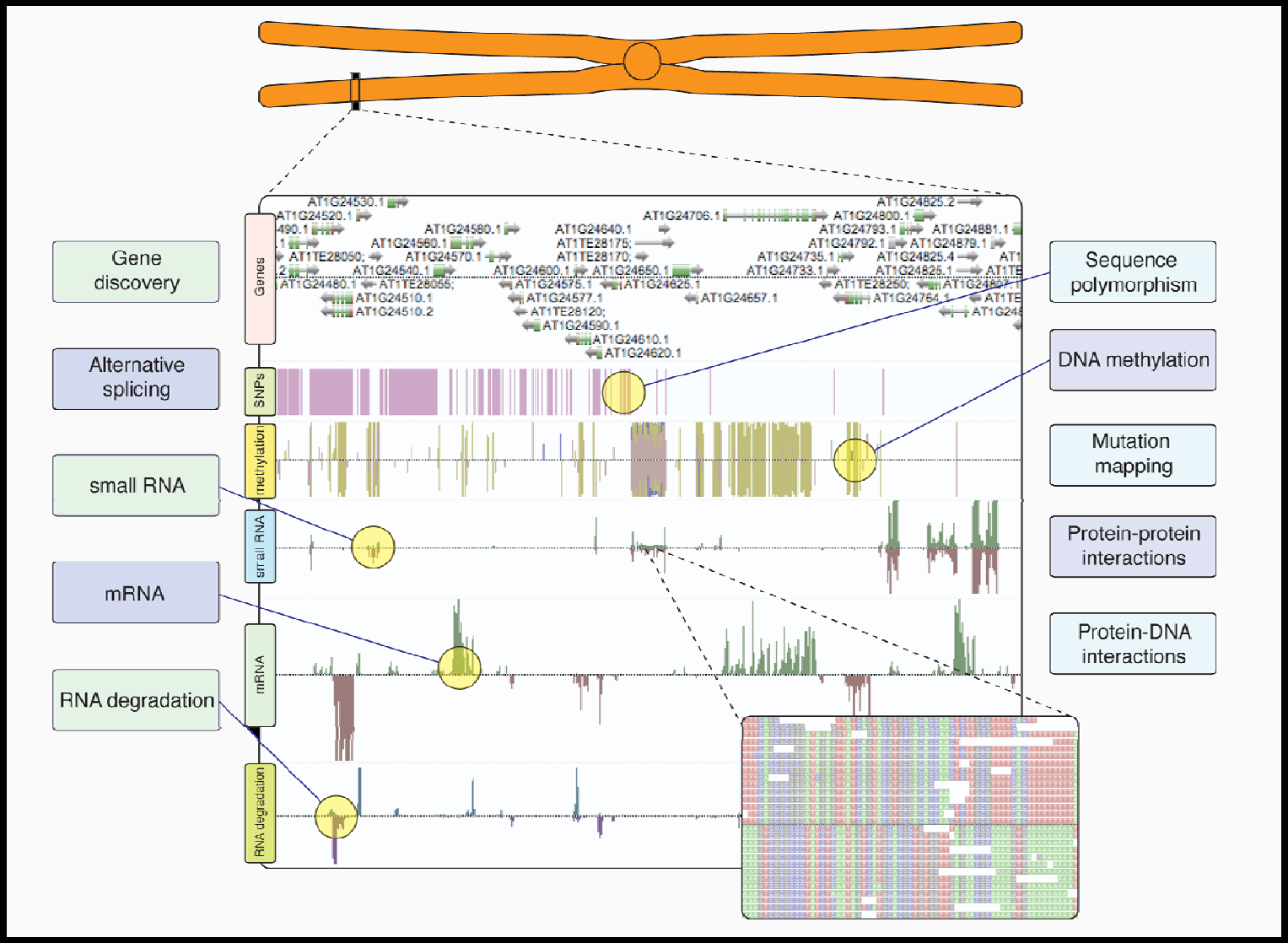
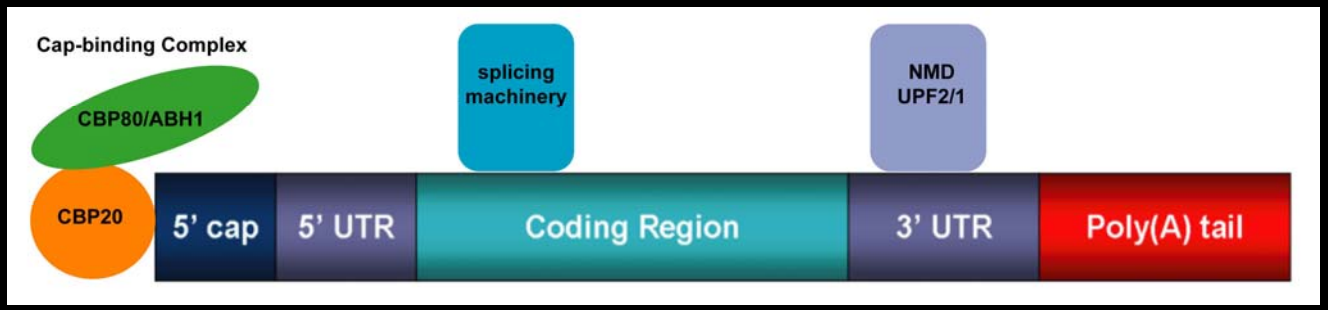
Diverse sequencing approaches to unravel eukaryotic cellular activities



The mRNA cap-binding complex

Arabidopsis ABH1 is the large subunit of the CBC

- Eukaryotic nuclear RNA cap binding protein, CBP80.
- Nonessential for plant growth and development
- *abh1* mutant plants demonstrate ABA-hypersensitive inhibition of seed germination.
- Lesions in the *abh1* gene can also suppress *FRIGIDA*-mediated delayed flowering in *Arabidopsis*.
- Found mostly in the nucleus of *Arabidopsis* guard cells.



Data from Hugouvieux, V. *et al. Cell* 106: 477-87 and Hugouvieux, V. *et al. Plant Phys.* 130: 1276-87

Phenotypes of *abh1-1* mutant plants

- ***abh1*** **mutant plants demonstrate a serrated leaf edge phenotype.**

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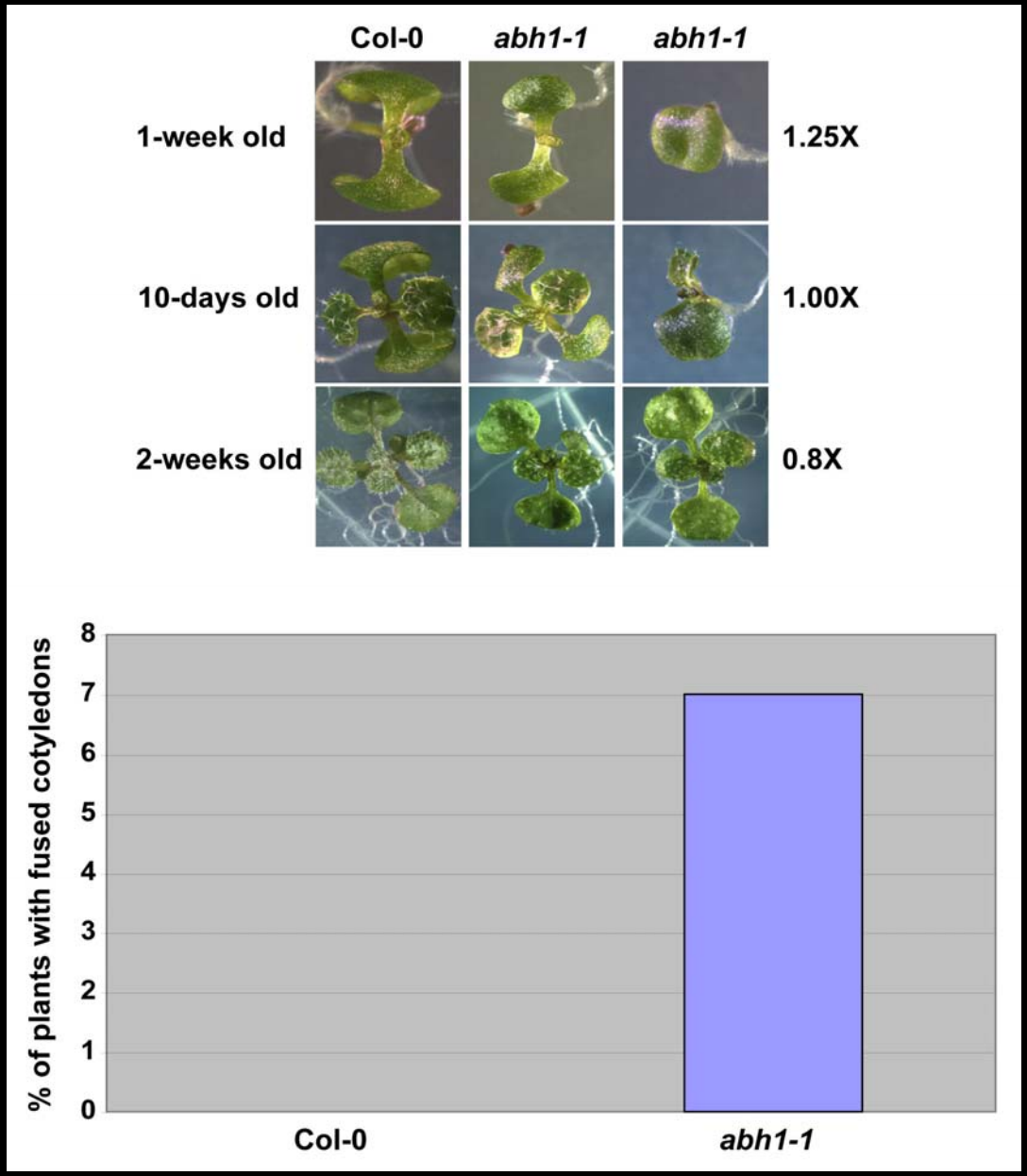
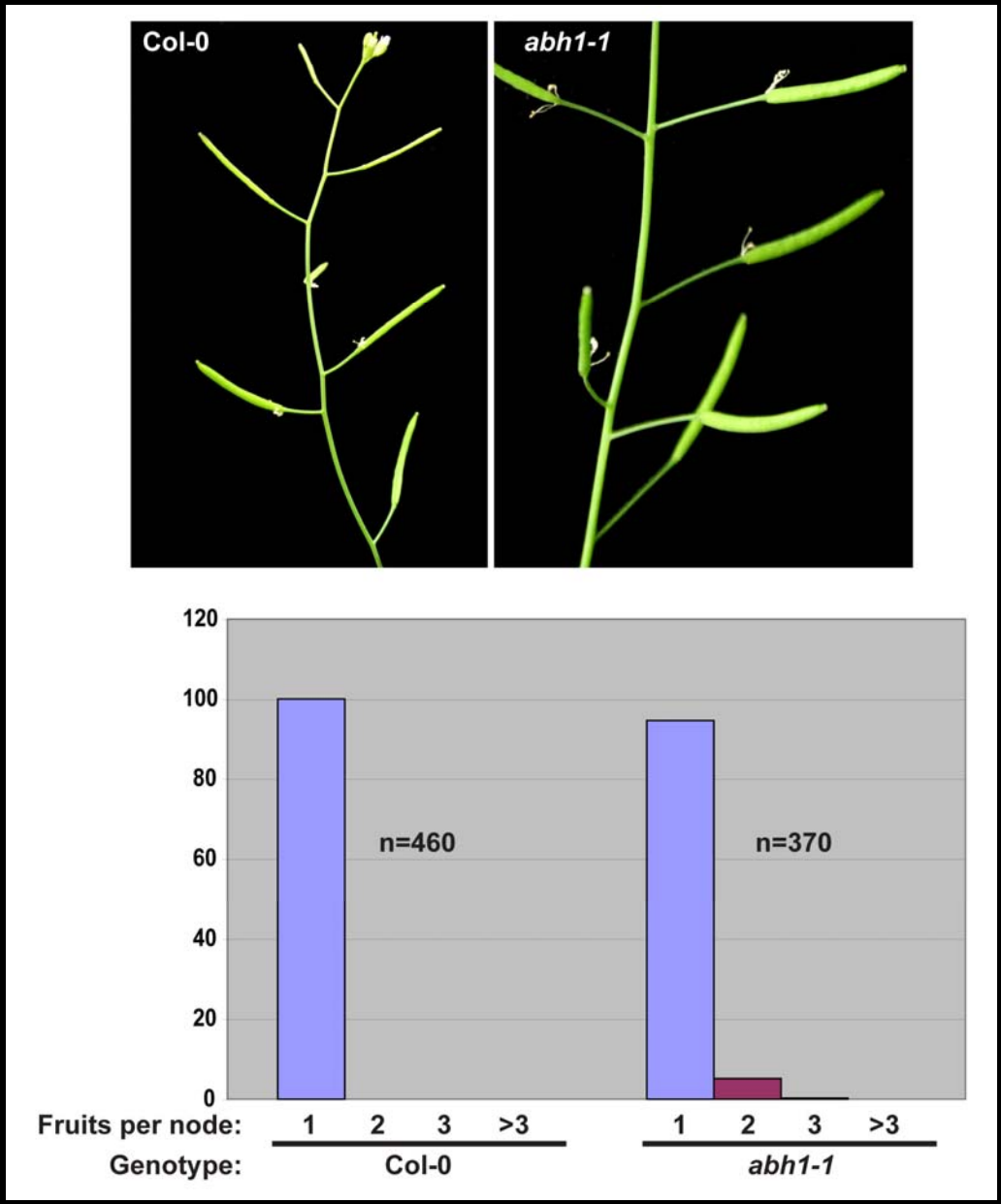
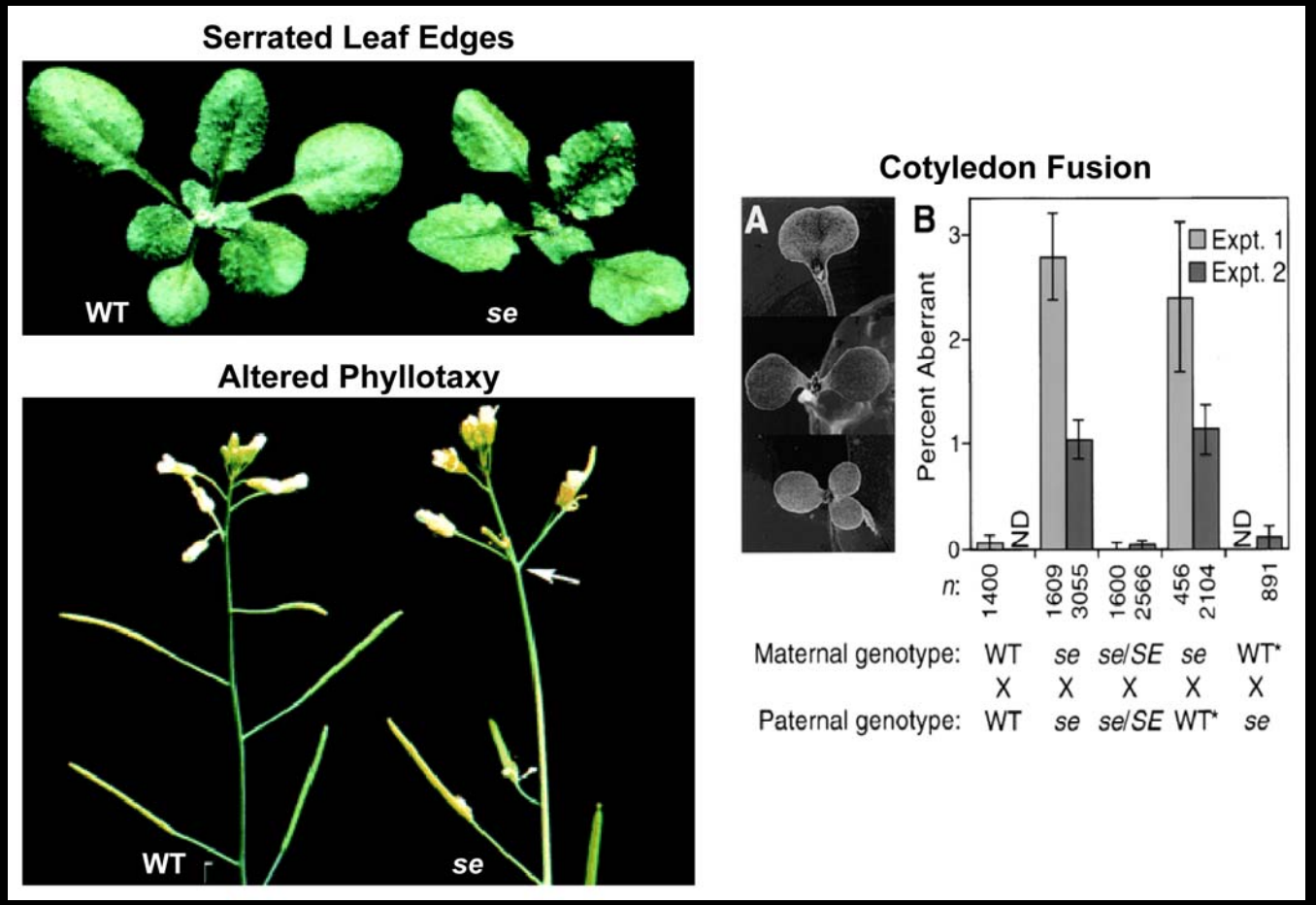
Phenotypes of *abh1-1* mutant plants



Altered phyllotaxy in *abh1-1* mutant plants



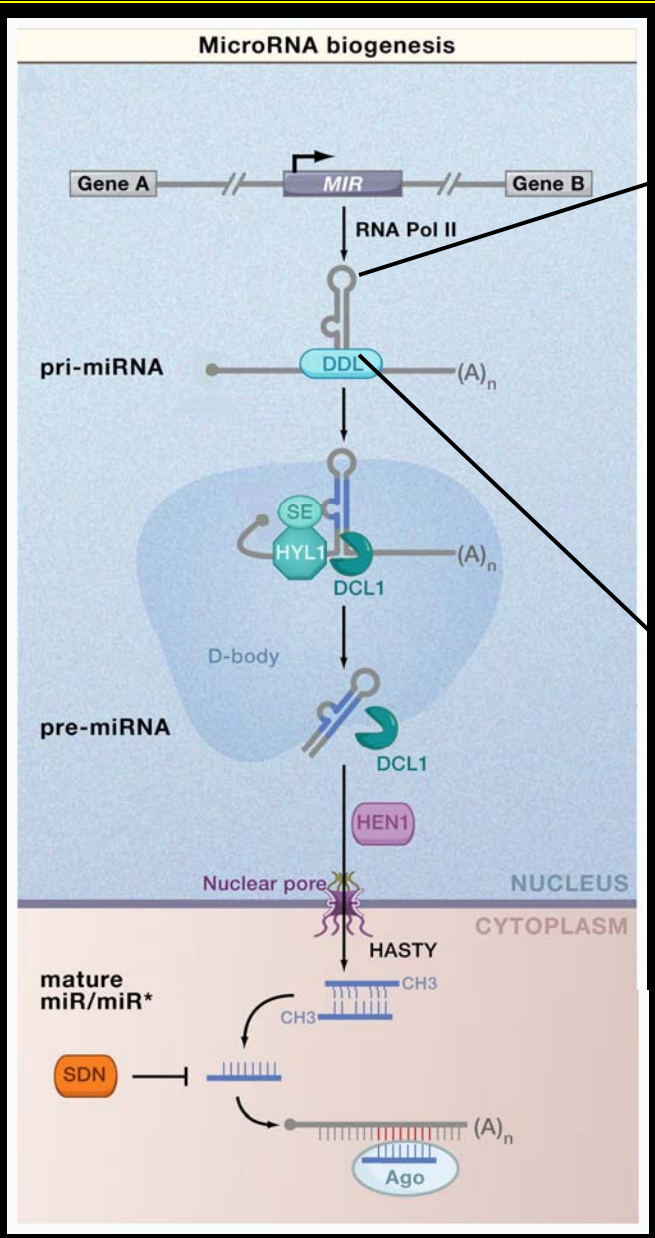
Developmental defects manifested in plants harboring a mutation in *SERRATE*, which encodes a protein recently demonstrated to be required for proper miRNA biogenesis

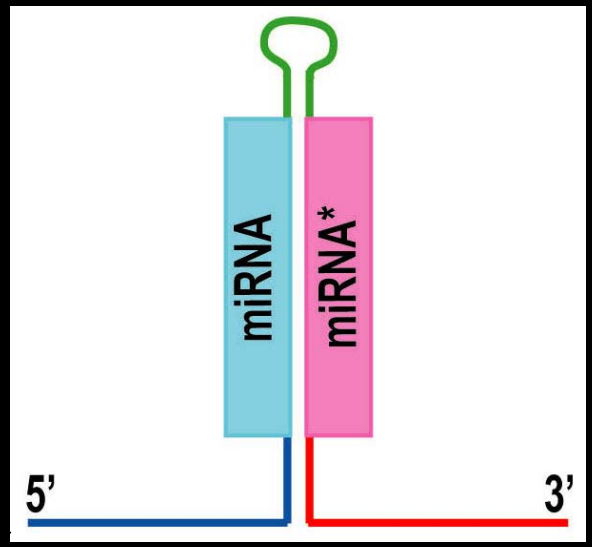


- Lesions in the *se* gene can suppress *FRIGIDA*-mediated delayed flowering in *Arabidopsis*.

From Prigge, M.J. and Wagner, D.R. *Plant Cell* 13: 1263-80

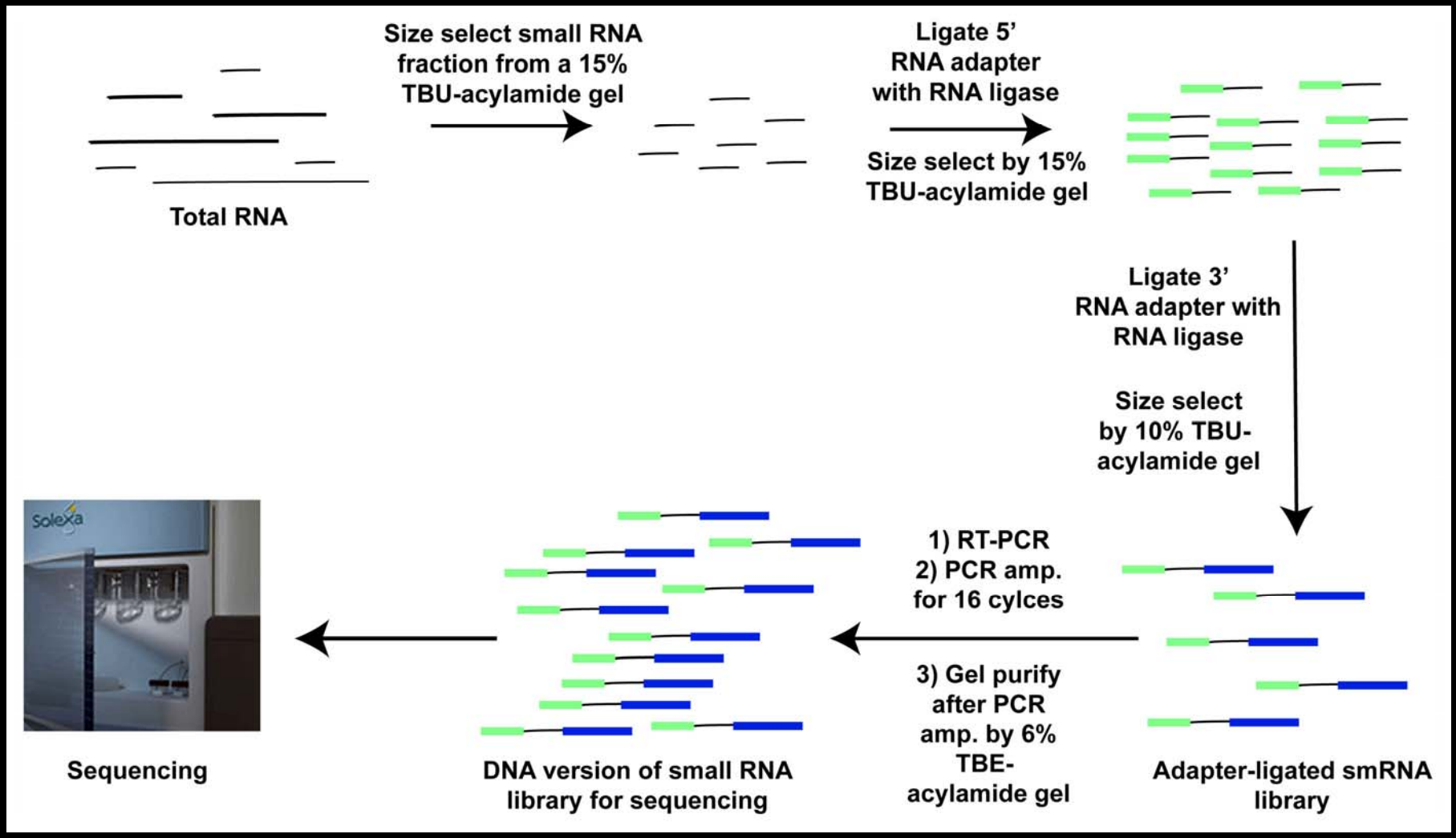
Model for RNA silencing by microRNAs (miRNAs)





Modified from Voinnet, O. *Cell* 136: 669-687

High-throughput sequencing of the smRNA-component of the Arabidopsis transcriptome using an Illumina Genetic Analyzer II



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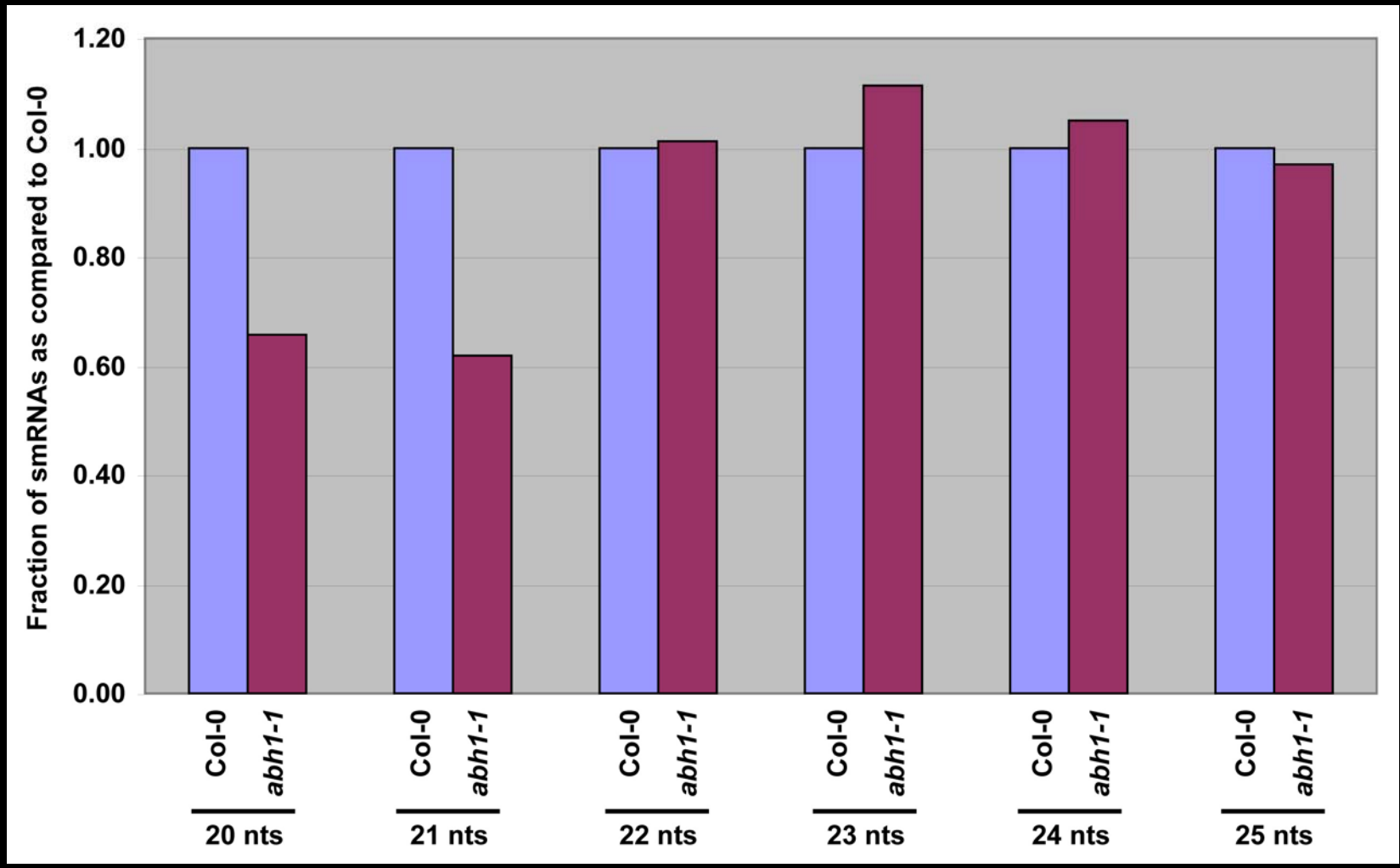
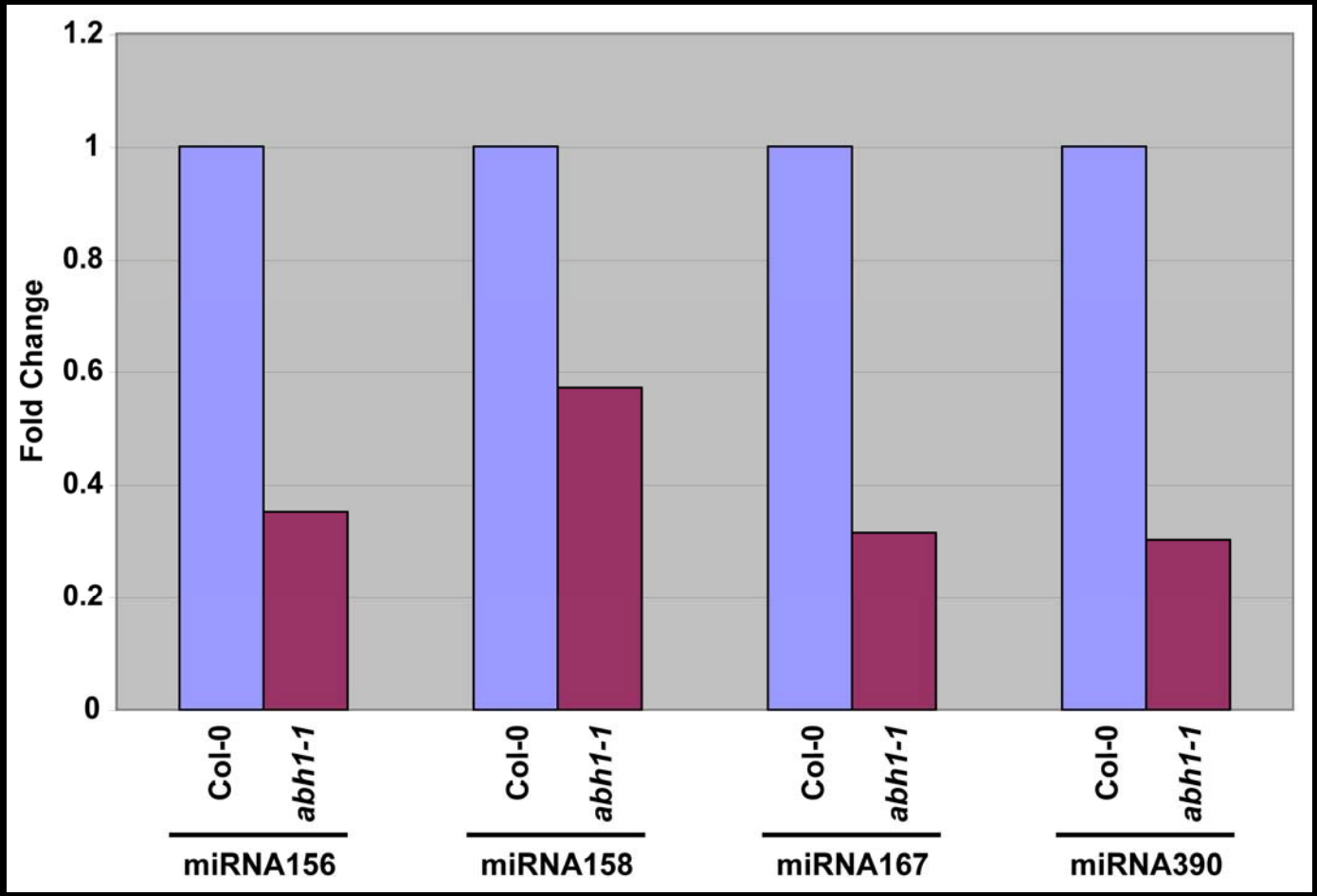


ABH1 is required for proper mature miRNA levels



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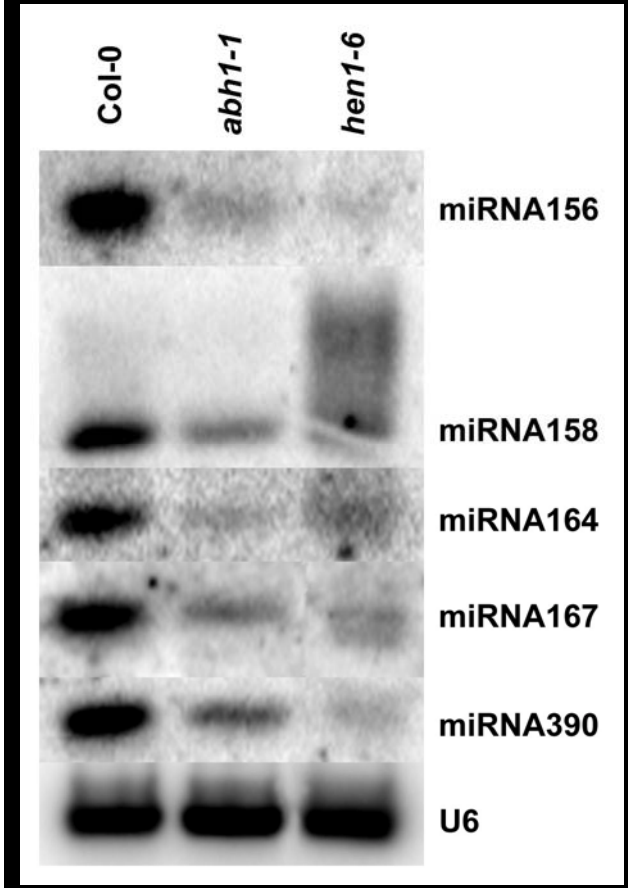
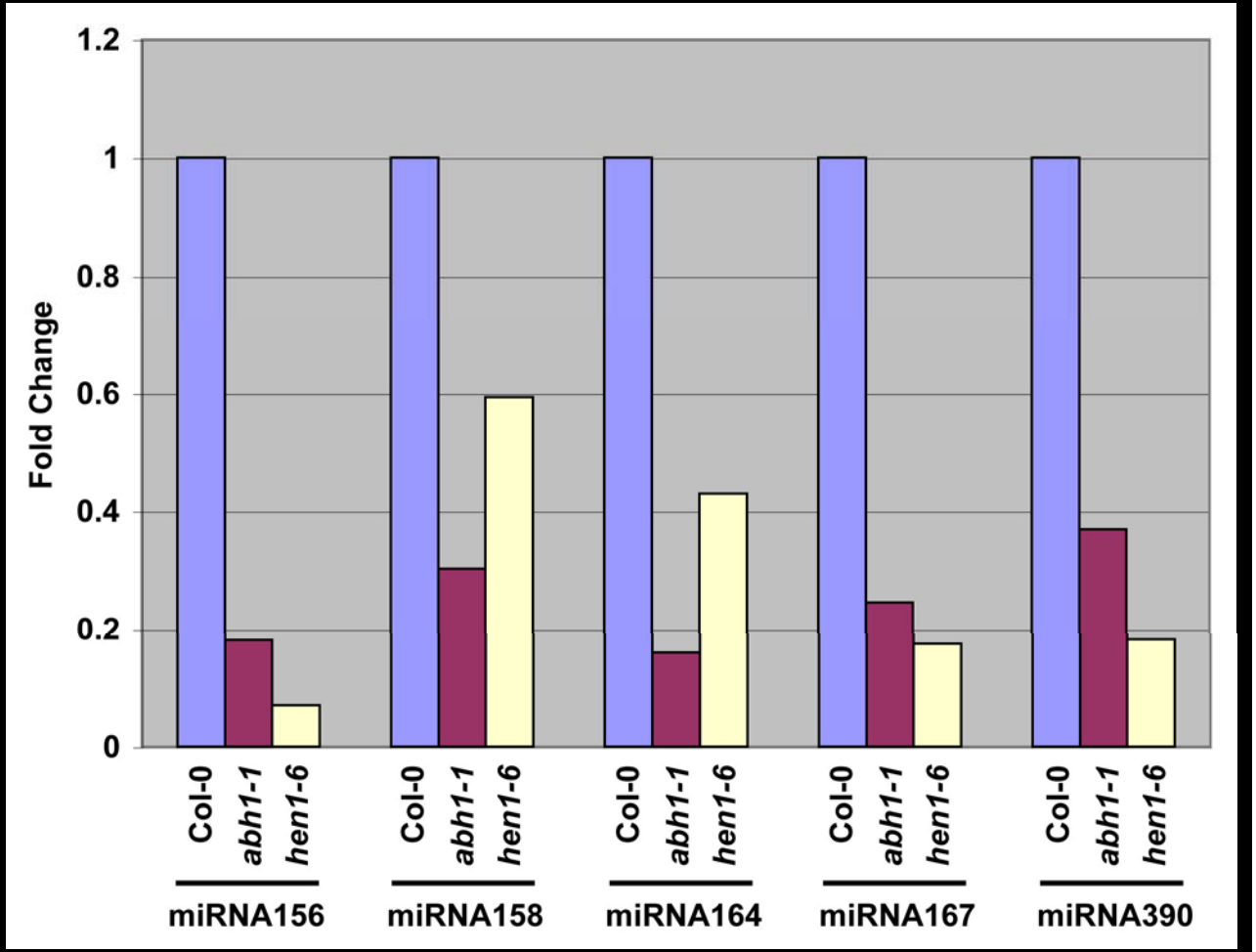
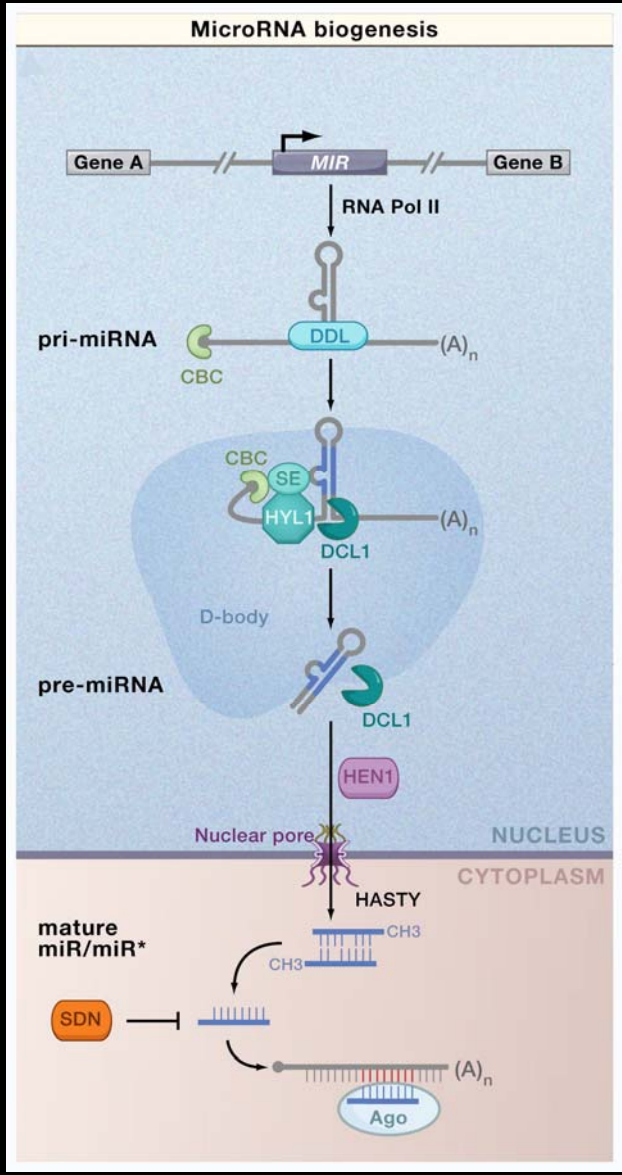




Conclusions



From Voinnet, O. *Cell* 136: 669-687

• ABH1, the largest subunit of the cap-binding complex, is a component of the miRNA biogenesis pathway

- Northern blotting and high-throughput smRNA sequencing on an Illumina Genetic Analyzer II confirm mature miRNA levels are deficient in *abh1-1* mutant plants

- miRNA-directed cleavage is decreased in *abh1-1* mutant plants

- The *abh1-13* mutation can enhance the developmental defects of a hypomorphic allele of *AGO1* (*ago1-27*)

• Plants maintain a cache of miRNAs far greater than what is required for basic growth, development, maturation, and reproduction

-As evidenced by the mostly normal development of *abh1-1* and *abh1-13* plants which contain significantly diminished miRNA populations.

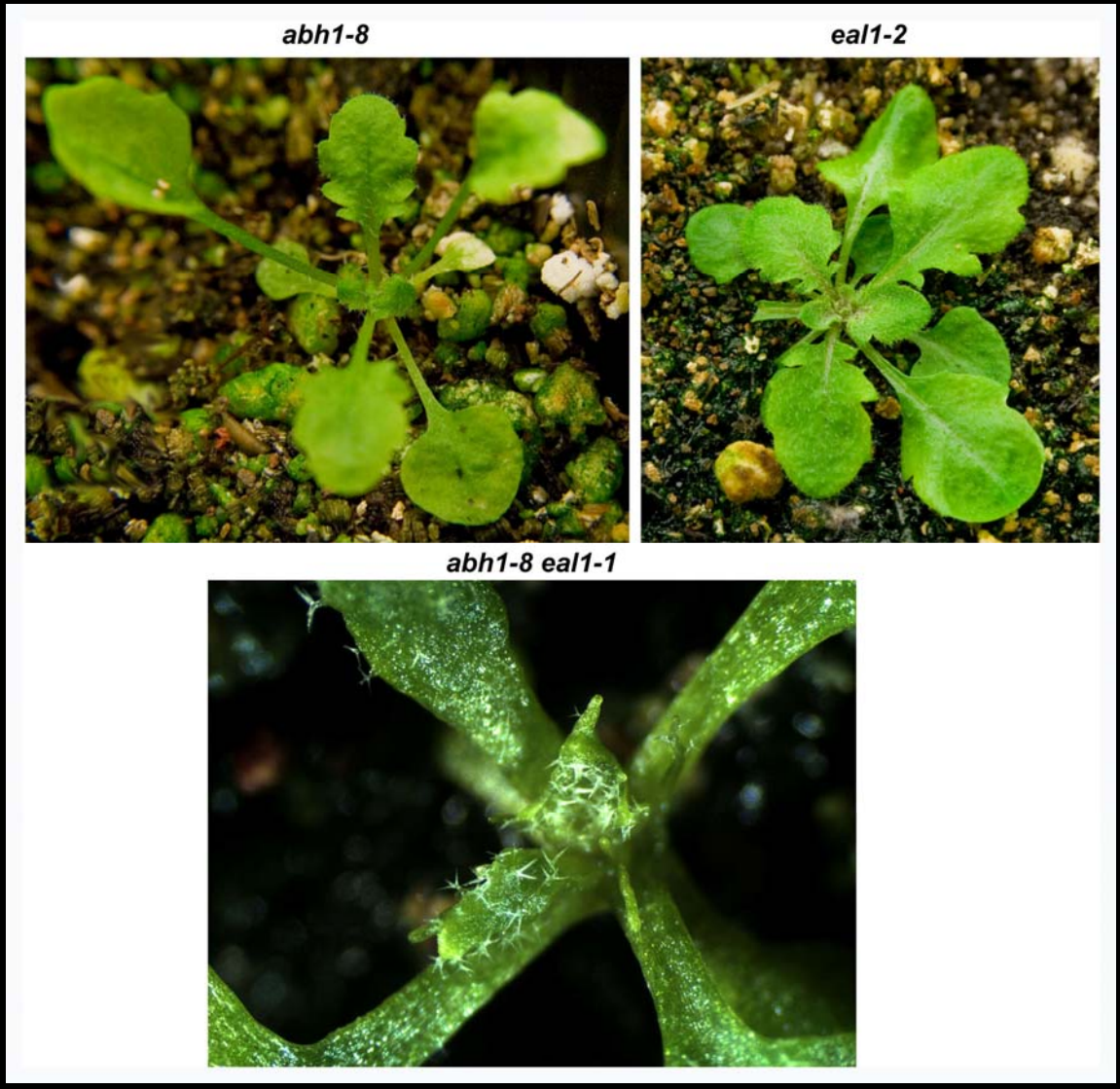
- Furthermore, the decreased miRNA levels in *abh1-1* plants does not significantly affect the overall levels of mRNAs targeted by miRNAs for down-regulation

A forward genetic screen to identify novel components and regulators of RNA silencing pathways

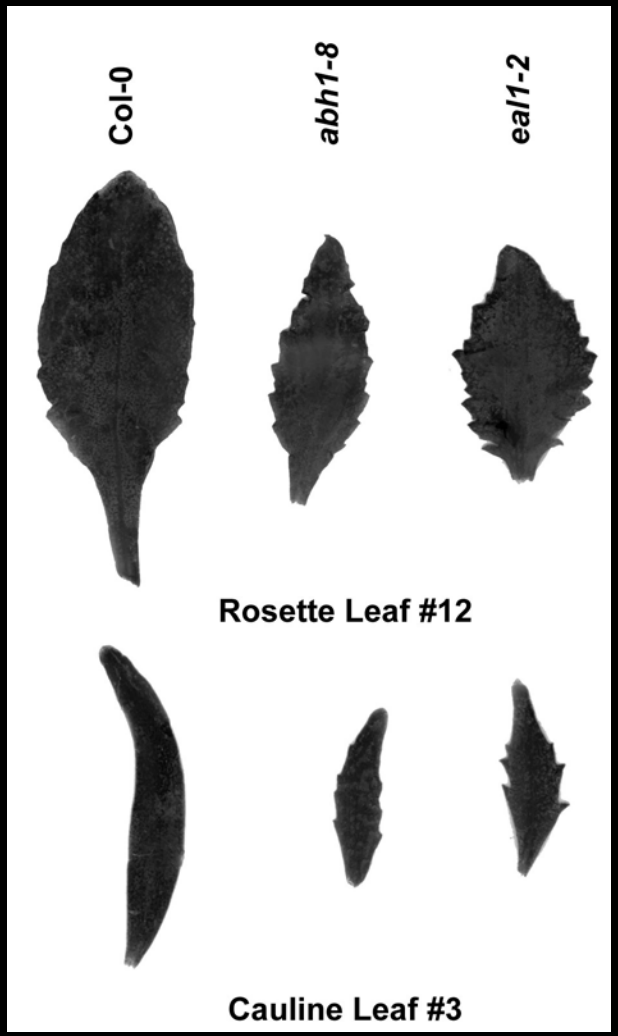
A screen for extragenic enhancer mutants of *abh1-8* plants

Annotated Gene Function
HDAC
PHP domain-containing protein
CLAVATA1-related receptor kinase-like protein
B3 Transcription Factor
Methyltransferase
F-box protein
WRKY Transcription Factor
SWAP Domain RNA Binding Protein
Lsm Domain RNA Binding Protein (EAL1)

eal1 an extragenic enhancer mutant of *abh1-8* plants



abh1-8 extragenic enhancer *eal1* has similar leaf phenotypes as *abh1* mutant plants



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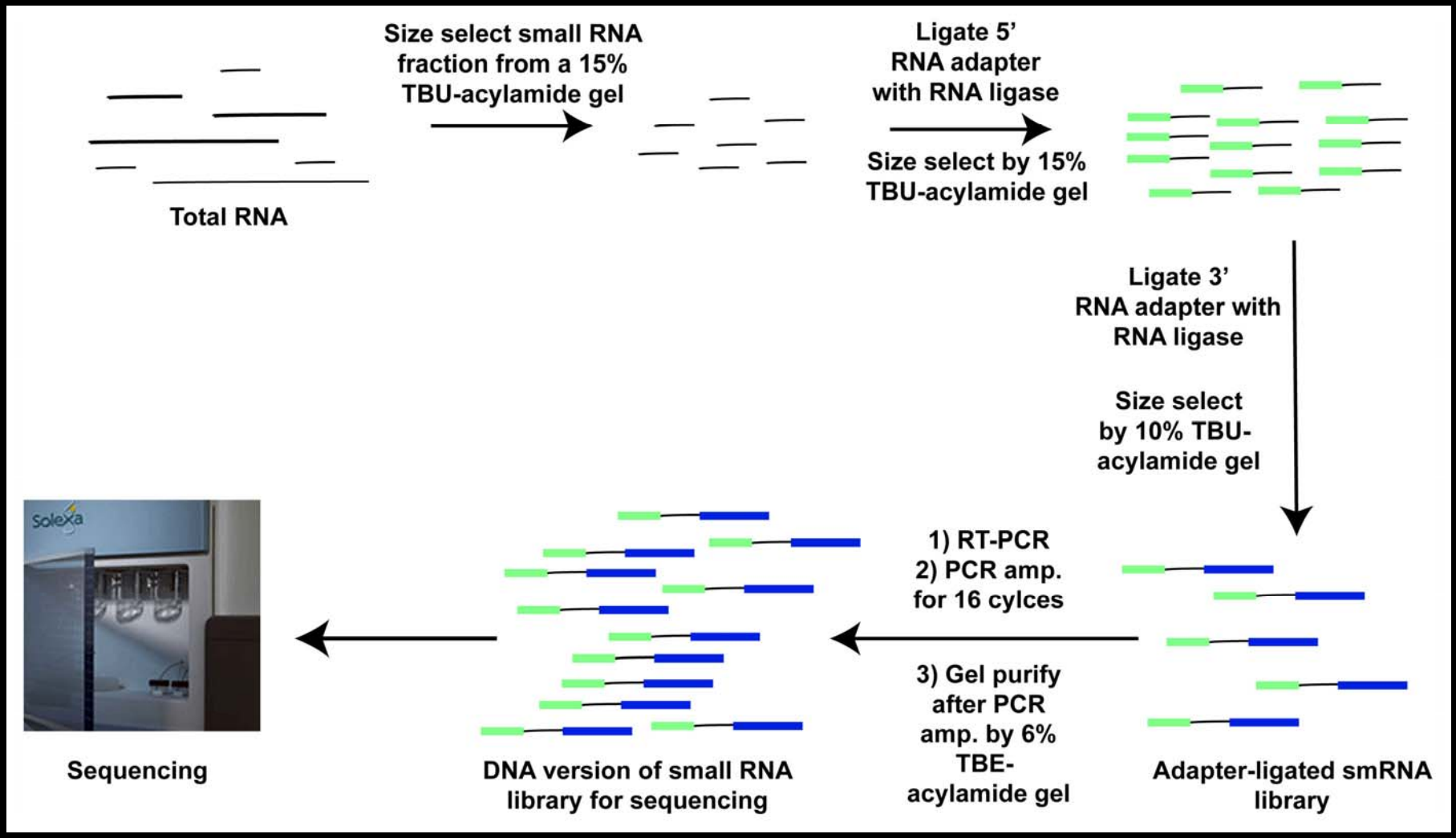
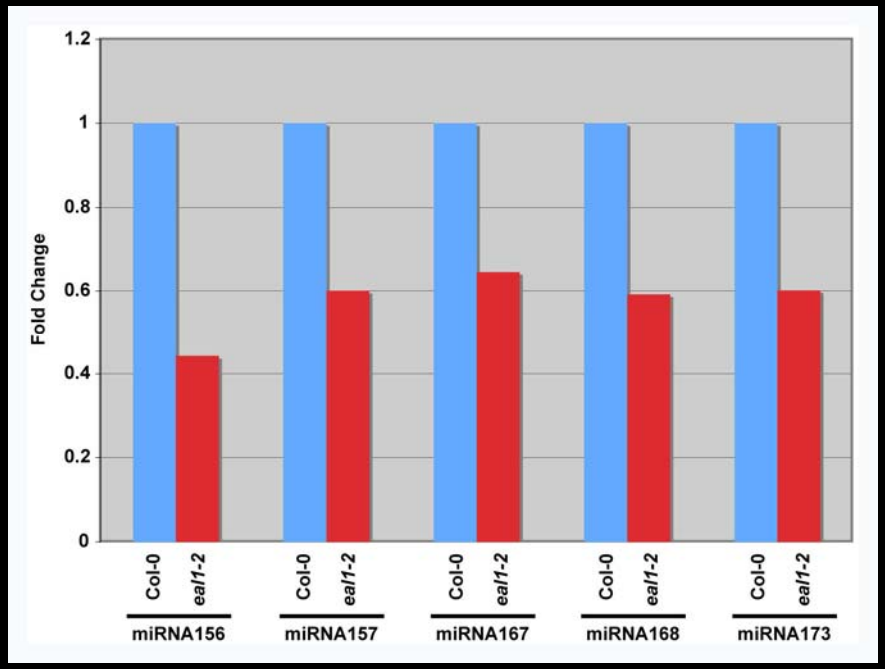
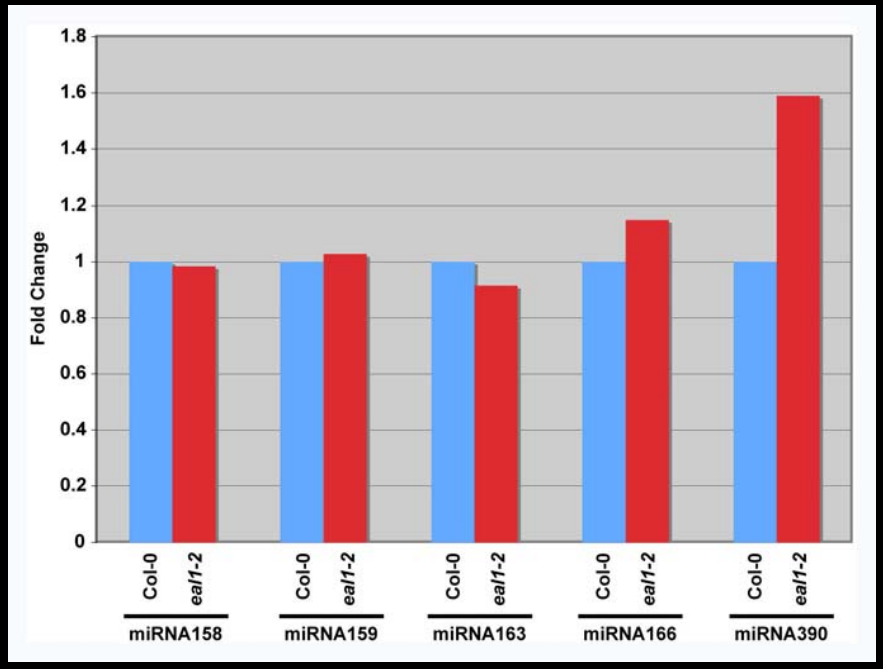
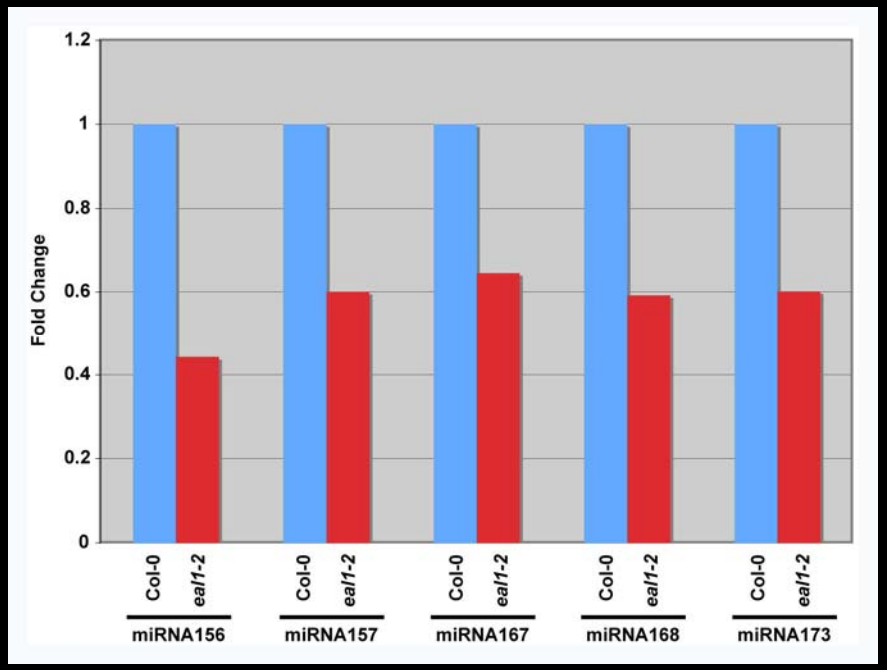


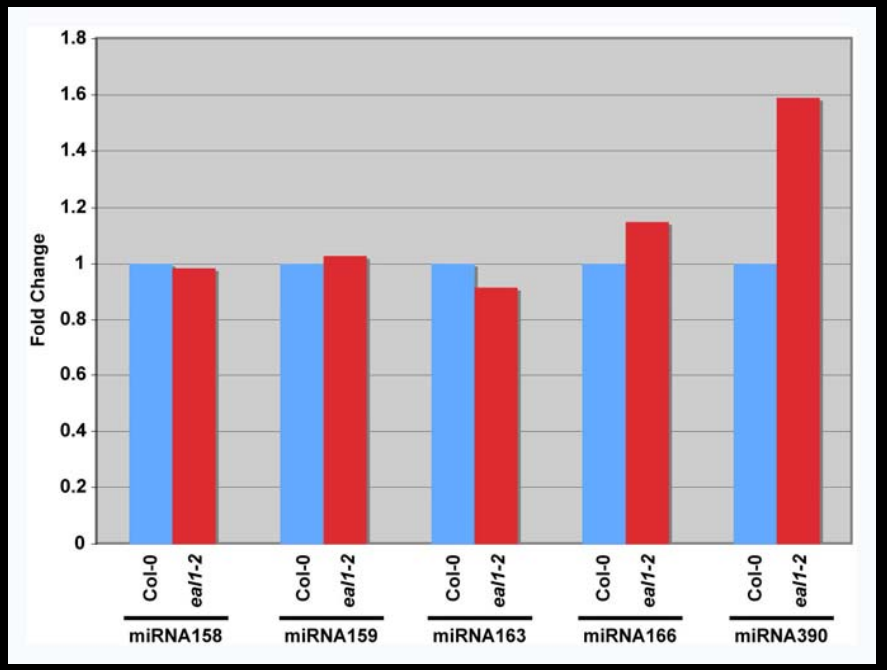
High-throughput sequencing of the smRNAome suggests EAL1 is required for the proper accumulation of specific mature miRNAs

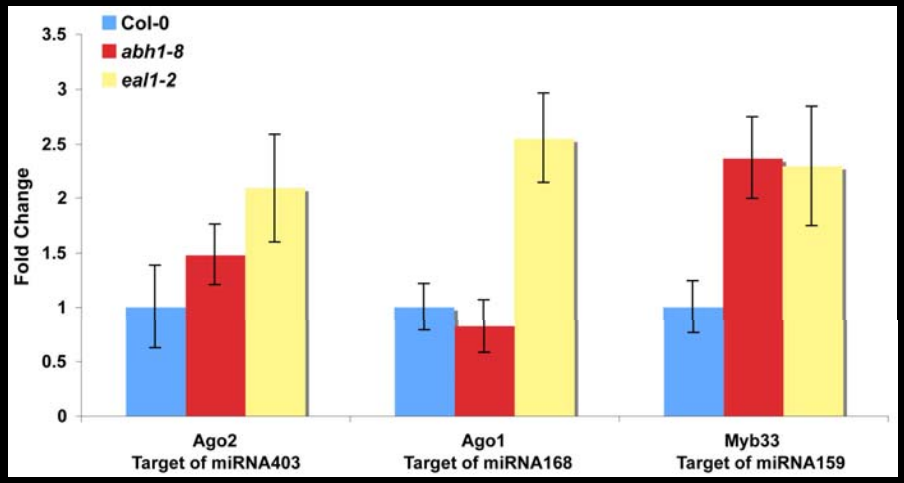


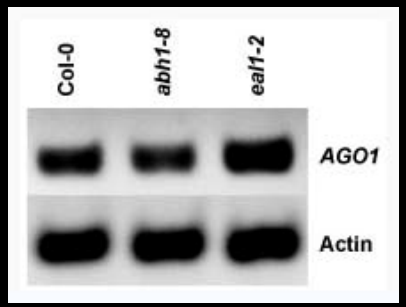


Loss of EAL1 function results in upregulation of numerous miRNA-target mRNAs

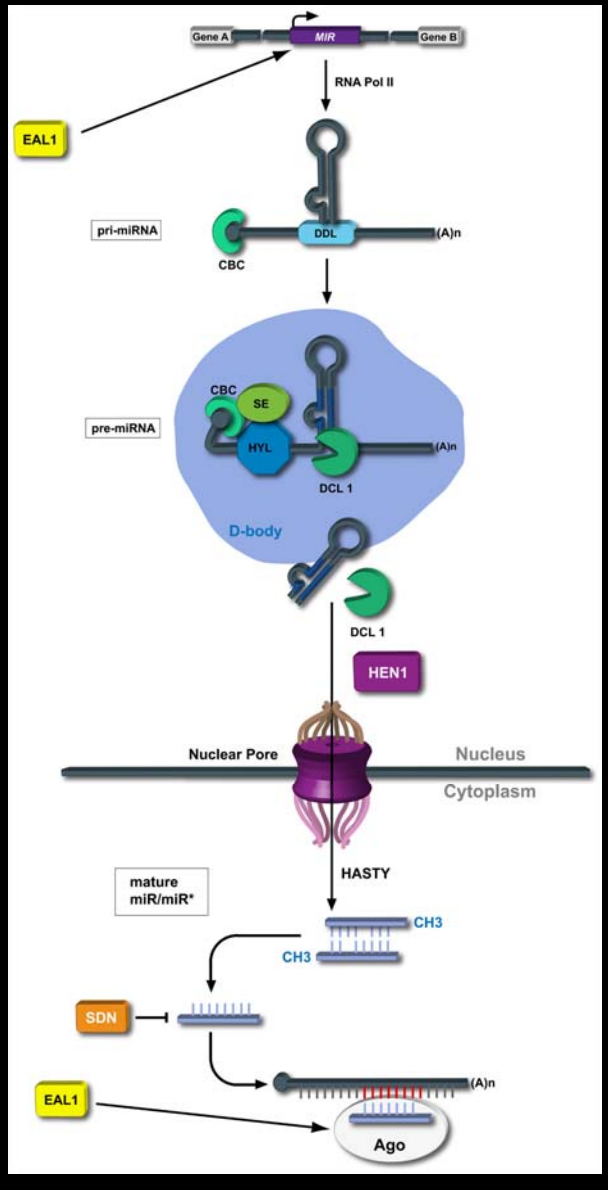








The functions of EAL1 in the miRNA-mediated RNA silencing pathway



• EAL1 is a novel component of the miRNA-mediated RNA silencing pathway that affects the functional silencing of a number of miRNA target mRNAs and the levels of specific mature miRNAs

- *eal1-2* mutant plants are hypersensitive to the plant hormone ABA just like *abh1* mutant plants

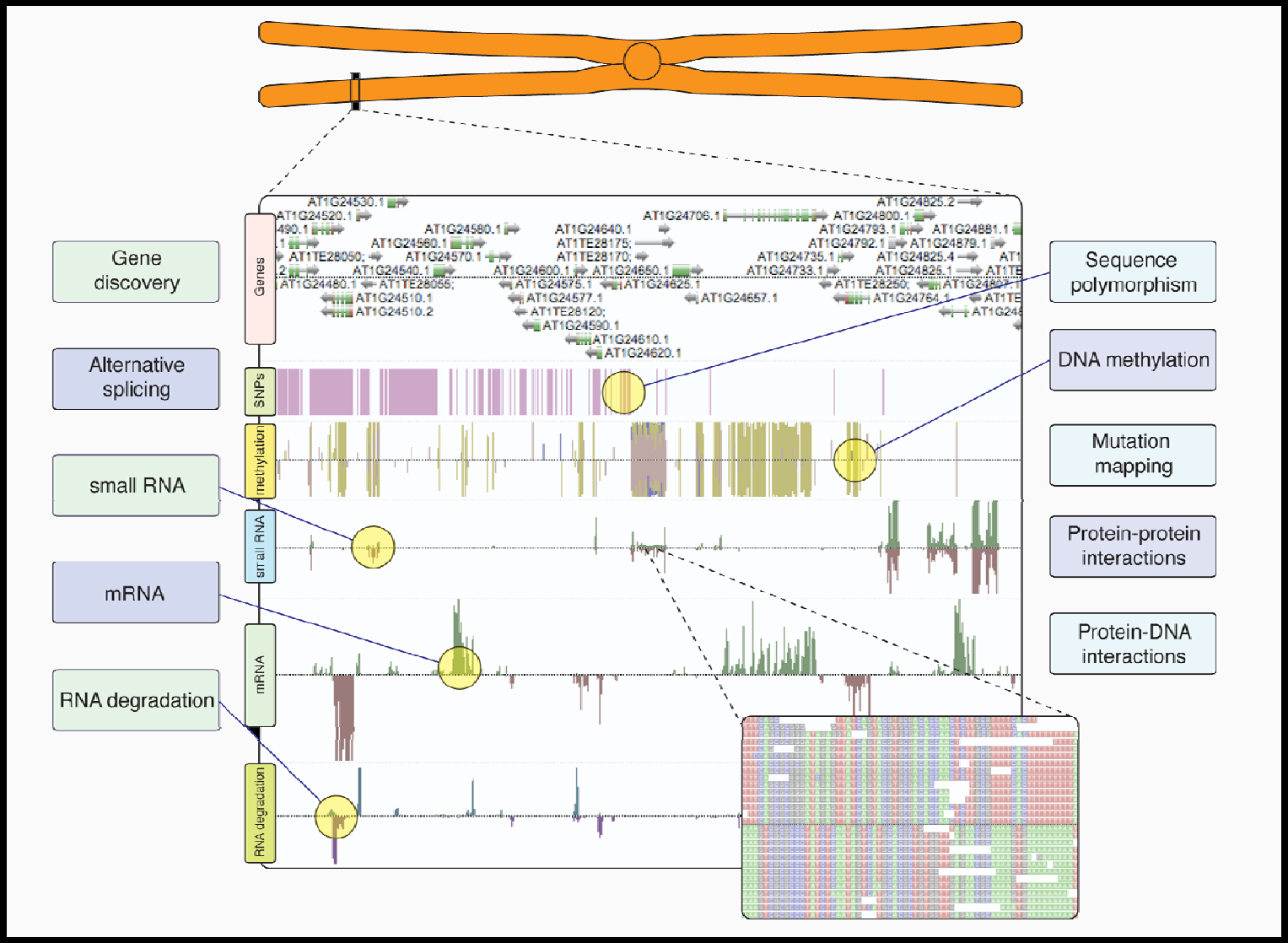
- High-throughput smRNA sequencing demonstrates specific mature miRNA levels are deficient in *eal1-2* mutant plants

- The primary transcript levels of the miRNAs deficient in *eal1-2* mutant plants are also decreased in this genetic background

- Numerous miRNA target mRNAs are upregulated in *eal1-2* mutant plants, even those that are not silenced by miRNA's whose levels are dependent on EAL1 function

  -The ABA hypersensitive phenotype of *eal1-2* mutant plants is likely due to the upregulation of MYB33 and 101, which are targeted by miRNA159 a miRNA that does not require EAL1 function for its proper accumulation

Diverse sequencing approaches to unravel eukaryotic cellular activities



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