Maternal AFB1 and AFB5 Positively Regulate Seed Dormancy in Arabidopsis

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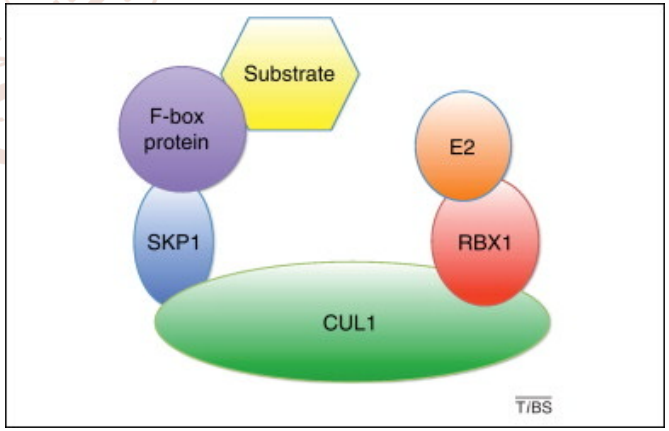
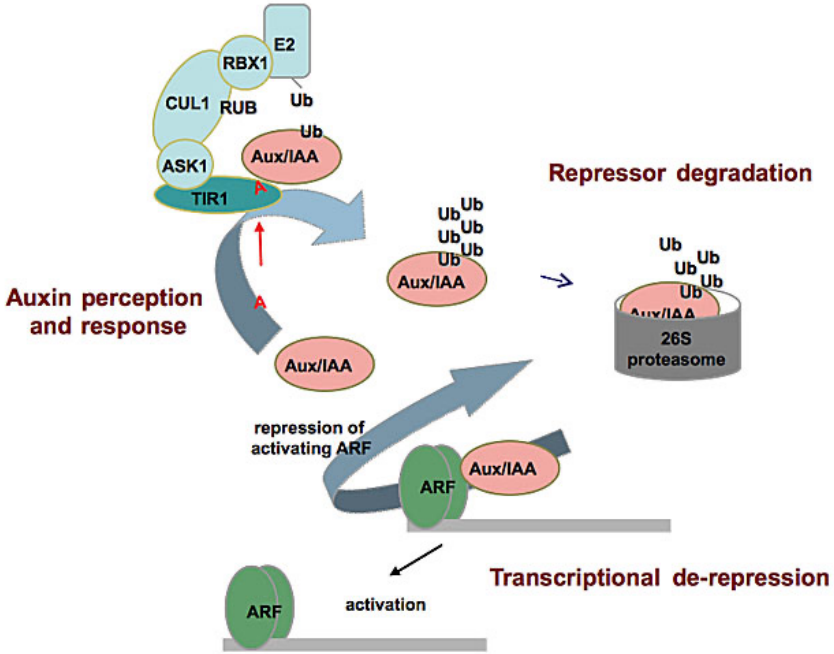
Skp1-Cullin-F-box protein (SCF) ubiquitin ligases



Silverman et al., Trends in Biomedical Science, 37: 66-73, 2012.

Substrates are recruited to the complex by SKP1 and a variable F-box protein that determines substrate specificity. In Arabidopsis, the primary SKP1 is ASK1.

The model of auxin signaling involving SCF^{AFB}-IAA/AUX



Mockaitis and Estelle, Annual Review of Cell and Developmental Biology 24:55–80. 2008

Six AFBs in Arabidopsis: TIR1 and AFB1-5

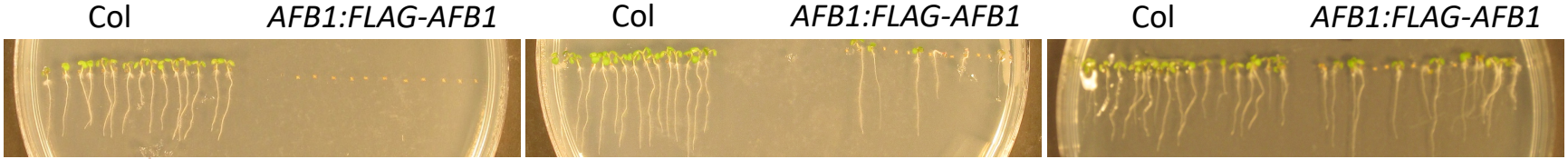
Our findings

AFB1 and AFB5 are the most reliably identified AFBs in the Arabidopsis inflorescence by immunoprecipitation and mass spec

- FLAG-ASK1 was used as the bait in young inflorescences (no open flower)
- AFB1 and AFB5, not the other AFBs (including TIR1), were identified every time in four independent experiments

Each of four *AFB1* transgenes can cause a seed germination defect

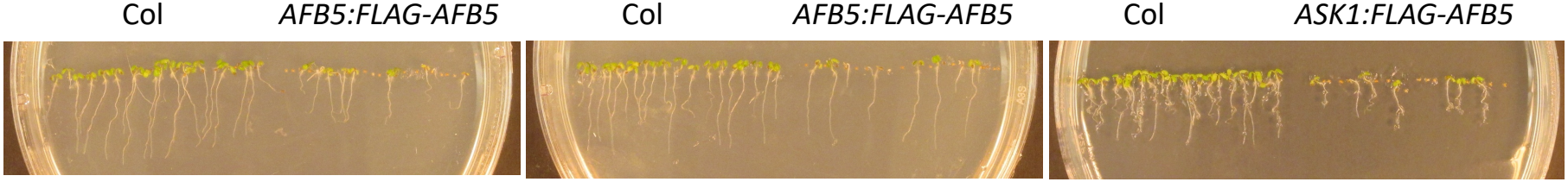
- Transgenes tested in a likely knockout mutant of *afb1* (expression level < 1% of wild-type level by RT-qPCR): *AFB1:FLAG-AFB1, AFB1:AFB1-FLAG, ASK1:AFB1-FLAG, and ASK1:FLAG-AFB1*
- Most severe phenotype: No T_2 seeds germinated after 3 weeks on MS agar medium, which indicates that the defect was likely caused by the maternal tissue in T_1 plants since segregation for the transgene is expected in T_2 seeds



Different severity levels of seed germination defect in independent T_2 lines

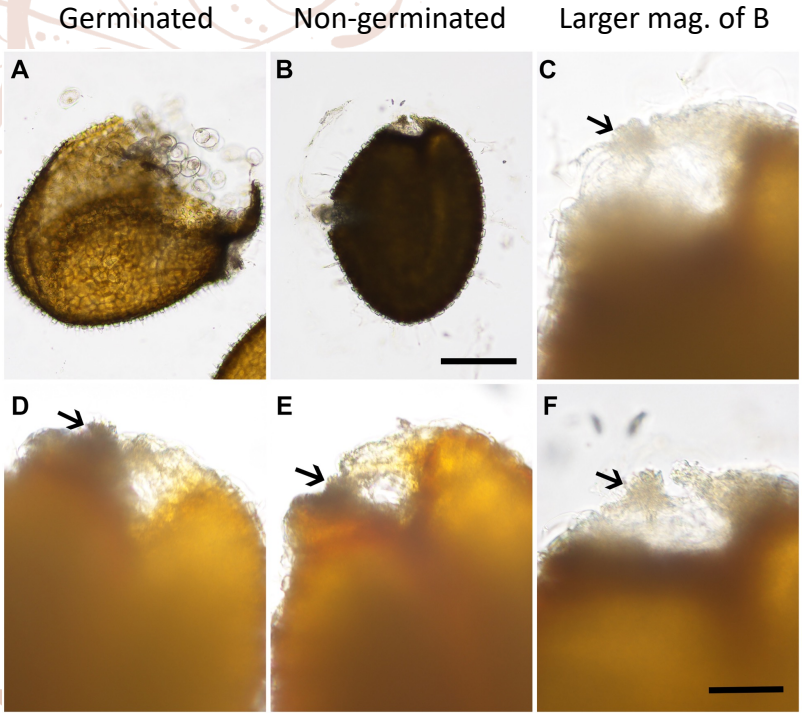
Each of Four *AFB5* transgenes can cause a seed germination defect

- Transgenes tested in a knockdown mutant of *afb5* (expression level ≈ 70% of wild-type level by RT-qPCR): *AFB5:FLAG-AFB5, AFB5:AFB5-FLAG, ASK1:AFB5-FLAG, and ASK1:FLAG-AFB5*
- Most severe phenotype: Few T_2 seeds germinated after 3 weeks on MS agar medium, which again indicates that the defect was likely caused by the maternal tissue in T_1 plants



Different severity levels of seed germination defect in independent T_2 lines

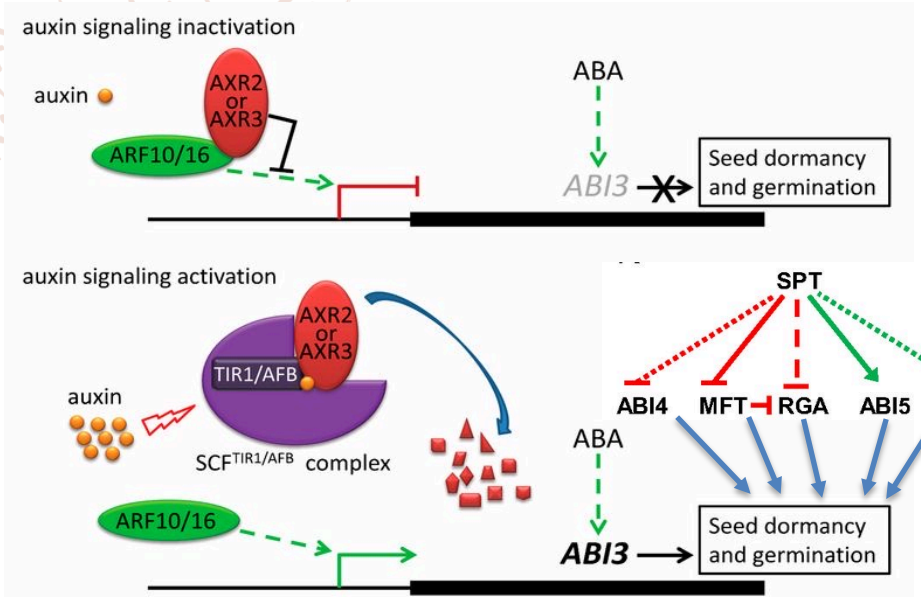
Non-germinated seeds of *AFB1:FLAG-AFB1* can be imbibed—suggestive of a defective signaling event



- Non-germinated seeds could have ruptured seed coat after long imbibition
- Inner part of hilum on non-germinated seeds could swell and project outward

- A-C and F, 20 days on MS agar medium
- D and E, ~5 minutes in water
- Arrows indicate outer part of hilum
- Bar in F for A-C and F = 50 µm, and bar in B for A and B = 200 µm

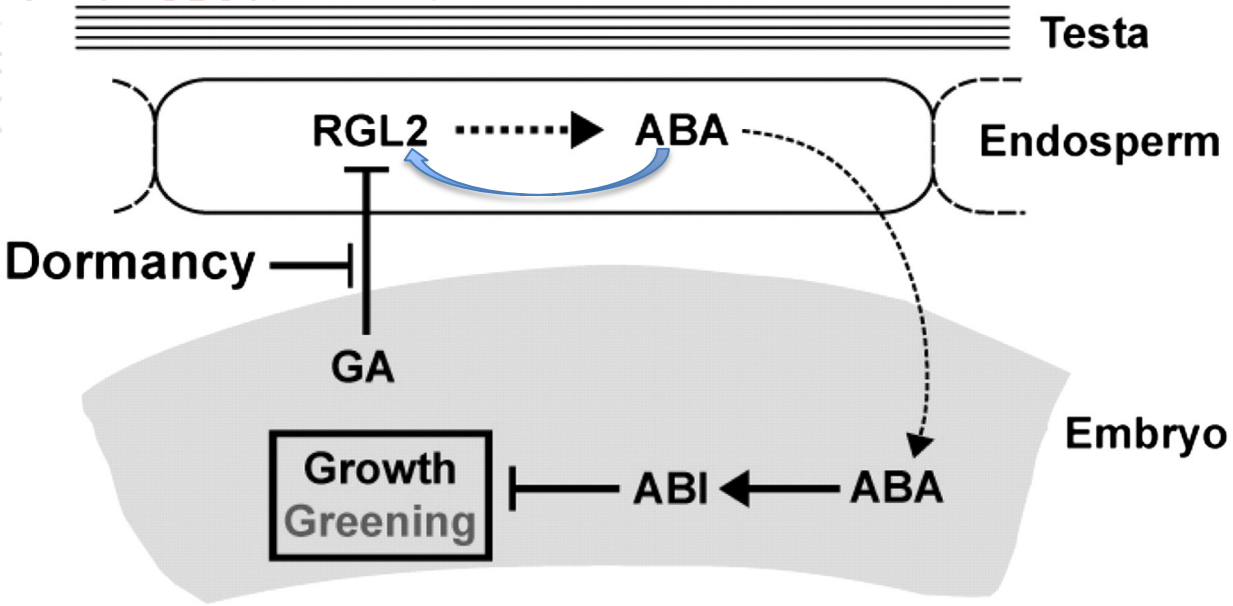
Auxin, ABA, and GA regulate seed dormancy



Xiaodong Liu et al. PNAS 2013;110:15485-15490

Fabián E. Vaistij et al. PNAS 2013;110:10866-10871

Model for seed coat- and ABA-dependent repression of dormant seed germination



Keun Pyo Lee et al. PNAS
2010;107:19108-19113

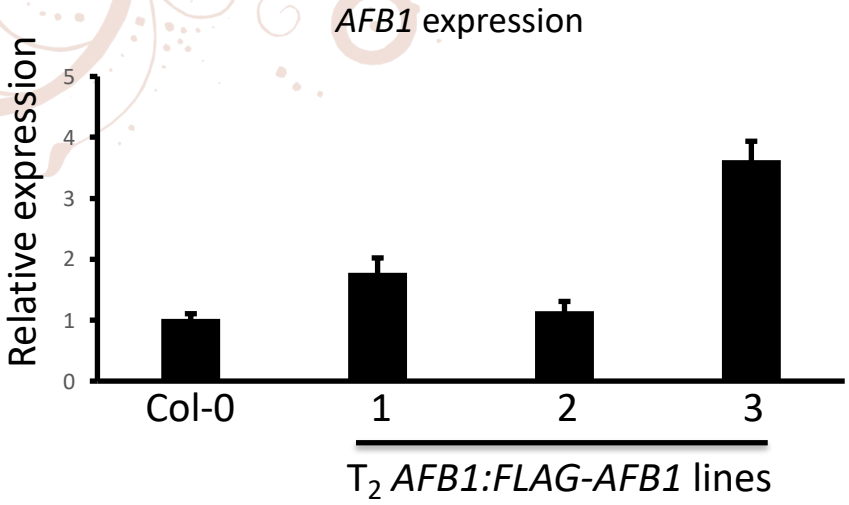
Hypothesis

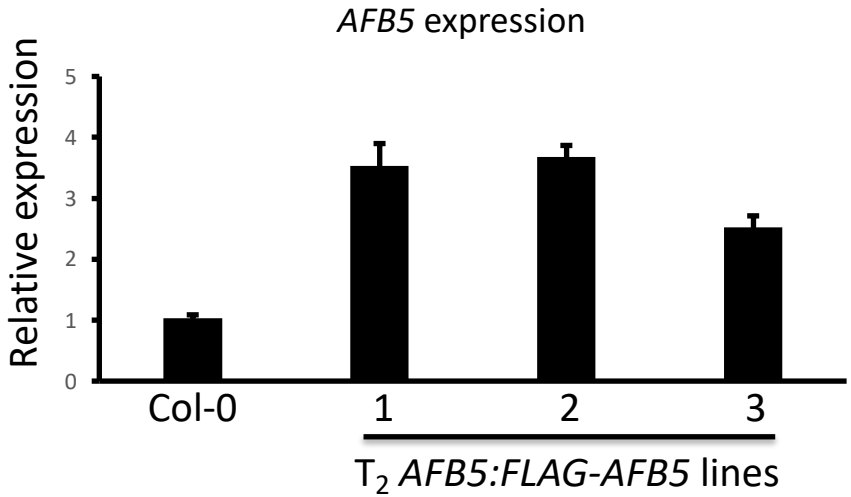
The seeds of the transgenic plants cannot germinate or germinate in a delayed fashion because of abnormally high levels of auxin signaling in the seed coat.

Predictions

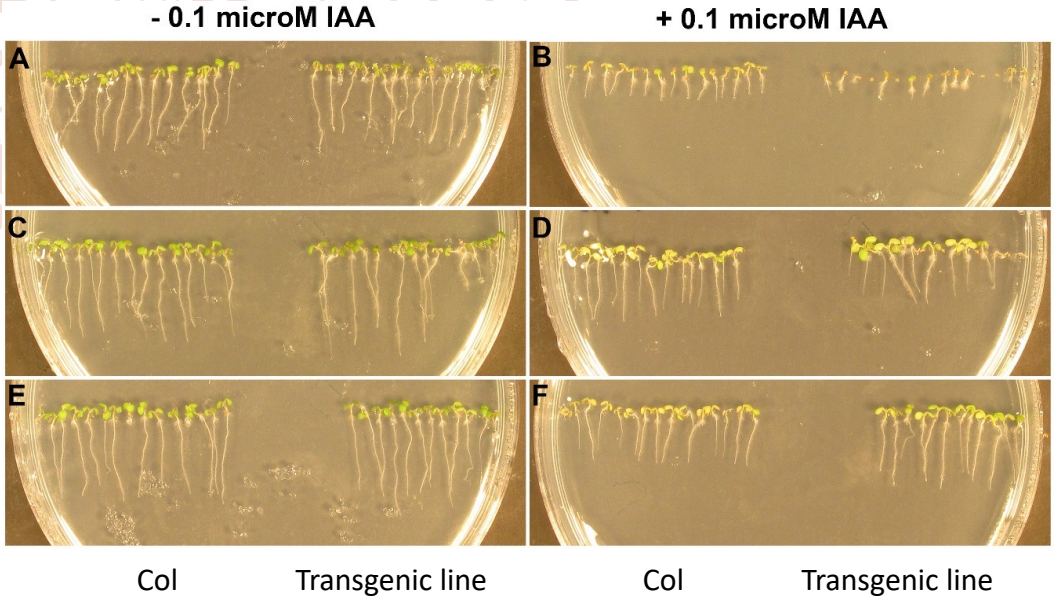
- *AFB1* or *AFB5* expression levels in at least some transgenic lines are higher than that in the wild type
- At least some *AFB1* or *AFB5* transgenic lines are more sensitive to IAA than the wild type is
- Seed germination defects in *AFB1* or *AFB5* transgenic lines are inversely correlated with their sensitivity levels to IAA

Compared to in Col-0, *AFB1* and *AFB5* are expressed at higher or similar levels in *AFB1:FLAG-AFB1* and *AFB5:FLAG-AFB5,* respectively.





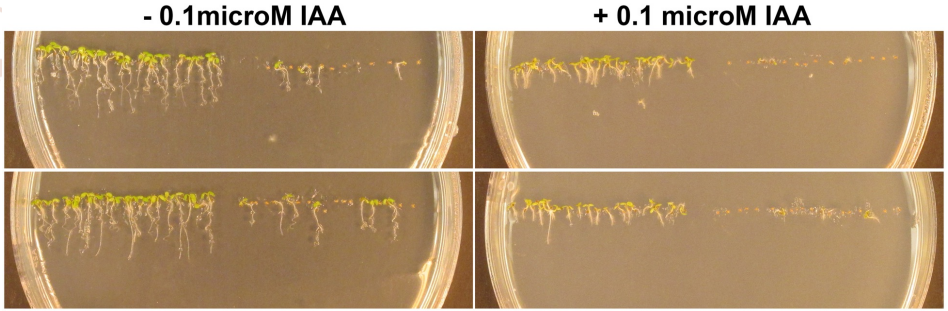
AFB1 transgenic lines are either more sensitive or approximately equally sensitive to IAA compared to the wild type



In 15 lines investigated (T_2 *AFB1:FLAG-AFB1* or homozygous T_3 *AFB1:AFB1-FLAG)*

Number of lines	Seed germination defect obvious on MS agar?	Sensitivity to IAA
8	Yes or no	≥ wild type
8	No	≈ wild type

AFB5 transgenic lines also exhibit higher or approximately equal sensitivity to IAA compared to the wild type

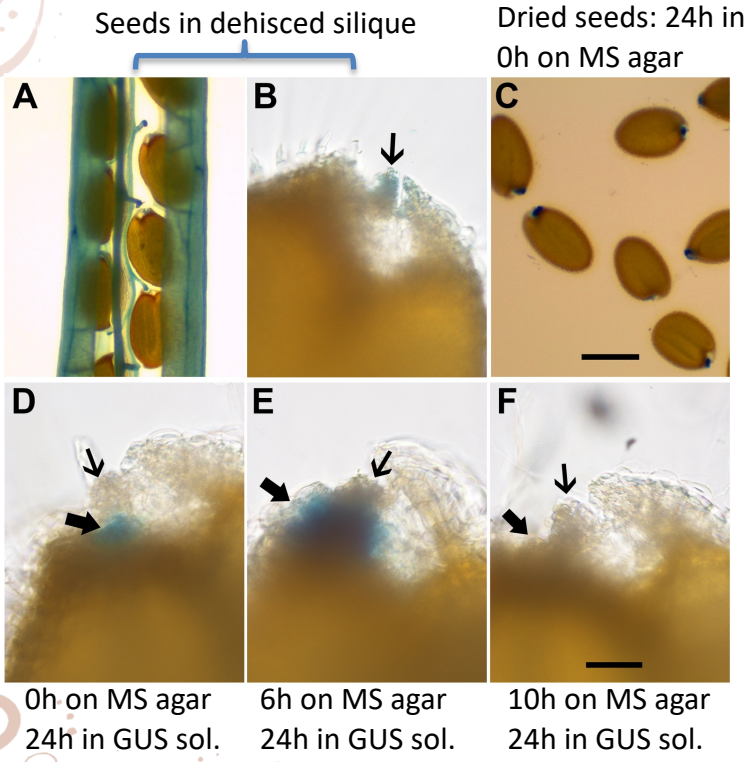


T_2 *AFB5:FLAG-AFB1* or T_2 *ASK1:FLAG-AFB1* lines were investigated.

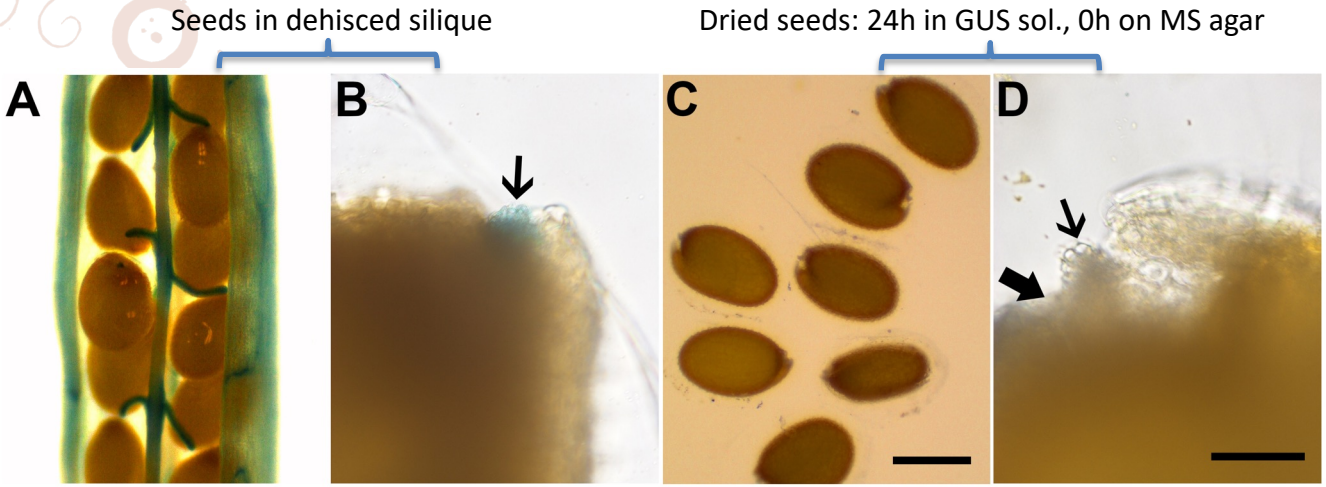
Conclusions

- Auxin signaling mediated by either AFB1 or AFB5 likely promotes seed dormancy in maternal tissue
- Seed dormancy is very sensitive to levels of AFB1 and AFB5
- *AFB1* likely plays a greater role in seed dormancy than *AFB5* does

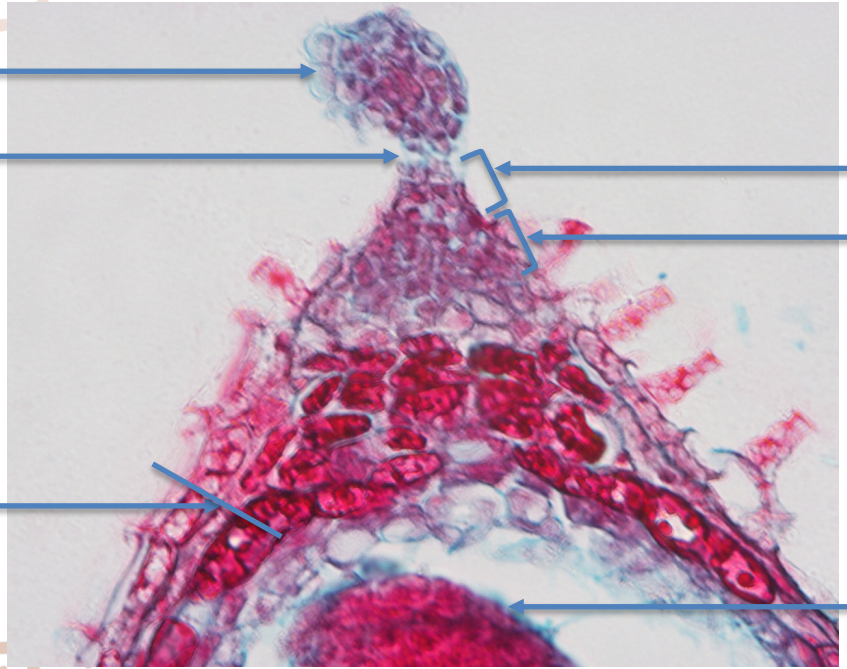
AFB1 is expressed in the funiculus and outer part of hilum in mature fruit and in the inner part of hilum during imbibition of dried seeds



AFB5 is expressed in the funiculus and outer part of hilum in mature fruit and not in the hilum during imbibition of dried seeds



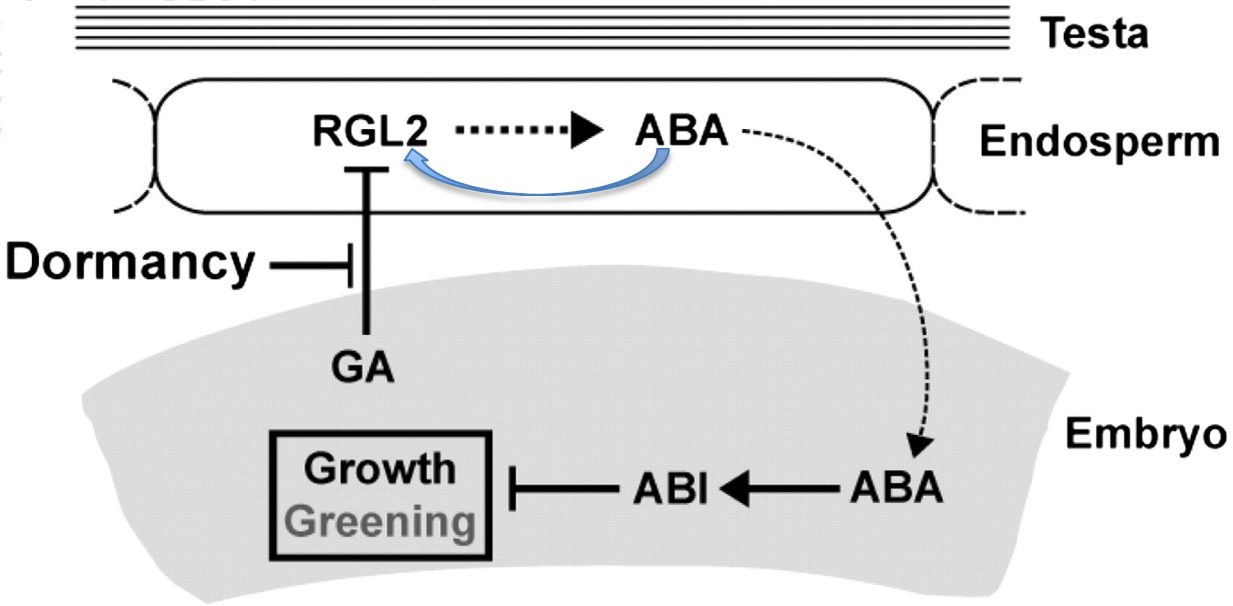
The structure of Arabidopsis seed coat at the hilum region



Summary of findings

- Maternal AFB1 and AFB5 promote seed dormancy, with AFB1's role being greater than AFB5's
- Higher-than-normal levels of auxin signaling is inversely correlated with seed dormancy
- *AFB1* and *AFB5* are expressed in an overlapping fashion in the funiculus and outer part of the hilum in nature fruit and that *AFB1* is also transiently expressed in the inner part of the hilum during the early hours of imbibition
- Transient maternal expression of *AFB1* and *AFB5* has a lasting impact on seed dormancy even when they are no longer expressed

Model for seed coat- and ABA-dependent repression of dormant seed germination



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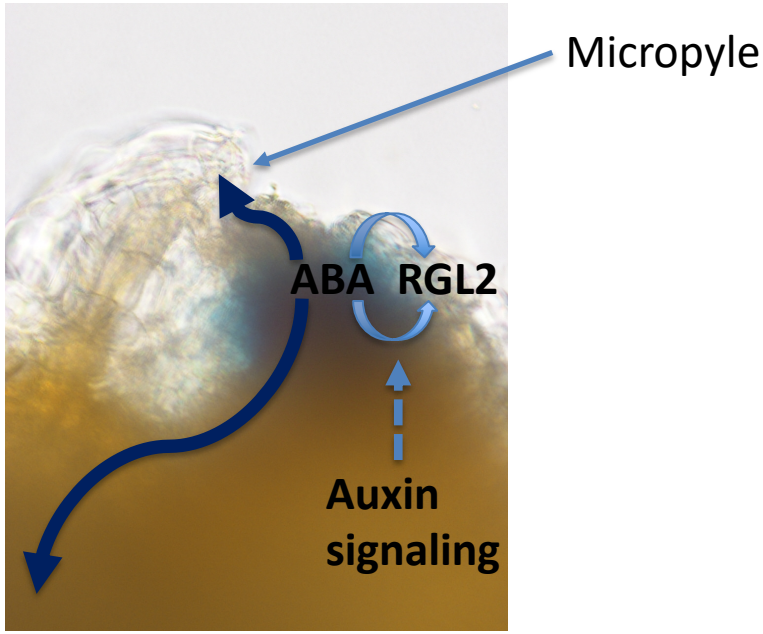
A model of auxin signaling in promoting seed dormancy



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