Functions of SCF components in reproductive processes in Arabidopsis

Ming Yang

Department of Plant Biology, Ecology, and Evolution

Oklahoma State University

Outline of my talk

- Brief introduction of research areas in my laboratory
- The function of ASK1 in meiosis
- The functions of AFB1 and 5 in seed germination and growth
- Summary and acknowledgements

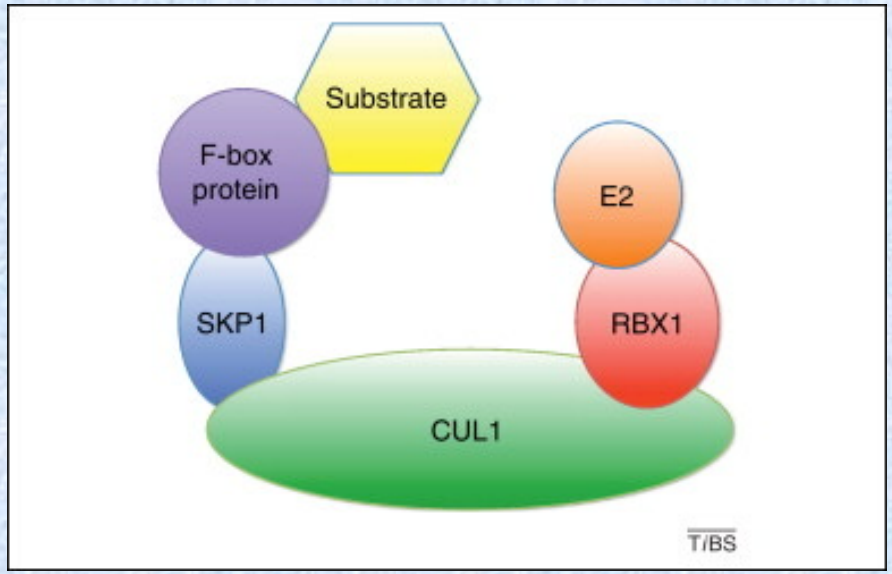
Research areas in my laboratory

- Meiotic cell cycle progression
- Cell morphogenesis
- Theoretical biology

Our work on meiotic cell cycle progression

- The function of the ARABIDOPSIS SKP1-LIKE1 (ASK1)
- The function of the TARDY ASYNCHRONOUS MEIOSIS (TAM)/CYCA1;2

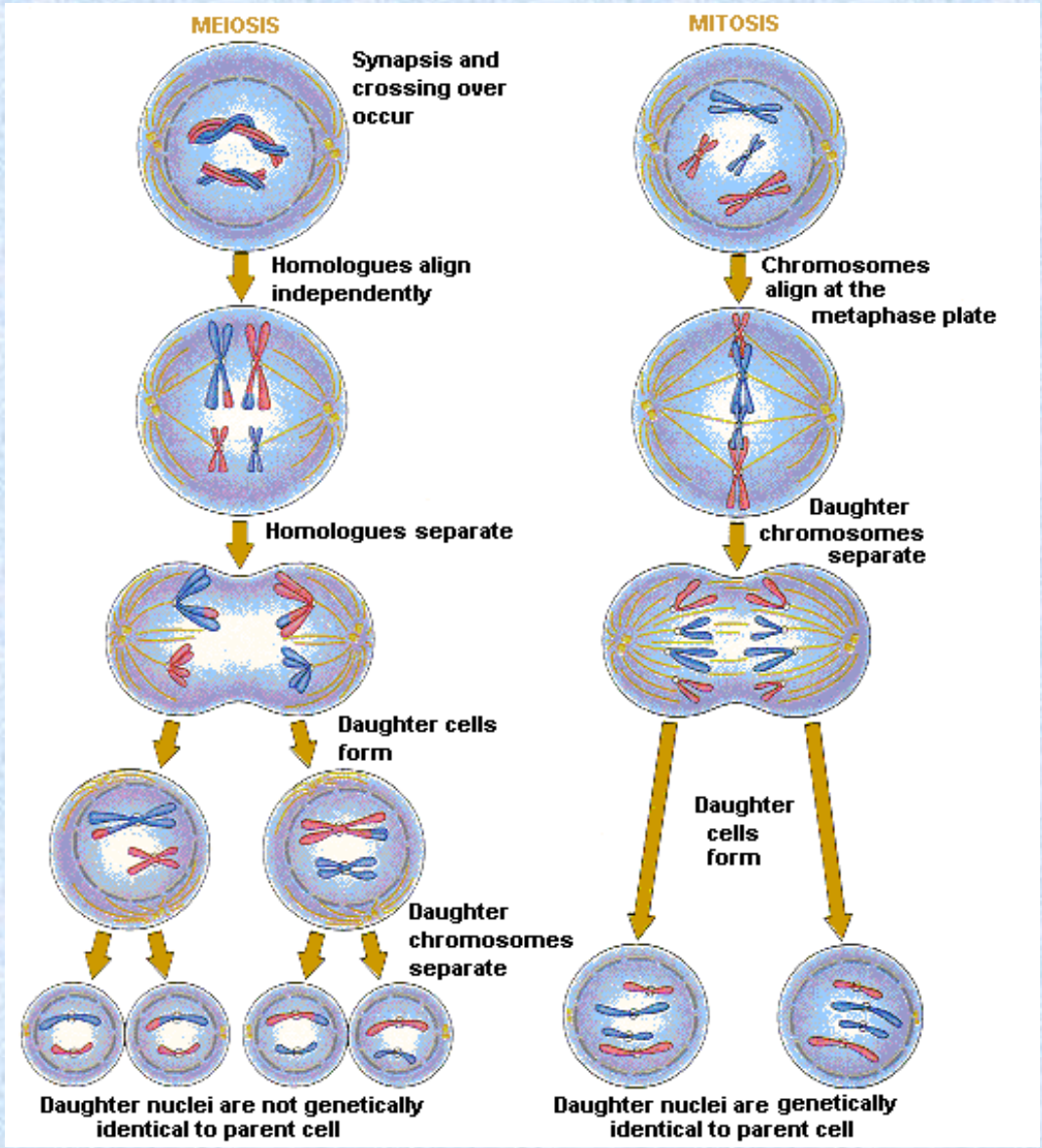
Skp1-Cullin-F-box protein (SCF) ubiquitin ligases

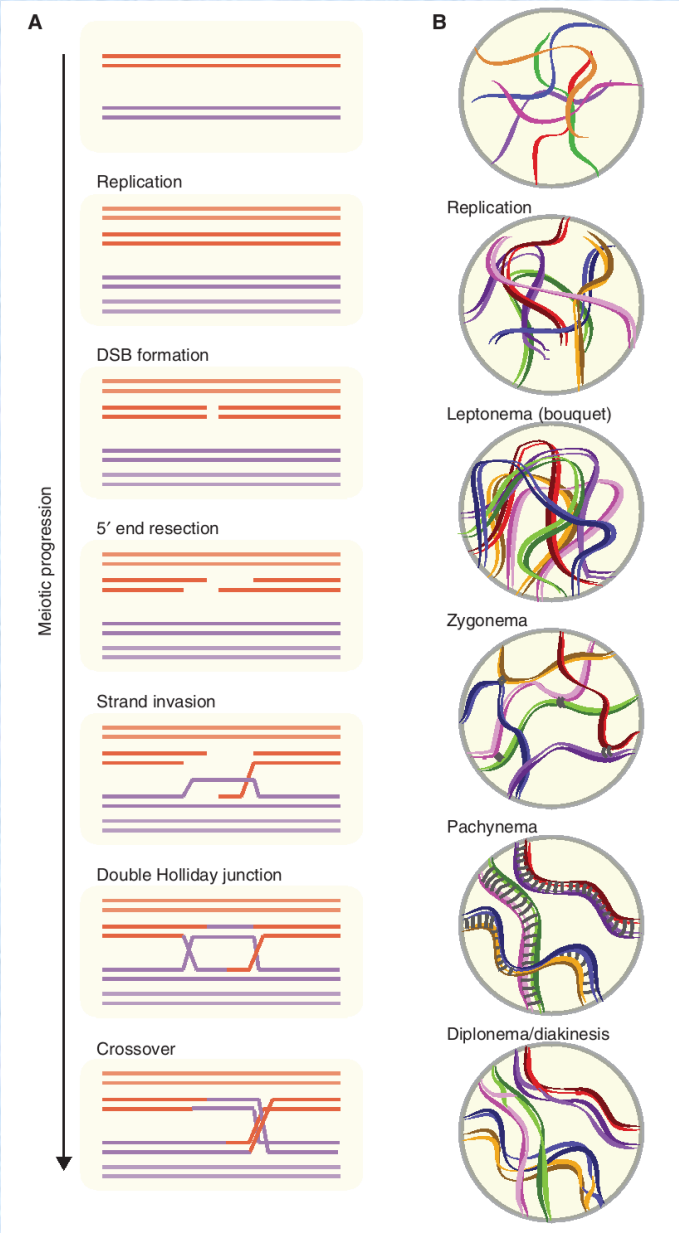


Silverman et al., Trends in Biomedical Science, 37: 66-73, 2012.

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.

Meiosis vs. mitosis





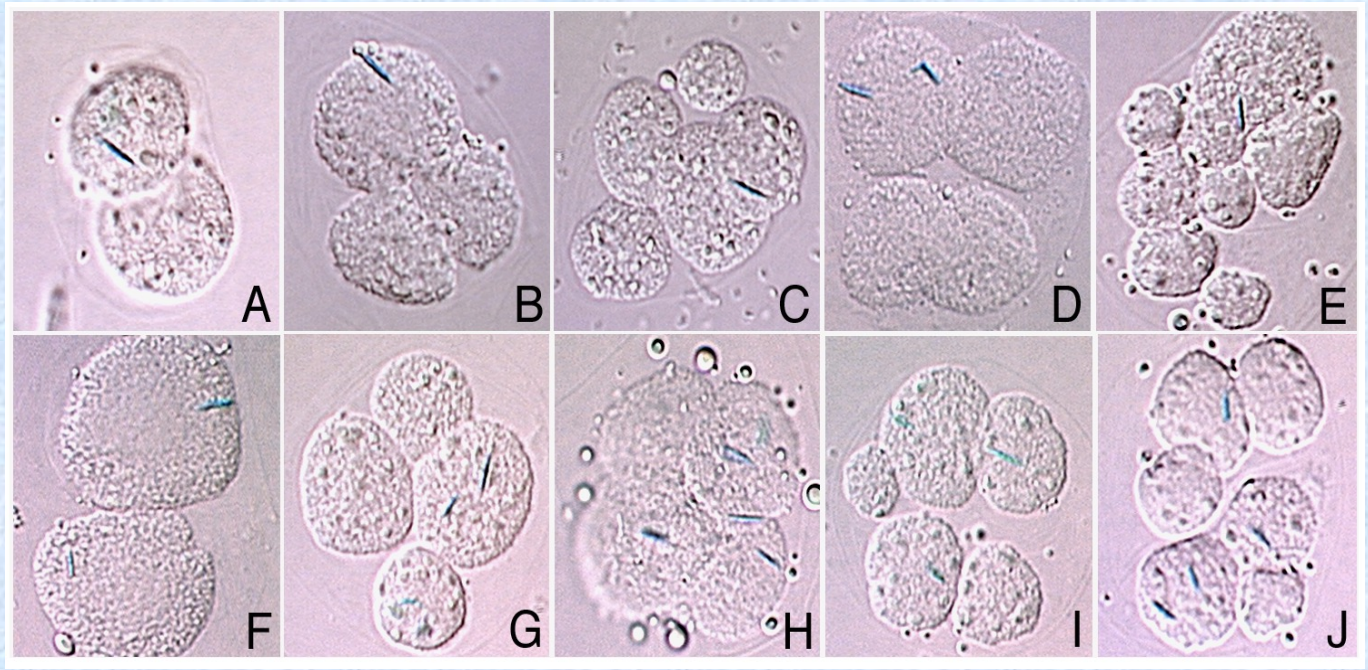
← S phase and G_2 phase

Prophase I

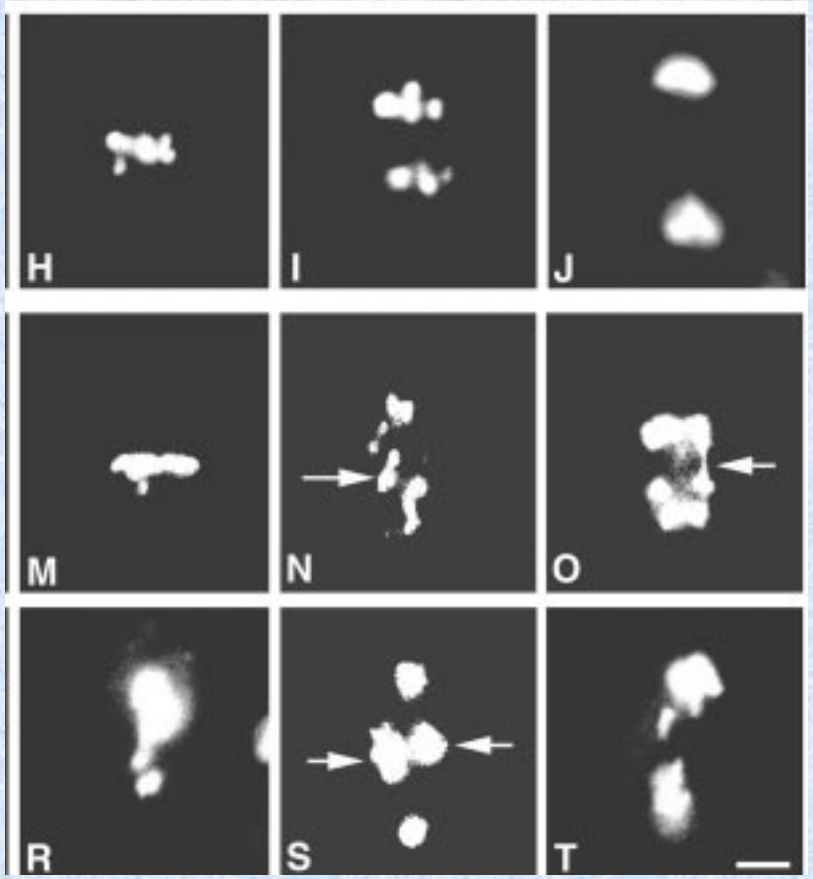
Adapted from Subramanian and Houchwagen, Cold Spring Harb Prospet Biol, 6: a016675, 2014

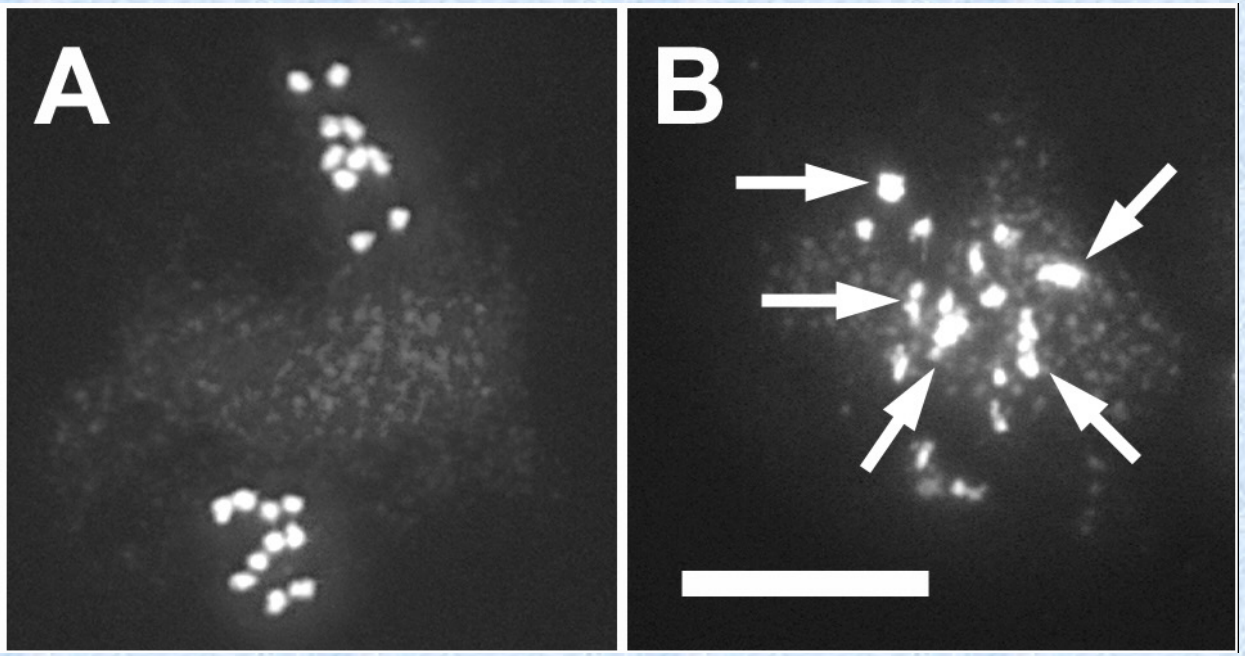
The *ask1-1* mutant

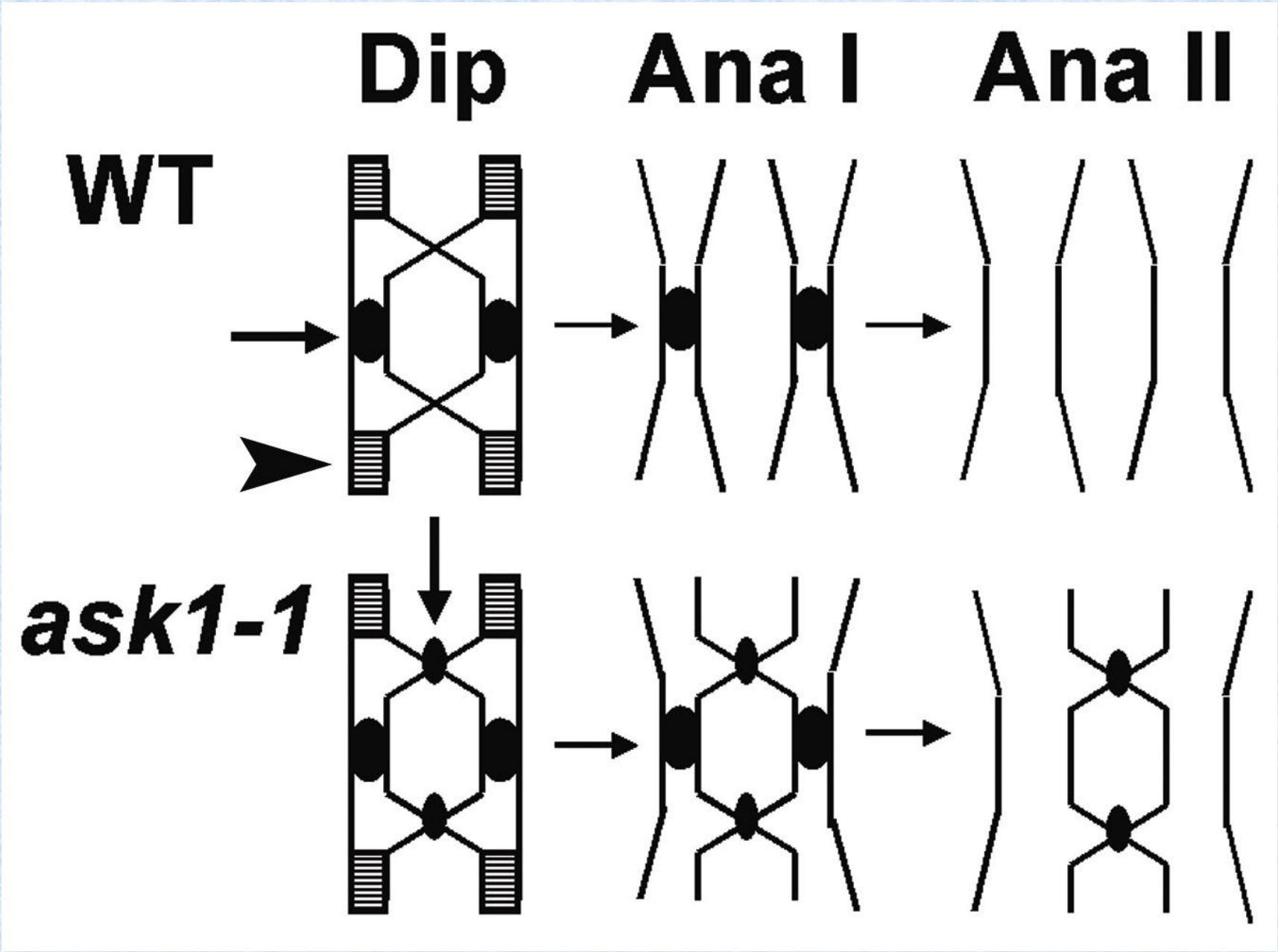
- A null mutant generated by transposon insertion
- Male sterile
- Mild defects in vegetative growth and floral organ formation



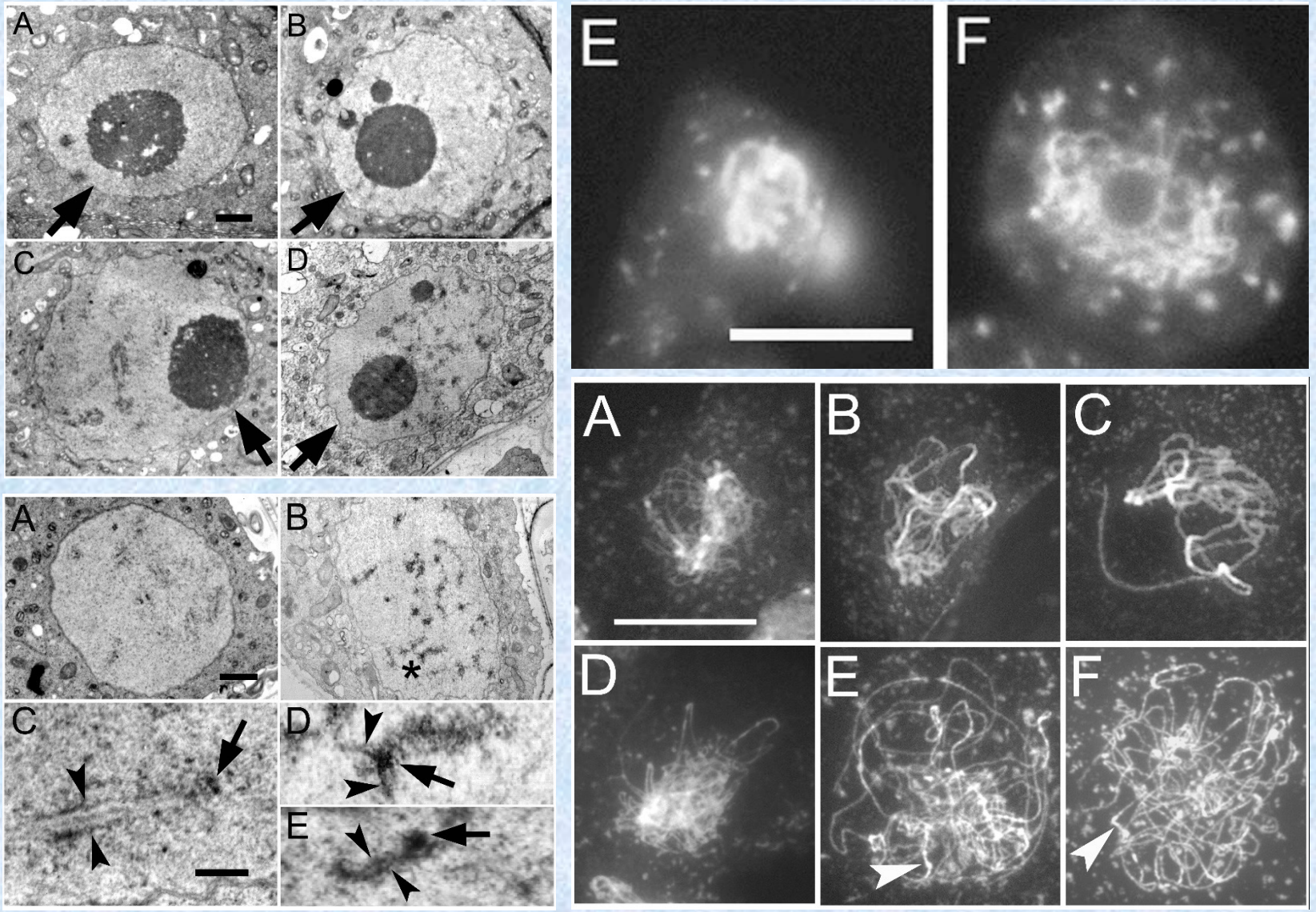
Chromosome nondisjunction occurs in anaphase I and persists into meiosis II



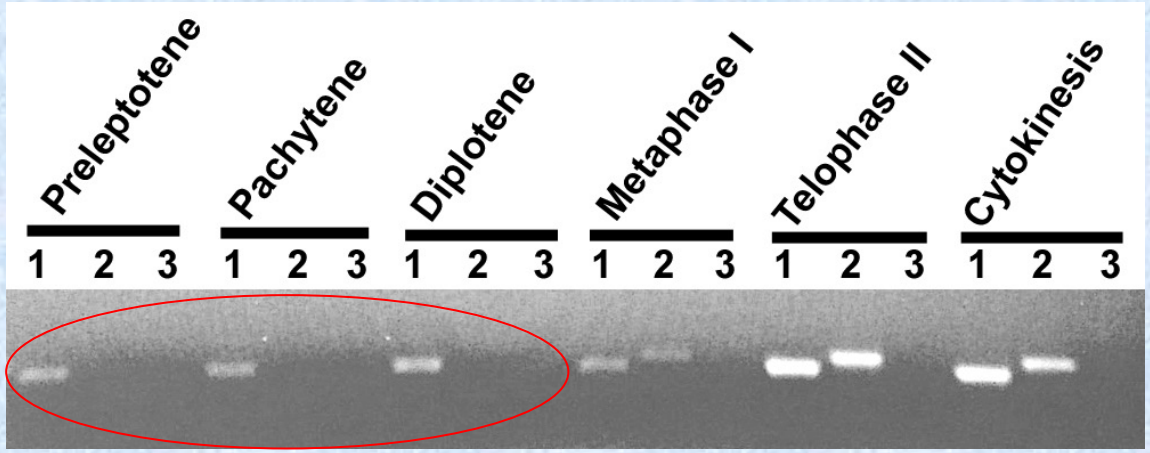



Dip
Ana I
Ana II
WT
ask1-1

Prophase-I defects indicate both cell cycle progression and synapsis are affected in *ask1-1* male meiosis



Expression of *ASK1* and *ASK2* at different stages of male meiosis

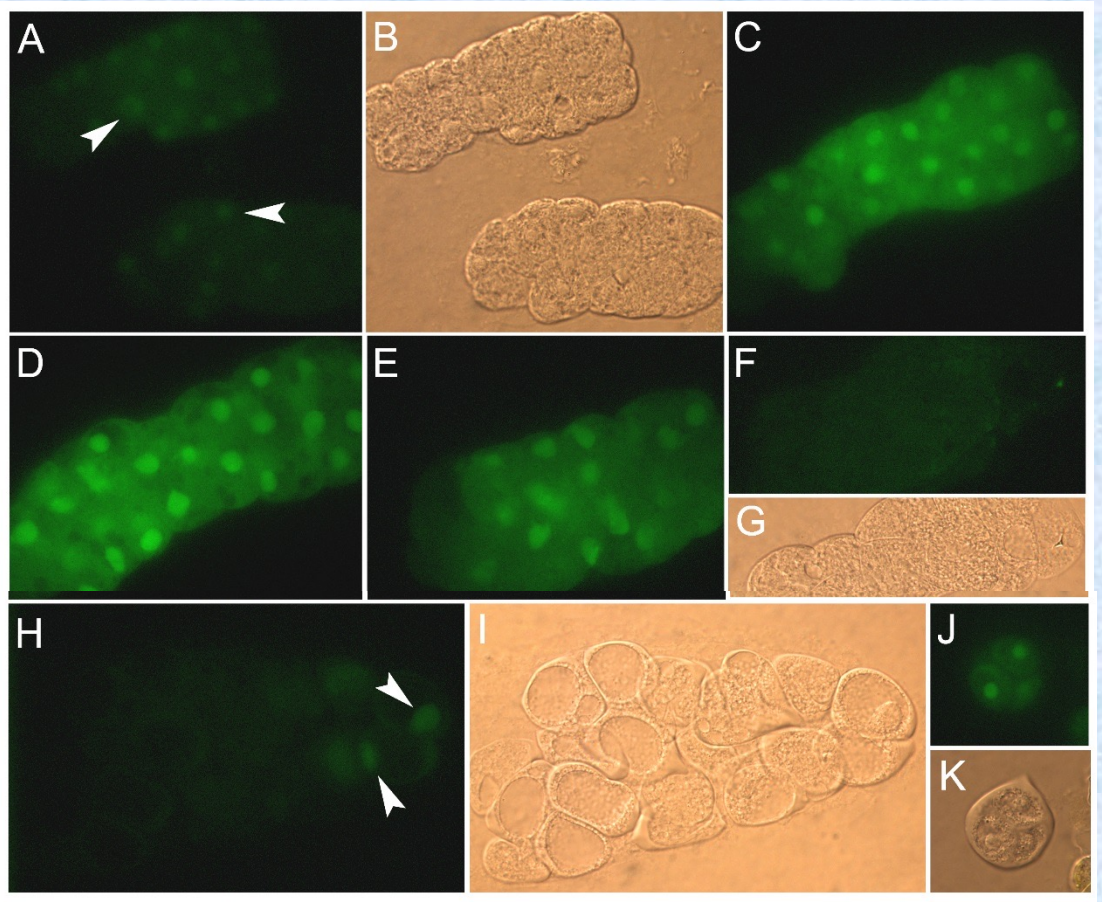


1 – *ASK1*
2 – *ASK2, ASK1* homolog
3 – *ATA1*, positive control for tapetal cell contamination

Increase in recombination frequency

ASK1/ask1-1 : *ASK1/ASK1* = 6.7 (assuming *ask1-1* only affects male meiosis) or = 2.6 (assuming *ask1-1* affects both male and female meiosis)

Levels of ASK1-GFP in WT microsporocytes



A-B Preleptotene **C** Leptotene **D** Zygotene **E** Pachytene **F-G** Zygotene, negative control **H-I** Diplotene **J-K** Tetrad

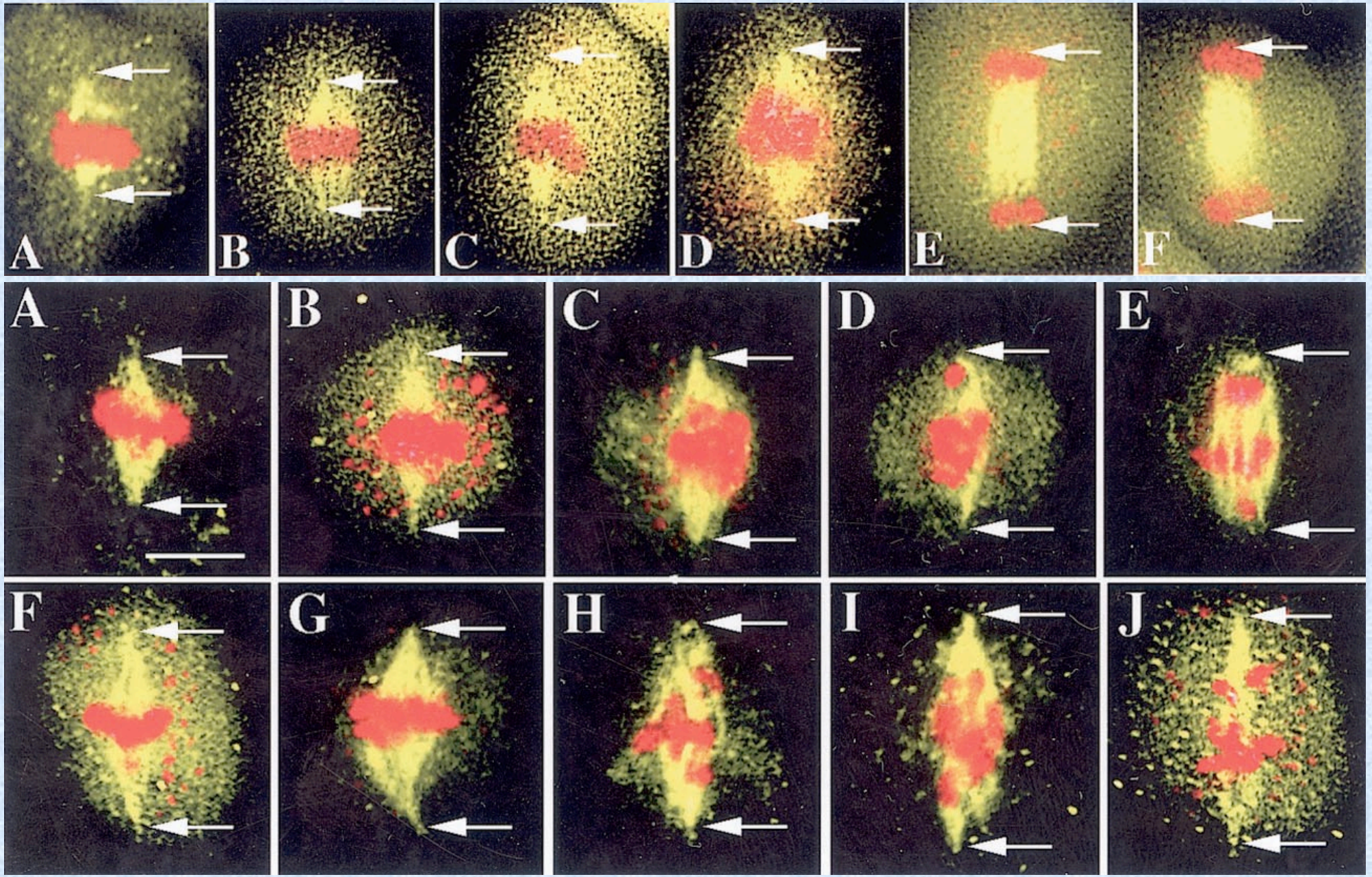
The conserved function of Skp1 in meiosis: phenotypic similarities between *ask1-1* and fission yeast *skp1* mutants

- Meiotic products with the numbers of microspores ranging from two to more than four
- Similar morphology and behavior of chromosomes during meiosis I
- Similar elongated spindle morphology
- Persistence of proteins on chromosomes

Meiosis-I spindles

WT

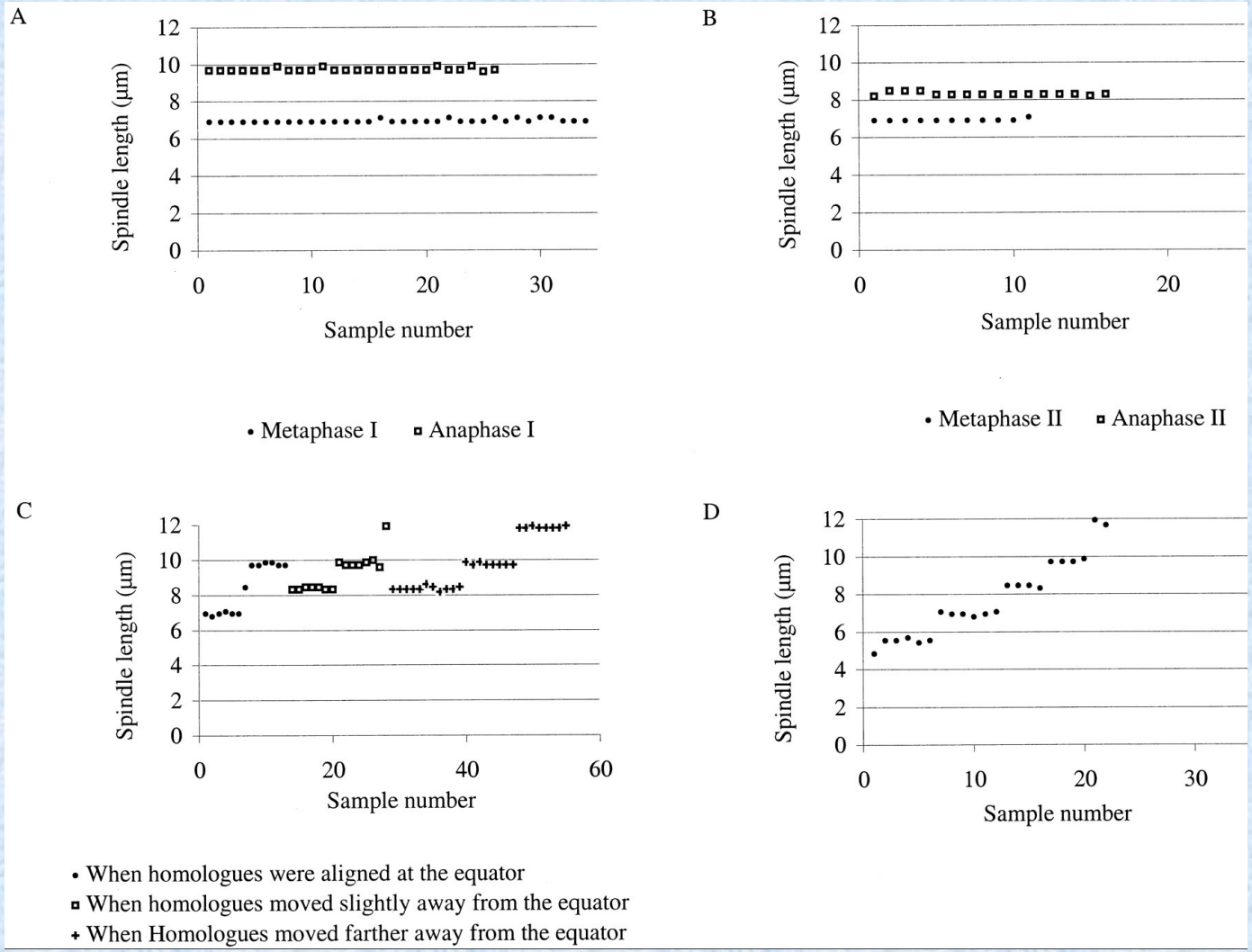
ask1-1



Distribution of WT and *ask1-1* spindle lengths in meiosis I and meiosis II

WT

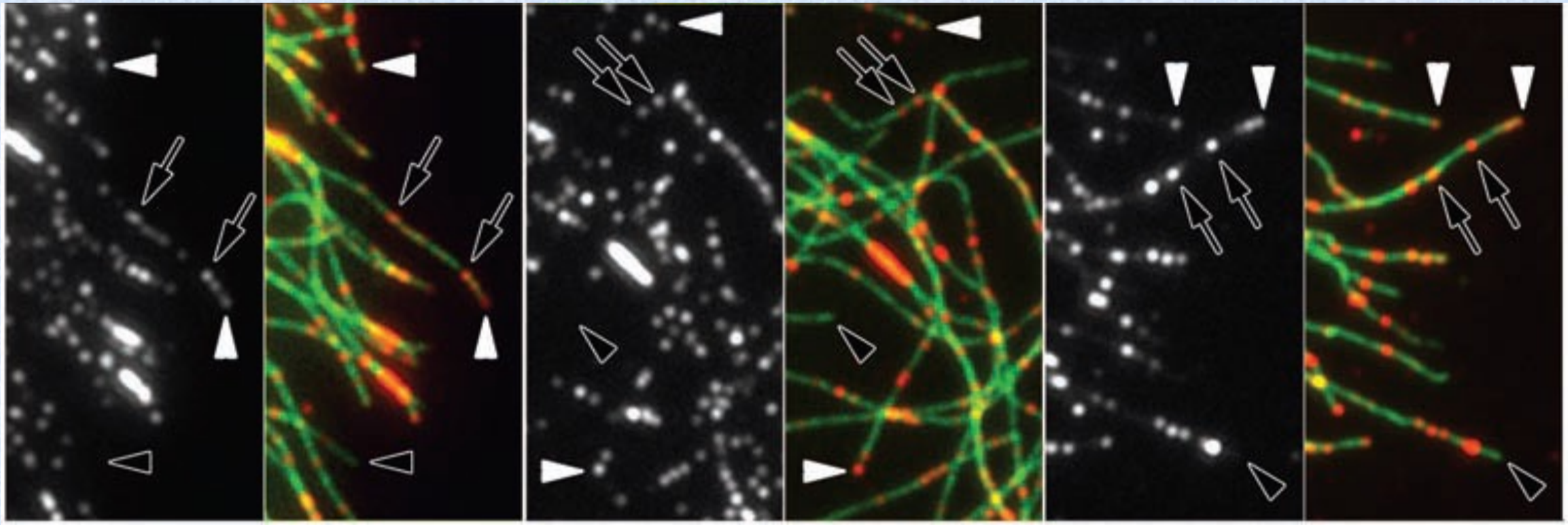
ask1-1



Spindle length differences (µm) in four other organisms

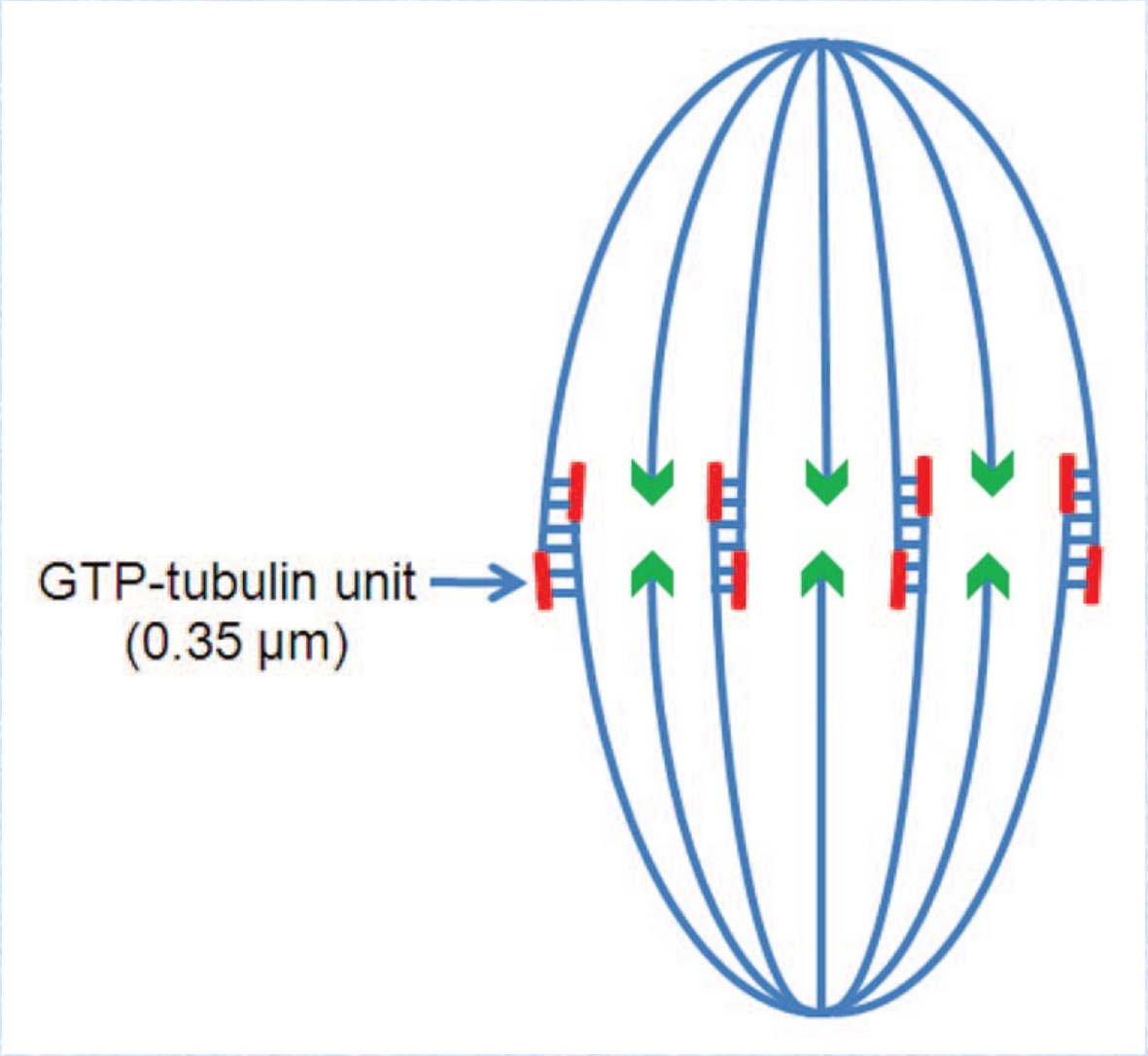
Organism	Length	Length Difference
S. cerevisiae (Winey et al., 1995)	L1 0.7 ± 0.1 (n = 4) L2 1.4 ± 0.1 (n = 6)	L2 - L1 = 0.7
F. capucina (Tippit et al., 1978)	L1 1.3 ± 0.1 (n = 2) L2 2.6 ± 0.1 (n = 25)	L2 - L1 = 1.3
Slime mold (Moens, 1976)	L1 2.1 ± 0.1 (n = 3) L2 4.9 ± 0.3 (n = 6)	L2 - L1 = 2.8
Rat kangaroo (PtK1 cells; Armstrong and Snyder, 1989)	L1 13.2 (n = 5) L2 17.4 (n = 5)	L2 - L1 = 4.2
Rat kangaroo (PtK1 cells; Snyder et al., 1986)	L1 12.2 (n = 6) L2 16.4 (n = 6)	L2 - L1 = 4.2

Discrete lengths of GTP-tubulin segments on human microtubules



(Dimitrov et al., Science, 322: 1353-56, 2008)

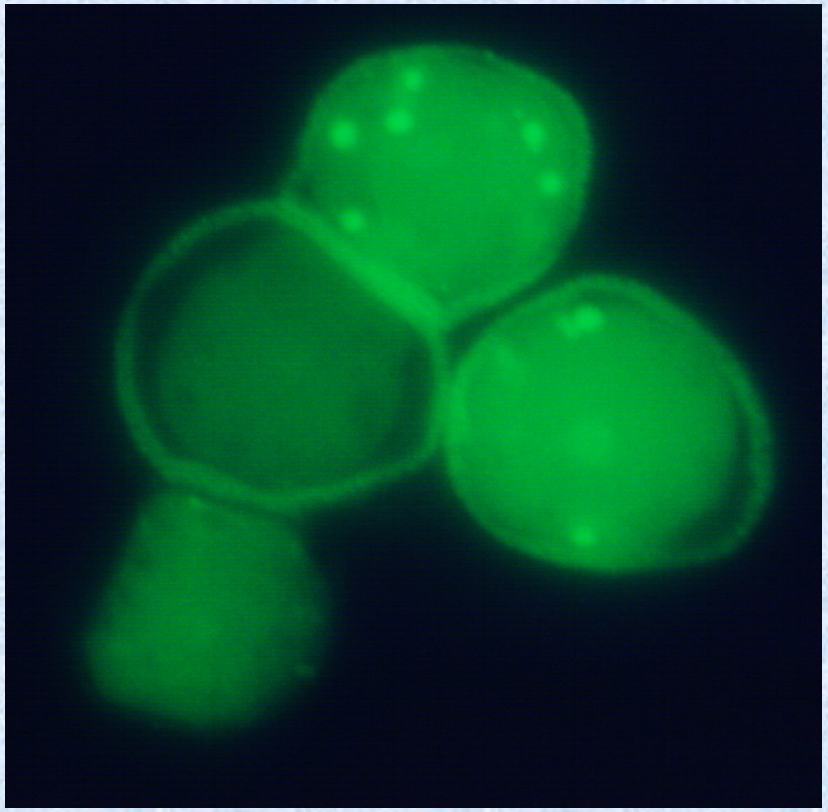
A model for discrete spindle elongation



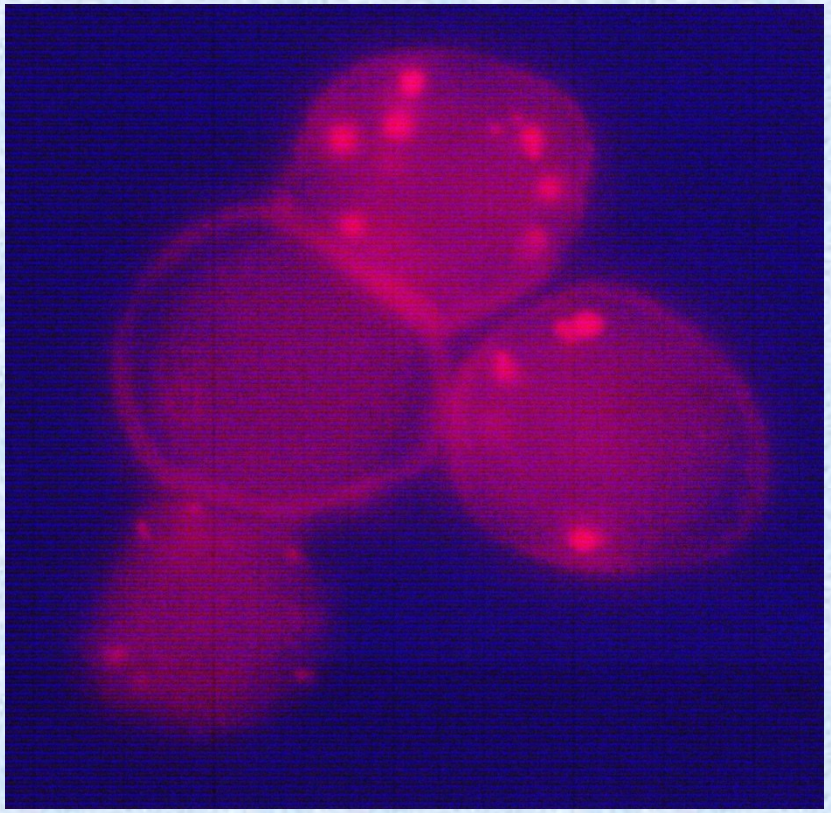
The spindle elongation studies led to investigations into the biophysical basis of biological rhythms: Slow diffusion, imprecision of biochemical reactions, and negative feedback are sufficient to generate sustained long oscillations.

Colocalization of β-tubulin and ubiquitin in cytoplasmic protein aggregates in microspores in *ask1-1*

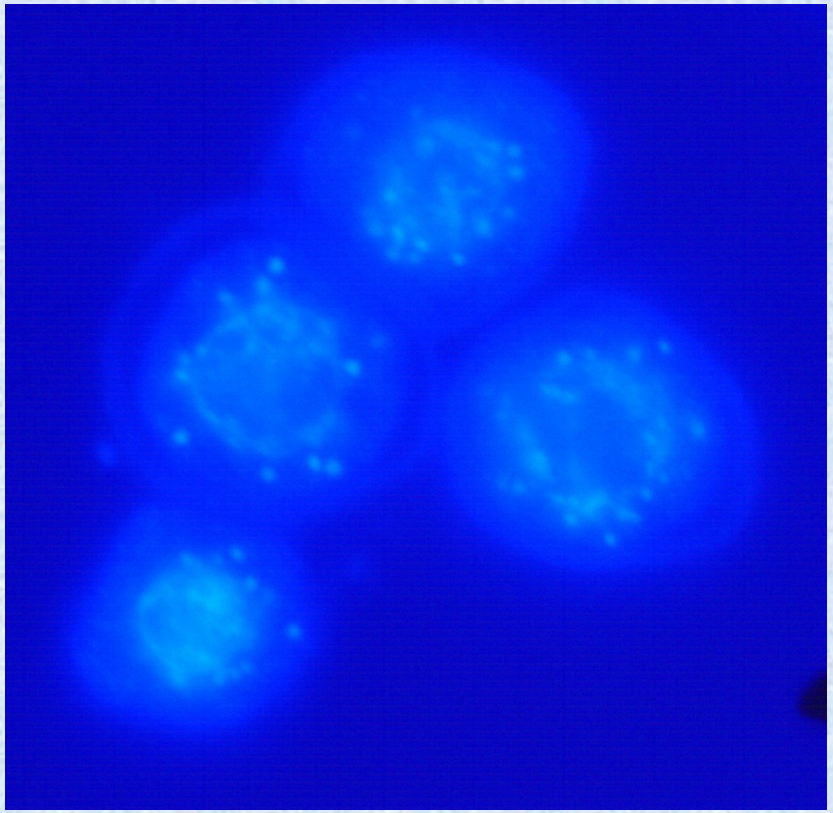
Anti-β-tubulin



Anti-ubiquitin



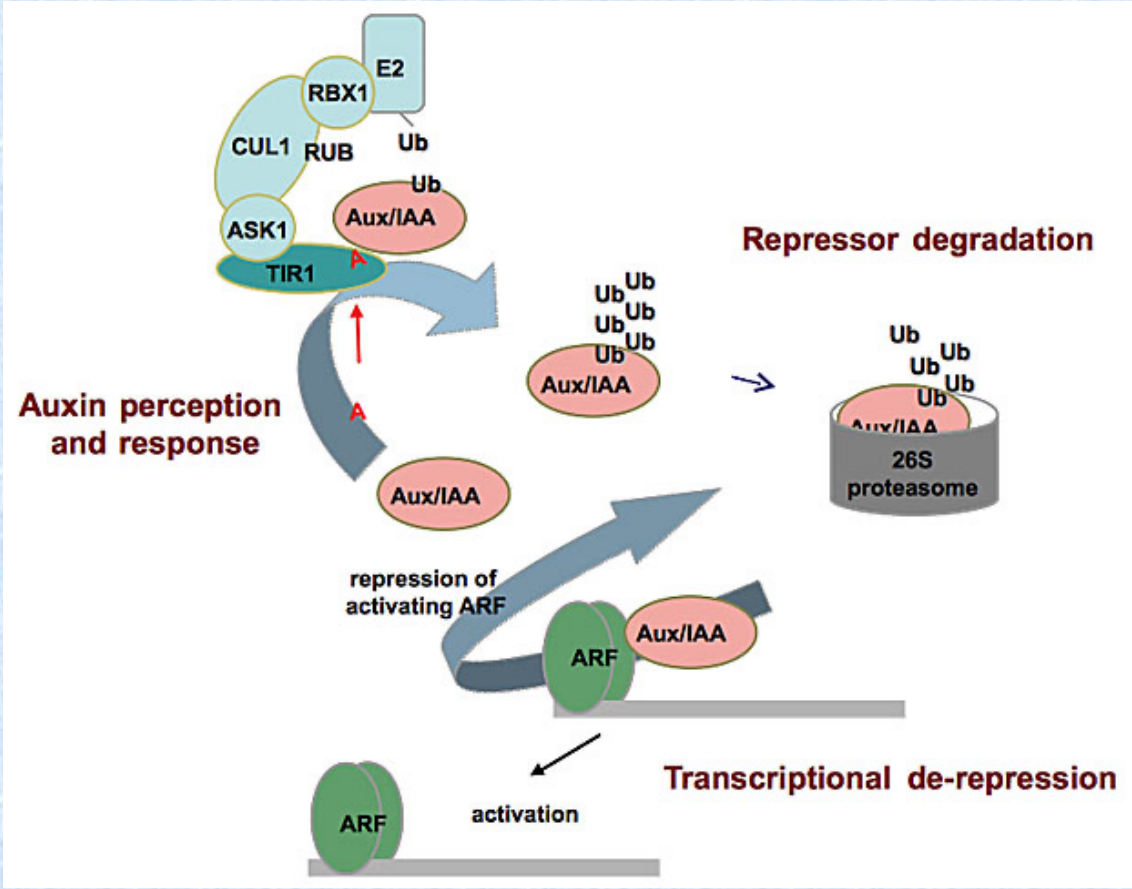
DAPI staining



Do these protein aggregates lead to cell death?

Is it similar to cell death in neurodegenerative diseases?

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

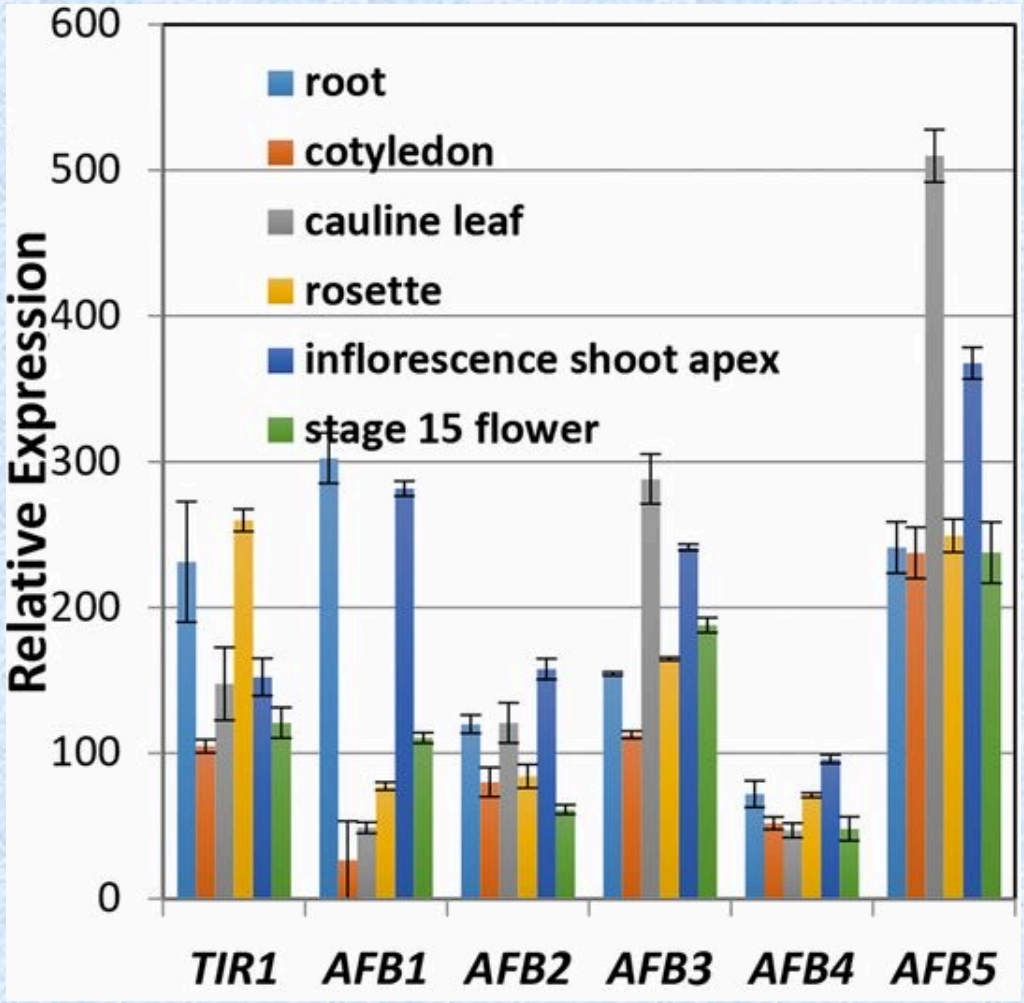
Our findings

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

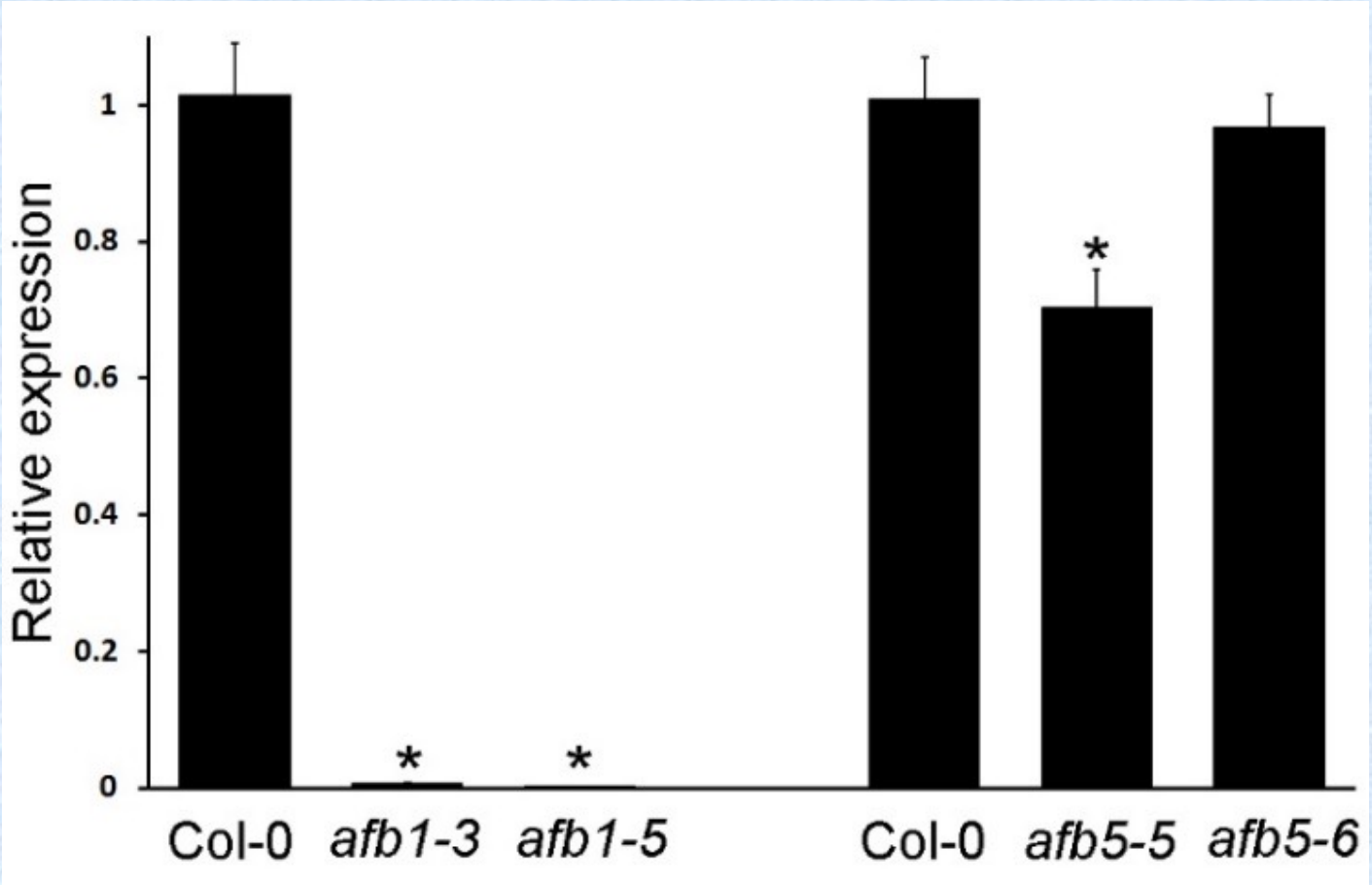
Expression of the *TIR1/AFB* genes in Arabidopsis



Michael J. Prigge et al. G3 2016;6:1383-1390

G3·Genes | Genomes | Genetics GSA

Confirmation of two mutant alleles for each of the *AFB1* and *AFB5* loci



* Statistically different from Col-0 (t-test, $P < 0.05$).

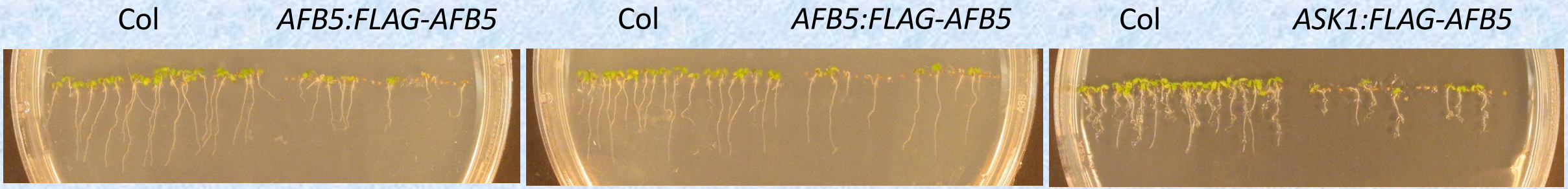
Each of four *AFB1* transgenes can cause a seed germination defect

- Transgenes tested in the likely knockout mutant *afb1-3*: *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

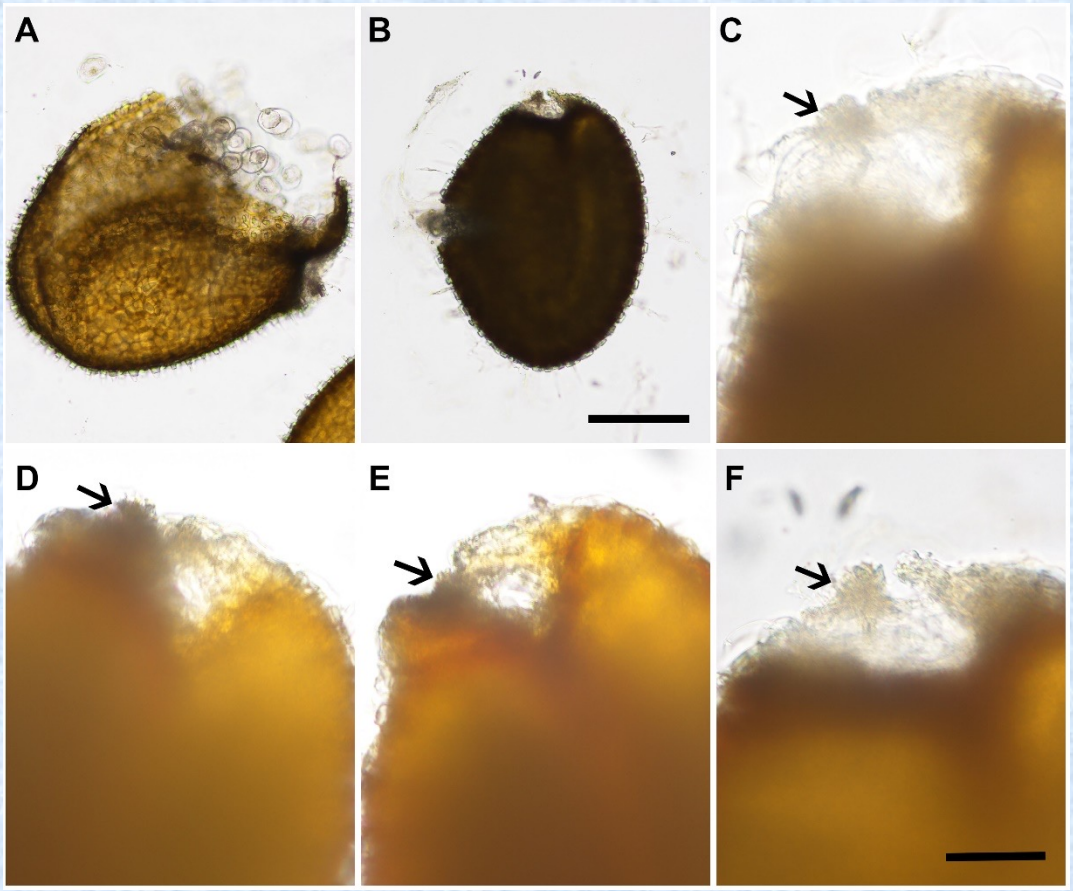
Each of Four *AFB5* transgenes can cause a seed germination defect

- Transgenes tested in the knockdown mutant *afb5-5*: *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



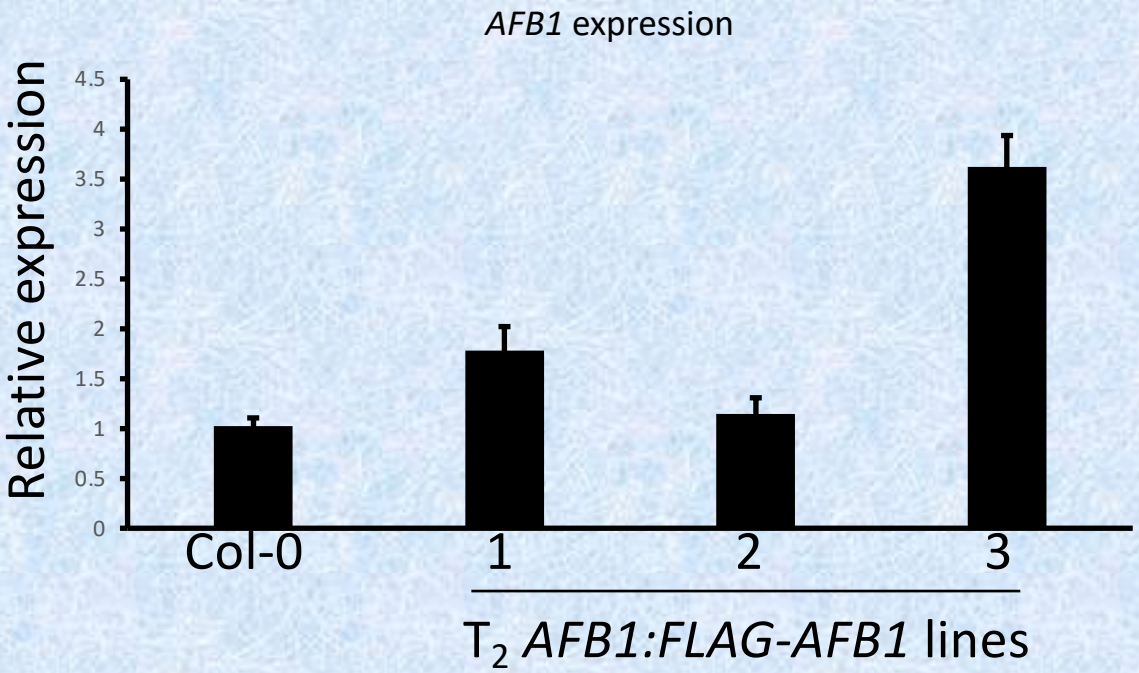
- Non-germinated seeds could have ruptured seed coat after long imbibition
- Inner part of hilum on non-germinated seeds could swell and project outward

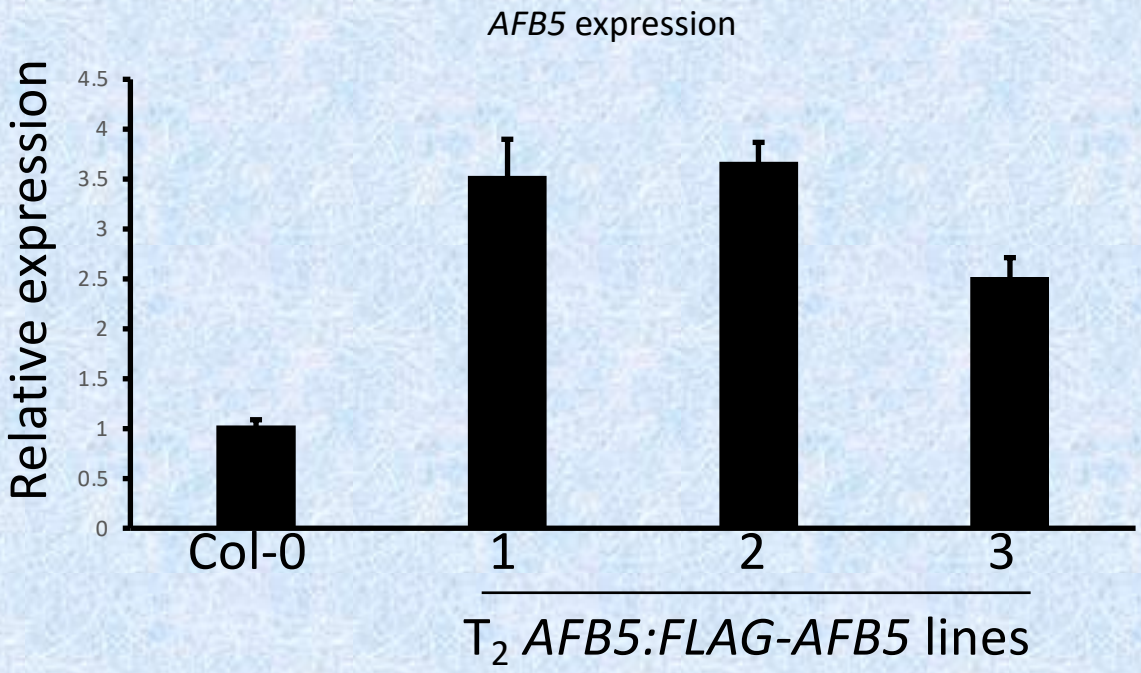
- A-C and F, 20 days on MS agar medium
- D and E, ~5 minutes in water
- Arrows indicate outer part of hilum
- Bar in F for A-C and F = 50 µm, and bar in B for A and B = 200 µm

Hypothesis

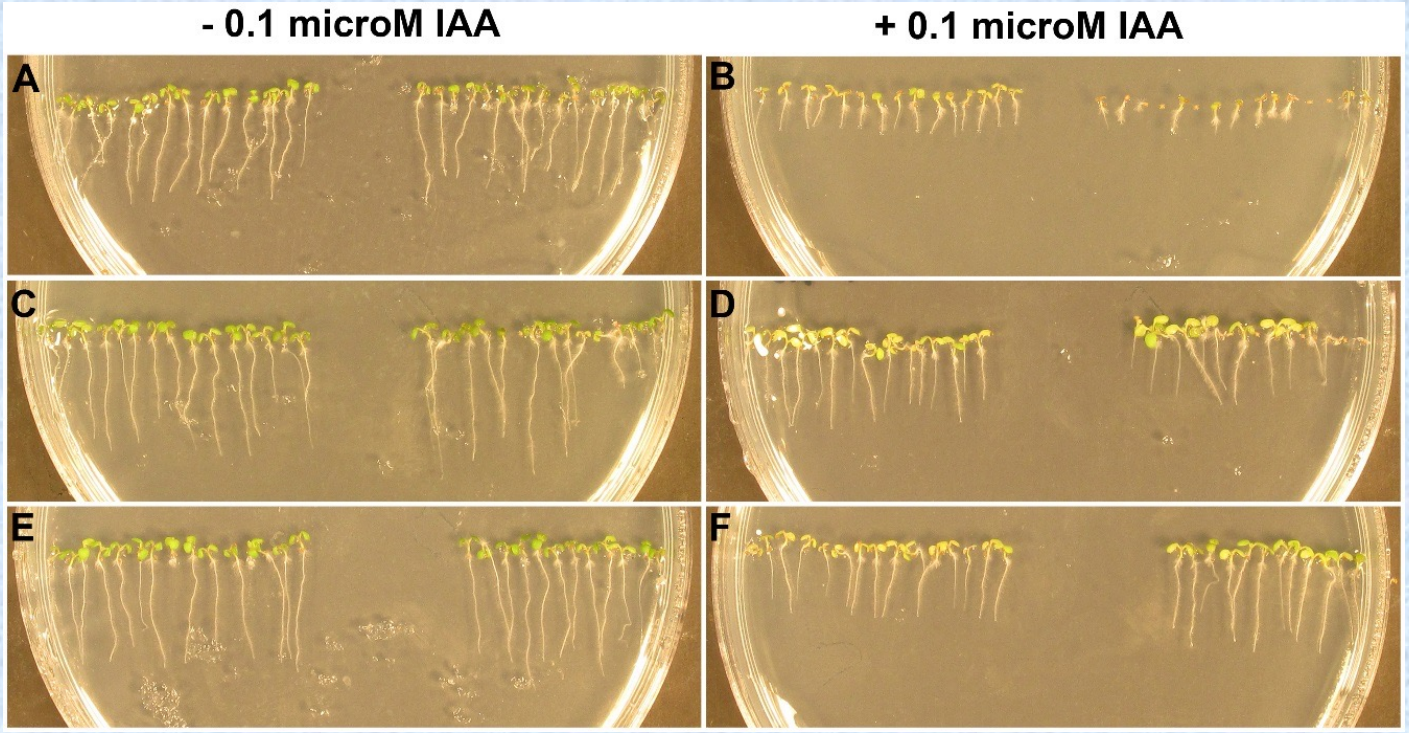
The seeds of the transgenic plants cannot germinate or germinate in a delayed fashion because of abnormally high levels of auxin signaling in the seed coat.

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





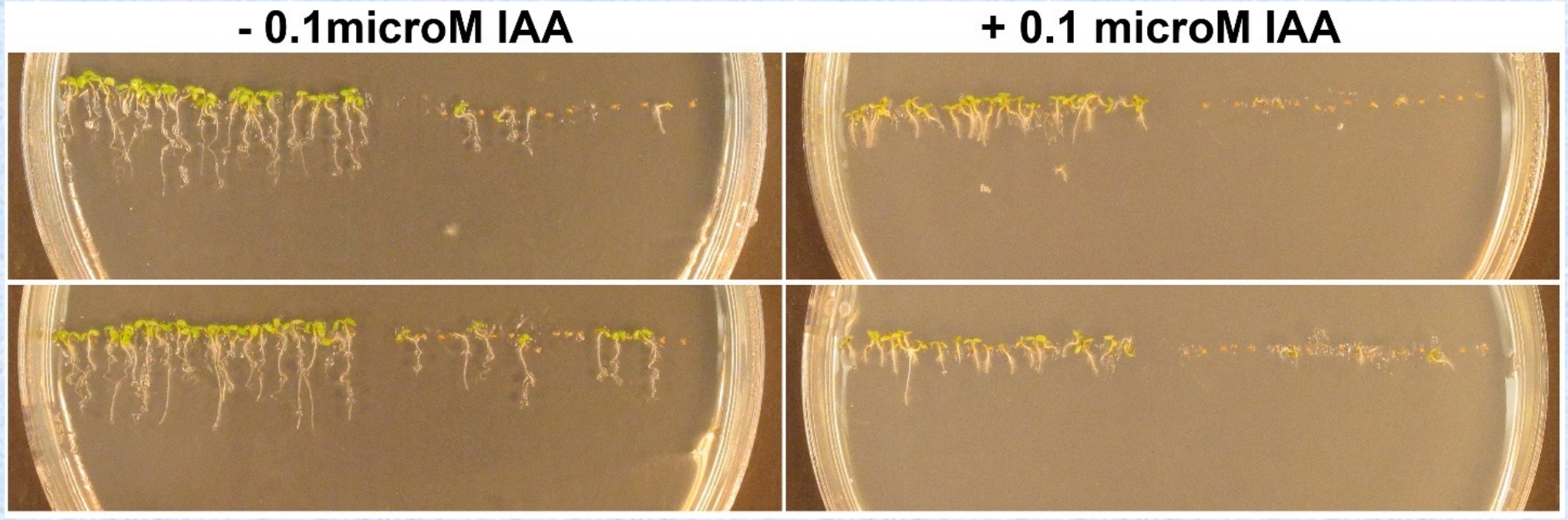
AFB1 transgenic lines are either more sensitive or approximately equally sensitive to IAA compared to the wild type



In 16 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

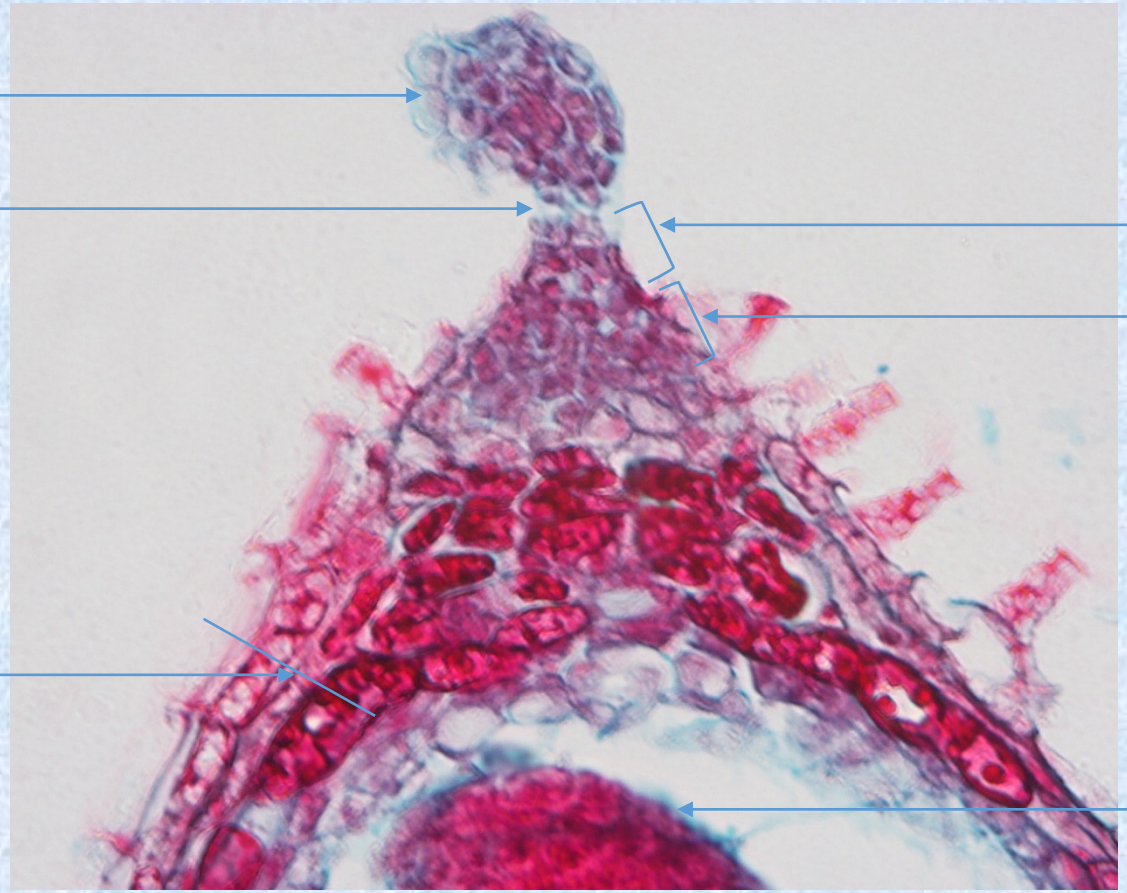


5 days after seeds T_2 *AFB5:FLAG-AFB5* or T_2 *ASK1:FLAG-AFB5* were sewn

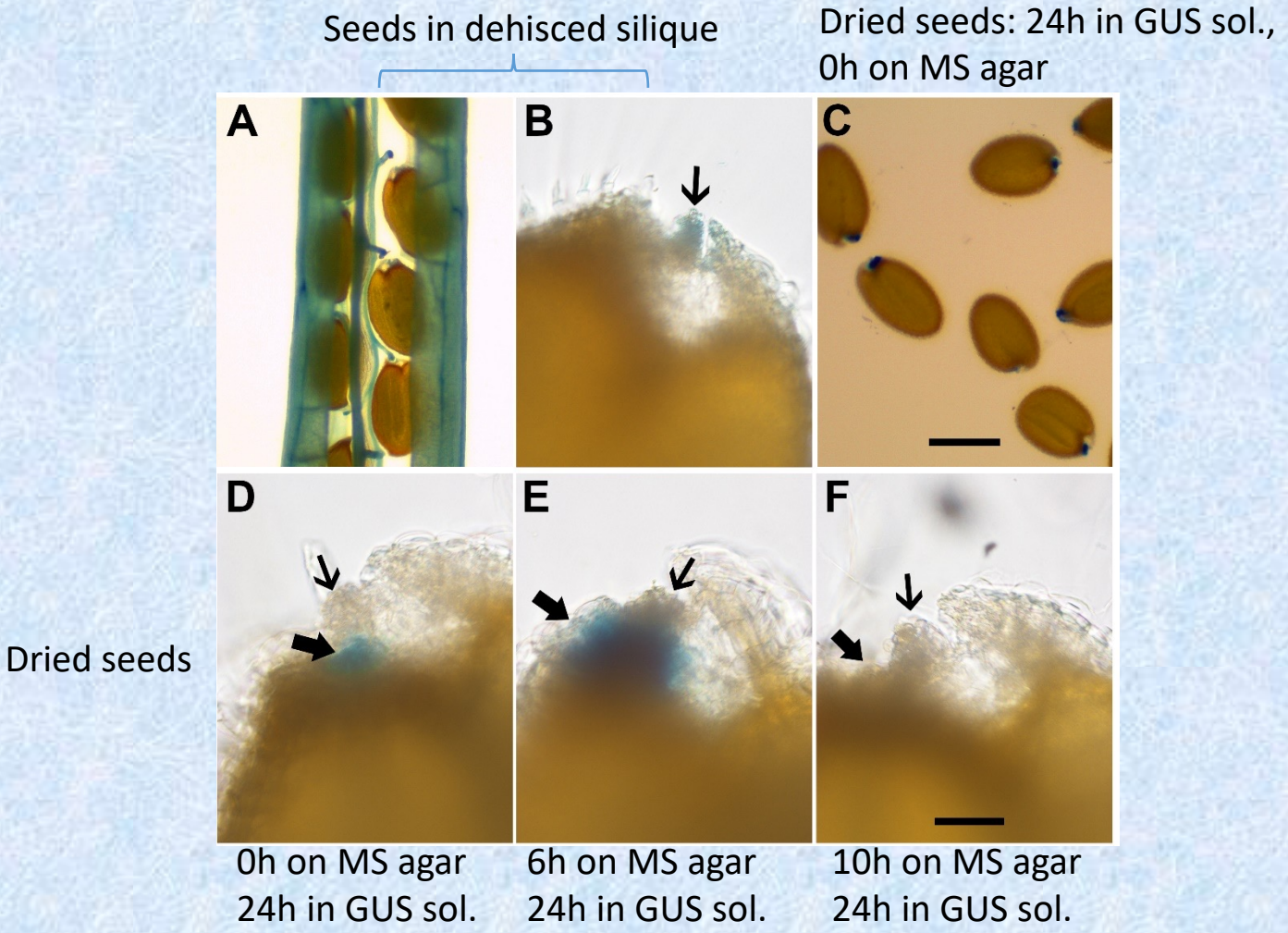
Conclusions

- 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

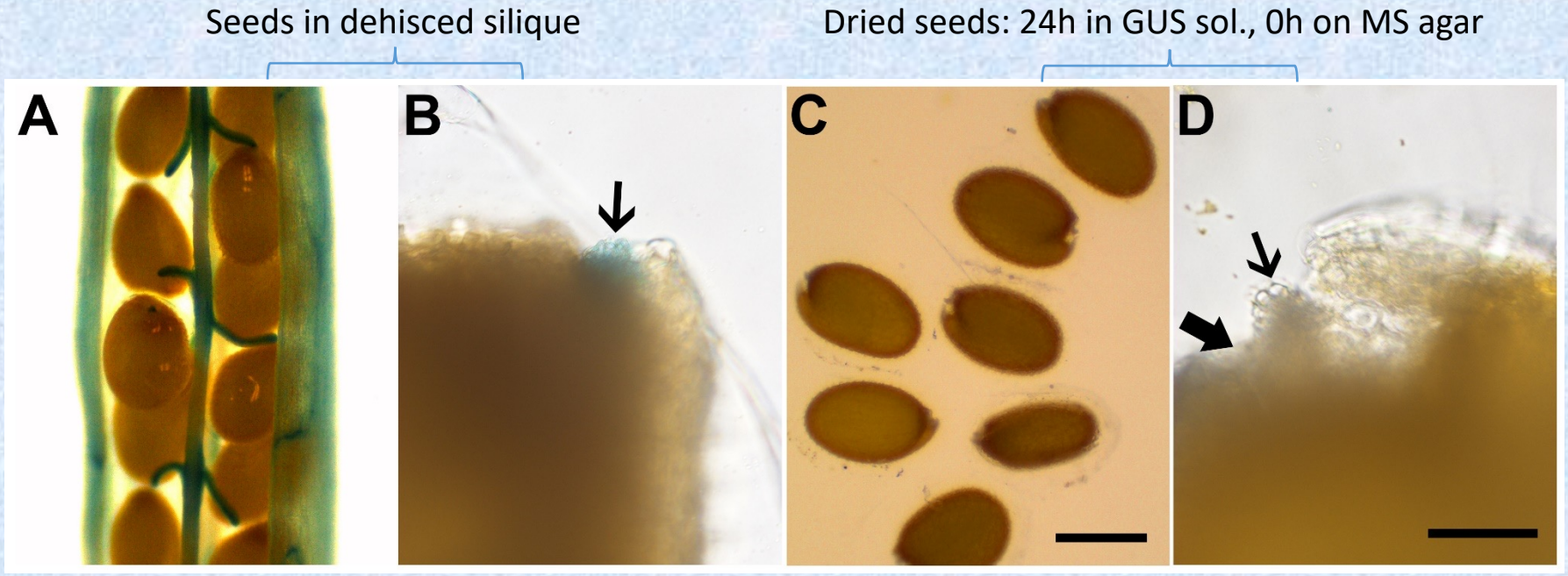
The structure of Arabidopsis seed coat at the hilum region



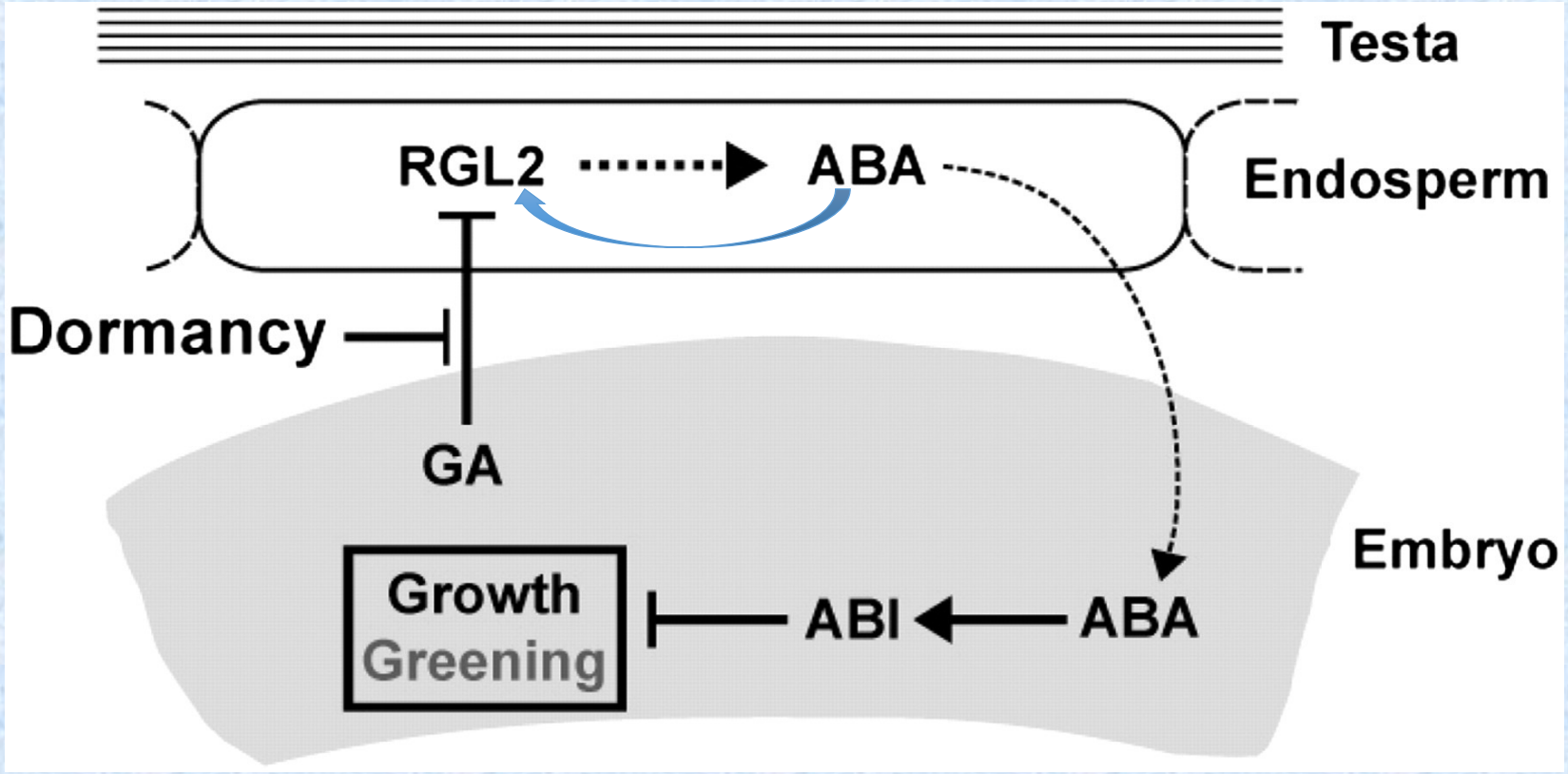
AFB1 is expressed in the funiculus and outer part of hilum in mature fruit and in the inner part of hilum during imbibition of dried seeds



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

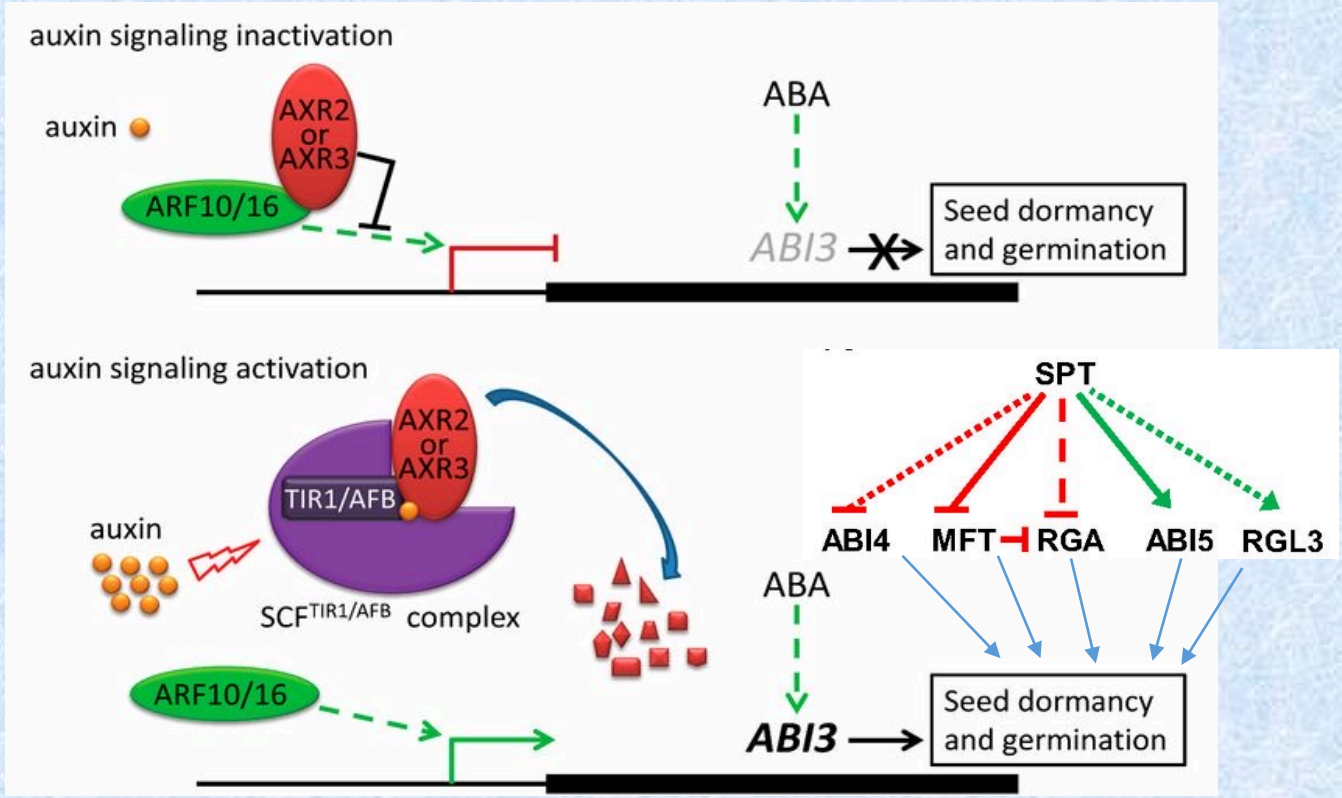


Model for seed coat- and ABA-dependent repression of dormant seed germination



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

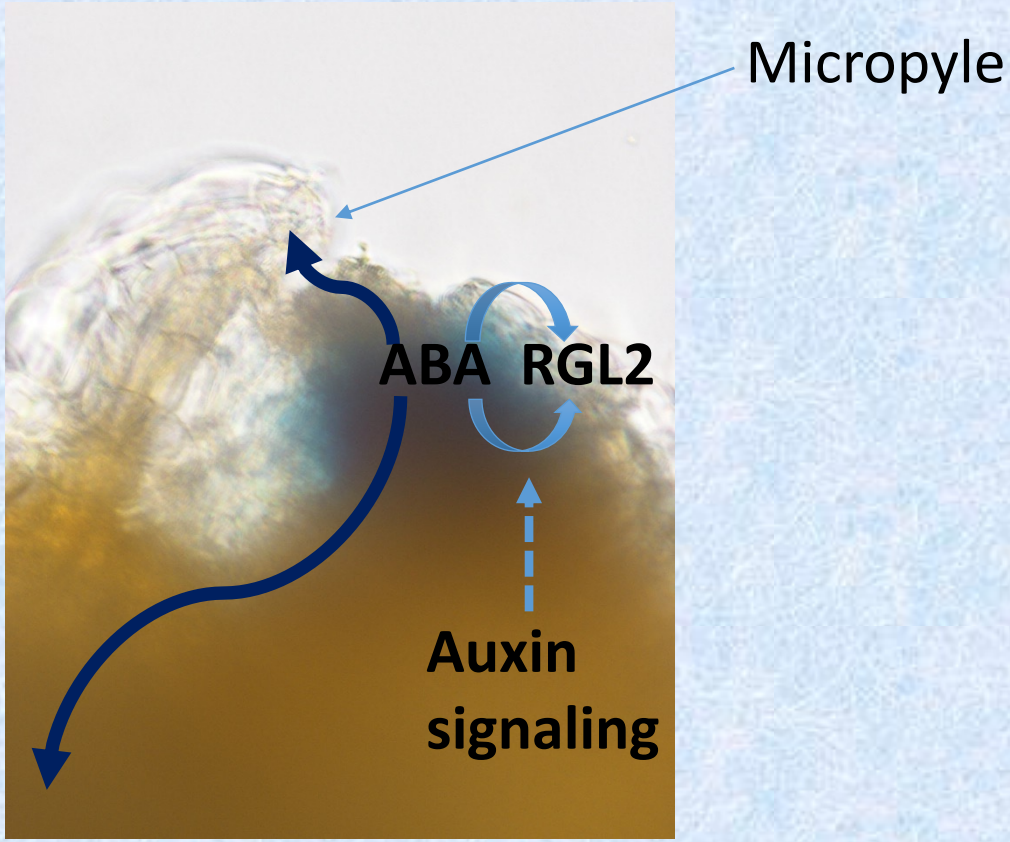
Auxin, ABA, and GA regulate seed dormancy



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

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

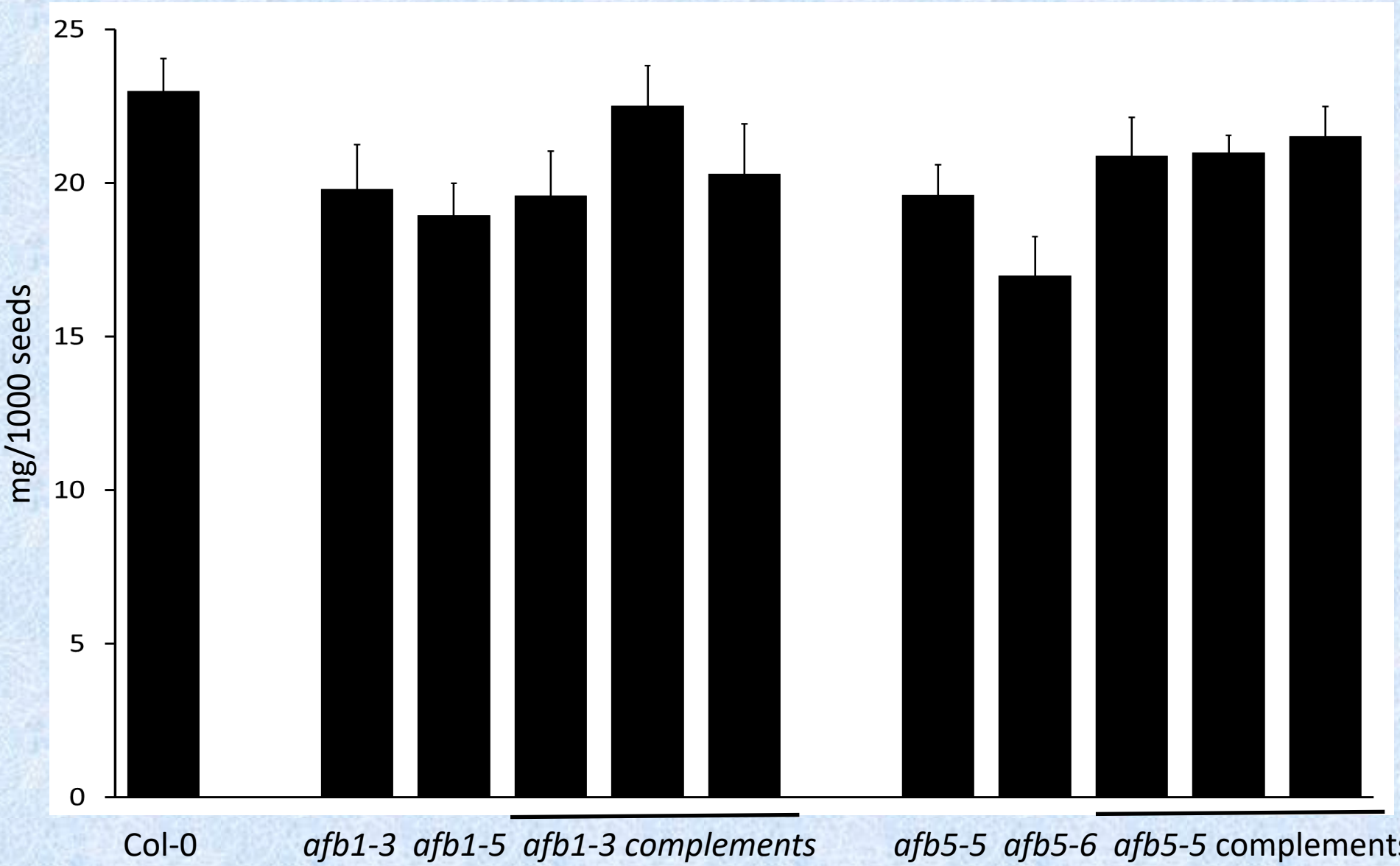
A model of auxin signaling in promoting seed dormancy



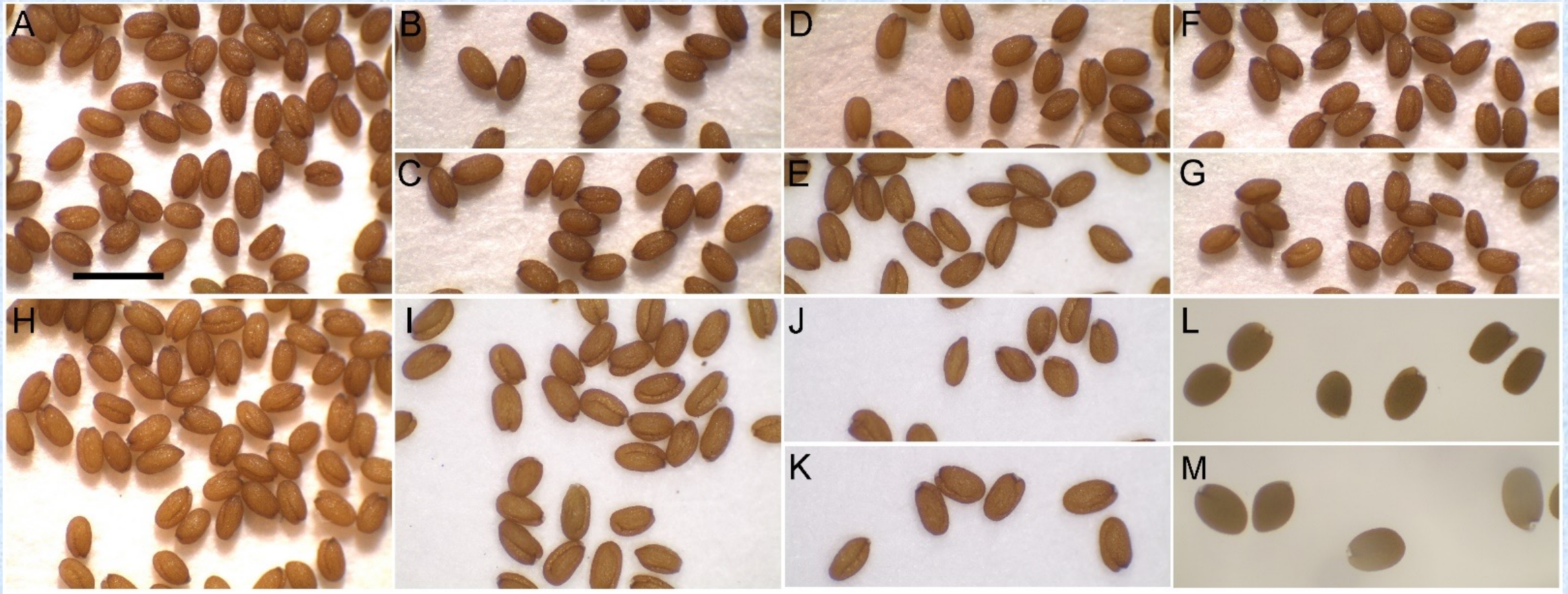
Summary of findings

- 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

AFB1 and AFB5 also promote seed growth



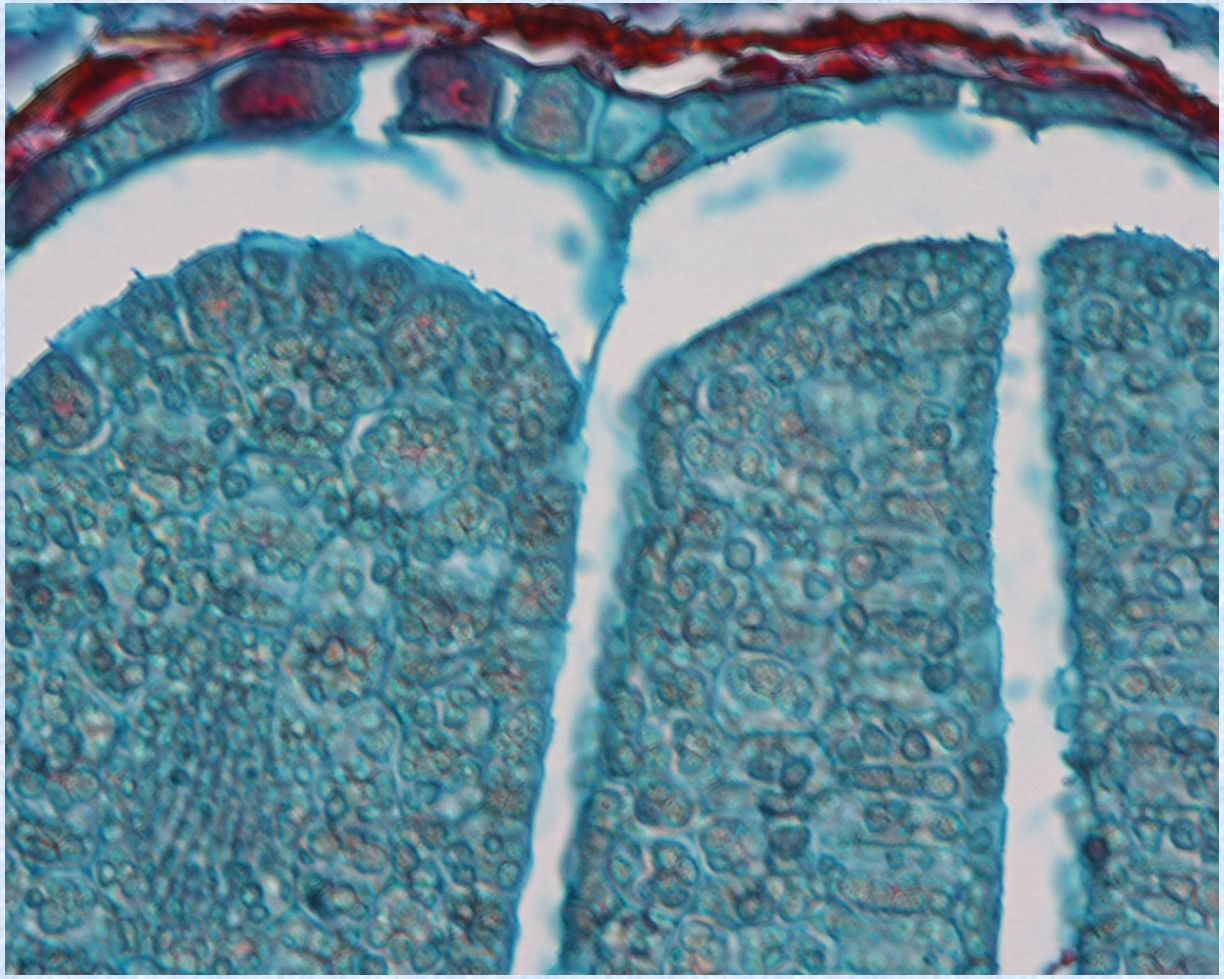
Maternal AFB1 and 5 affect seed size



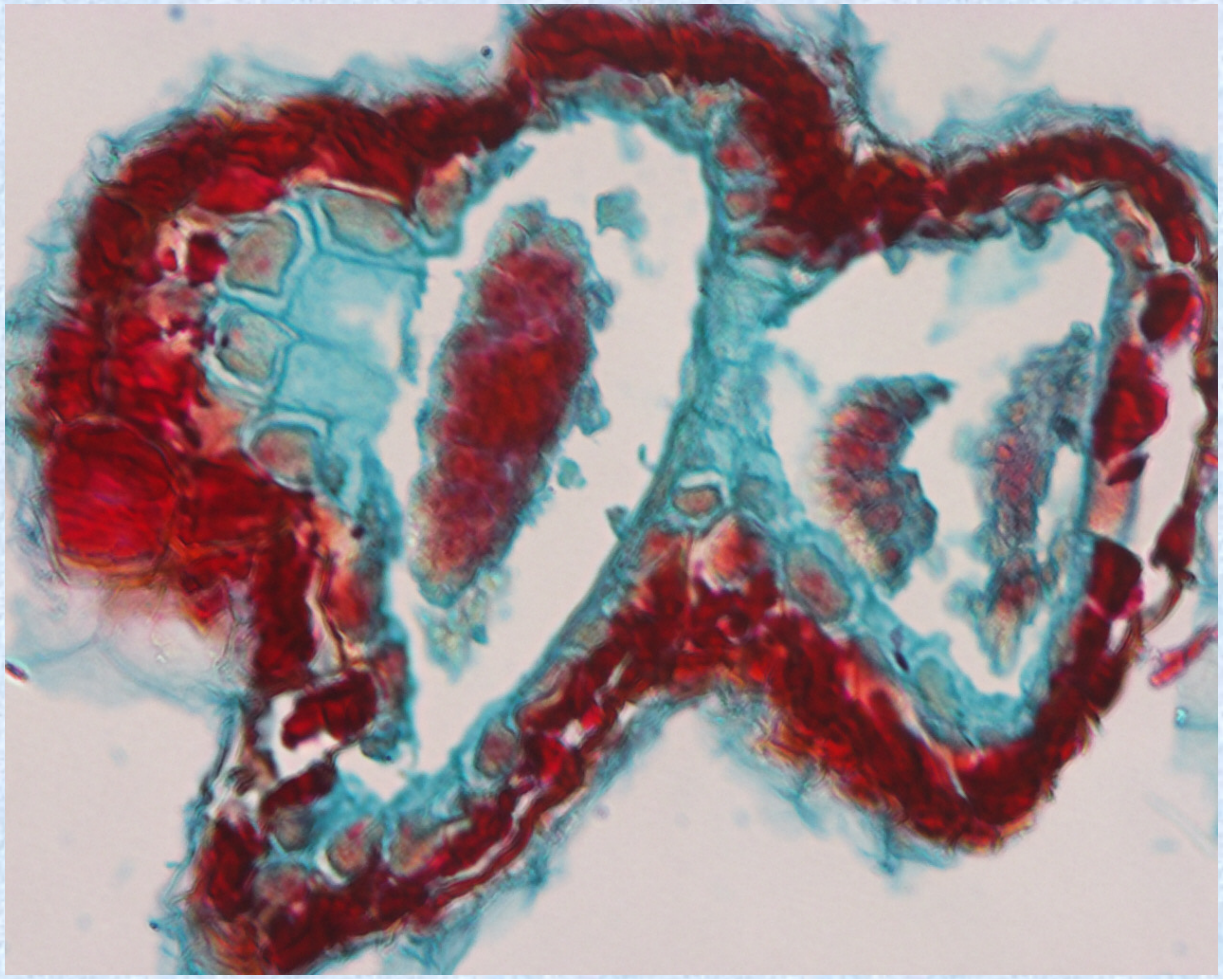
(A) Col-0. (B) *afb1-3*. (C) *afb1-5*. (D*) afb1-3/AFB1:AFB1*. (E) *afb1-3/ASK1:AFB1*. (F) *afb5-5*. (G) *afb5-6*. (H) *afb5-5/AFB5:AFB5*. (I) *afb5-5/ASK1:AFB5*. (J) F_1 seeds of female Col-0 x *afb5-5/ASK1:AFB5*. (K) F_1 seeds of female *afb5-5/ASK1:AFB5* x Col-0. (L) and (M) F_1 seeds of female Col-0 x *afb5-5/ASK1:AFB5* and female *afb5-5/ASK1:AFB5* x Col-0 after imbibition, respectively. Bar in (A) for all images = 1 mm.

Comparison of seed morphology between Col-0 and *afb5-6*

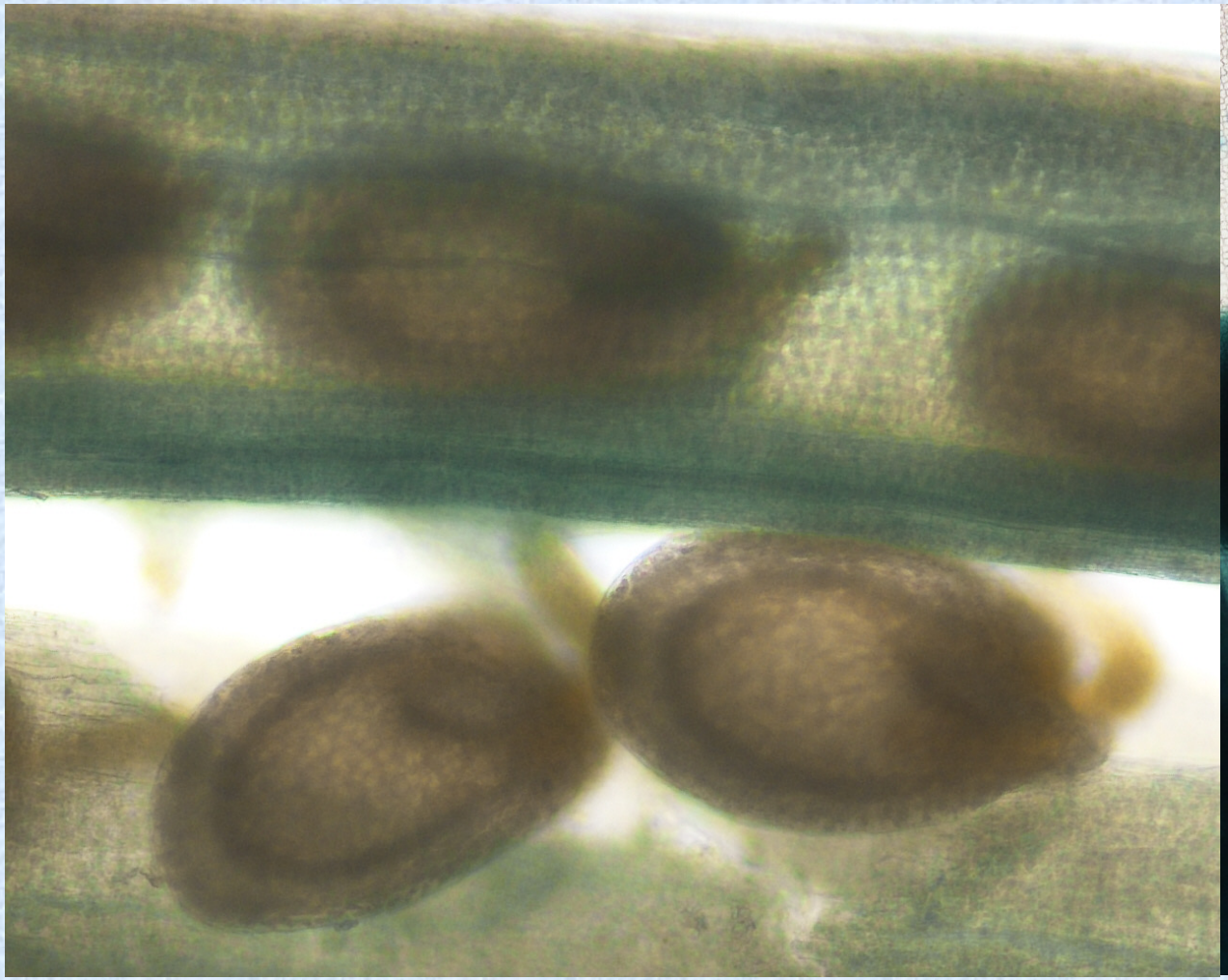
Col-0

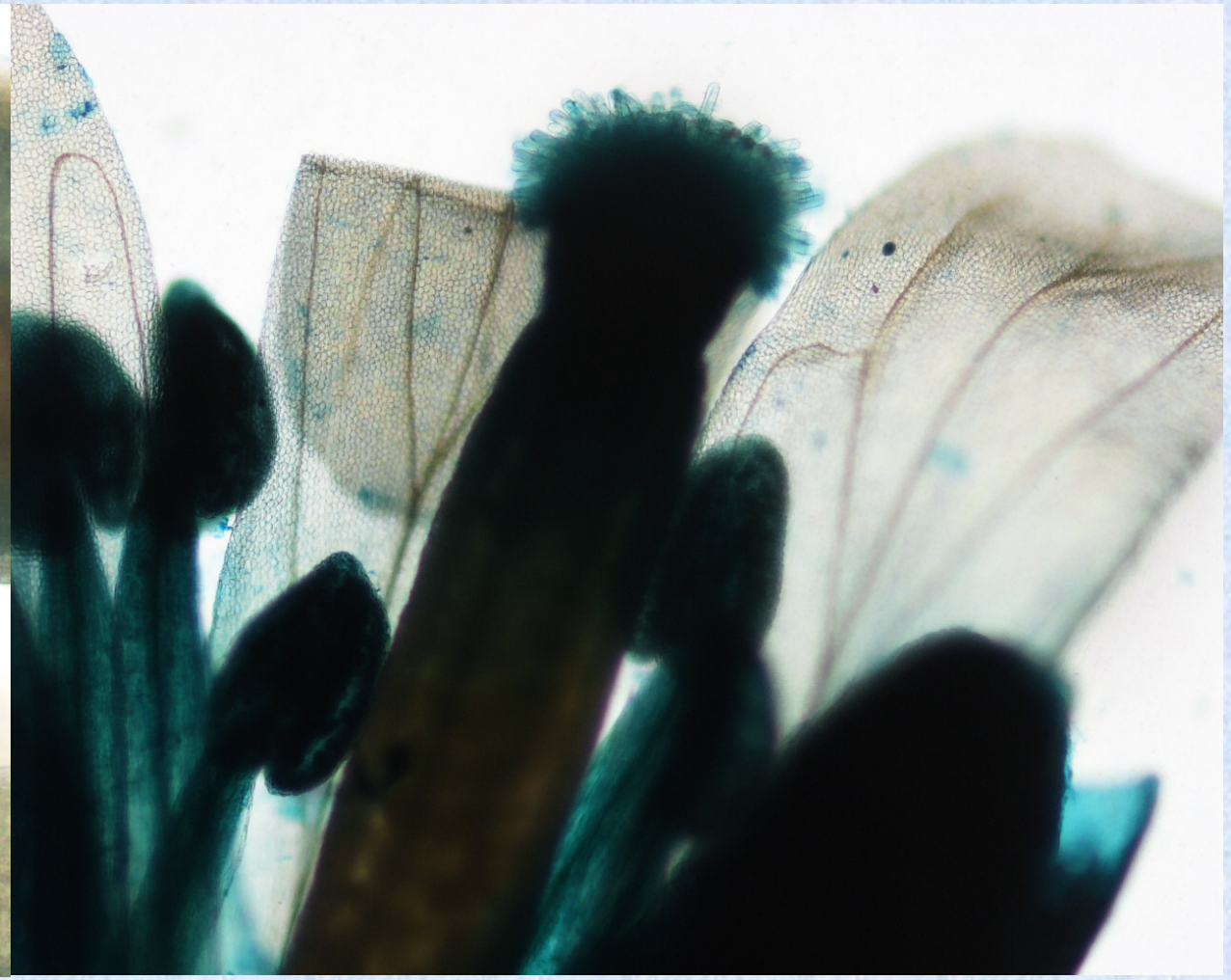


afb5-6



AFB1:GUS is expressed in almost all tissues but not in the seed





Conclusion

Maternal AFB1 and 5 outside the seed promote seed growth

Summary

- ASK1 regulate positively cell cycle progression and synapsis and negatively recombination
- The above regulation may be conserved in diverse species
- Spindle elongation occurs in discrete steps and possibly reflects rhythmicity of biochemical reactions
- Protein aggregates seen in *ask1-1* microspores may be similar to those in animals that are linked to cell death
- AFB1 and 5 are the primary AFBs in inflorescence tissues
- Transient expression of AFB1 and 5 in the hilum inhibits seed germination
- AFB1 and 5 outside the seed promotes seed growth

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Hong Wu

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John Doonan

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