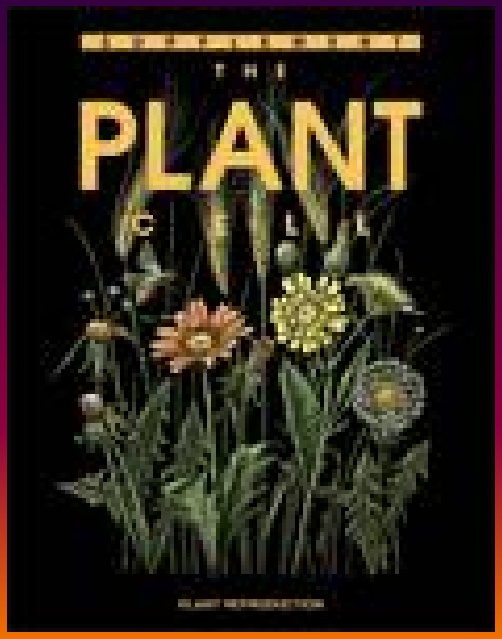
PMP

2025

3) Plant reproduction

d) Formation of gametes
e) Mutations in the development of gametophyte
f) Pollination, fertilization

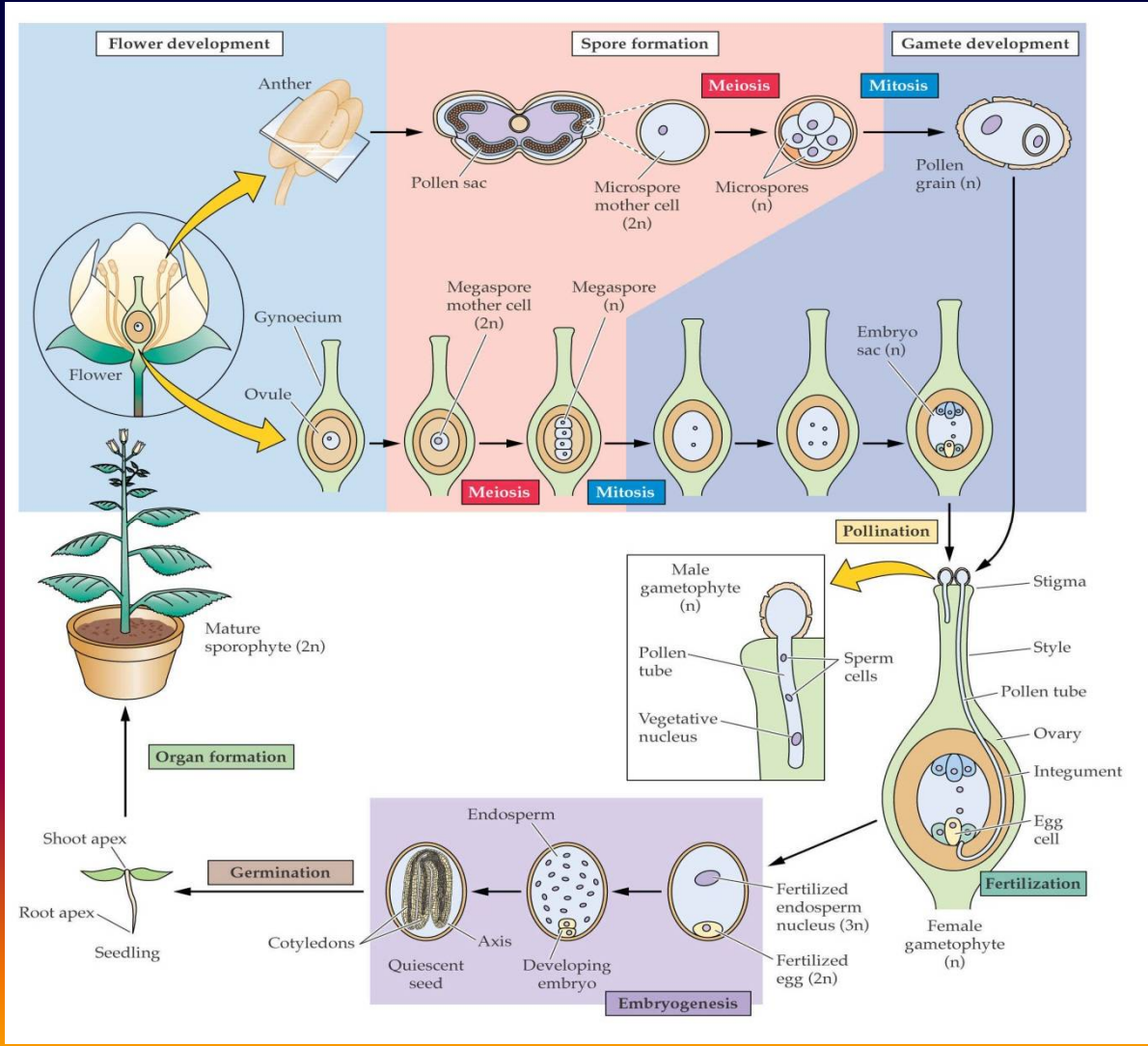


d) Formation of gametes

Microsporogenesis

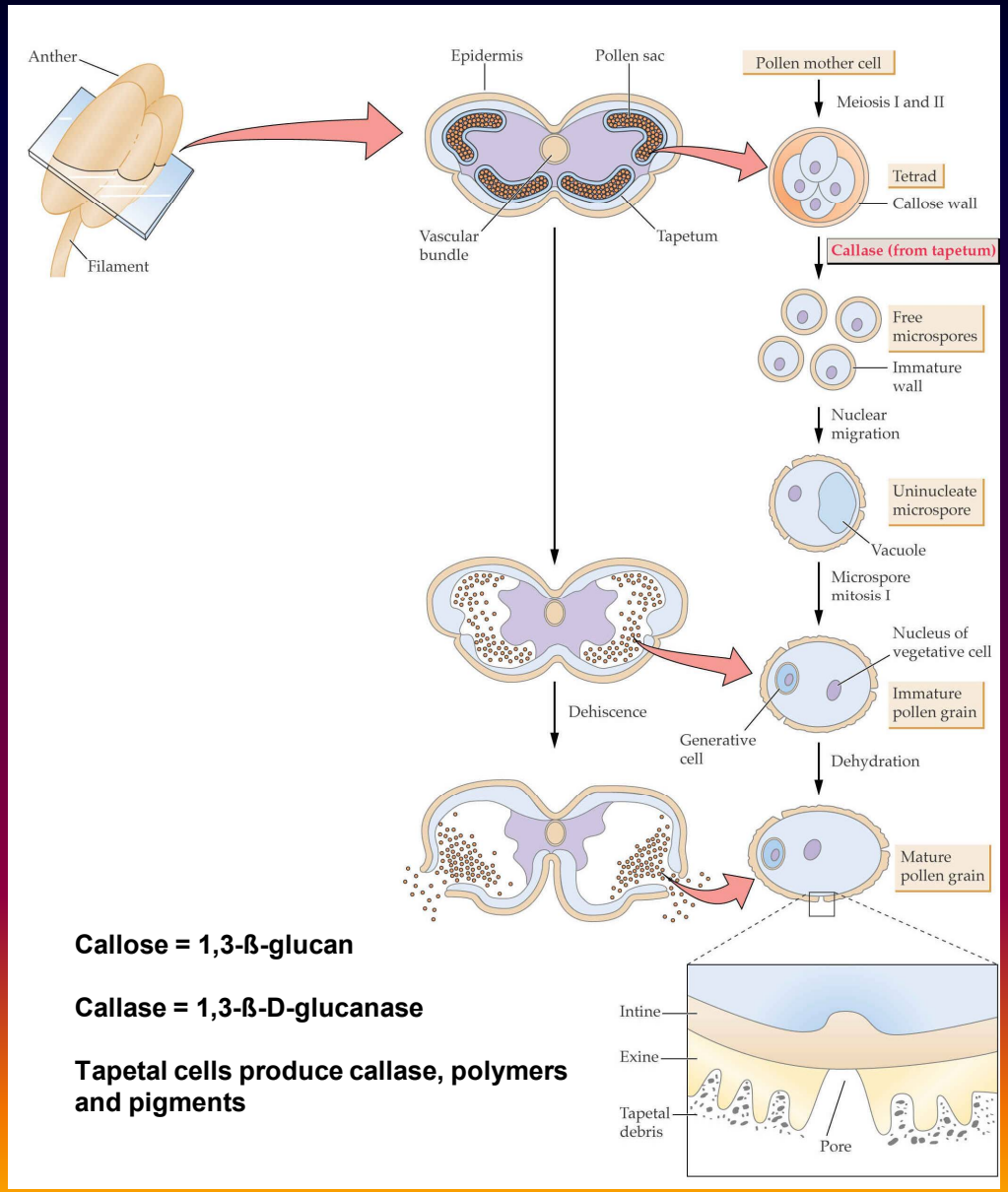
Megasporogenesis

Plant life cycle



PMP

Development of male gametophyte - microsporogenesis



Callose = 1,3-ß-glucan

Callase = 1,3-ß-D-glucanase

Tapetal cells produce callase, polymers and pigments

Microsporogenesis = formation of male gametophyte = pollen grain

Sporophytic cell

Tapetal initiation cell → **Tapetum**

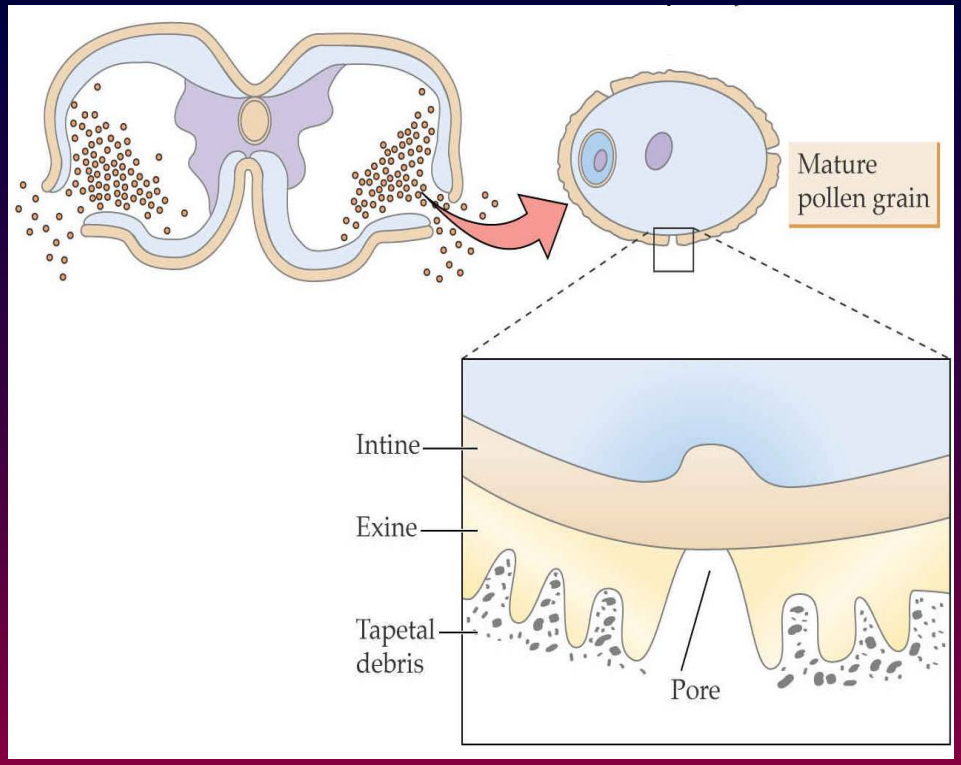
Sporogenous initiation cell (pollen mother) → **Meiosis (2x)** → **Tetrade of haploid cells**

→ **4 free microspores**

Mitosis → **Vegetative cell**, **Generative cell**

Generative cell → **Mitosis** → **2 sperm cells**

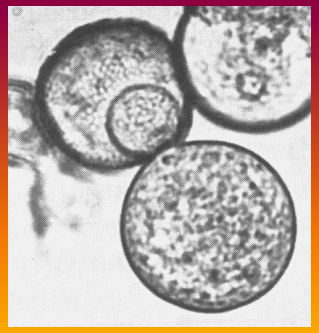
Male gamete – pollen grain

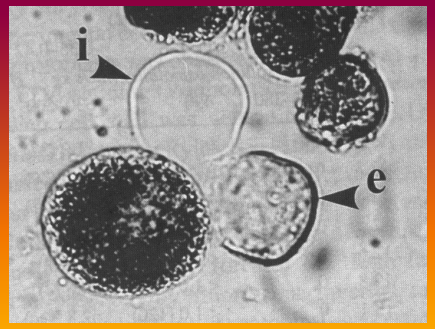


Exine = sporopolenin = polymer of phenols; extremely resistant to chemicals; genes which codes for exine formation are not known.

Fosill records of pollen grains

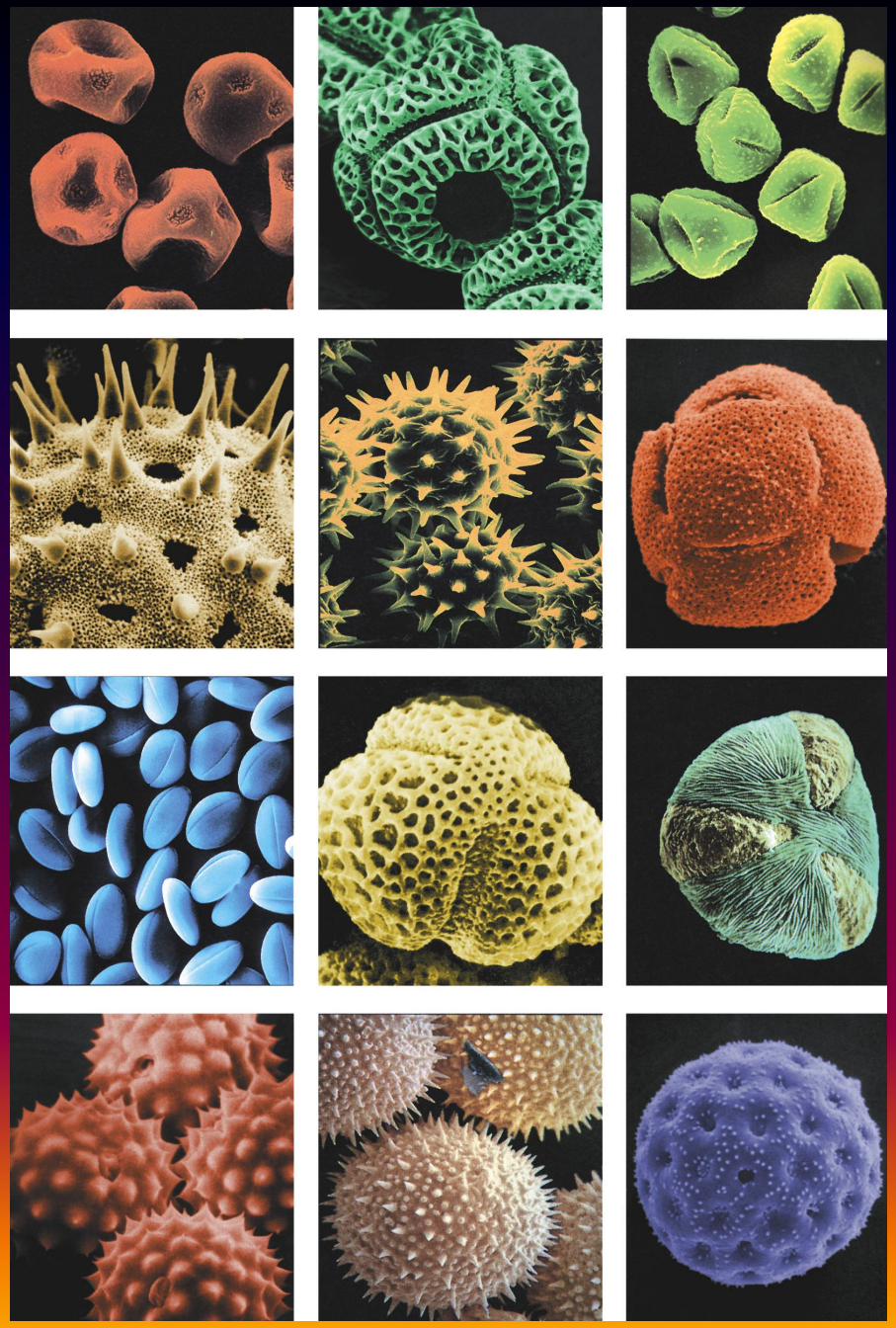
Developmental biology





Pollen protoplasts

Fellner M (1995) Plant Cell, Tissue and Organ Culture 42: 157-162.

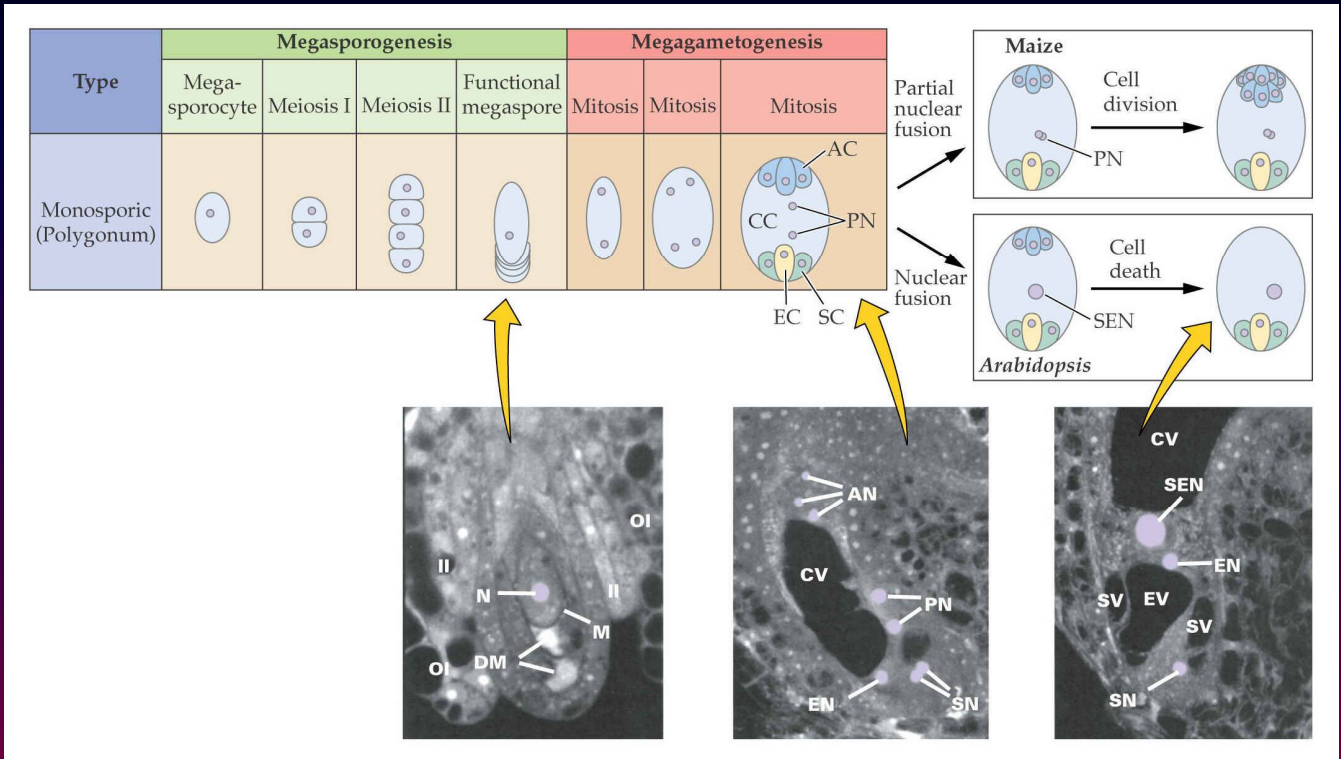


Varied forms of exine in pollen grains

Practical use
(e.g. criminology)

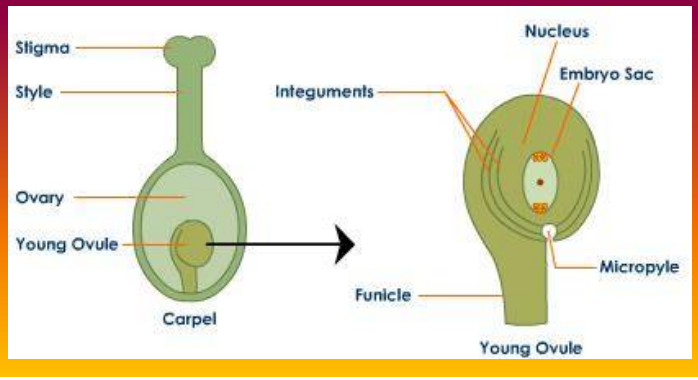
PMP

Development of female gametophyte - megasporogenesis



Megasporocyte → (Meiosis 2x) → **Megaspore** → (Mitosis 3x) → **Embryo sac: 7 cells**

- 3 antipodal (AC)
- 2 synergid (SC)
- 1 central (CC)
- 1 egg (EC)



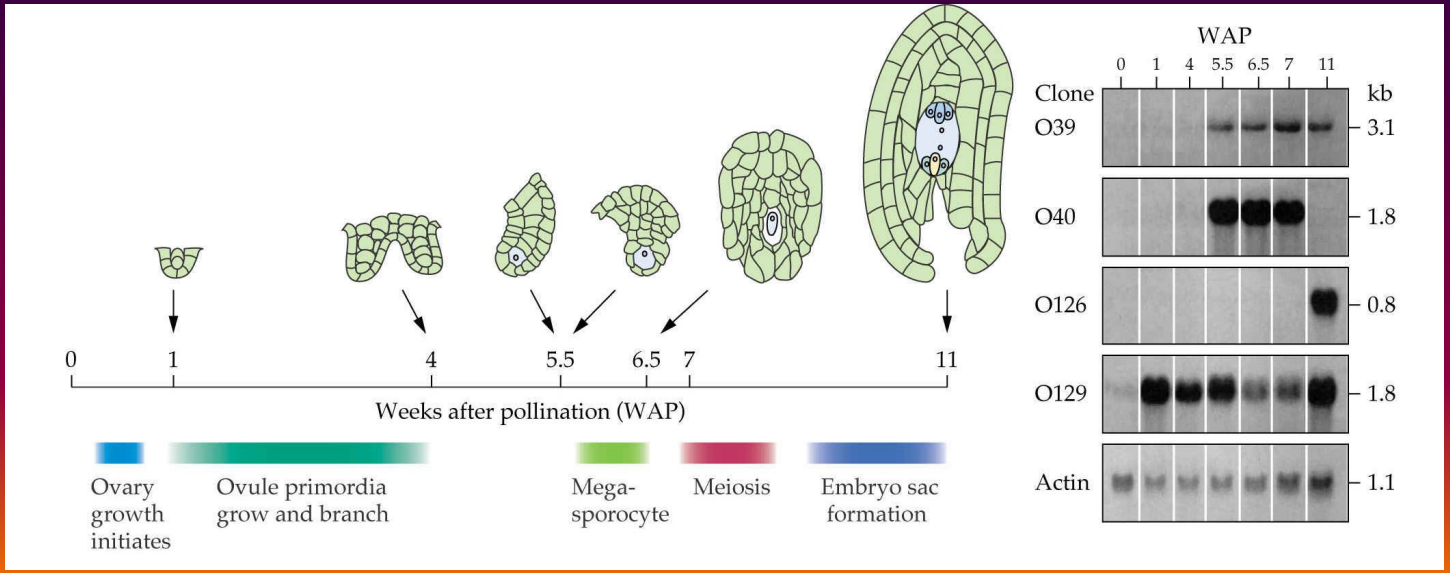
2 polar nuclei (PN)

Secondary endosperm nucleus (SCN)

It is complicated to reveal genes specifically expressed in female gametophyte: a problem to isolate ovule from sporophyte tissue => difficult to isolate mRNA and to build cDNA libraries.

Orchid – experimental plant for study of specifically expressed genes in ovule

- Synchronized development of the ovules
- Ovules develop for long time (11 weeks) => the possibility to isolate ovules in different stages of development => mRNA in different stages of development => the possibility to determine expression during development



Nadeau JA et al. (1996) Plant Cell 8: 213-239

e) Mutation in the development of gametophyte

Analysis of sterile mutants → gene identification

Pollen sterile mutants with a defect in pollen development – mostly recessive, homozygous

- defect in meiosis, do not form pollen
- defect in development of tapetal cells
- deformation of anthers – pollen is not released or too late
- defect in pollen pore – pollen grain cannot germinate
- defect in development of sporophyte – pollen grain cannot germinate

Sterile mutants with the defect in the ovule development

- defect in development of sporophyte
- defect in development of megaspore

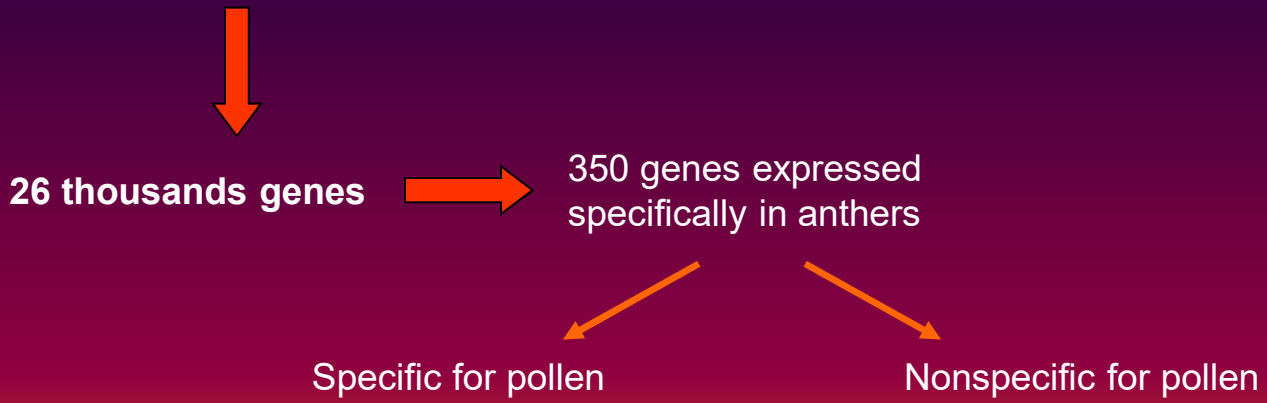
} - affected ovule development and fertilization

- defect in development of embryonic sac and ovule – affected fertilization

Mutation in development of male gametophyte

60 –90% of genes expressed in the gametophyte is expressed even in sporophyte

Sequencing of *Arabidopsis* genome (2000) **(see PMP1)**



WT *tdf1* Trangen *TDF1*

TDF1
(DEFECTIVE IN TAPETAL DEVELOPMENT AND FUNCTION1) **– transcription factor MYB; key regulator of tapetum development**

Zhu J et al. (2008) Plant J 55: 266 - 277

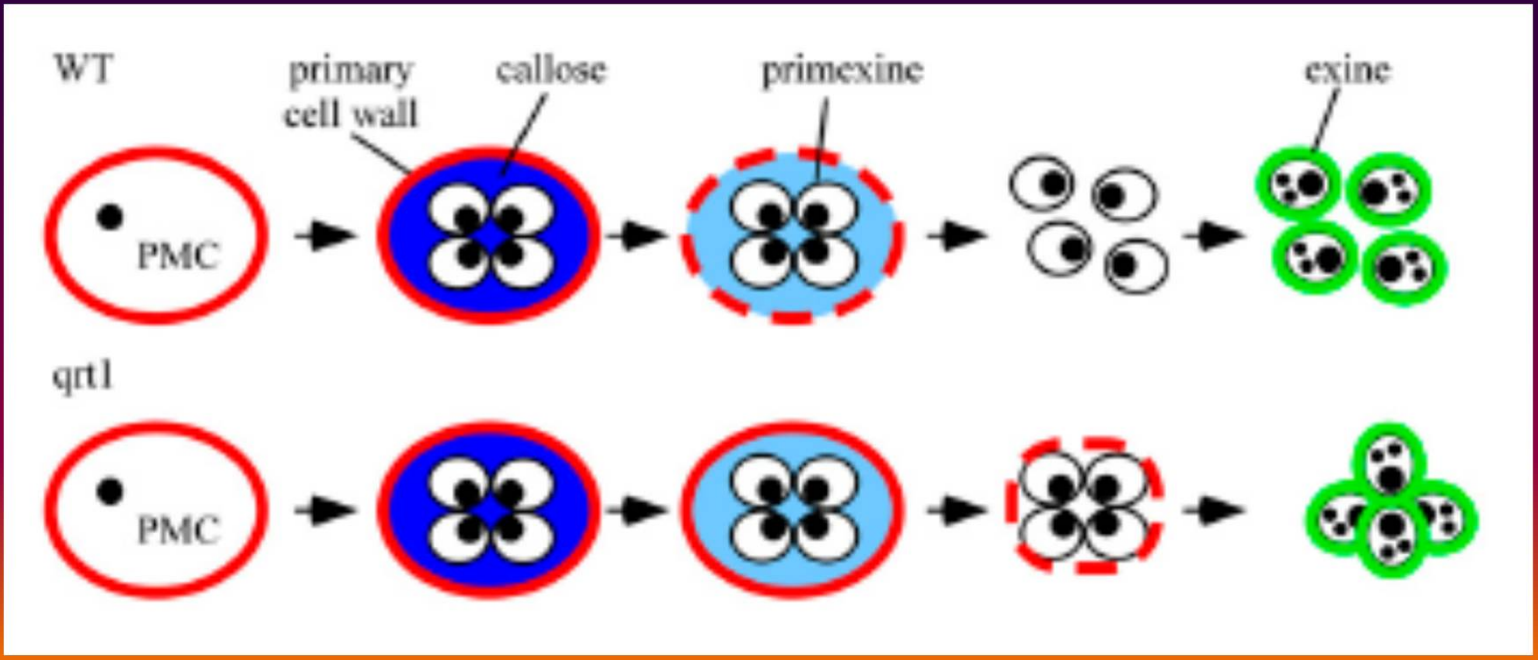
fkp1 **– pollen grains lacking the coating**

FKP1 (FLAKY POLLEN 1) – codes for 3-hydroxy-3-methylglutaryl-coenzym A synthase = enzyme of mevalonate (MVA) pathway involved in biosynthesis of sterols

MVA is important for development of organelles in tapetal cells => pollen grains *fkp1* lack coating, which is formed from disturbed tapetal cells.

***quartet* (*qrt*) – tetrads do not split up and they release from the anther**

***QRT* – codes for enzyme with pectin methylesterase activity (PME); expressed in the anther tissues before the termination of meiosis**



T-DNA mutant *cals5* – impaired fertility, degenerated microspores

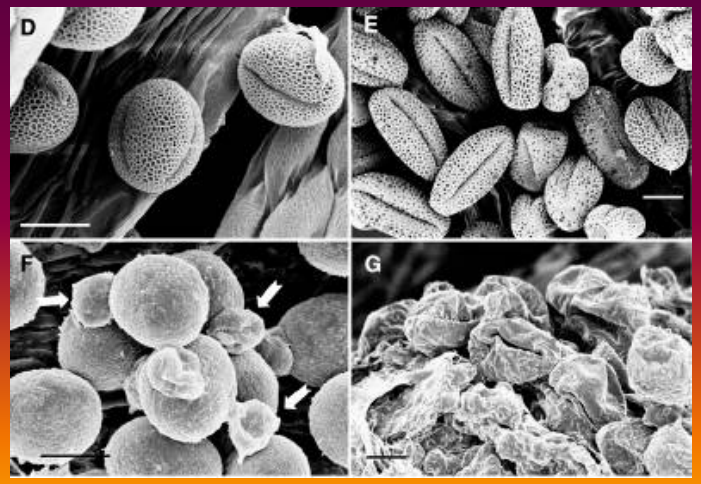
CALS5 codes for callose-synthase → callose synthesis → exine

Knockout mutant *CYP703A2* (cytochrom P450)
CYP703A2 – catalyses hydroxylation of lauric acid

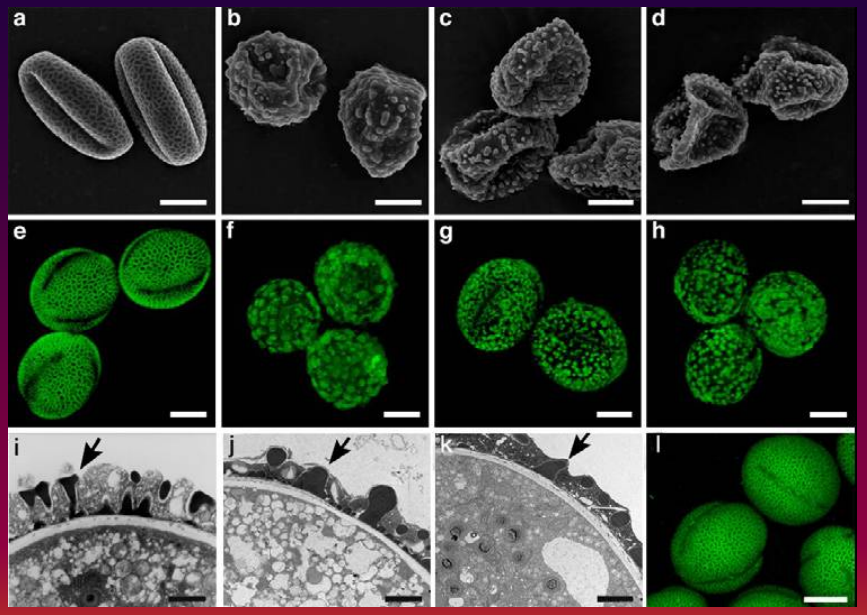
Sporopolenin – sections of hydroxylated lauric acid

WT

CYP703A2

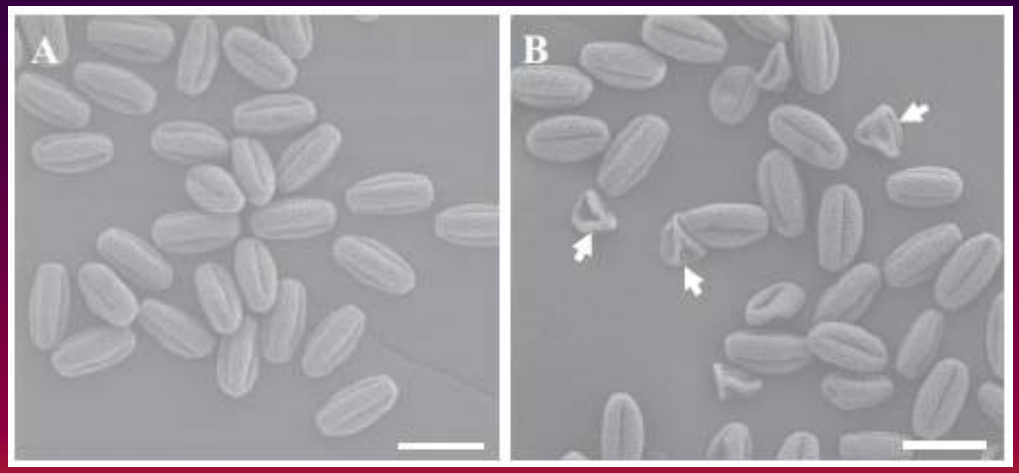


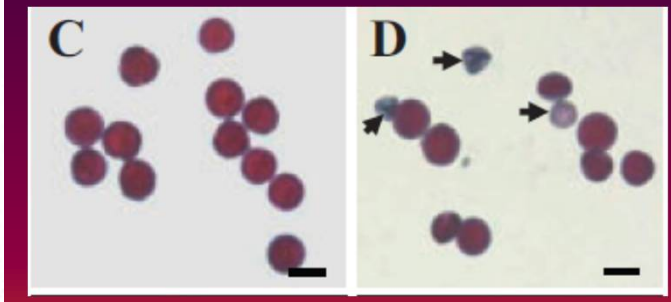
WT *cals5-3* *cals5-4* *cals5-5*



MGP1 **(*MALE GAMETOPHYTE DEFECTIVE 1*) – codes for F_ad subunit of mitochondrial F_1F_0-ATP synthase at *Arabidopsis*.**

mgp1 **mutant – destruction of mitochondria in pollen grains and destruction of pollen grains**

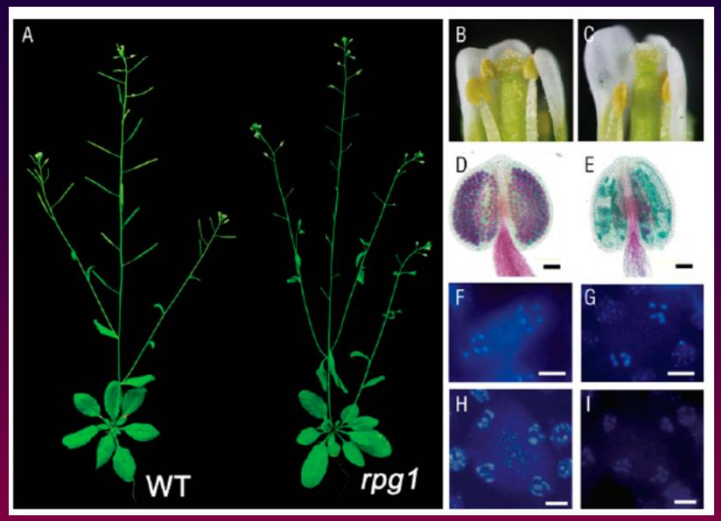




Li W-Q et al. (2010) Plant Cell Physiol 51: 923-935

MS1 **(*MALE STERILITY1*) – transcription factor regulating formation of exine, pollen cytosol and tapetum**

RPG1 **(*RUPTURE POLLEN GRAIN1*) – membrane protein essential for formation of exine**



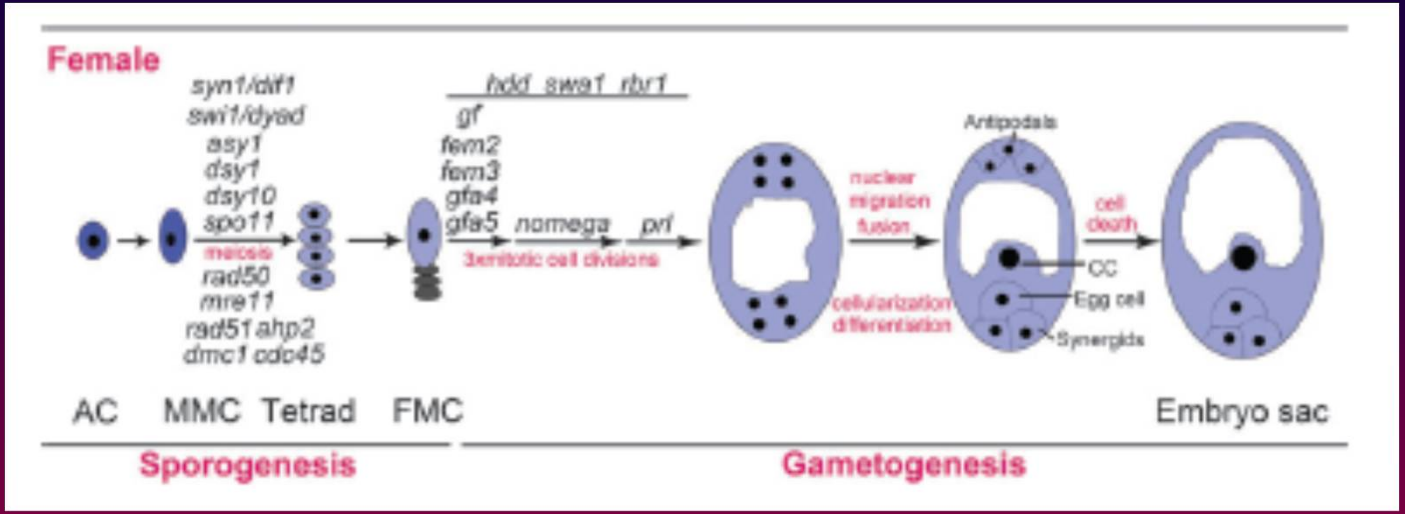
WT ***rpg1***

Guan Z-F et al. (2008) Plant Physiol 147: 852 - 863

TIR1, AFB1 – AFB3 **– auxin receptors; mutants generate short stamens of anthers and precocious maturation of pollen grains**

Mutation in development of female gametophyte

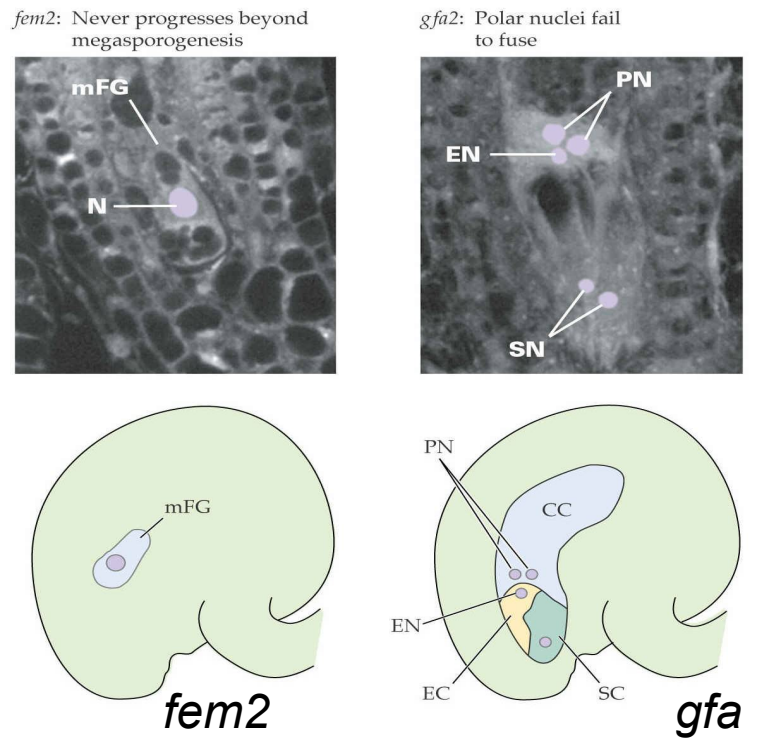
Mutations involved in meiotic and mitotic cell cycle – female gametophyte



23 genes (unknown functions)

- 14 nonspecific (♀+♂)
- 9 specific (♀)

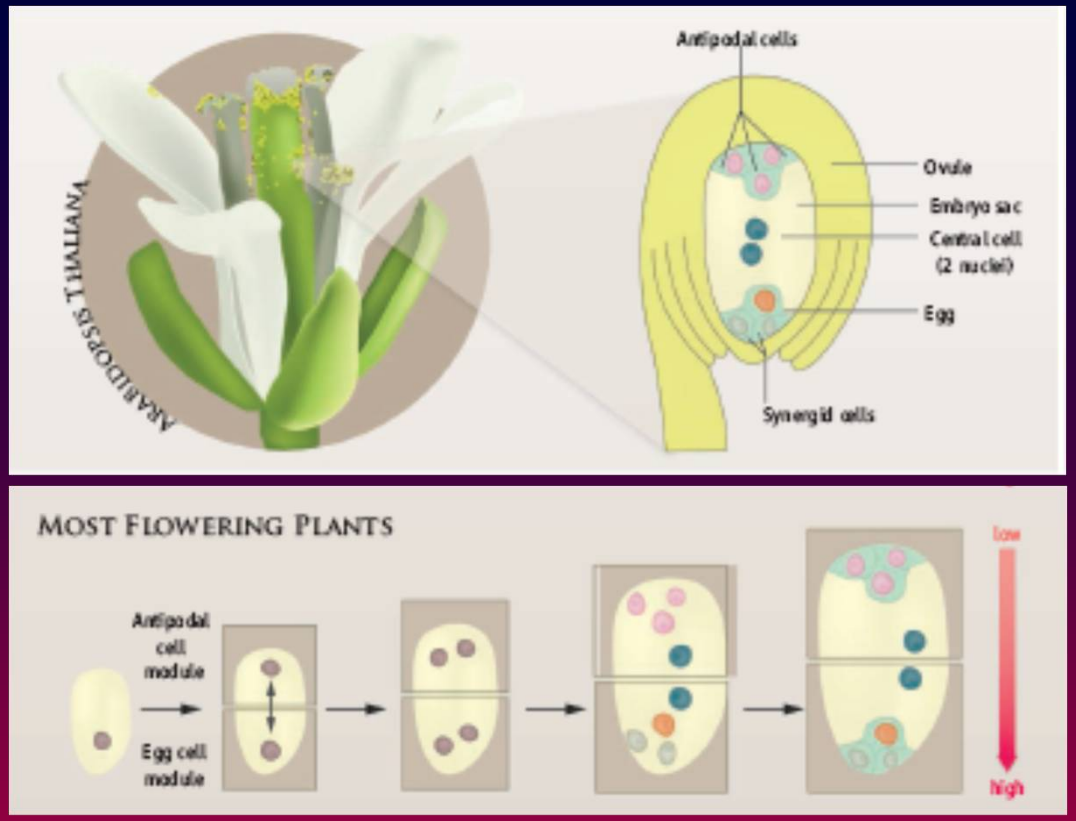
Specific genes



Arabidopsis mutant *fem2* – development of the ovule is arrested before megasporogenesis

Arabidopsis mutant *gfa* – fusion of nuclei of the central cells does not appear

Pagnussat GC et al. (2009) Science 324: 1684-1689



Auxin distribution in embryo sac is polarized – auxin gradient develops. Based on this gradients, auxin determines cell identity:

High auxin concentration → Synergid cells and egg cell

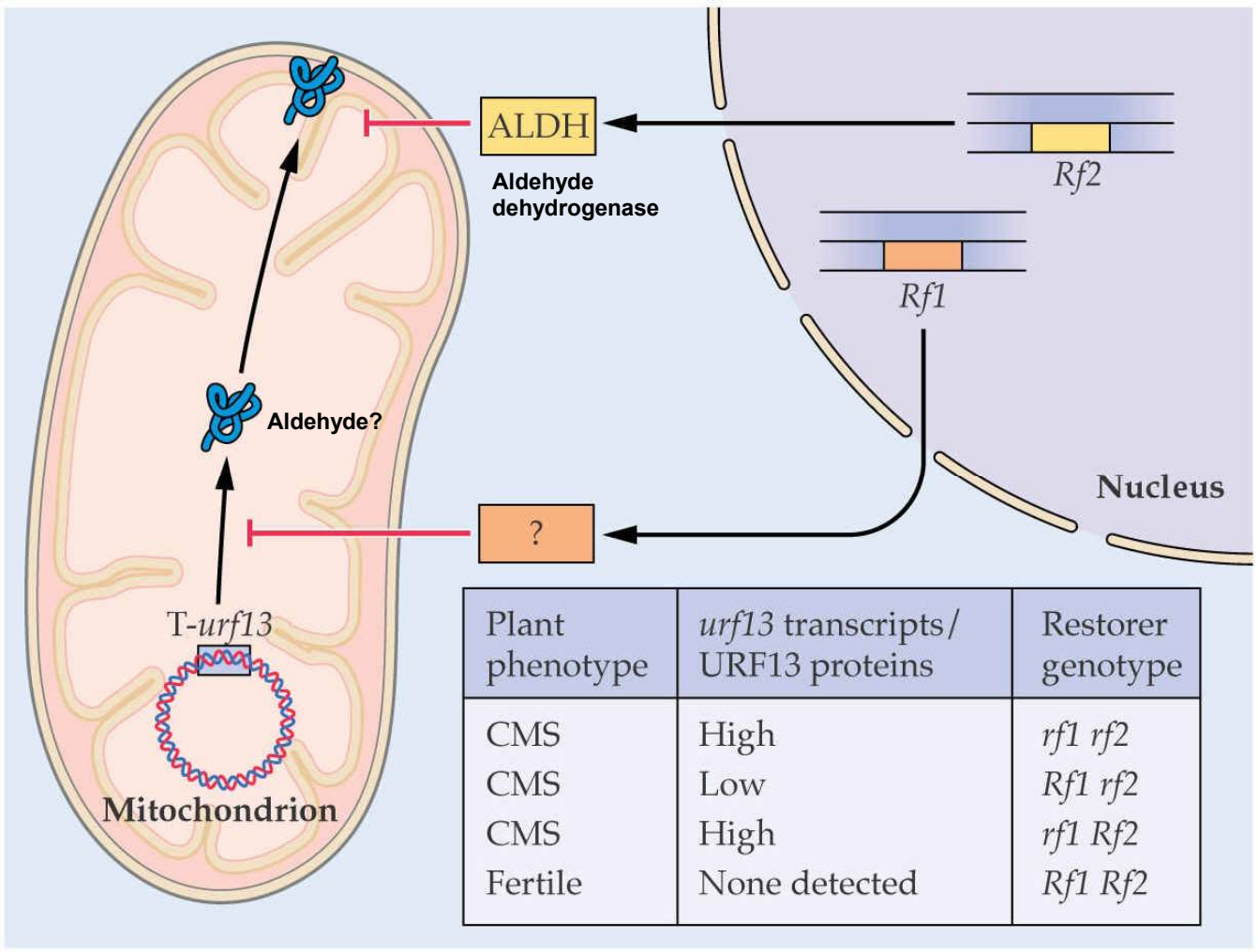
Low auxin concentration → Antipodal cells

Cytoplasmic male sterility (CMS) = pollen sterility transferred only by female organs

- Responsible genes are mostly part of chloroplast or mitochondrial genome.
- In all known cases, CMS is caused by expression of abnormal proteins in mitochondria of anthers.
- Mechanism, by which abnormal proteins influence mitochondria, is not known.
- Mitochondria in anthers influence pollen development.
- If the expression of abnormal protein is reduced, the fertility is restored
- In all CMS systems, nuclear genes exist. They suppress expression of abnormal proteins in anthers.

CMS-T system in maize – abnormal mitochondrial protein URF13

Restoration of fertility requires 2 nuclear genes: *Rf1* and *Rf2*



Plant phenotype	*urf13* transcripts / URF13 proteins	Restorer genotype
CMS	High	*rf1 rf2*
CMS	Low	*Rf1 rf2*
CMS	High	*rf1 Rf2*
Fertile	None detected	*Rf1 Rf2*

Pollen germination

For the germination, dry pollen grain requires humidity.

1) Plants with humid stigma – pollen grain uses the humidity from the stigma

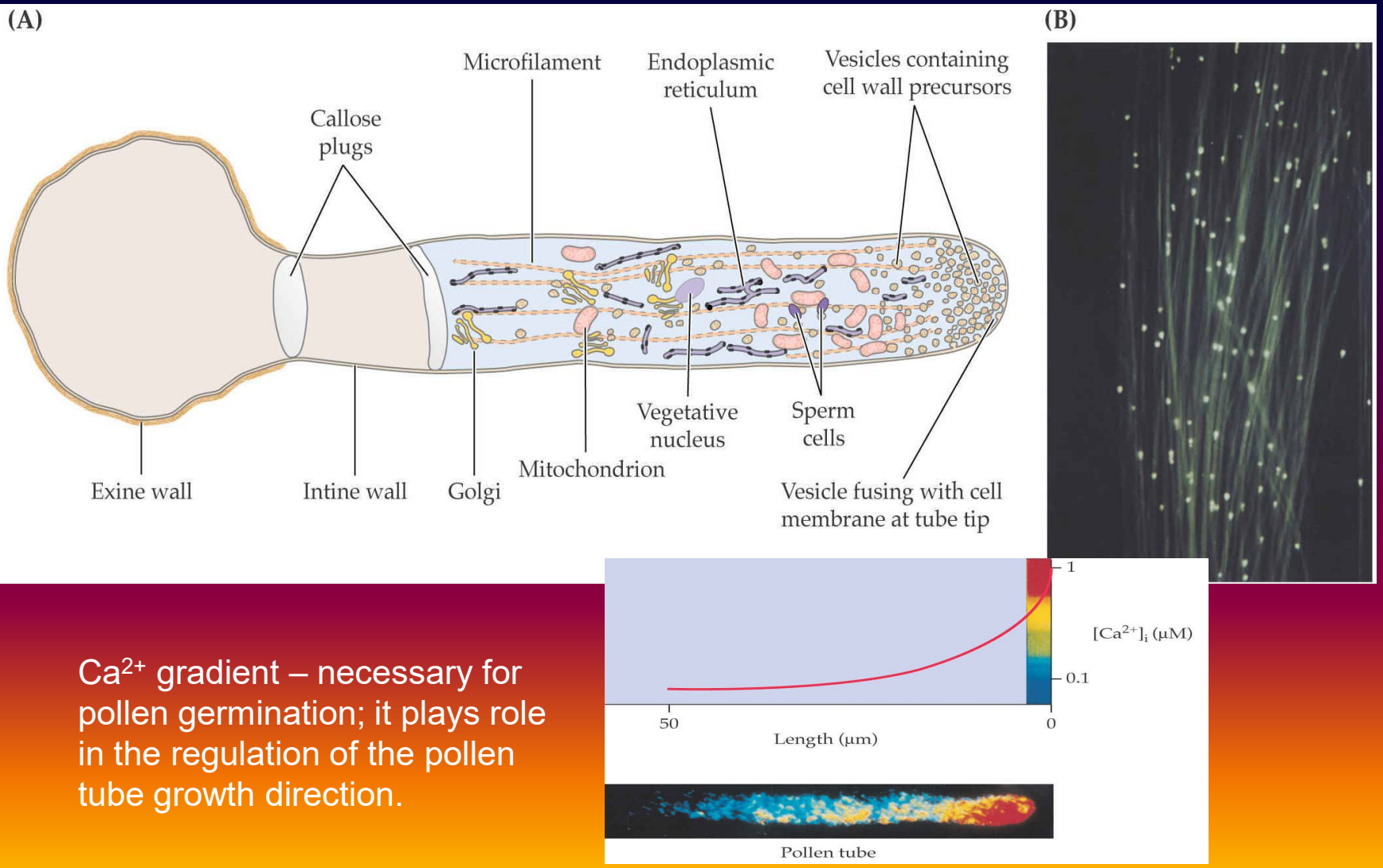
2) Plants with dry stigma – humidity is catered by lipids from the surface of the pollen grain. Lipids play important role in pollen germination.

Arabidopsis mutant ***cer*** – it has defective lipid layer – it germinates only at extreme humidity

Arabidopsis mutant ***fiddlehead*** –the epidermal leaf cells contain different type of lipids (high molecular ones). Pollen grains of WT germinate on these leaves!!!

Flavonoids on the surface of the pollen grain play important role in pollen germination. Maize plants mutated in the gene coding for enzyme involved in flavonoid biosynthesis are self-sterile.

Mechanisms of pollen germination is still not known very well.



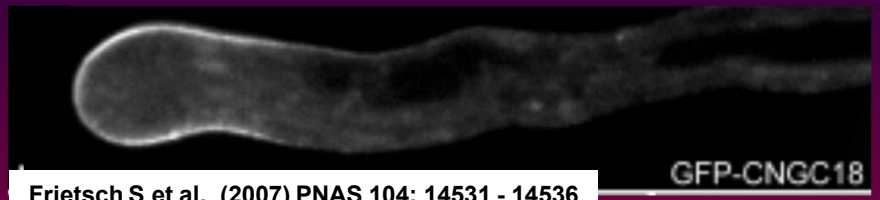
Ca^{2+} gradient – necessary for pollen germination; it plays role in the regulation of the pollen tube growth direction.

It is not known, how Ca^{2+} signal is translated to final response of polen tube elongation.

cngc18 – null mutant carrying pollen sterility

CNGC18 – codes for cation channel regulated by cyclic nucleotides

GFP:CNGC18 analysis



Frietsch S et al. (2007) PNAS 104: 14531 - 14536

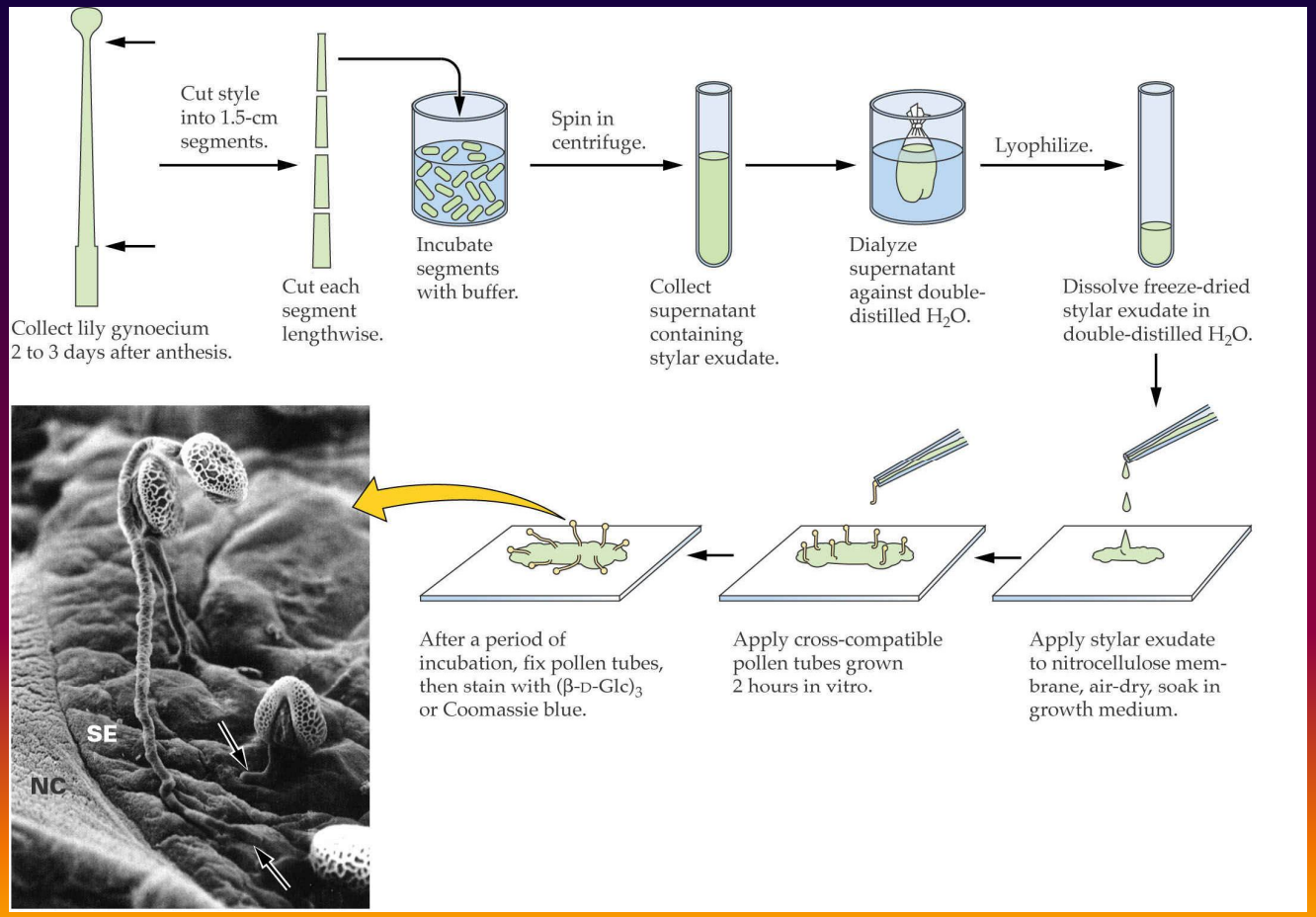
cngc18: complemented with GFP:CNGC18

Heterologous expression in *E. coli* – temporal- and concentration-dependent accumulation of Ca^{2+}

Pollen germination is possible to induce in conditions *in vitro* on the medium containing sucrose, boric acid and Ca^{2+}

Germination *in vivo* is always faster than *in vitro*

Other factors (**?**) originating from the stigma and functioning in pollen germination



Other factors affecting growth of pollen tube – plant hormones

Gibberellins – stimulate elongation

(Swain and Singh 2005, TIPS 10: 123-129)

GA-deficient mutants
Mutants with defect in GA-signaling
} Dwarf growth, defect in development of anthers and pollen

Overexpression of the enzyme deactivating GA → Inhibition of pollen tube growth

Brassinosteroids – stimulate elongation

Mutant *cpd* – *CPD* codes for cytochrome P450 (BR biosynthesis)

Mutant *bri1* – *BRI1* codes for BR receptor

Pollen tube growth stopped

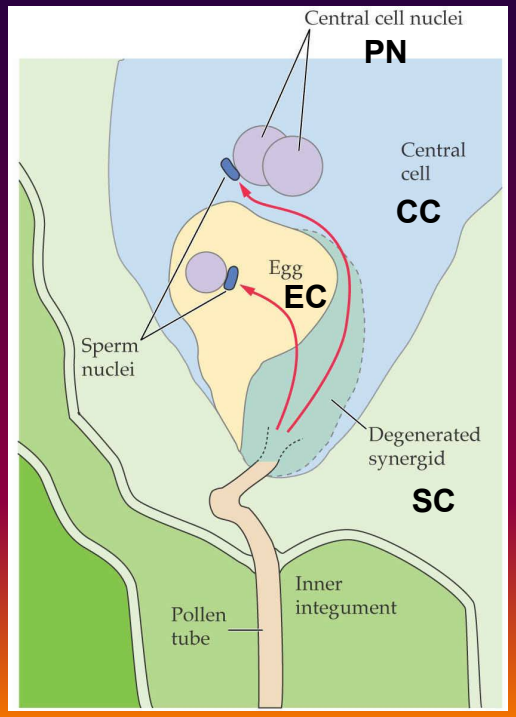
BRs and BR signaling are necessary for pollen tube growth

Clouse et al. (1996)
Plant Physiol 111: 671-678

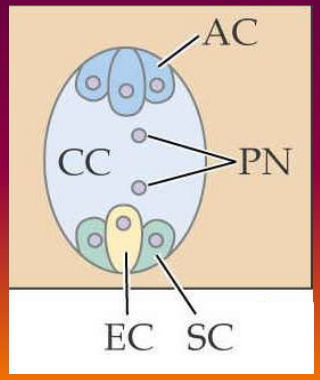
f) Pollination, fertilization

Both sperm cells penetrate one of the synergids (SC). Double fertilization occures:

1st fertilization:
1st sperm cell fertilizes haploid egg cell (EC) => **diploid zygote**

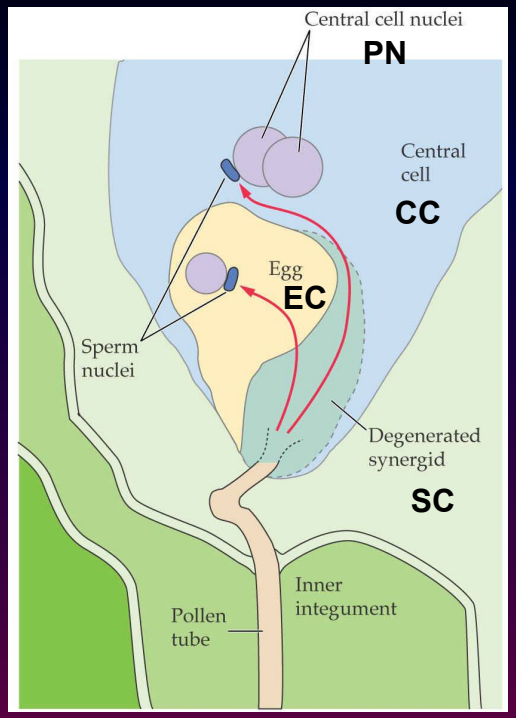


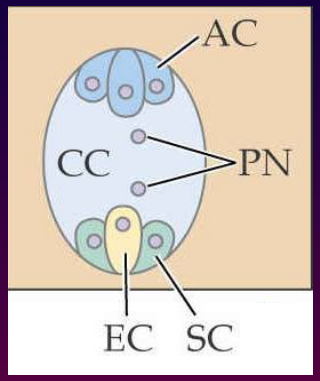
2nd fertilization:
2nd sperm cell fertilizes diploid central cell (CC) = fuses with nuclei (PN) => **triploid endosperm**



Key question:

What is the thing that forces and directs pollen tube to embryo sac?





HAP2 **– expressed in haploid sperm cells; it is necessary to the pollen tube can reach the ovule => sperm cells direct actively pollen tube to the ovule**

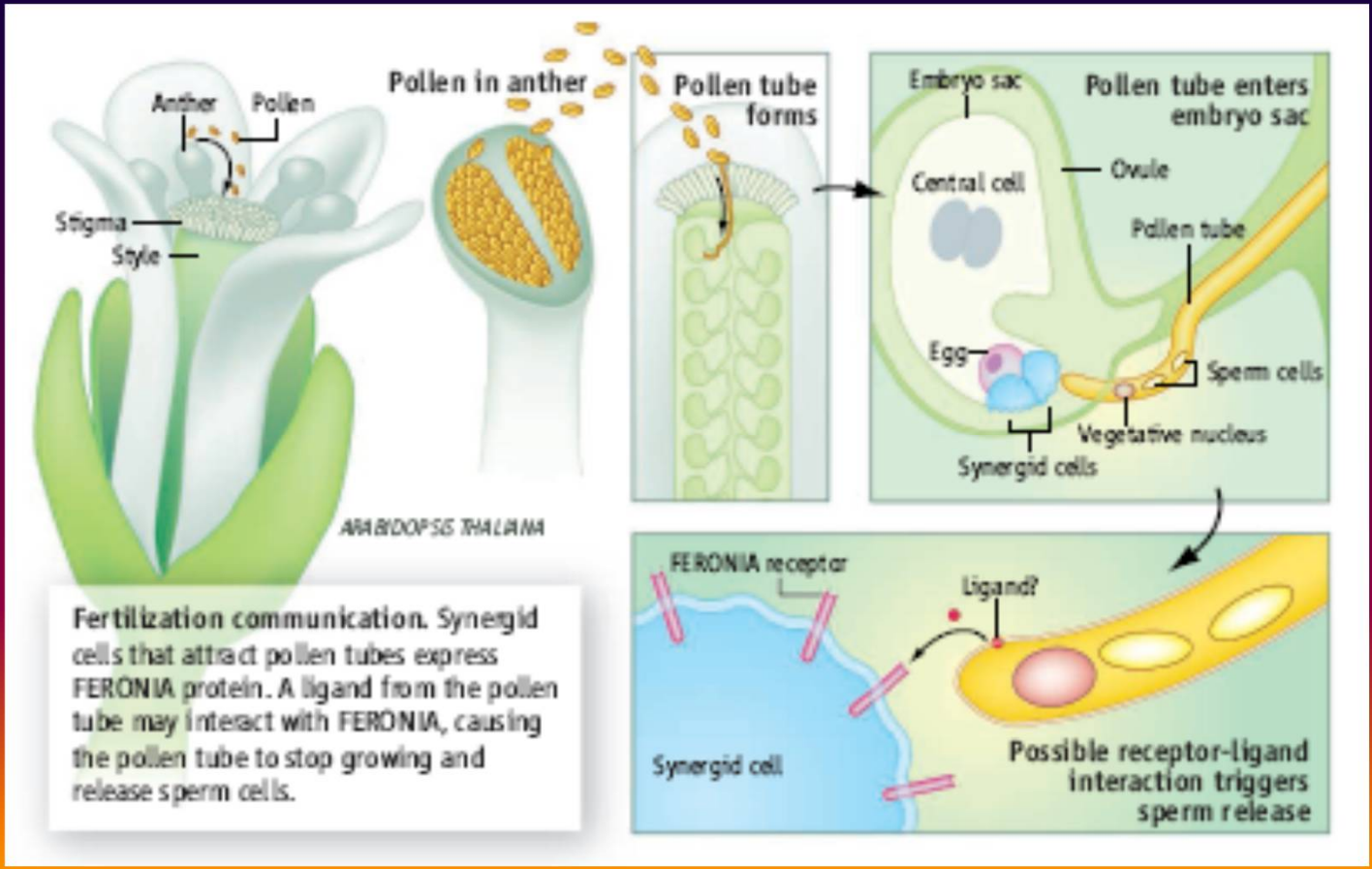
GEX3 **– correct expression of the gene in the egg cell and sperm cells – directing the pollen tube growth to the ovule**

MAA3 (MAGATAMA3) **– codes for enzyme helicase, which is necessary for RNA metabolism. It plays a role in correct dirrection of the pollen tube to the micropyle**

feronia **– pollen tube enters synergid, but it does not rupture and does not release sperm cells**

Synergids express protein FERONIA (receptor-like kinase) – attract the pollen tube

Unknown ligand + receptor FERONIA → **Growth of pollen tube stops, sperm cells are released**



anx1anx2 **– pollen tube ruptures before reaching the female gametophyte**

Genes *ANXUR1* and *ANXUR2* – homologous to the gene *FERONIA*

Function of ANX1 and ANX2? : constitutive inhibition of pollen tube rupture and releasing of sperm cells; specifically expressed in the pollen tube

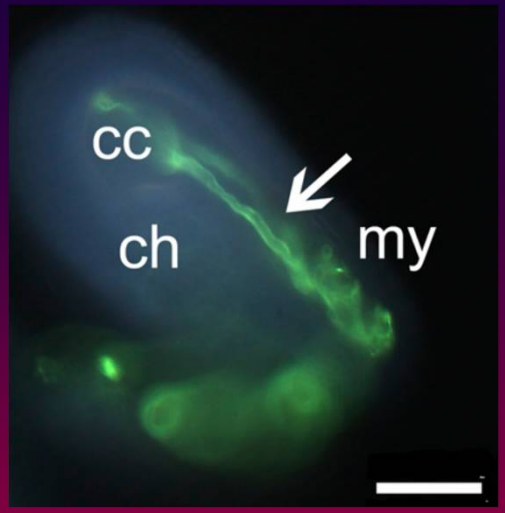
Pollen tube reaches female gametophyte.

Activation of FERONIA pathway **Inactivation of ANX pathway**

Rupture of the pollen tube and releasing sperm cells

Mutant *lorelei* - defective in releasing of sperm cells

After reaching embryo sac, the pollen tube of the mutant does not rupture. Instead, it continues in the growth in the embryo sac towards the central cell (CC). However, at CC it turns and grows back to the micropyle.



cc – central cell

my – micropylar end

ch – chalazial end

LORELEI (*LRE*) – expressed in synergids

LORELEI is glucosylphosphatidylinositol (GPI)-anchored protein – it allows female gametophyte to distinguish the intrusion of compatible pollen tube and to release the sperm cells.