Bi8940 Developmental Biology
Lesson 6

Plant Reproduction

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Outline of Lesson 6

Plant Reproduction

- Sexual and asexual plant reproduction
- Plant life cycle
- Initiation of flowering
- Determination of floral organ identity
- Microgametogenesis
- Megagametogenesis
  - Female gametophyte patterning
- Pollen tube growth, guidance and fertilization
- Endosperm and seed formation

Outline of Lesson 6

Plant Reproduction

- ☐ Sexual and asexual plant reproduction
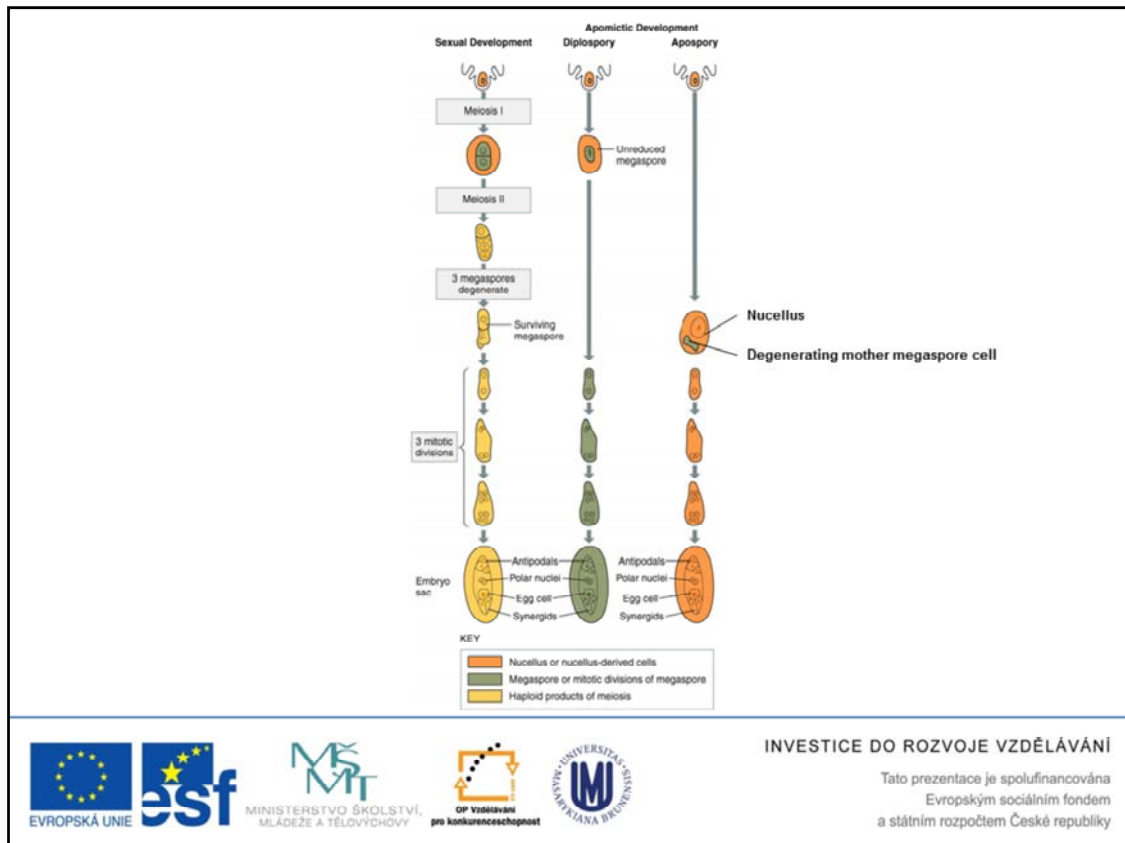
Besides the sexual reproduction, plants are quite often able to form embryo even via asexual reproduction.

There is a number of developmental variations of the asexual embryo (seed) formation, called *apomixis*.

Diplospory is a formation of female gametophyte from the unreduced megaspore, i.e. the megaspore, which did not underwent meiosis.

Apospory is the formation of female gametophyte from the cell of the sporophyte, e.g. nucellus, the mother megaspore cell degenerates.

However, the non-sexual reproduction will be in detail described in the lectures of prof. Vyskot in frame of his course "Bi0580 Developmental genetics". Here, only the sexual reproduction will be discussed in more detail.
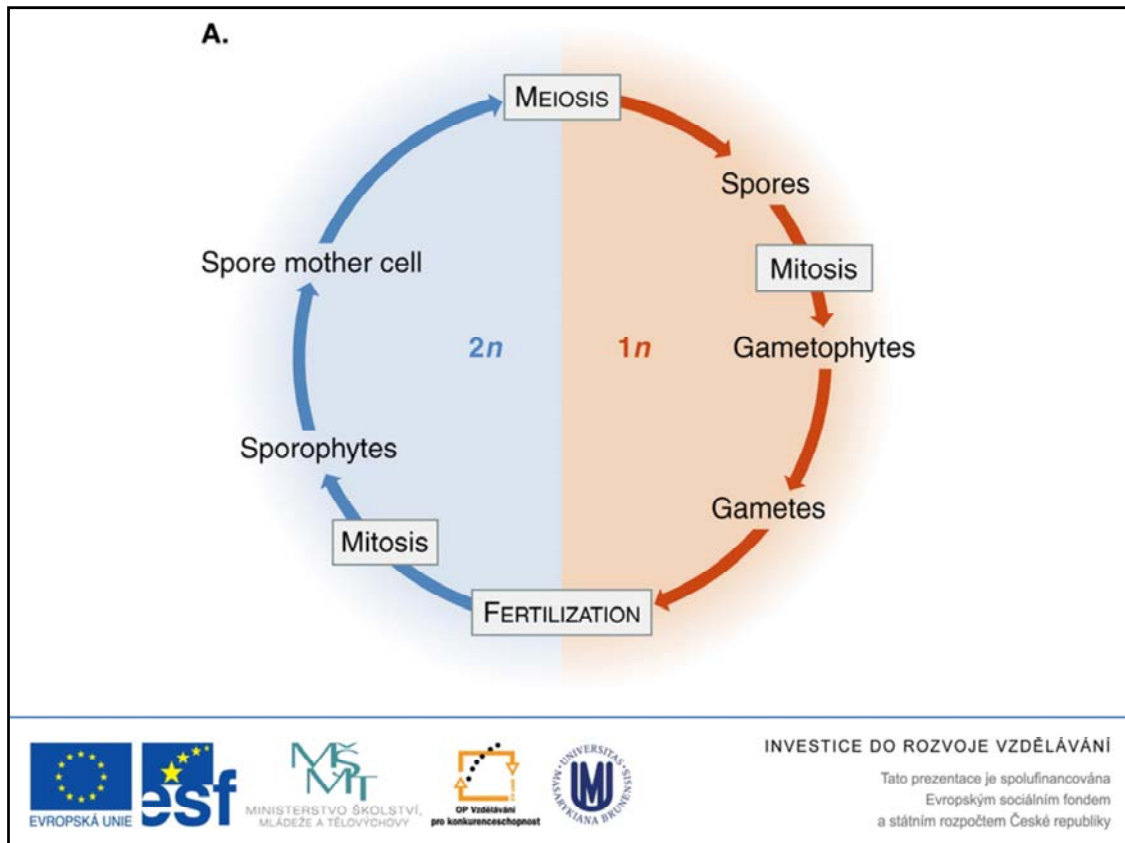
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Plant Reproduction

- Sexual and asexual plant reproduction
- Plant life cycle

A.

In plants there could be distinguished two phases of their life cycle: The phase, when ONLY diploid or haploid cells of the sporophyte or gametophyte, respectively, are present.

Two alternating generations can be recognized in the plant life cycle: a haploid phase, known as the *gametophytic generation* and a diploid phase, known as the s*porophytic generation.* The gametophyte reproduces by means of *gametes,* but it does not reproduce itself directly. Instead, the zygote resulting after gamete fusion develops into the sporophyte.

The sporophyte, similarly, is not reproduced directly, but forms reproductive cells known as *spores*, which develop into gametophytes*.*

Thus, the gametophytic and the sporophytic generations alternate and reproduce each other.

The relative lengths of the sporophyte and the gametophyte generations have changed during evolution from a dominant autotrophic (selffeeding) gametophyte and a nutritionally dependent sporophyte to a dependent gametophyte and a dominant autotrophic sporophyte.

Life Cycle in Mosses
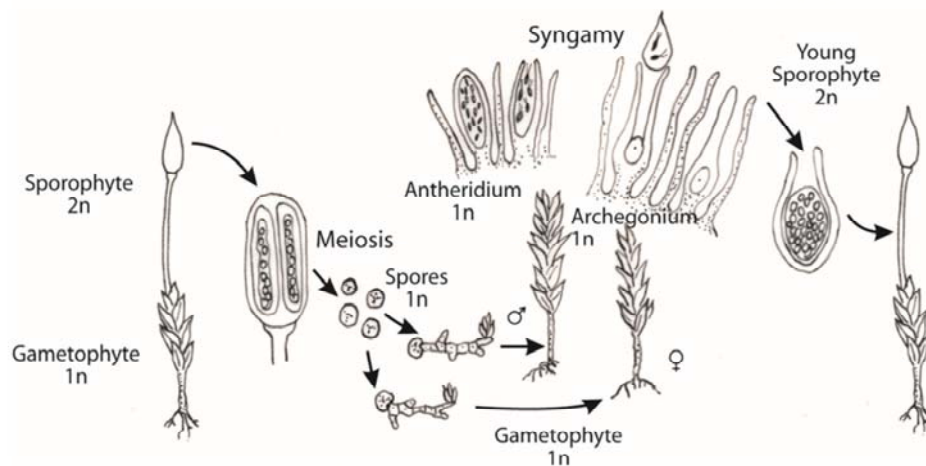
Dubova, Hejatko, Friml (2005)

In mosses, both the embryo and the mature sporophyte are dependent on the photosynthetic gametophyte for nutrition. Meiosis within the capsule of the sporophyte yields haploid spores that are released and germinate to form a male or female green gametophyte.

Differentiation of the gametophyte produces *antheridia* with sperm cells in males and *archegonia* with eggs in females*.*

Fertilization occurs after transfer of sperm cells in drops of water, and the sporophyte generation develops into sporangium that remains attached to the gametophyte (Dubova, Hejatko, Friml, 2005).

Life Cycle in Ferns
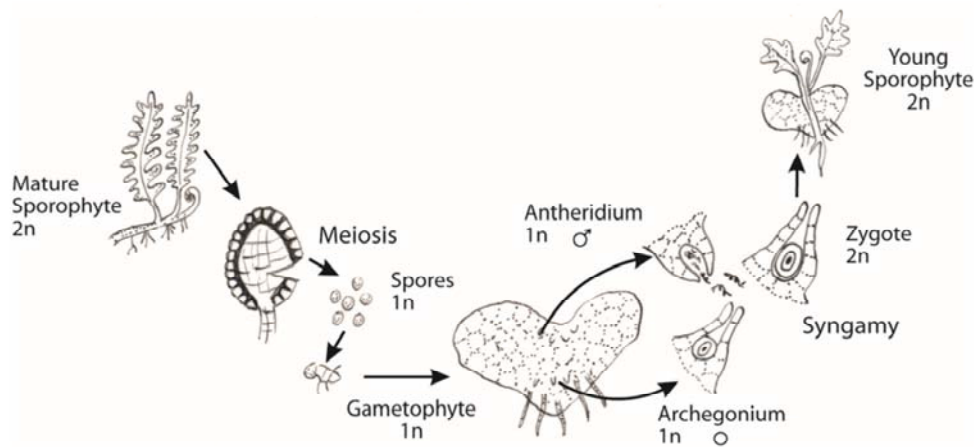
Mature Sporophyte 2n

Meiosis

Spores 1n

Gametophyte 1n

Antheridium 1n ♂

Archegonium 1n ♀

Young Sporophyte 2n

Zygote 2n

Syngamy

Dubova, Hejatko, Friml (2005)

Both the gametophyte and the sporophyte of ferns photosynthesize and are thus autotrophic; the shift to a dominant sporophyte generation is apparent.

Life Cycle in Angiosperms
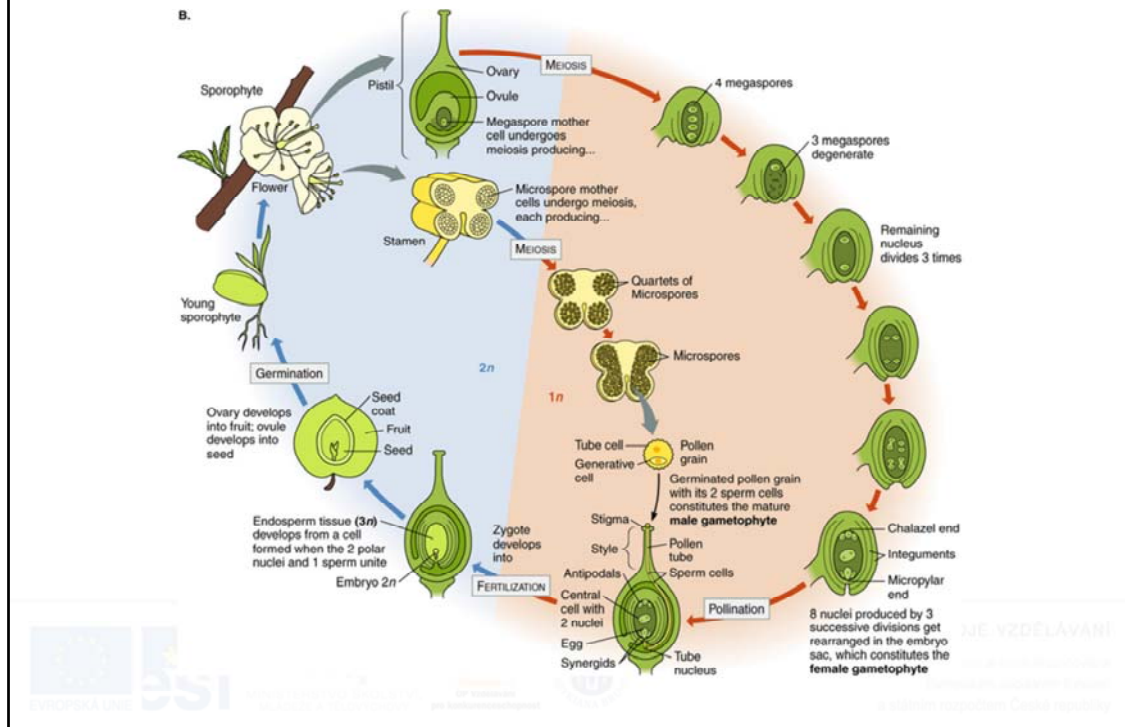
Further reduction of the gametophyte generation during evolution led to the highly reduced gametophyte of angiosperms.

The **male gametophyte** is represented only by the *pollen grain* consisting of the *vegetative cell* that develops a *pollen tube* and the *generative cell,* which produces male gametes – *the sperm cells.*

The **female gametophyte** of angiosperms is represented by the *embryo sac,* which often contains eight haploid cells, the most important of which is the female gamete – *the egg cell (EC).*
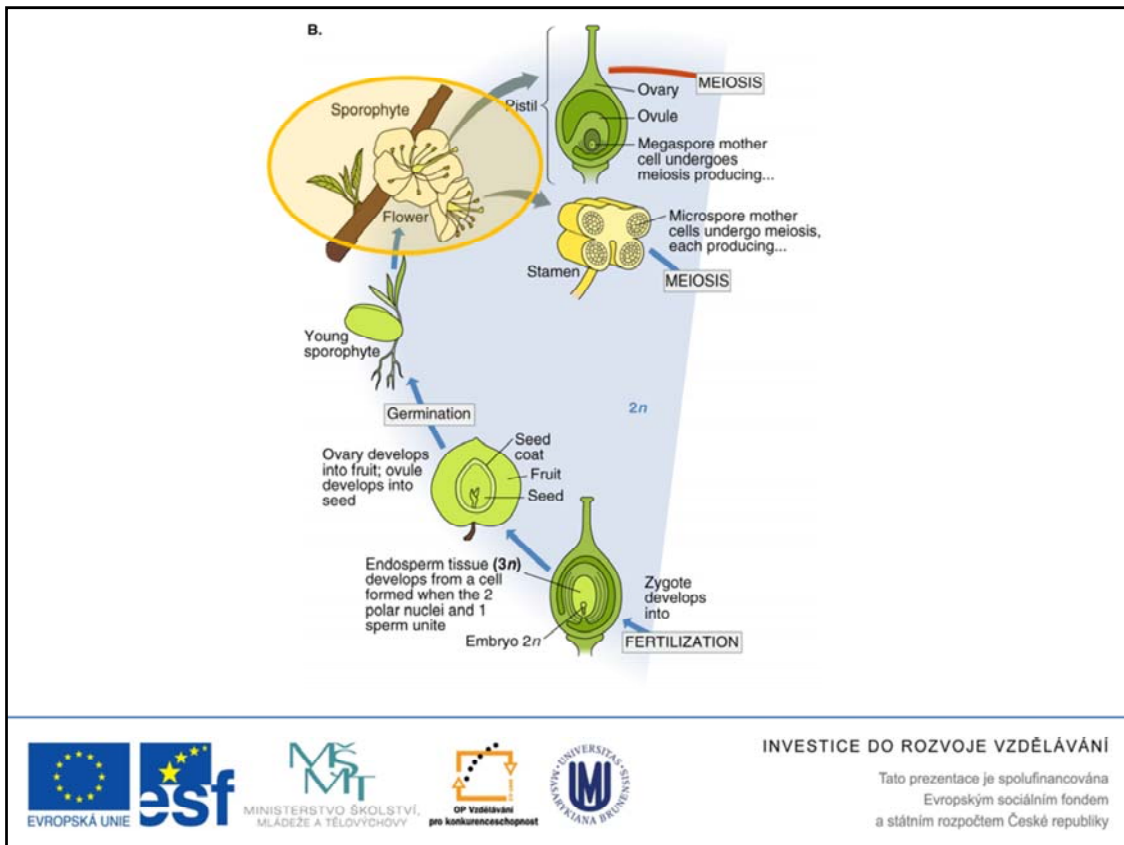
Let's start with the description of the life cycle of angiosperms in a moment of initiation of flowering.

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- Sexual and asexual plant reproduction
- Plant life cycle
- Initiation of flowering

Figure: A, Antirrhinum (snapdargon), B, Arabidopsis thaliana (thale cress).

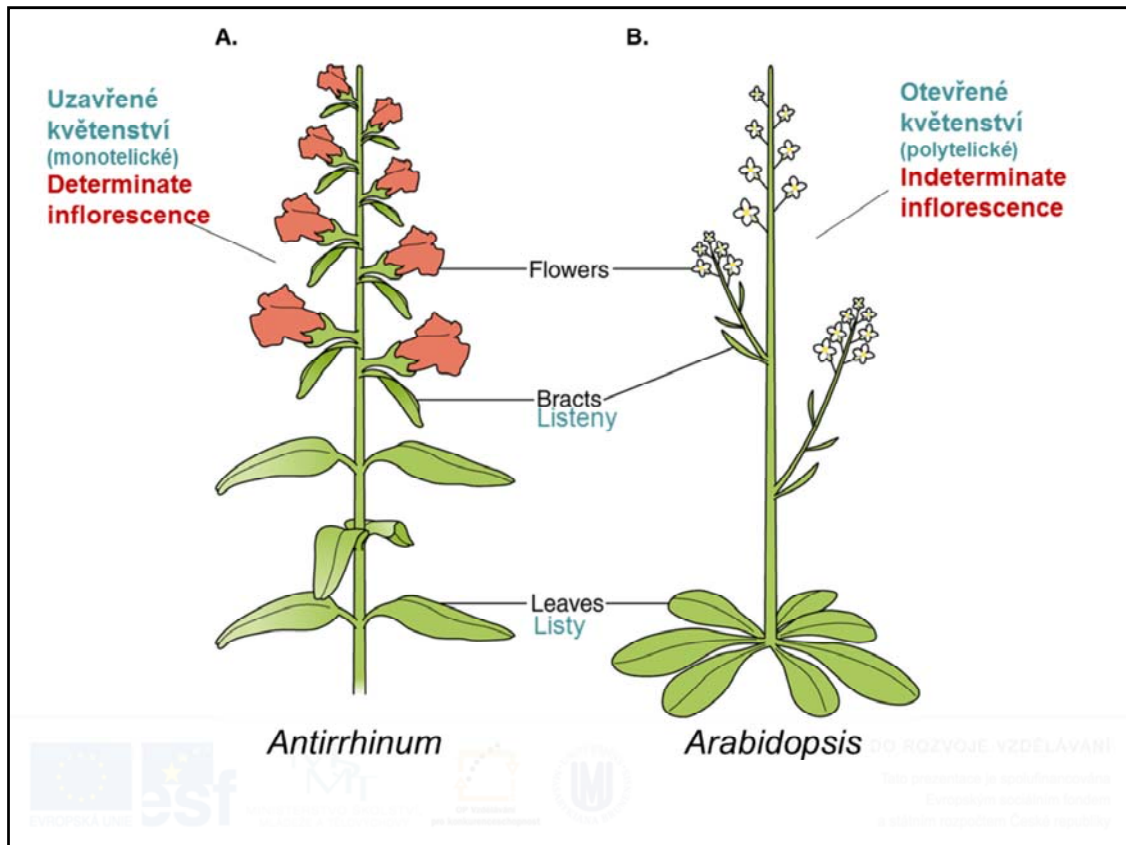Onset of flowering is characterized by the transition from the **shoot apical meristem (SAM)** that produces *leaves* to the **inflorescence meristem (IM)** that produces bracts and **floral meristems (FMs)**, which produce individual floral organs of flowers.

Bracts are modified or specialized leaves, associated with a reproductive structures such as a flower, inflorescence axis, or cone scale. Bracts are often (but not always) different from foliage leaves, for example being smaller, larger, or of a different colour or texture.

On the figure, plants with determinate inflorescence (forming only single flowers on each branch), while indeterminate inflorecsences form many flowers at each branch. A, *Antirrhinum* (snapdargon), B, *Arabidopsis thaliana* (thale cress).
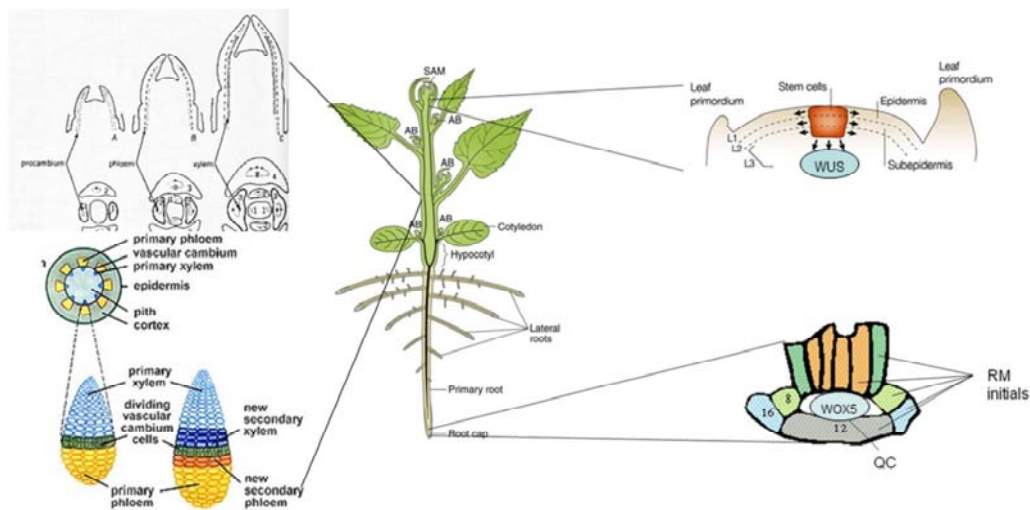
The postembryonic plant growth is mediated by the cell division and differentiation occurring in specialized structures called meristems.

There are three main types of plant meristems-two apical meristems - shoot apical meristem (SAM) and root apical meristem (RAM) that allow plant growth in a longitudinal directions. The lateral growth is due to action of lateral meristems, procambium, which allows primary vascular tissue formation and that later differentiates into cambium, leading to secondary plant thickening.

The detailed structure of meristems and their crucial role in plant development will be discussed in later lectures.
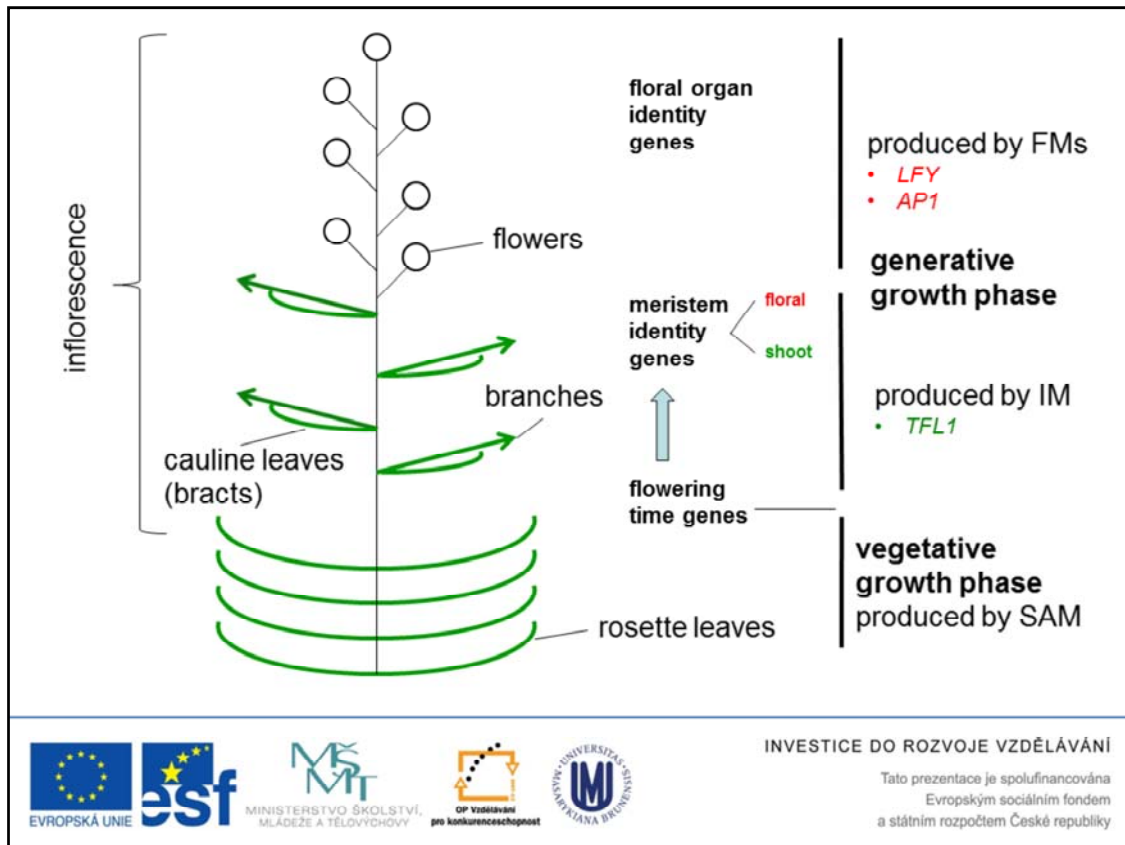
The initiation of flowering requires several switches in the meristem identity.

In indeterminate species (like *Arabidopsis* and similar plants with raceme inflorescence), flowers are formed in inflorescence, growing out from the rosette of pedestrian leaves in *Arabidopsis*. Thus, induction of flowering in *Arabidopsis* involves two transitions:

(1)   formation of inflorescence (otherwise known as bolting) from inflorescence meristem (IM) and

(2)   formation of individual flowers from floral meristems (FMs) growing out from the *inflorescence* (see the scheme above).

In determinate species, the inflorescence meristem differentiates into a single floral meristem leading to formation of a single terminal flower.

Multiple endogenous and environmental stimuli lead to the switch from vegetative to reproductive growth (see the following slides).

After bolting is induced, secondary inflorescence meristems, *axillary meristems,* are formed giving rise to lateral inflorescences (coflorescences) on the axils of 2 to 5 *cauline leaves.* It is still not completely clear whether SAM directly switches to the inflorescence apical meristem or whether there is some interphase between the vegetative and reproductive phases.

Induction of flowering is controlled by *flowering time genes,* which control the activity of *meristem identity genes.*

There are two subclasses of the meristem identity genes in *Arabidopsis*: shoot meristem identity genes and floral meristem identity genes.

The activity of shoot meristem identity genes (e.g. *TERMINAL FLOWER1 (TFL1))* specifies the inflorescence apical meristem as indeterminate and nonfloral.

The second subclass, floral meristem identity genes (e.g. *LEAFY (LFY) and APETALA1 (AP1)),* mediate transformation of the lateral ends of the inflorescence apical meristem into floral meristems, where *floral organ identity genes* are activated (see following slides; Dubova, Hejatko, Friml, 2005).

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The signal for the initiation of flowering, i.e. for the transition of the SAM to the IM originates in leaves. There is several experimental evidence for that.

First, for the induction of flowering, there is more important the **dark phase**. Thus, the length of the dark phase is decisive. In the short-day induced plants, if just one leave is covered so that it is under short-day conditions, it is sufficient to induce flowering via production of yet unknown signal. As shown in panel A, in the short-day induced flowering plants , the short-day period that is delimited to single leaf (by cover) can induce flowering in the entire plant.

Second, mutation in the *INDETERMINATE1* (*ID1*) gene in maize leads to the late flowering and production of more leaves (up to 50 in comparison to 12 in WT). *INDETERMINATE1* encodes zinc-finger TF that is expressed in leaves. Again, ID1 is probably not the signal, but it might induce the signal formation (see the next slide).

Third, when the SAM of maize is removed after it formed 14 leaves and only two leaves are left, the isolated meristem forms 18 leaves as necessary in WT to reach the flowering induction. However, when 6 leaves are left, the isolated SAM produces only 12 remaining leaves, suggesting communication among leaves and the meristem.

The signal is transmissible by grafting, as shown in the panel B. Stock from the short-day induced flowering plants can induce flowering in graft from the non-induced plant.
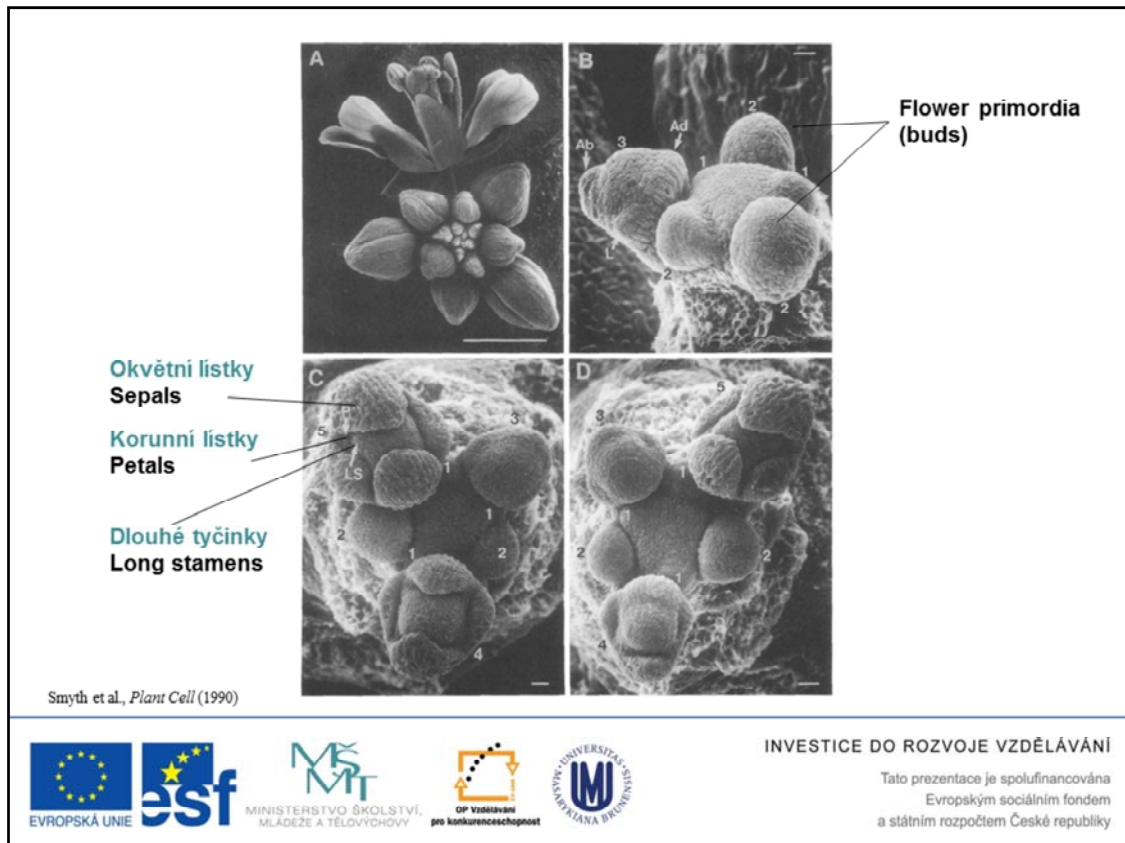
In the *id1* mutant, there is late no induction of flowering, as apparent in the left figure. While WT forms 13 leaves and starts flowering, in *id1* mutant there are 20 laves and there are no signs of flowering. On the middle figure (B), there is apparent tassel-like structure, where plantlets emerge from every spikelet, instead of male flowers (tassel, compare with the inset).

In the right-hand figure, there is result of *in situ* localization of *ID1* mRNA. Two concentric leaves are shown wrapped within the whorl; *ID1* staining is evident in the inner leaf whorl. Scale bar 5 300 mm.

In the SAM, no expression was found, while using *KNOTTED1* anti-sense probe (see next lectures) shows clear signal in the SAM (positive control).
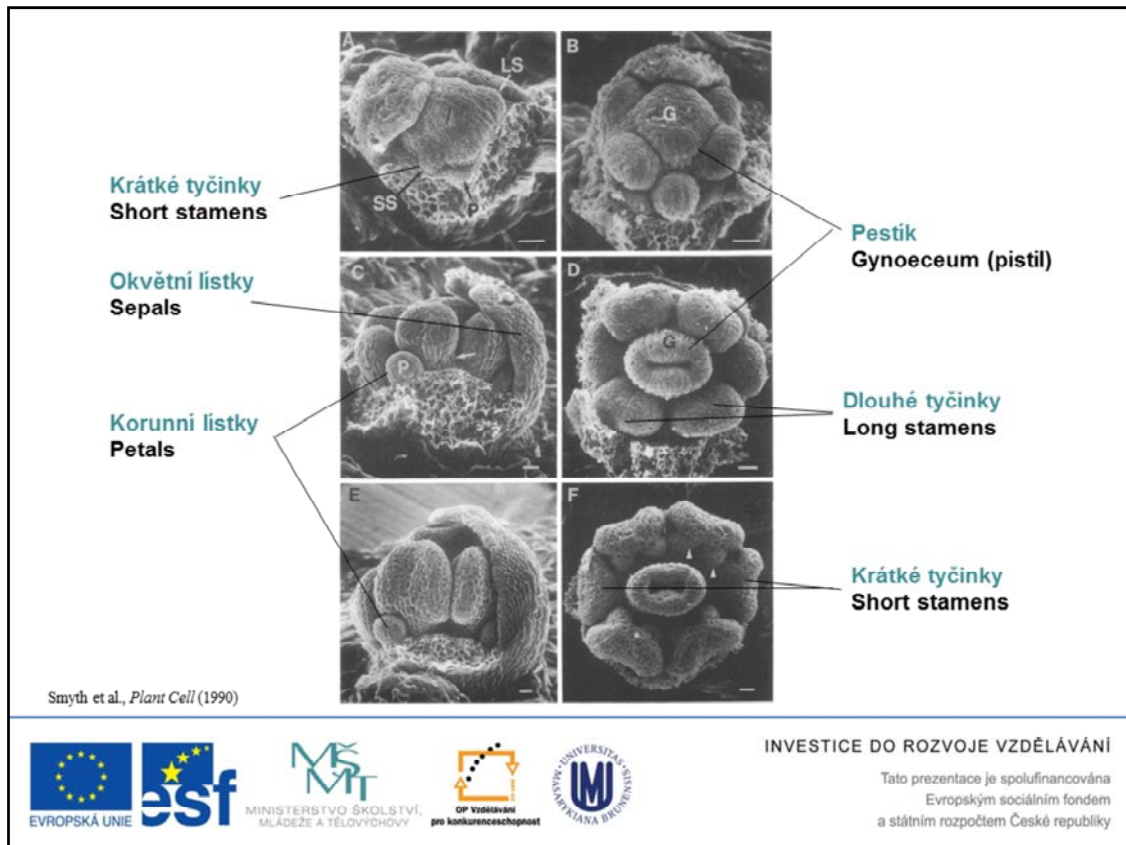
Okvětní lístky
Sepals

Korunní lístky
Petals

Dlouhé tyčinky
Long stamens

Flower primordia (buds)

Smyth et al., *Plant Cell* (1990)

Flower formation in *Arabidopsis thaliana*.

After transition form the inflorescence to the floral meristem (FM), individual flower primordia are produced in spirals on the FM.

First buds form as an undifferentiated outgrows. Later on, the most lateral floral organs*, sepals* differentiate, followed by male gametes producing *long stamens* and *petals*.
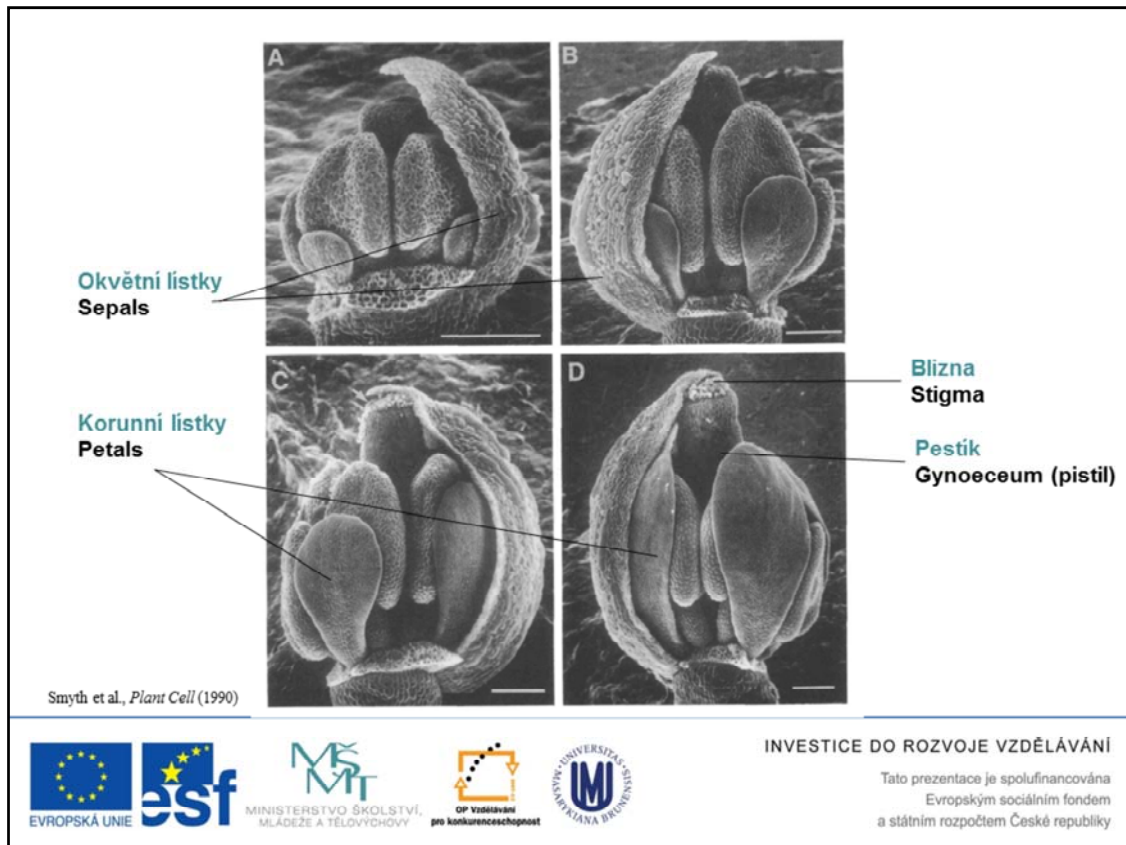
Krátké tyčinky
Short stamens

Okvětní lístky
Sepals

Korunní lístky
Petals

Pestík
Gynoeceum (pistil)

Dlouhé tyčinky
Long stamens

Krátké tyčinky
Short stamens

Smyth et al., *Plant Cell* (1990)

Later, inner-most female reproductive organ, *gynoeceum* forms and *short stamen* primordia became recognizable, too.

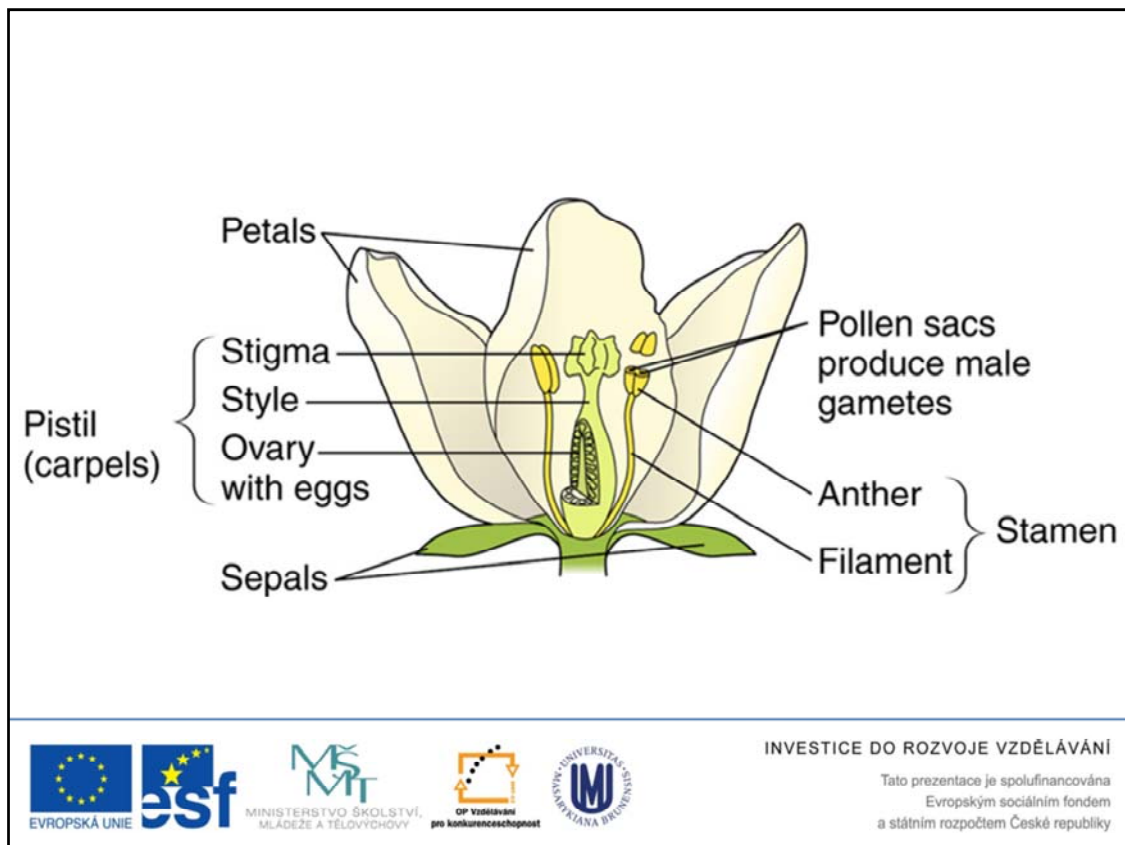Smyth et al., *Plant Cell* (1990)

In the final stages of the flower development, gynoeceum initiates its growth, followed by the elongation of filaments.
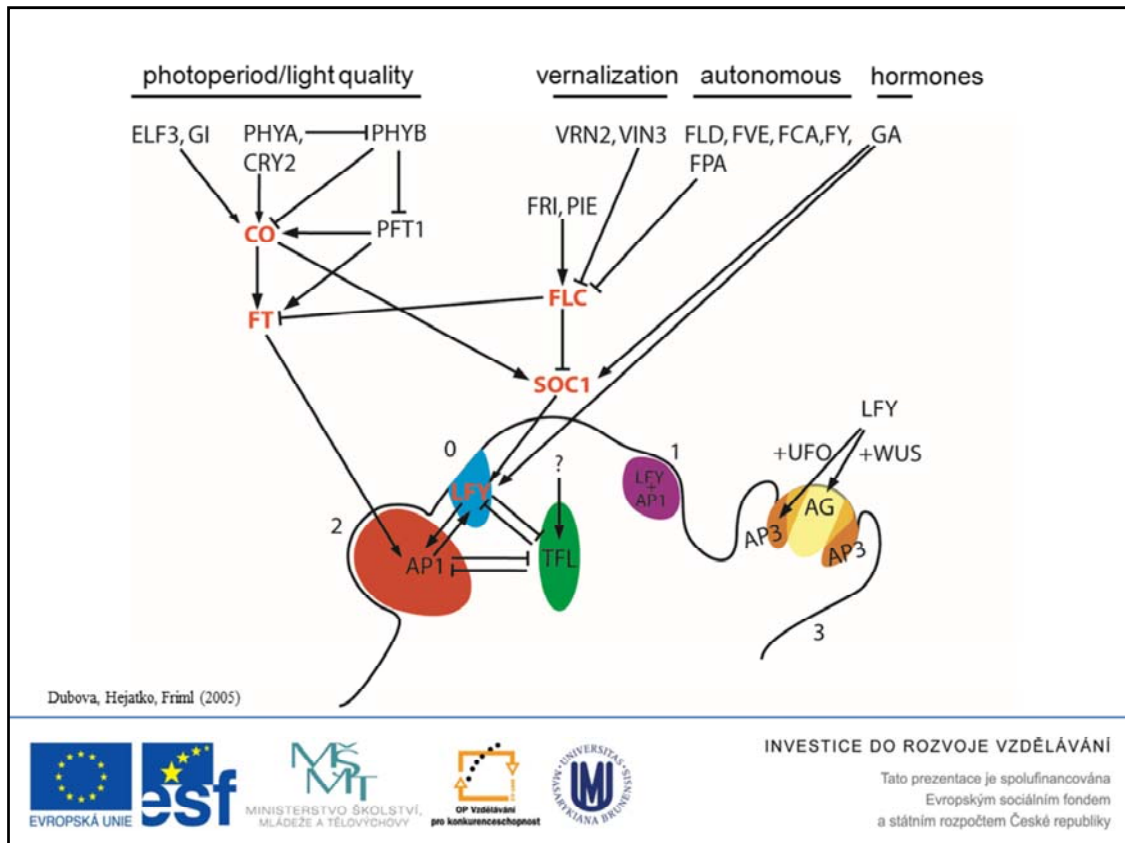
The prototypic flower of angiosperms with (from the outside) sepals, petals, stamens (consisting of filament and anther) and pistil (composed from ovary containing eggs, style and pollen accepting stigma).

Dubova, Hejatko, Friml (2005)

There is quite complicated network of external and internal stimuli, involved in the regulation of the flowering time. See some of them in the scheme above (Dubova, Hejatko, Friml, 2005). The different signals are integrated at the key players, e.g. CONSTANS (CO; similar to zinc finger TFs), FLOWERING LOCUS T (FT; long-range protein signaling molecule), FLOWERING LOCUS C (FLC; MADS-box TF) or SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1; MADS-box TF).

There are, however, several key players in the complicated signalling cascade that will be discussed in more detail later.

The complicated signaling network leads to the **LEAFY (LFY)** upregulation that is one of the first determinants of the newly induced flower primordia.

Schematic representation of gene interactions during flower development in *Arabidopsis*.

Selected genes acting in four major pathways of flowering induction and their interactions are shown. Both direct and indirect as well as positive (arrows) and negative interactions are marked; floral pathway integrators are in red. PHYA and CRY2 have similar effects on flowering, but they act via independent pathways. However, for the sake of simplicity, they are depicted as sharing the same one.

The colored areas in the schematically depicted inflorescence apical meristem represent approximate areas of the respective gene activities (expression patterns): *AP1 (*red), *LFY* (blue), *TFL* (green), *AP3 (*brown), *AG* (yellow). *LFY* is first activated in the so-called floral anlagen (floral stage 0) and it precedes the expression of *AP1* (for the definition of anlagen see Lesson 7).

Later on, the expression patterns of *AP1 and LFY* are overlaid (stages 1 and 2). On the left-hand side, developing floral meristem (stage 2) is shown, on the right-hand side, there are developing flowers at stages 1 and 3. Numbers label the individual stages of flower development (0–3).

Overlapping activities of B and C gene classes *AP3* and *AG,* respectively, in the whorl 3 (see later) are schematically shown (light brown). At that stage of flower development, expression patterns of *UFO* and *AP3* are similar*; LFY* is expressed all over the developing flower at stage 3 (not shown). *AP1* is expressed all over the flower meristem at stages 1 till early stage 3 and after *AG* induction at stage 3, *AP1* expression is repressed in *AG* domain (whorls 3 and 4), but persist in whorls 1 and 2 (not shown).

Ectopic overexpression of *LFY* leads to premature onset of flowering irrespective of the photoperiod length.

II. Floral *meristem identity acquisition* (LFY, AP1)

III. Activation of *floral organ identity* genes (whorl-specific)

IV. Activation of *floral organ building* genes

I. Inflorescence meristem formation (switch from vegetative to reproductive growth)

Dubova, Hejatko, Friml (2005)

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována Evropským sociálním fondem a státním rozpočtem České republiky

Developmental processes leading to flower formation could be divided into four basic steps occurring in a temporal sequence:

(1)   switch from vegetative to reproductive growth leading to inflorescence meristem formation,
(2)   floral meristem identity acquisition by activation of *floral meristem identity genes*,
(3)   activation of floral *organ identity genes*, and finally,
(4) activation of flower ''organ building'' genes.

Molecular control of all of these processes integrates outputs of many signaling pathways.

The major activators of floral organ identity genes are *LFY and AP1.*

As mentioned earlier, *LFY and AP1 are fl*ower meristem identity genes, which are necessary for flower meristem identity acquisition. In combination with other factors, LFY and AP1 also act as activators of the flower meristem identity genes *AGAMOUS (AG,* C class*)* and *APETALA3* (*AP3,* B class).

LFY and *AP1* are necessary to specify a meristem as floral, but they do not act independently of each other. *LFY* directly activates *AP1* and *AP1* is necessary for the function of *LFY* in floral promotion.

There is a mutual repression between *LFY and AP1* on one hand and *TFL1* on the other. This is consistent with the apparently opposite role of both groups of the genes. *TFL1* maintains the shoot meristem indeterminate and non-floral and negatively regulates flowering, whereas *LFY1* and *AP1* promote the formation of floral meristem and flowering.

LFY1 and AP1 are transcription factors, which might directly regulate *TFL1. TFL1 is* similar to FT (see above), but the molecular mechanism of their opposite functions is unclear.

LFY and *UNUSUAL FLOWER ORGANS (UFO):* LFY is able directly to bind to the *AP3* promoter *in vitro;* UFO is an F-box protein and it functions as a part of the SKP1-cullin-F-box (SCF) complex that targets proteins for ubiquitin-mediated degradation. A recent model suggests a role for UFO in the degradation of the putative *AP3* repressor in whorls 2 and 3.

The central role of LFY in the activation of floral organ identity genes is further supported by its role in co-activating class C gene *AG.* Together with the homeodomain protein *WUSCHEL (WUS), LFY* binds to the regulatory region of *AG* in its second intron. The expression of *WUS* in precursor cells of whorls 3 and 4 seems to be responsible for spatial specificity of the LFY/WUS-mediated *AG* activation.

Interaction of specific genes expression leads to *floral meristem zonation*, i.e. differentiation of what is called *whorls*.
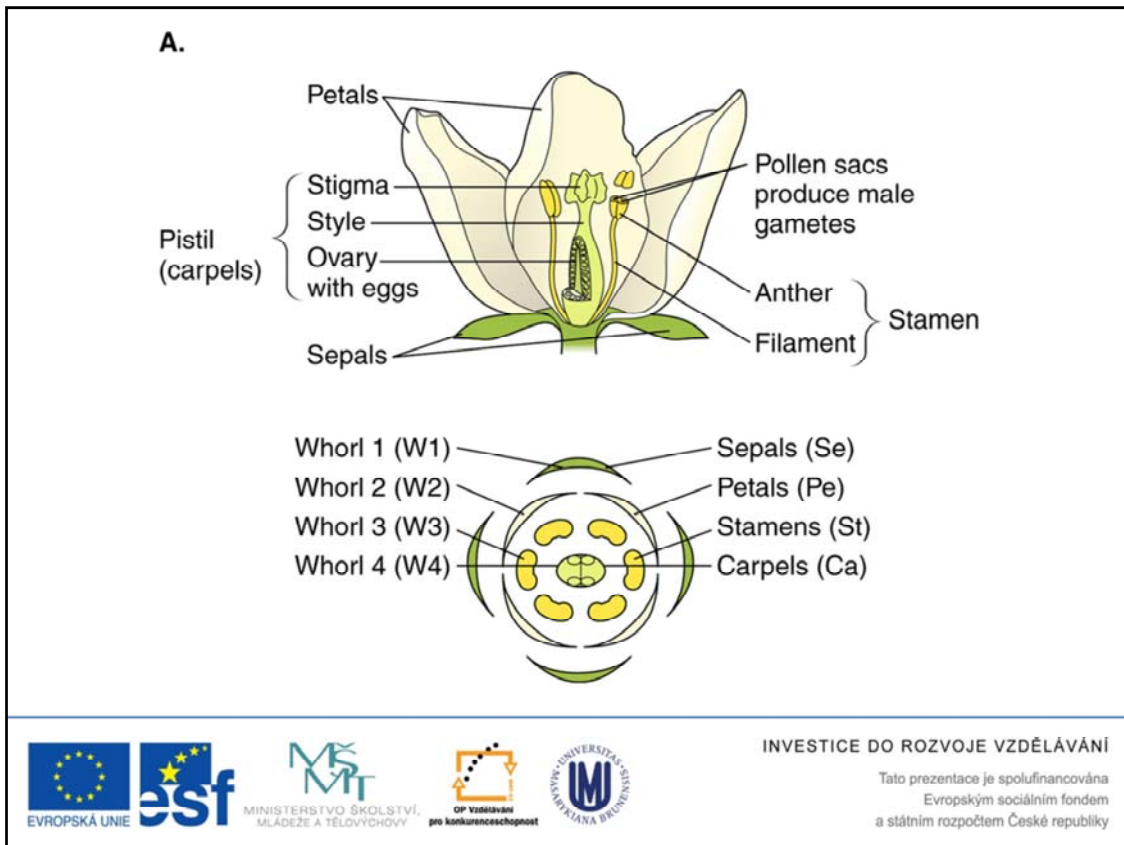
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- Sexual and asexual plant reproduction
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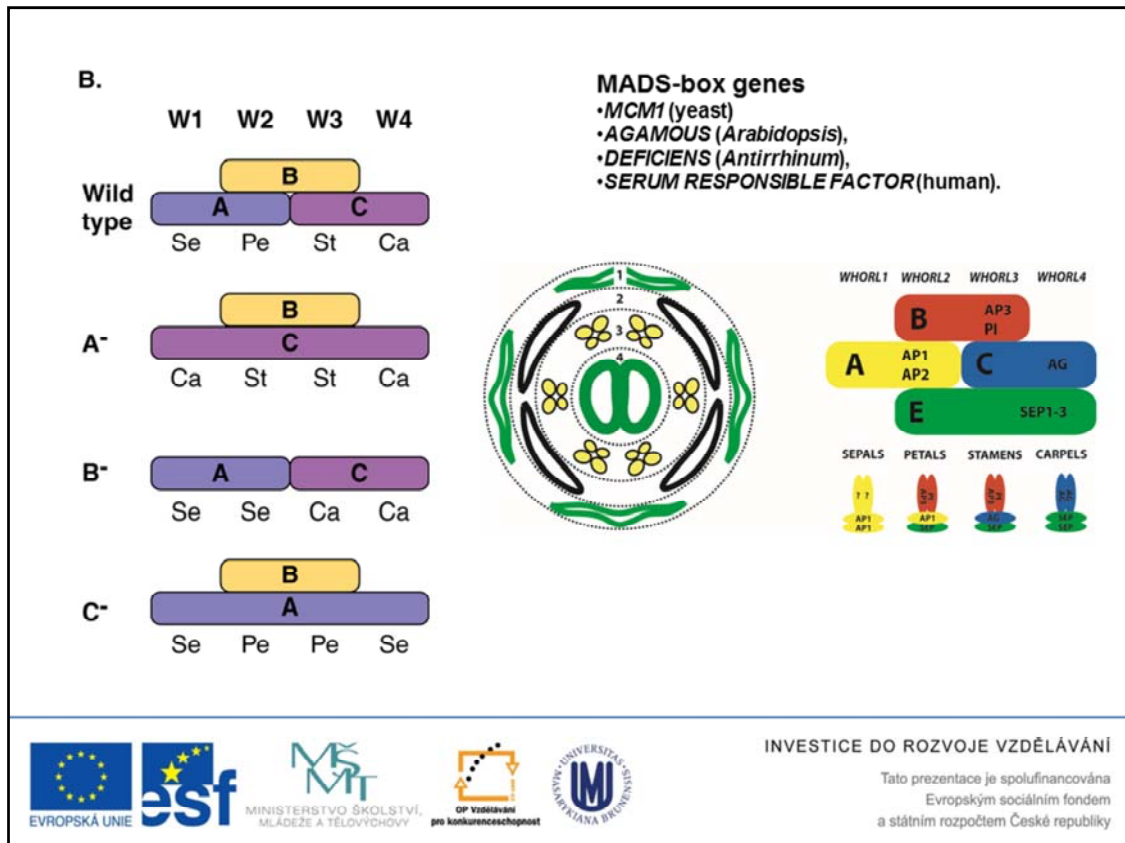- **Determination of floral organ identity**

The identity of individual whorls and thus, the identity of individual floral organs, is specified by the expression of *organ identity genes.*

These genes belong to the group of regulatory genes called *homeotic genes*. Complex interactions occur among those genes, which allow final floral organ formation.

Individual flowers are formed by floral meristems growing out from the lateral parts of the inflorescence meristem.

With the exception of *APETALA2* (AP2), a member of a small plant-specific family of transcription factors, products of ABCE genes are MADS-transcription factors.

The MADS transcription factors are analogs of animal homeotic genes, which, instead of homeodomain, contain the so-called *MADS-box,* found in MCM1 (yeast), AGAMOUS (*Arabidopsis*), DEFICIENS (*Antirrhinum*), and SERUM RESPONSIBLE FACTOR (SRF, human).

Additionally, there are D-class genes (e.g. *SEEDSTICK (STK), SHATTERPROOF1* (*SHP1,* and *SHP2), which are* important for specification of ovule organ identity.

Molecular nature of those regulations will be discussed in the more detailed lecture of prof. Vyskot in frame of his course "Bi0580 Developmental genetics".

Author of ABC model, professor Elliot Meyerowitz (middle) during his visit of MU campus
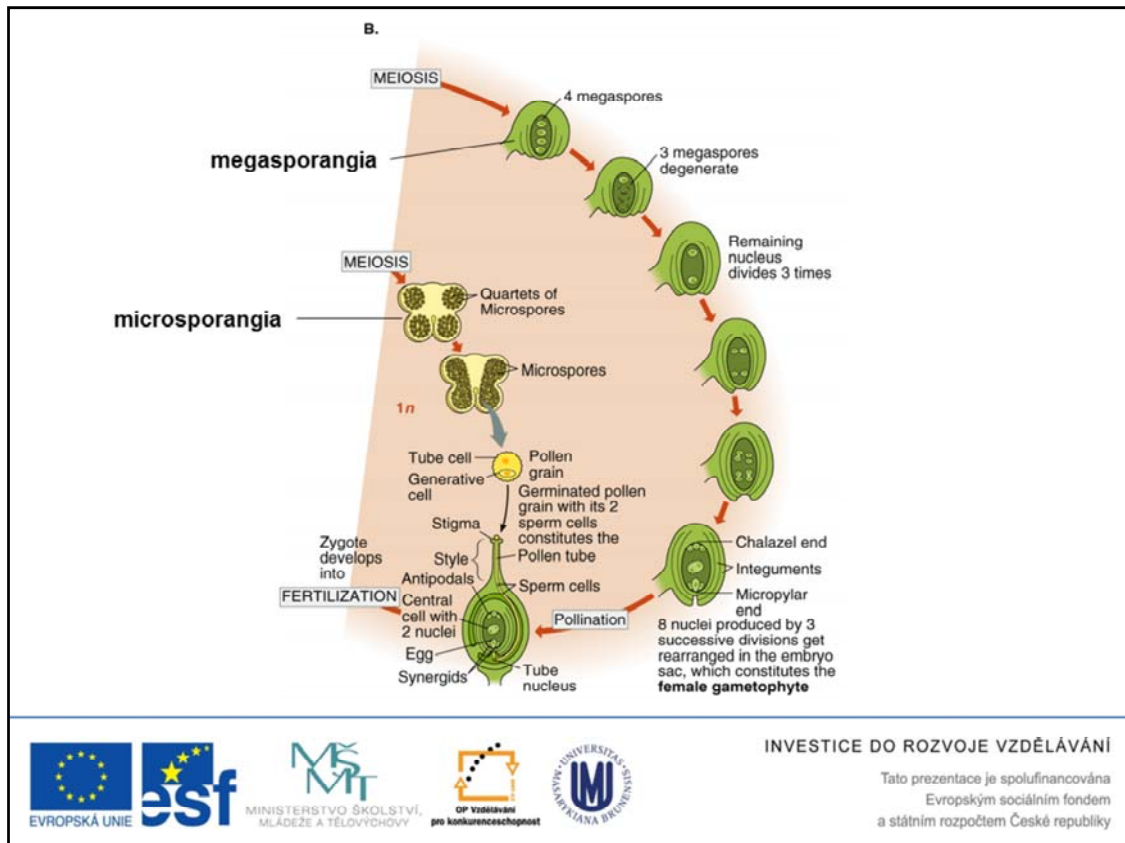
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- **Microgametogenesis**

In higher plants, male and female gametes, the **sperm** and **egg cells**, are formed in special flower tissues, so-called **sporangia**.

Microsporangia, located in **anthers**, produce a large number of **microspores** that differentiate into mature **male gametophytes (microgametophytes)**, the **pollen grains**.

Megasporangia, so-called **ovules**, are located in the ovary of the pistil (gynoecium). In contrast to microsporangia, during megasporogenesis in a majority of plant species only one of four megaspores differentiates into a functional **megaspore** that develops into a **female gametophyte (megagametophyte)**, the **embryo sac**.

Two **sperm cells** develops from each pollen grain whereas there is only one **egg cell** in each embryo sac. Both micro- and megagametophytes (i.e. pollen grains and embryo sacs) are multicellular structures that undergo specific developmental processes.

Dubova, Hejatko, Friml (2005)
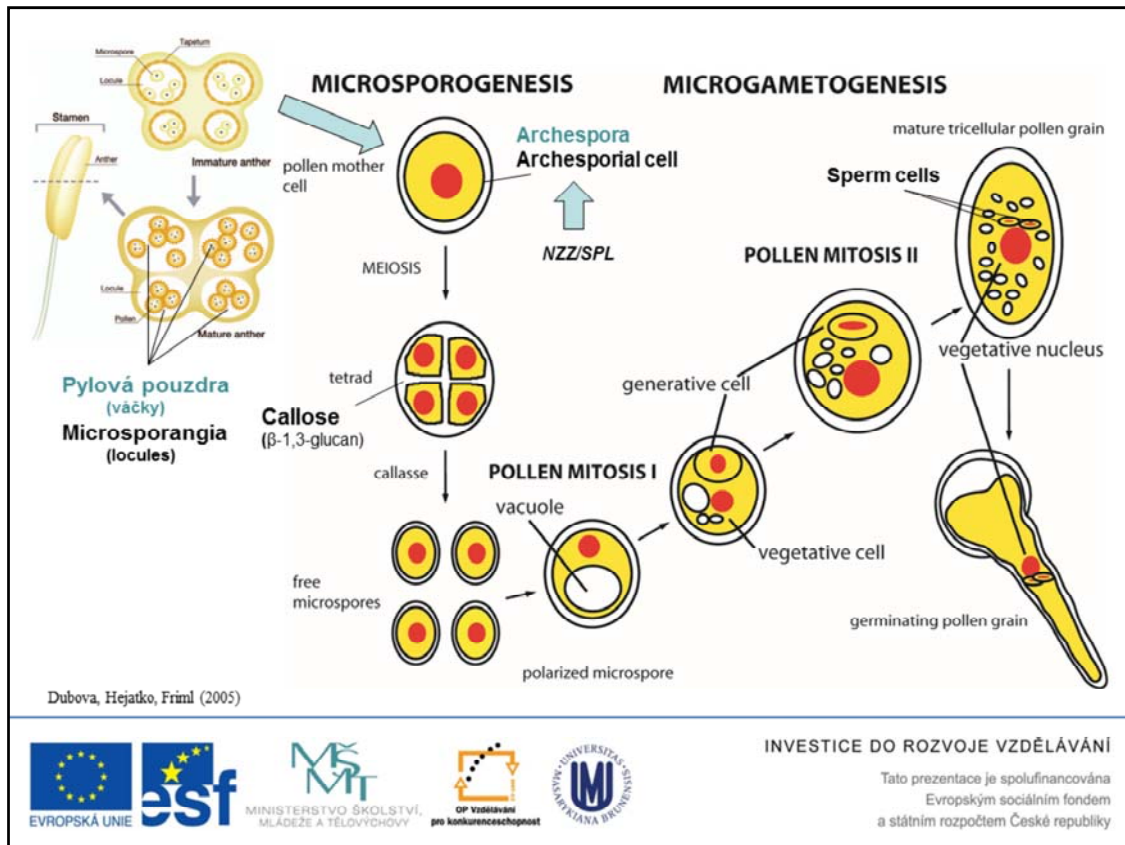
The formation of pollen in *Arabidopsis thaliana* is schematically shown. In each of four developing *locules* (microspororangia) formed in the anther, the **archesporial cells** differentiate.

The archesporial cell is specified from one of the subepidermal cells originating from the L2 layer. In *Arabidopsis* **NOZZLE/SPOROCYTELESS** (*NZZ/SPL*) encodes a putative transcription factor similar to MADS-box proteins, necessary for the specification of archesporial cell. *NZZ/SPL* was recently shown to be under direct control of **AGAMOUS (AG),** one the homeiotic genes involved in the control of floral organ identity.

After each meiotic division in species with *successive cytokinesis* (e.g. most of monocots) or after meiosis II in species with *simultaneous cytokinesis* (e.g. most of eudicots including *Arabidopsis*), the cell wall is formed between individual microspores. The cell wall is composed of **callose**, a β-1,3-glucan.

Control of the division plane in plant male meiosis is achieved by an array of radial microtubules, surrounding newly formed nuclei. At the interface of those microtubule arrays the callose wall is formed either centrifugally (in case of *successive cytokinesis*) or centripetally (in *simultaneous cytokinenesis*).

Individual microspores are released by the action of the enzyme **callase,** comprising endo- and exoglucanase activities, which is produced by the *tapetum*.

Formation of haploid microspores completes **microsporogenesis** that is followed by **microgametogenesis**. The individual microspores enlarge and a single large vacuole is formed, which is associated with polarization of the microspore that subsequently undergoes two rounds of mitosis.

During the first, asymmetric cell division also called **pollen mitosis I**, the nucleus is attached to the cell wall and the spindle orientation leads to the formation of a large vegetative and a small generative cell. The nucleus of the vegetative cell generally maintains irregular shape with dispersed chromatin, while the nucleus of the generative cell is highly condensed, often spindle shaped.

The generative cell detaches from the cell wall (leading to the formation of the "cell in cell" structure) and undergoes a further round of mitosis, **pollen mitosis II** that gives rise to the mature, three-celled male gametophyte consisting of the large **vegetative cell** and two small **sperm cells**.
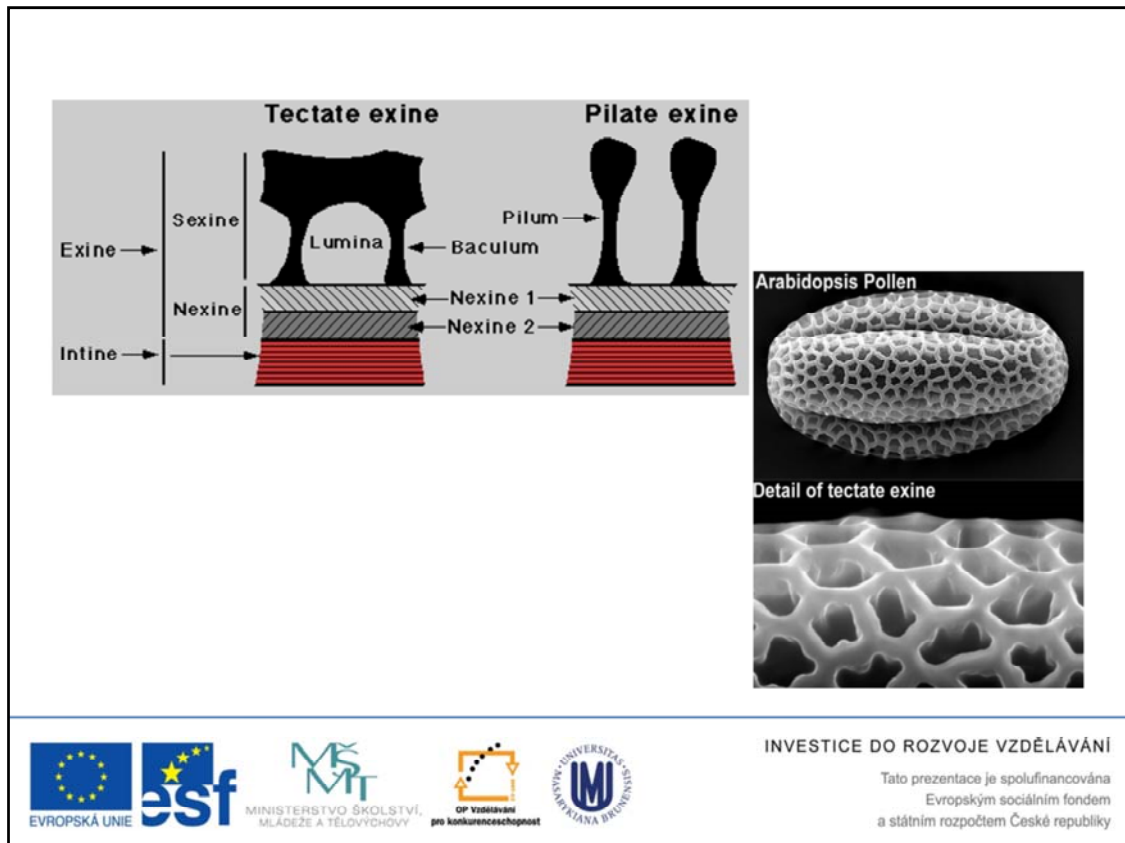
Structure of the pollen wall (Twell lab, University of Leicester; http://www2.le.ac.uk/departments/biology/people/twell/lab/pollenis/wall).

The wall of pollen grains is a complex, multilayered structure consisting of the innermost pectocellulosic **intine** and outer **exine**. The outer exine is further divided into the two-layered **nexine** (nexine I and II) with nexine I adjacent to the intine, and outer **sexine** that consists of **columella** and the outermost **tectum**.
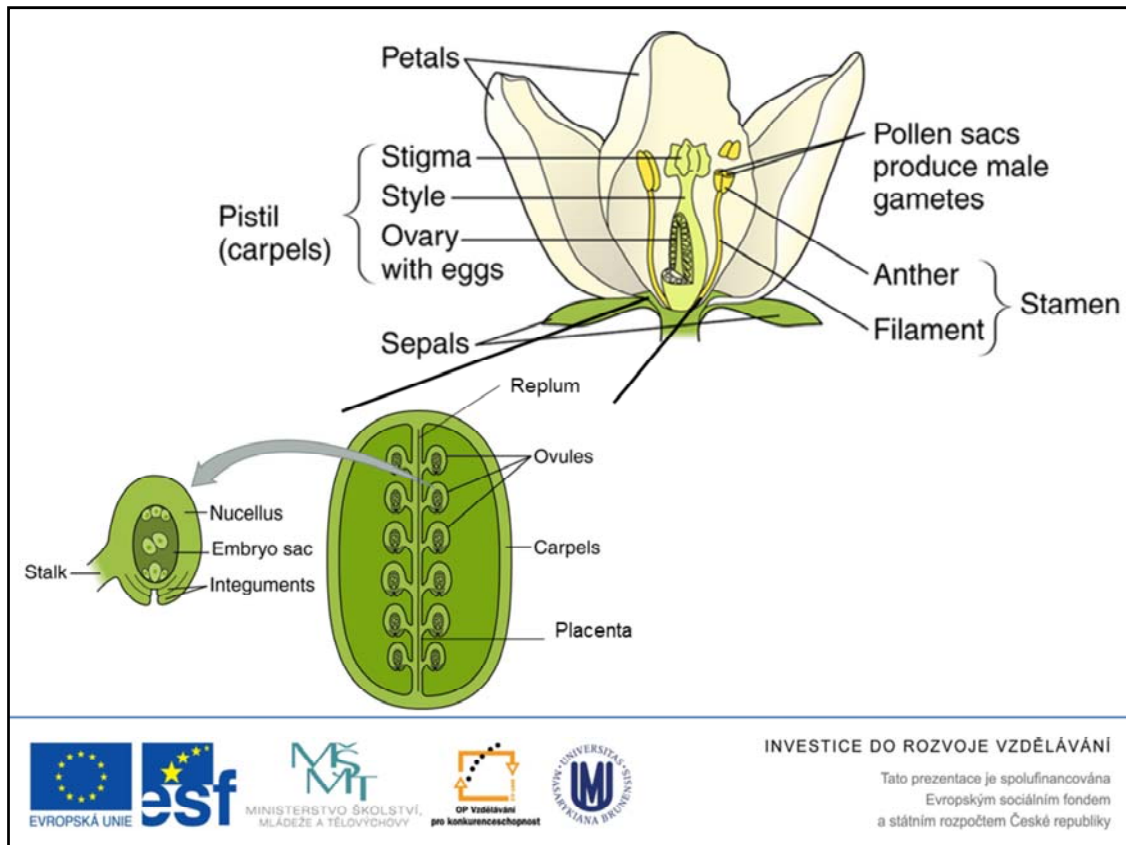
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- **Megagametogenesis**

Female gametophyte develops in angiosperms as a tiny structure called embryo sac.

Embryo sac develops in the **pistil** of the flower.

In angiosperms, the key structure where the pre- and postzygotic development takes place is the *ovule.*

The female gametophyte is represented by the so-called *embryo sac,* the multicellular, mostly haploid and highly differentiated tissue, surrounded by diploid mother (sporophytic) tissue of the ovule.

Similarly to animal embryos, there is communication supposed to occur between the developing gametophyte and surrounding sporophytic tissue.
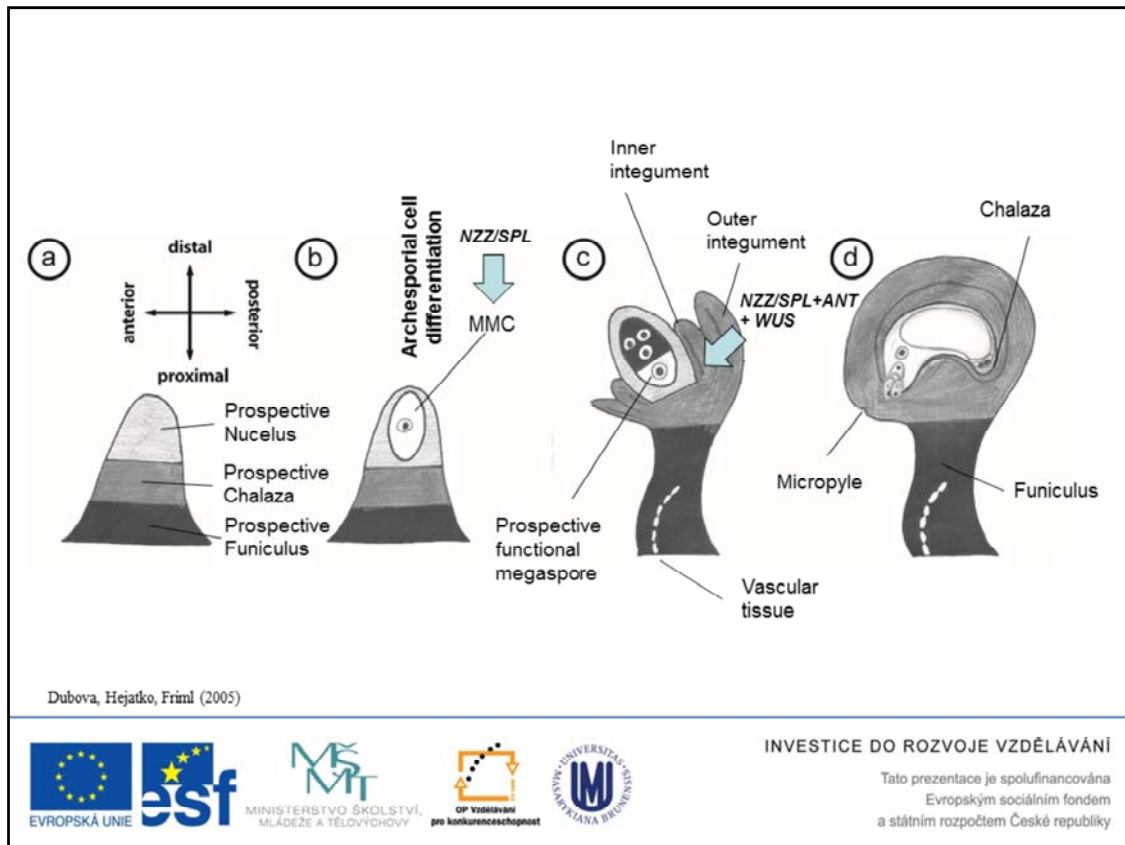
However, in plants, the molecular determinants involved in that type of communication are still mostly unknown.

In *Arabidopsis*, typically 60 ovules are placed along the *replum in the female* reproductive organ, the gynoecium (the pistil). Early in flower development, *ovule primordia* differentiate from the placenta.

Dubova, Hejatko, Friml (2005)

Female reproduction organ, the **ovule**, development in *Arabidopsis*.

During ovule development, three anatomically distinguishable and functionally distinct parts of the ovule are formed (see the figures).

The **nucellus**, located at the distal pole of the ovule, contains the sporogenic tissue.

The growth of **inner and outer integuments** is initiated in the **chalaza**, located approximately in the middle of the ovule primordium.

Soon after integument differentiation, elongation growth is initiated and the integuments cover the nucellus of the mature ovule except a small part at the distal pole, forming a gate for the pollen tube entry, the **micropyle**.

The extended growth of the outer integument at the outer (posterior) side of the ovule results in a characteristic bending and finally the placement of the micropyle along the base (the stalk) of the ovule.

The position of the integument outgrowth defines the most proximal part of the ovule, which differentiates into the tissue connecting the maternal placenta tissue, the **funiculus**.

(a)   Primary, non-differentiated protrusion of the placenta; the proposed diversification of three future ovule regions (nucellus, chalaza, and funiculus) is depicted by different gray grades.

(b)   Megaspore mother cell (MMC) is differentiated.

In the early phases after *Arabidopsis* ovule primordia growth initiation, an **archesporial cell** of subepidermal origin directly differentiates into the megasporocyte**, the megaspore mother cell** (MMC).

The archesporial cell is originally established as a non-polar, rather large cell (approx. 17 μm in diameter), while the MMC just before meiosis is already polarized, with the majority of the organelles located at the functional pole. In a majority of species including *Arabidopsis*, the functional pole points towards the distal pole of the ovule primordium.

The polarity of the megasporocyte is also reflected by the preferential accumulation of callose at the functional pole. However, the polarity is reversed in some species, e.g. *Oenothera* and *Endymion*, see also below. Activity of **NZZ/SPL** was shown to be crucial for archesporial cell differentiation in both male and female germ lines.

(c)   Inner and outer integuments are developed; asymmetrical growth of integuments on abaxial and adaxial sides is depicted. Three of four **megaspores** at the distal pole will degenerate, the most proximal one will form the *functional megaspore*; the developing vascular tissue of *funiculus* is schematically shown.

In *Arabidopsis*, the position of the inner integument outgrowth seems to be dependent on the spatial interactions of expression domains of **AINTEGUMENTA (ANT)**, encoding an AP2 domain transcription factor and **NZZ/SPL**, whose role was already mentioned in male archesporial cell differentiation.

The expression of **WUSCHEL** (*WUS*), encoding a homeodomain protein was also found to be involved in the positional specification of integument outgrowth. According to a recent model, the distal expansion of proximally located expression domain of *ANT* is negatively regulated by activity of *NZZ* and the spatial relationship between *WUS* expression in the nucellus and *ANT* in the chalaza determines position of inner integument outgrowth.

(d)   Fully developed ovule with two **synergid cells** and **egg cell** at the micropylar (distal) pole.

Drawings in a–d by Romana Dobešová (According to Grossniklaus, U., Schneitz, K. (1998) The molecular and genetic basis of ovule and megagametophyte development, *Semin. Cell Dev. Biol. 9, 227–238)* (Dubova, Hejatko, Friml, 2005).

Dubova, Hejatko, Friml (2005)
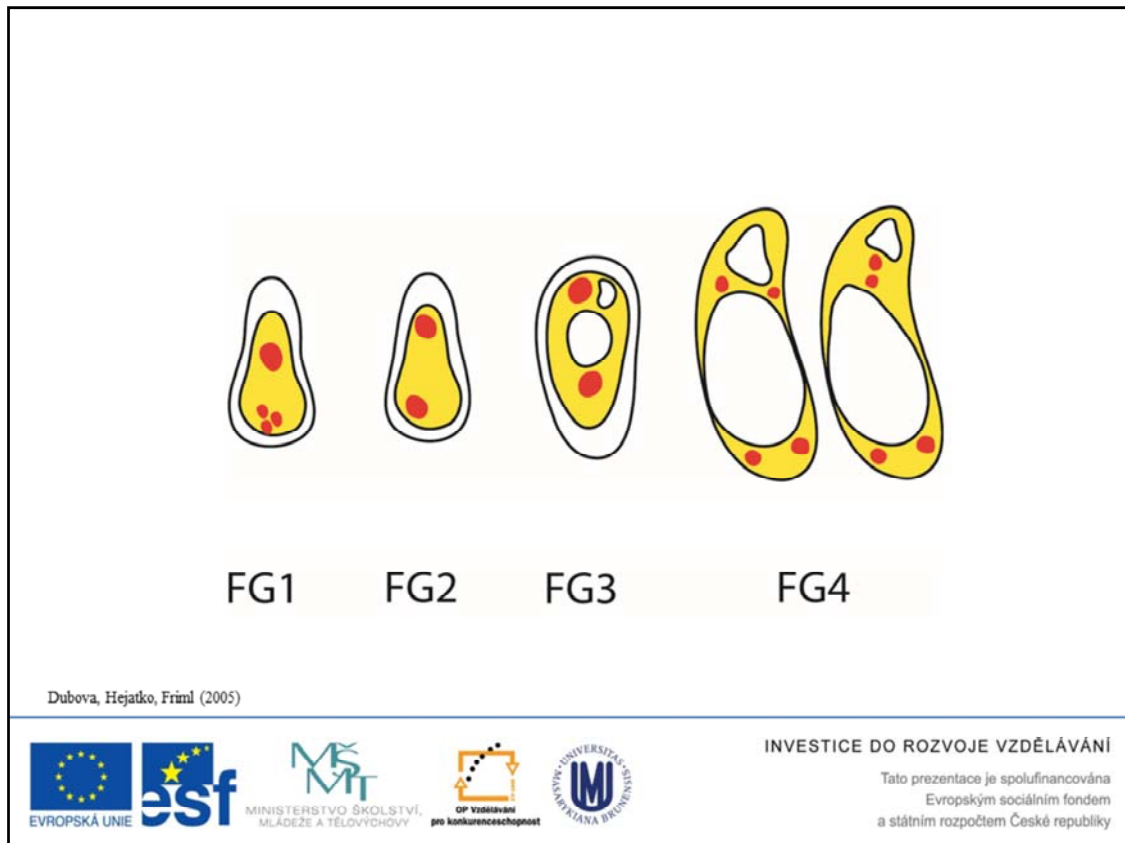
Cytokinesis in *Arabidopsis* occurs after meiosis is completed, which results in the formation of a multiplanar or linear tetrad.

The most proximal product of the megaspore mother cell meiosis survives in *Arabidopsis,* forming the **functional megaspore**.

In contrast to that, in *Oenothera* and *Endymion*, the most distal megaspore survives and in some other species the surviving megaspore is not determined by position at all.

The mechanisms of the functional megaspore selection are unknown, possibly both cytoplasmic determinants and cellular interactions with neighboring cells might contribute to this.

There are seven distinct stages of the female gametophyte development, the **megagametogensis**.

During the first step, **FG1** (the **F**emale **G**ametophyte 1), a functional megaspore is already differentiated; the remnants of the three degenerating meiotic products are still present. In the next development, the functional megaspore undergoes three rounds of mitosis, constituting the eight-nuclear embryo sac.

In **FG2,** the first round of mitosis results in a two-nuclear female gametophyte, with many small vacuoles all around the cytoplasm.

In **FG3**, the large central vacuole is formed and usually another (smaller) one at the proximal (chalazal) pole is also present.

In **FG4,** the second round of mitosis results in the formation of a four-nuclear gametophyte, with a well developed central vacuole. The four nuclei are organized in two pairs, located at opposite poles. The chalazal nuclei change their positions during FG4, as the line between them, originally orthogonal to the micropylar-chalazal axis, is flipped-over to become parallel with the micropylar-chalazal axis at the end of FG4.

Dubova, Hejatko, Friml (2005)
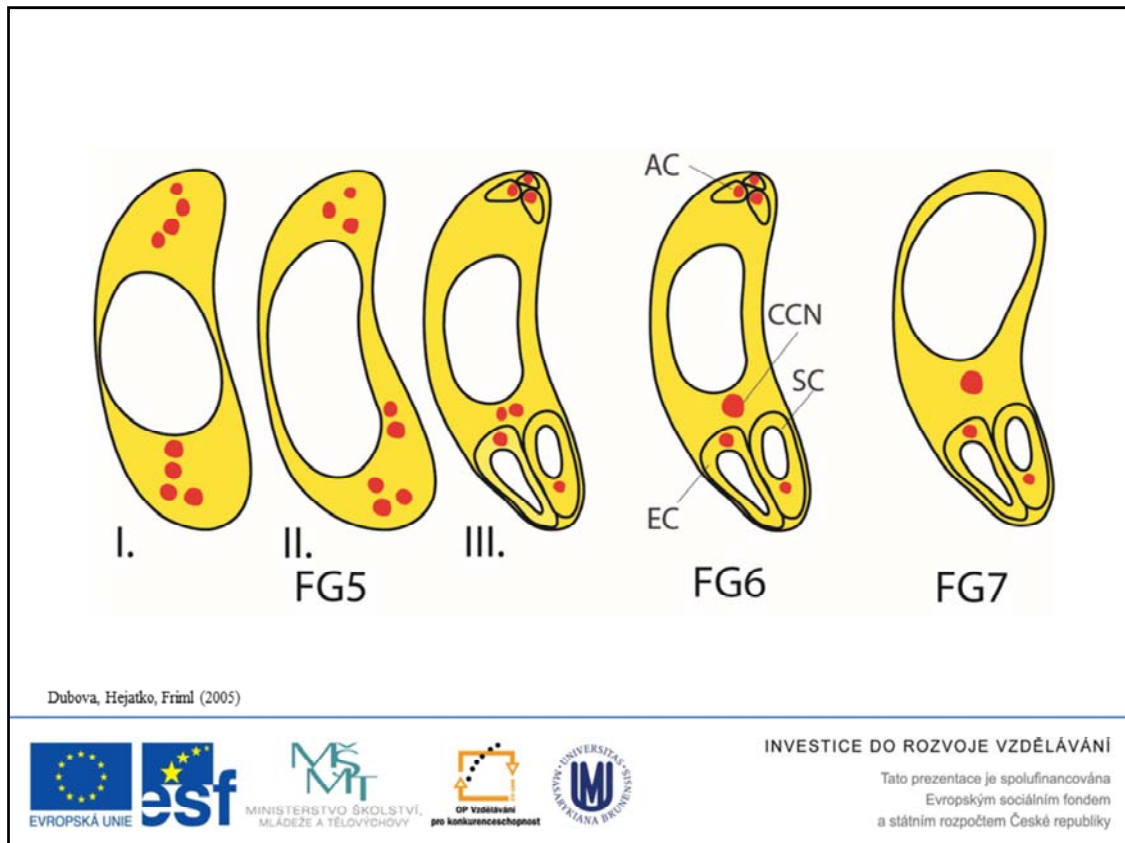
After the third (and final) round of mitosis, the eight-nuclear gametophyte is formed, defining the beginning of the most complex stage, **FG5**. This stage is characterized by dramatic changes in the position of nuclei; cellularization and cell differentiation takes place during FG5.

FG5 was originally considered to be a rather uniform stage, however, based on our results we suggested its further division into three sub-phases FG5 *I* - FG5 *III*. In the first sub-phase FG5 *I*, the embryo sac is a syncitium of eight nuclei in two groups of four, separated by the central vacuole. In FG5 *II*, the translocation of nuclei can be observed, resulting in the formation of two groups of three on both poles and two **polar nuclei** migrating towards the position of the future central cell nucleus. The polar nucleus of chalazal origin migrates faster, which results in the asymmetric location of the polar nuclei that underlines the already pre-established polarity of the developing embryo sac. Both these stages are rather short, as the frequency of the ovules found in both FG5 *I* and FG5 *II* is very low. In FG5 *III*, cellularization and cell differentiation occurs. At the end of the phase, the two **synergid cells** with well-developed vacuoles can be distinguished surrounding an **egg cell** on the **micropylar** (distal) **pole**. At the opposite, **chalazal** (proximal) **pole**, three **antipodal cells** are distinguishable; the **central cell** is still not fully differentiated. The end of FG5 is defined by the fusion of polar nuclei that leads to the formation of the diploid nucleus of the central cell.

In FG6, the antipodal cells undergo cell death, thus forming the four-celled mature female gametophyte (FG7), where double fertilization triggers embryo and endosperm development, giving rise to a fully functional seed. In some species (e.g. maize), the antipodal cells do not degenerate but proliferate, forming a cluster of up to 40 cells at the chalazal pole.
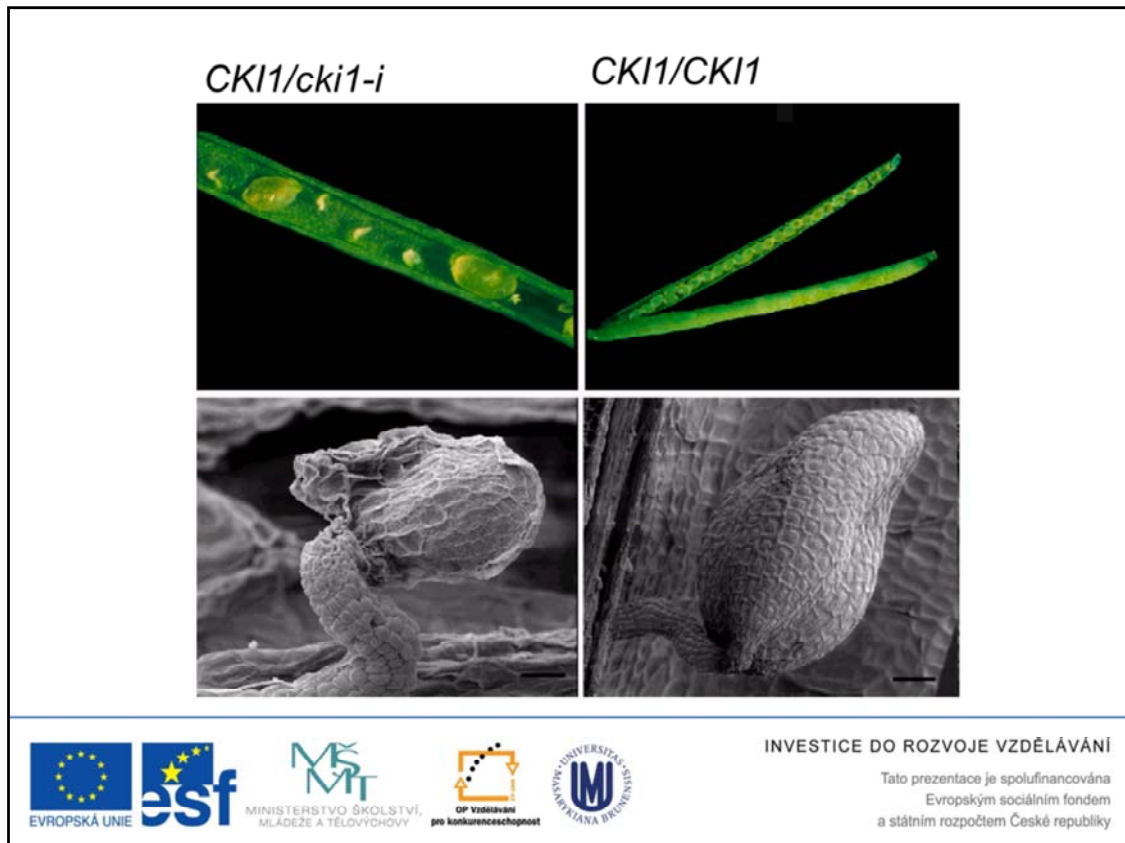
In spite of the crucial importance of the female gametophyte tissue in the entire plant life cycle, the factors necessary for proper formation and function of mature embryo sac are mostly still unclear. Just recently, extensive mutation screens specifically directed to identify female gametophyte mutations were performed.

Based on the process affected, female gametophytic mutations could be classified into six phenotypic categories and very recently, the molecular nature of the involved factors is being discovered.

Interestingly, the largest group of mutants defective in female gametophyte development or function seems to affect post-fertilization processes. That fact suggests importance of maternal control of post fertilization processes in *Arabidopsis* and possibly also in other plant species.

As an example, the **CYTOKININ INDEPENDENT-1 (CKI1)** will be discussed in more detail.

Mutants carrying the *knock-out allele* (what is also called *null allele*) were found to be semisterile when heterozygous.
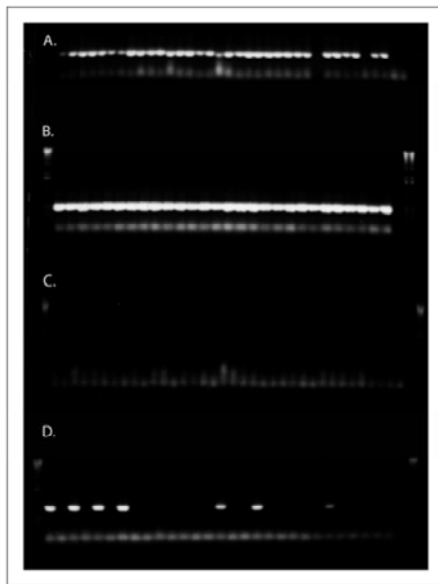
A. ♂ wt x ♀ *CKI1/cki1-i*

CKI1 specific primers (PCR positive control)

B. ♂ *CKI1/cki1-i* x ♀ wt

C. ♂ wt x ♀ *CKI1/cki1-i*

cki1-i specific primers

D. ♀ wt x ♂ *CKI1/cki1-i*

Importantly, plants homozygous for the cki1-I (cki1-i/cki1-i) were not identified in the segregating population. Via analysis Iof the progeny of reciprocal back-cross it has been found that the cki1-I allele can not be transduced through the female germ line.

However, the transduction through the male germ line was unaffected.

FG 0 — MMC

FG1 FG2 FG3 FG4

FG 1   FG 2   FG 3   FG 4

Hejátko et al., *Mol Genet Genomics* (2003)

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Tato prezentace je spolufinancována
Evropským sociálním fondem
a státním rozpočtem České republiky
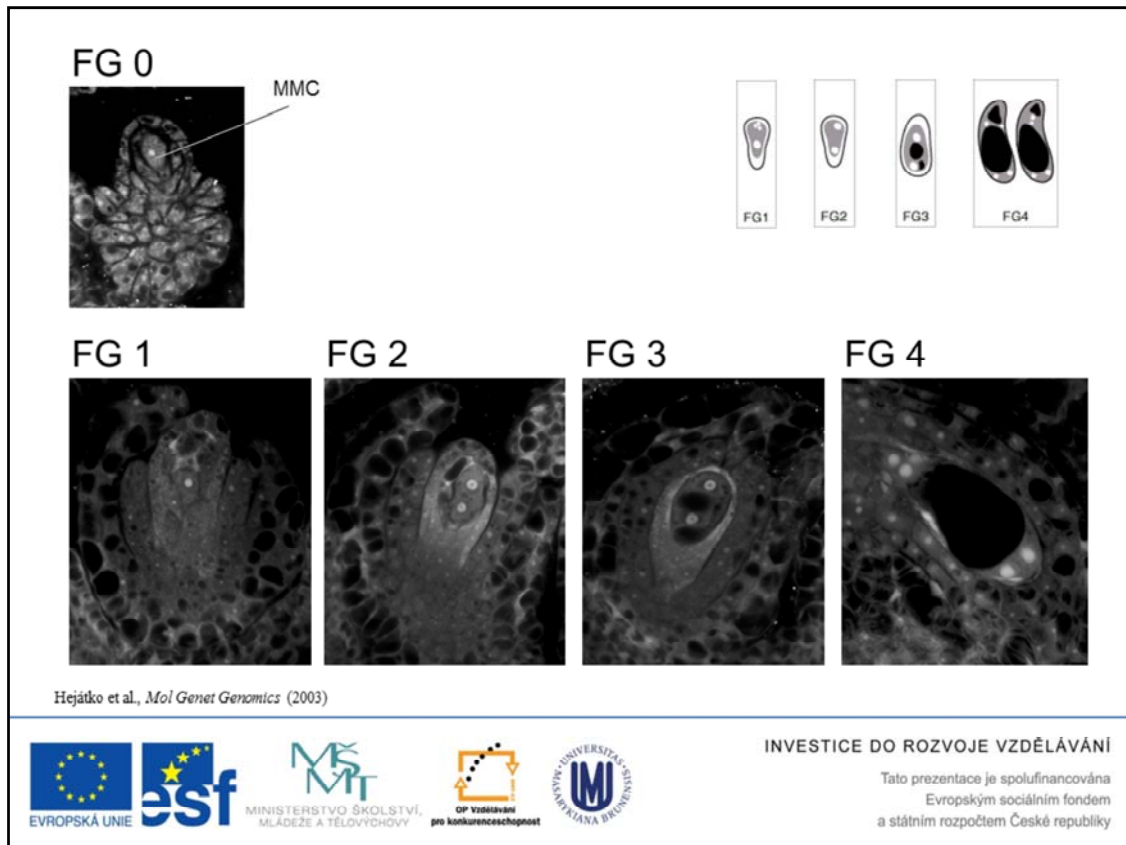
Detailed analysis of the megagametogenesis in the cki1-i/CKI1 siliques did not show any abnormalities during FG0-FG4 stages.

FG0 is sometimes defined as a stage just before meiosis, after the MCC is differentiated.

CKI1 late FG5   cki1-i 24 HAE

Hejátko et al., *Mol Genet Genomics* (2003)
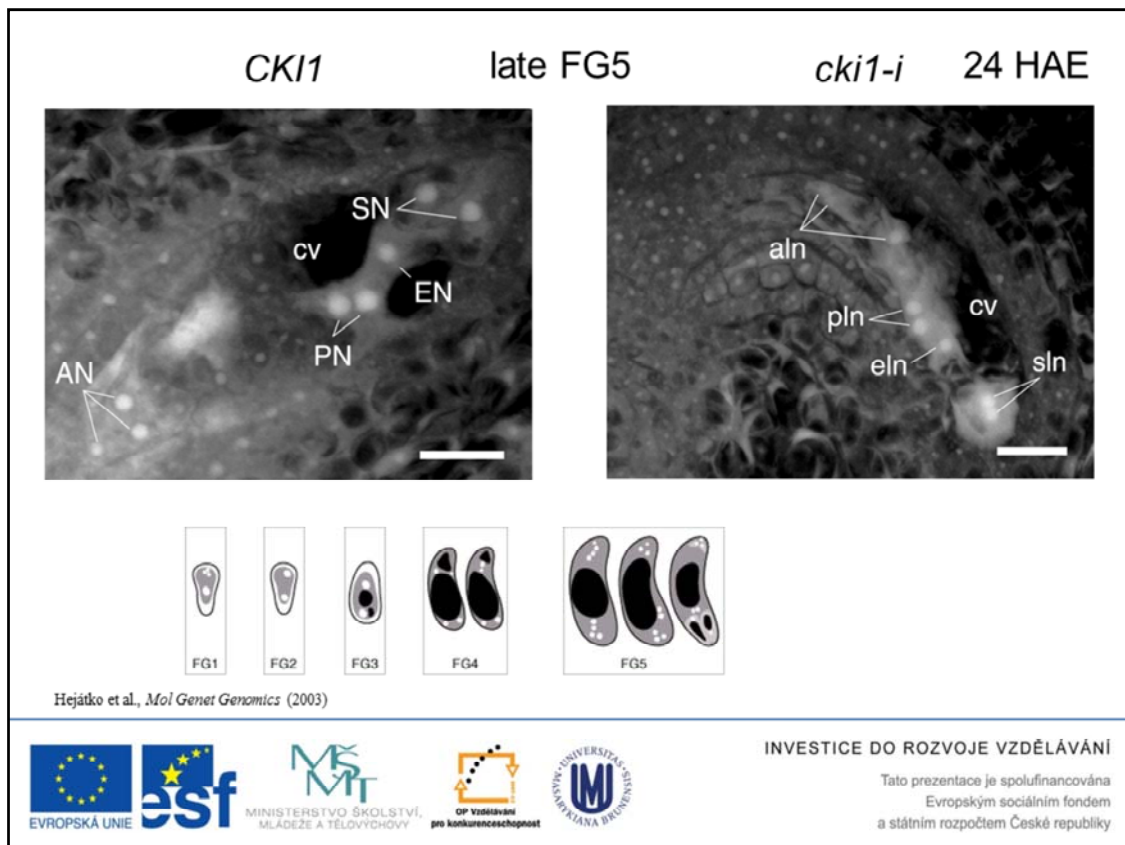
However, at the end of the FG5, severe defects were recognized in approximately half of the embryo sacs from *cki1-i/CKI1* pistils.

In the mutant embryo sacs, the central vacuole was partially collapsed and antipodal cells nuclei were improperly located.
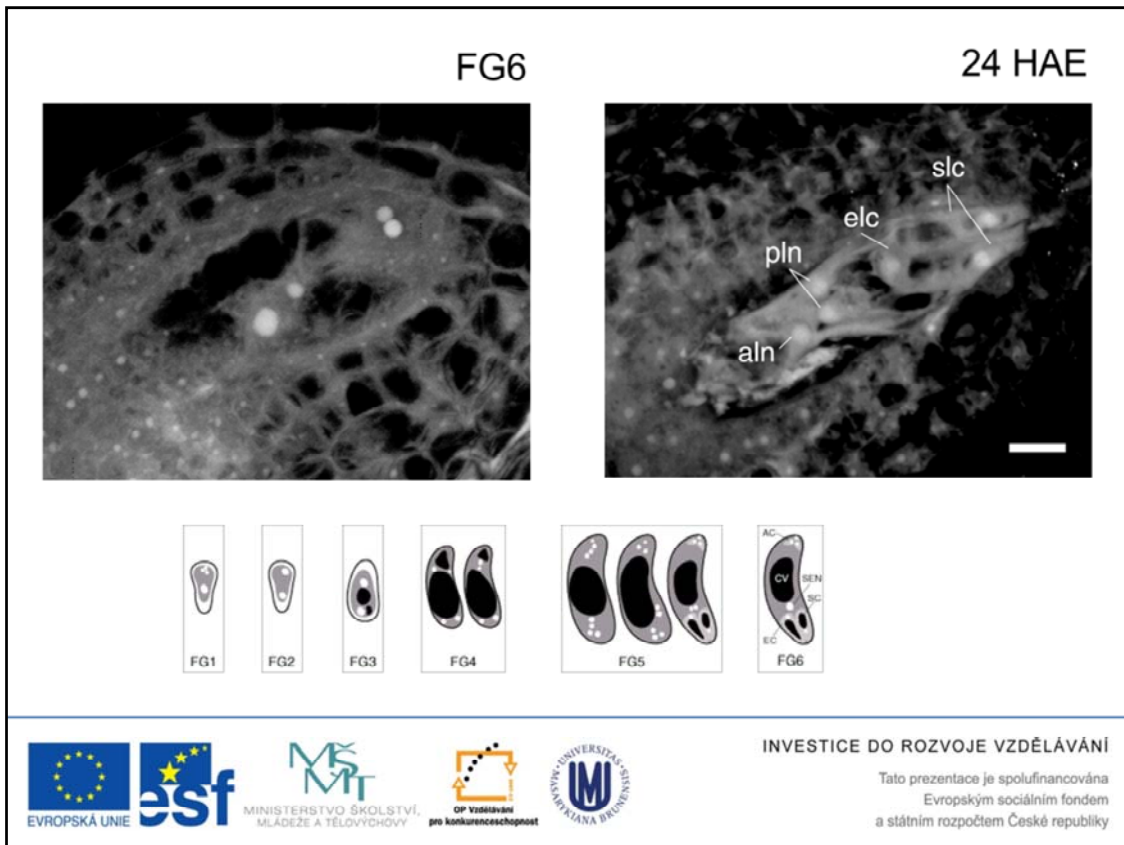
24 hours after flower emasculation (HAE), polar nuclei still did not fuse, central vacuole was collapsed.
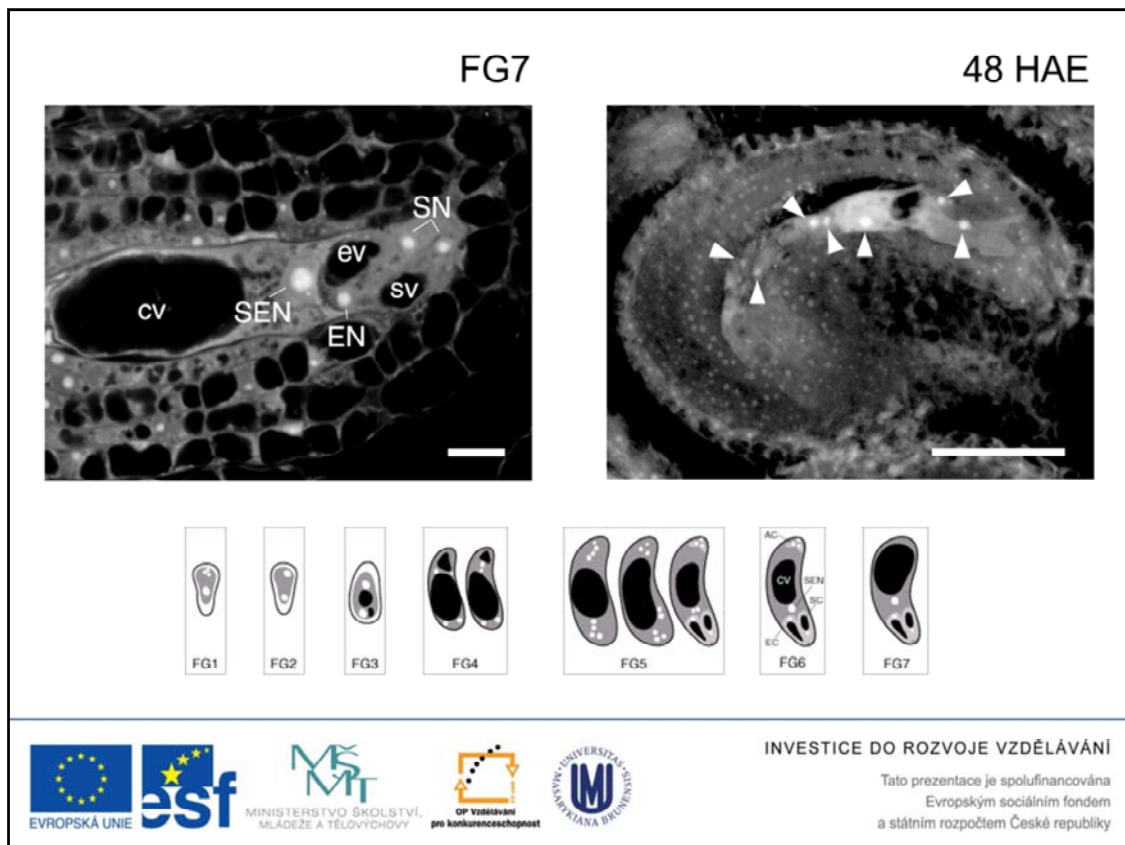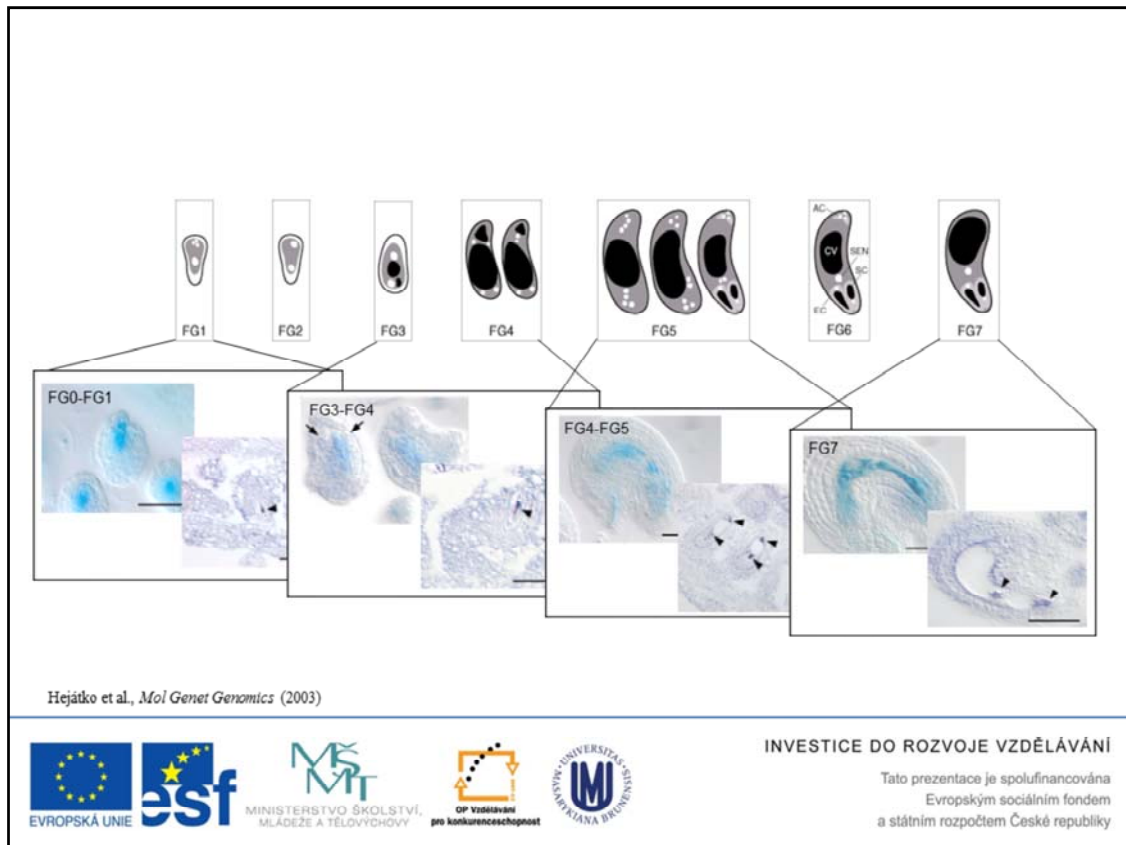
Finally, at 48 HAE, the female gametophytes revelaed different stages of degeneration and cellularization failed.

These results indicated that in the embryo sacs carrying the *cki1-i* allele, the development was broken at transition between stages FG5 and FG6.

Hejátko et al., *Mol Genet Genomics* (2003)

Using stable *Arabidopsis* transgenic lines carrying transcription fusion of the CKI1 promoter with GUS reporter gene, the expression of *CKI1* was identified in the developing embryo sac throughout megagametogenesis (stages FG1-FG7).

Thus, CKI1 is probably important for the transition between FG5 and FG6, however, the sequence of the developmental pathway might be triggered early during megagametogenesis.
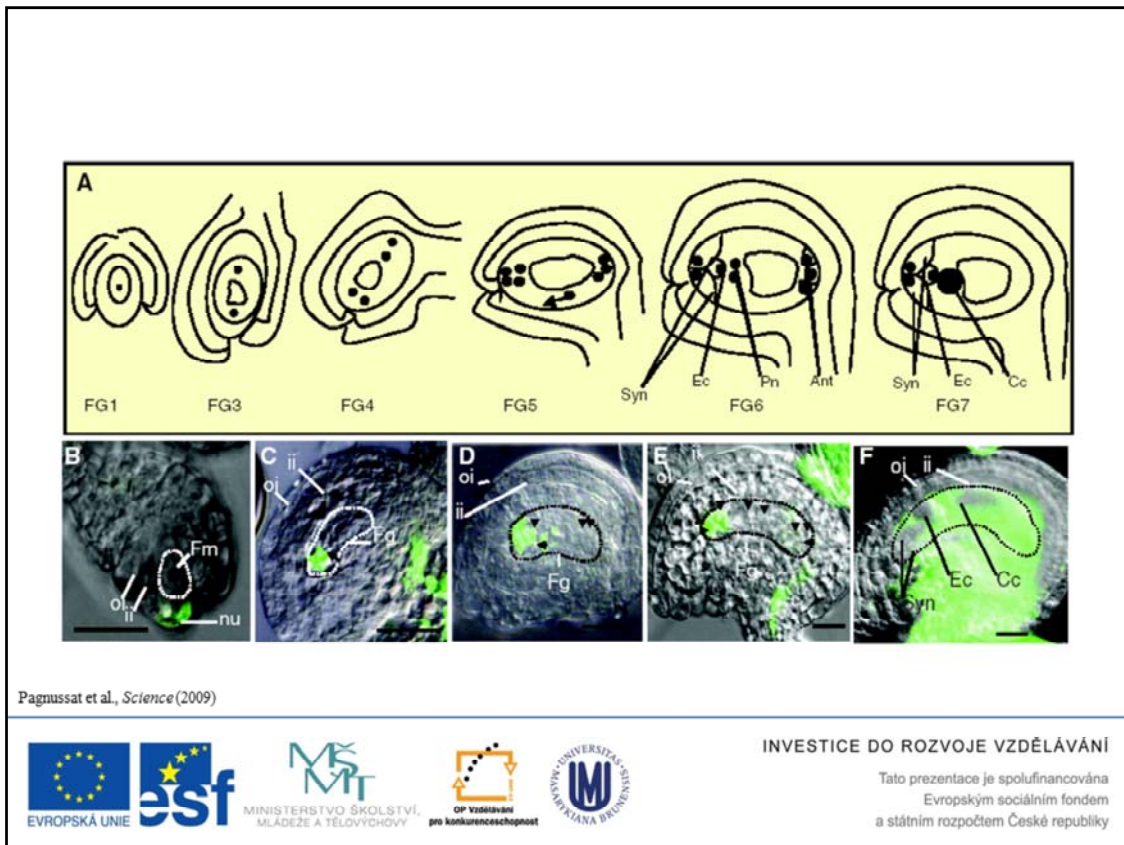
Outline of Lesson 6

Plant Reproduction

- Sexual and asexual plant reproduction
- Plant life cycle
- Initiation of flowering
- Determination of floral organ identity
- Microgametogenesis
- Megagametogenesis
 - Female gametophyte patterning

Auxin gradient regulates cell fate determination during female gametophyte formation in *Arabidopsis*.

Auxin gradient is formed during female gametophyte development. In the early stages of development (FG1), the auxin maximum (green signal) appears at the micropylar pole.

The auxin maximum is maintained at that position till the FG5. At FG6, the auxin maxima became less polarized.
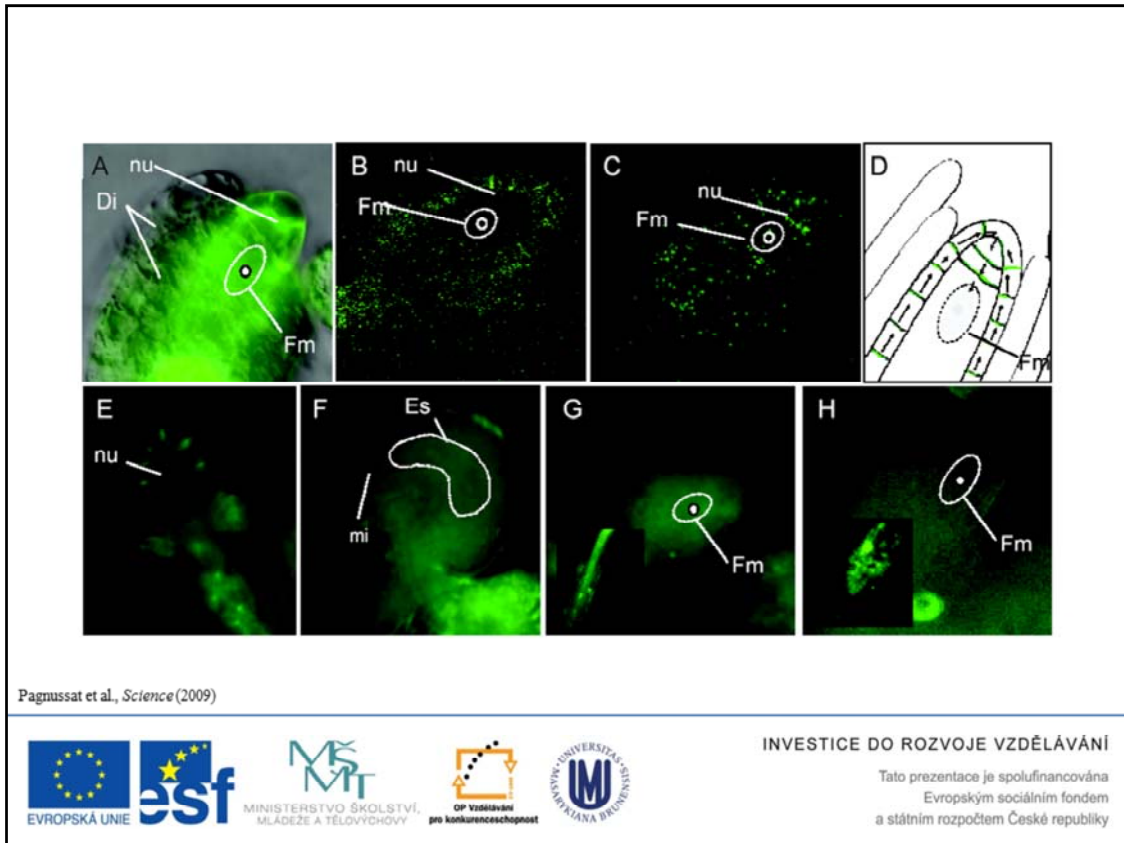
Auxin transporter, PIN1 protein was found to be produced in the nucellus at FG1 (A) and just before sporogenesis (E). PIN1 localization implies potential auxin flux, leading to the auxin maxima formation at the micropylar pole during FG1.
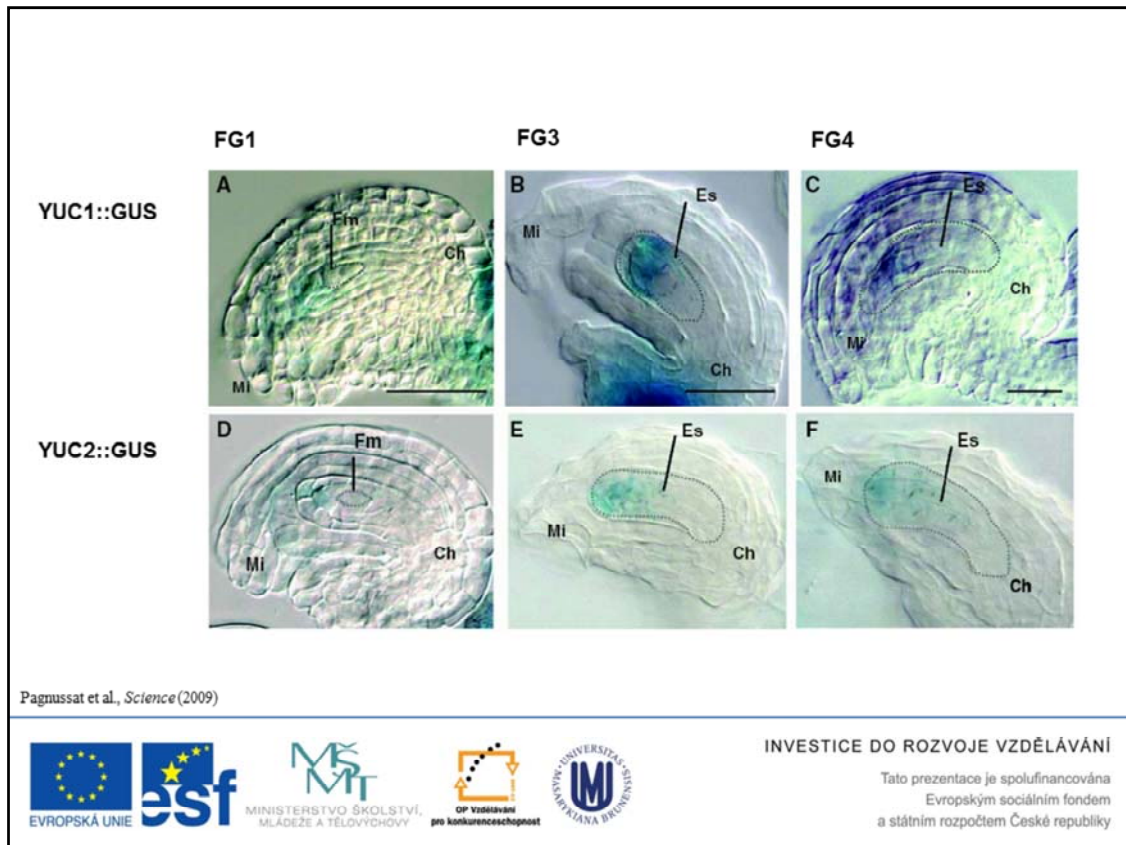
Expression of auxin biosynthetic genes, YUCCA1 (A-C) and YUCCA2 (D-F) suggest possible role of localized auxin biosynthesis in the later stages of the female gametophyte development (onwards FG2).

Pagnussat et al., *Science* (2009)
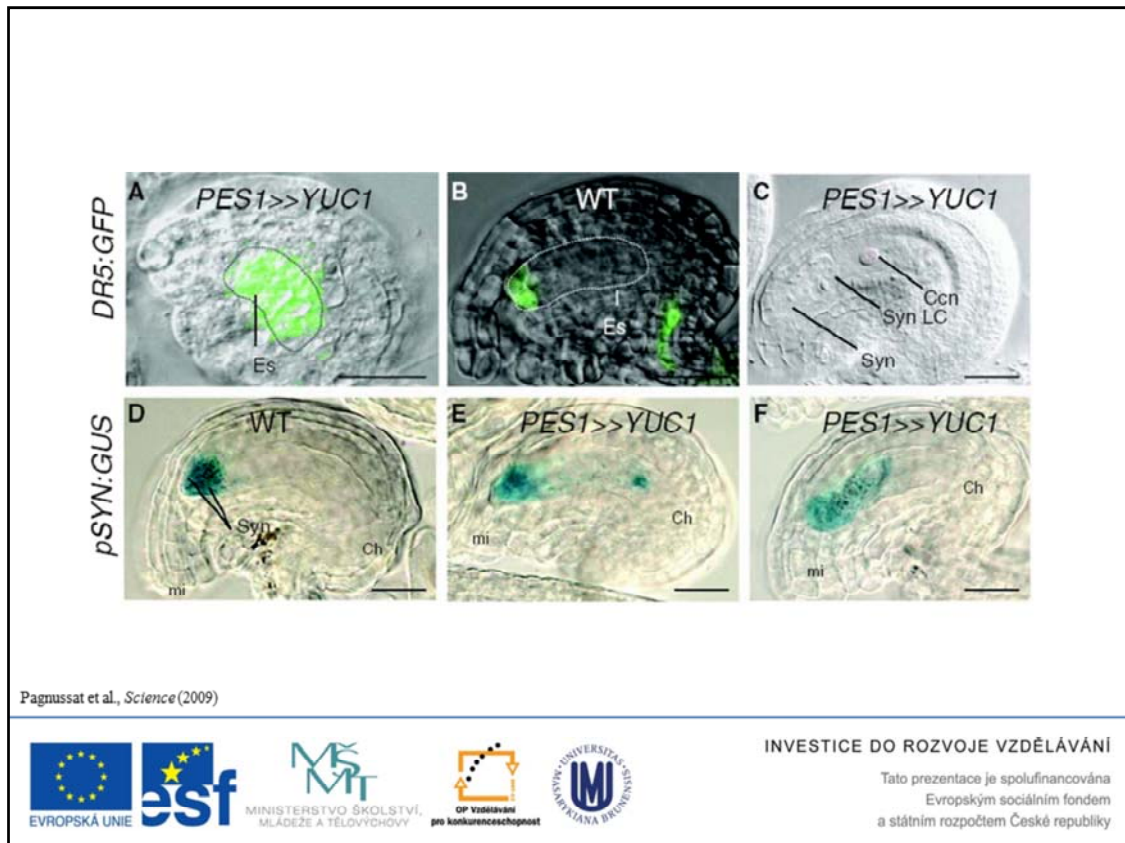
In the ovules with ectopic overexpression of YUC2 via transcription fusion with promoter of pES1, which is uniformly expressed during early female gametophyte development (stage FG1) to the mature embryo sac (stage FG7), the auxin maximum is disturbed.

In comparison to WT, where the auxin maximum is located at the micropylar pole at FG5 (B), auxin is equally distributed throughout the embryo sac (A).

The abortion of the located auxin maximum results into aberrant cell identity of the forming embryo sac, as shown by the expression of synergid cell marker in the antipodal cells (E) or in the central and egg cell (F).

Pagnussat et al., *Science* (2009)
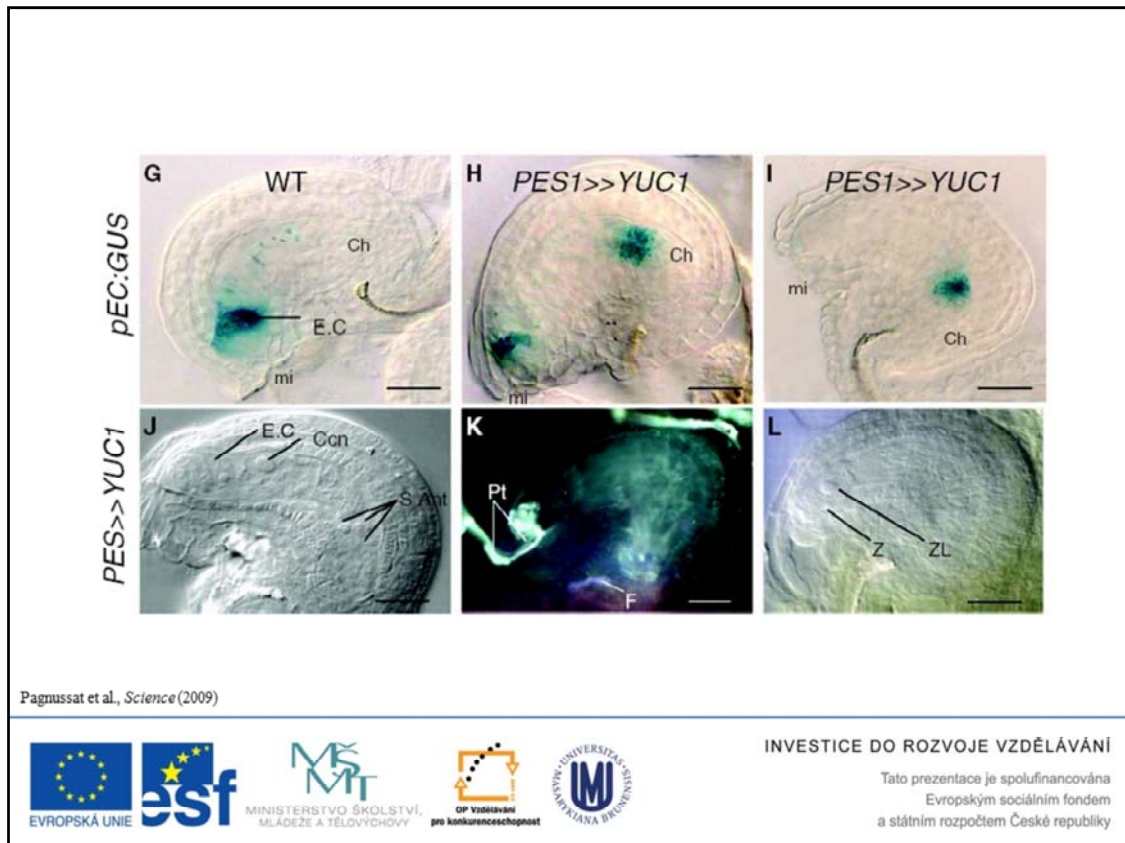
The developmental defects due to absence of proper auxin maxima can be further demonstrated by the ectopic expression of egg cell marker in the egg cell and antipodal cells (H) or only in the position of the antipodal cells (I).

Finally, antipodal cells do not degenerate in the PES>>YUC1 embryo sacs, two pollen tubes were found to penetrate in parallel into the embryo sac and zygote-like structure was found in a position of central cell. No endosperm development was apparent (see later).

(J) Mature and unfertilized YUC1-overexpressing embryo sac from an emasculated flower showing surviving antipodal cells (S Ant) 2 days after emasculation. (K) Two pollen tubes (Pt) enter the micropyle of a YUC1-overexpressing embryo sac and continue to grow within the embryo sac. F, funiculus. (L) Fertilized YUC1-overexpressing embryo sac showing no endosperm development and a structure that morphologically resembles a zygote at the position of the central cell. EcL, egg cell–like cell; Z, zygote, and ZL, zygotelike cell (Pagnussat et al., Science, 2009).

Sundaressan and Alandete-Saez, *Development* (2010)
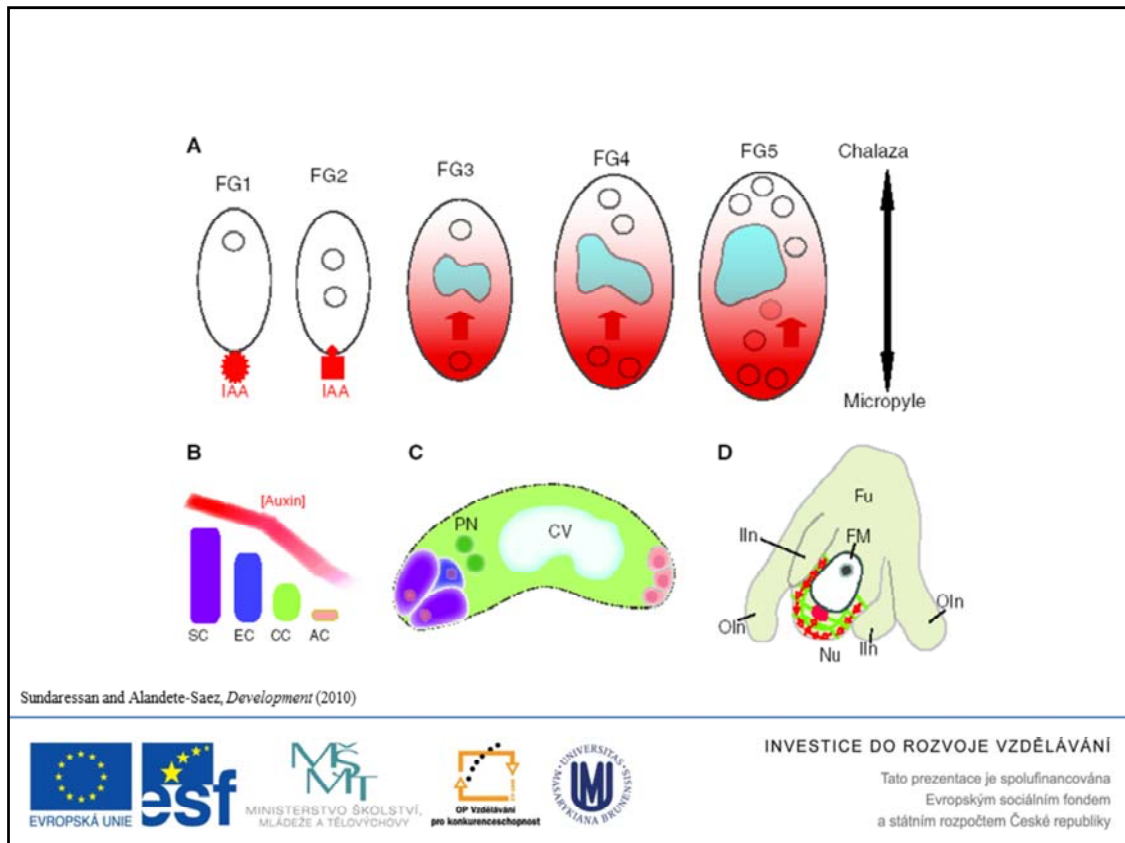
Taken together, the above mentioned findings let to the suggestion that auxin gradient determines the identity of cell during female gametophyte development.

In the early stages FG1 and FG2, the auxin originates from the maternal tissue of nucellus. In the FG3, the expression of YUCCA genes allows auxin biosynthesis located at the micropylar pole, leading to the auxin gradient formation (red), proper cell fate specification (B) and mature female gametophyte formation (C, at the cellularization stage FG6).

Directed intercellular auxin transport via PIN1 allows the initial auxin maxima formation at FG1 (D).
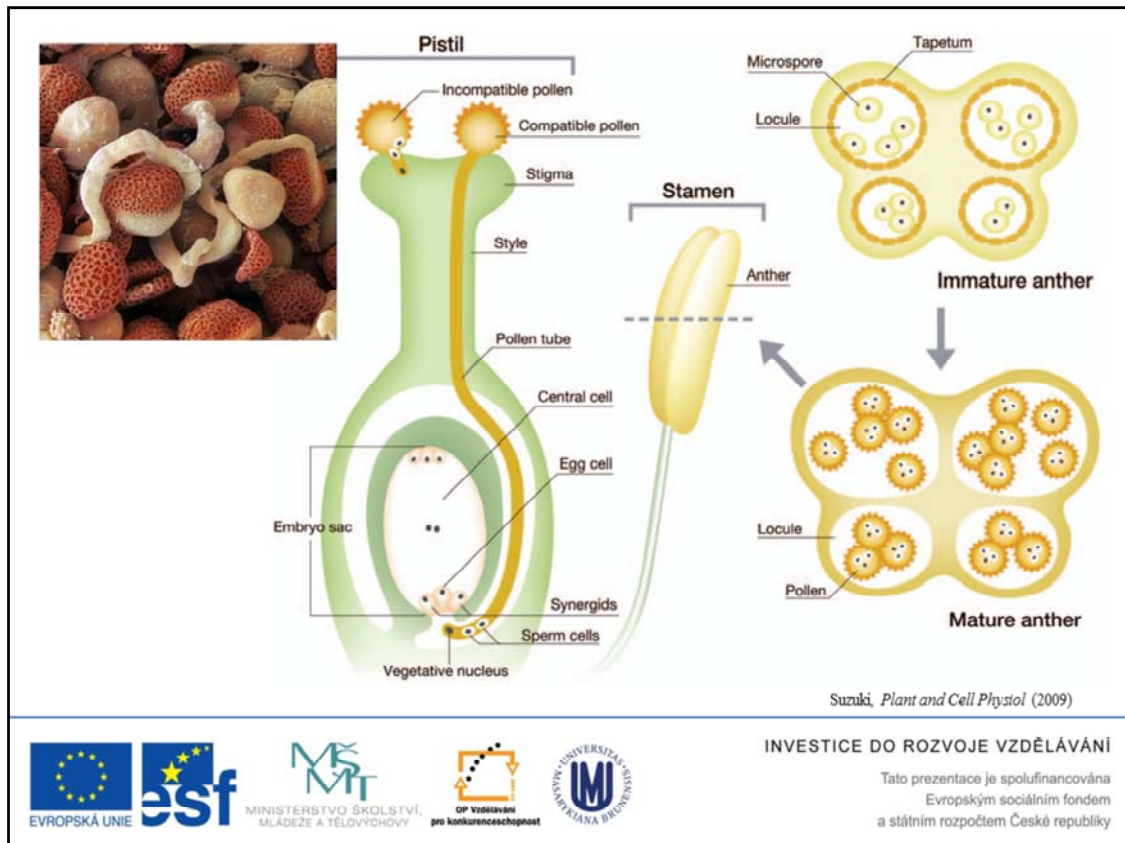
Outline of Lesson 6

Plant Reproduction

- Sexual and asexual plant reproduction
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- Initiation of flowering
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- Megagametogenesis
  - Female gametophyte patterning
- **Pollen tube growth, guidance and fertilization**

Suzuki, *Plant and Cell Physiol* (2009)

In angiosperms, the **pistil** (gynoecium) in the central part of the flower represents the pollen-accepting organ, which has multiple functions in plant reproduction: it decides whether to accept or reject the pollen, sustains pollen tube growth, and forms and protects the female gametophyte inside the ovule.

The pistil is composed of one or more fused **carpels** that bear the **ovules**.

Carpel fusion occurs very early in pistil development. Inner tissues of the developing upper pistil part differentiate to form the specialized secretory zone of the stigma at the top of the style and the transmitting tissue within the central cylinder of the style.

At flower maturity, when pollination takes place, the pistil is fully developed and composed of the **stigma**, the **style**, and the **ovary**.

The morphology of anthers and pistils co-evolved with the mode of pollen dispersal. Different mechanisms of pollen release, pollen transfer, and its deposition to the female sexual organs have developed in plants during evolution.

Numerous **interactions** between the sporophyte and gametophyte include signaling and nutrition.

The first interactions take place on the stigma surface after pollen grain landing, where the pollen grain hydrates and germinates forming a **pollen tube,** followed by the penetration of the pollen tube through the specialized tissues of the pistil - the stigma and the style.

Finally, the pollen tube bearing the two sperm cells reaches the female gametophyte, the **embryo sac**, located inside the **ovule** in the **ovary**. Pollen tube guidance at this stage is influenced by interactions between pollen tube and **micropyle exudates** and the **filiform structures** of the **synergids**.

Inset (left up): Asiatic lily (Lilium asiatic) stigma detail showing germinating pollen grains, coloured scanning electron micrograph (SEM). Magnification x465 at an image size of 10 cm.

Kost., *Trends in Plant Science* (2008)
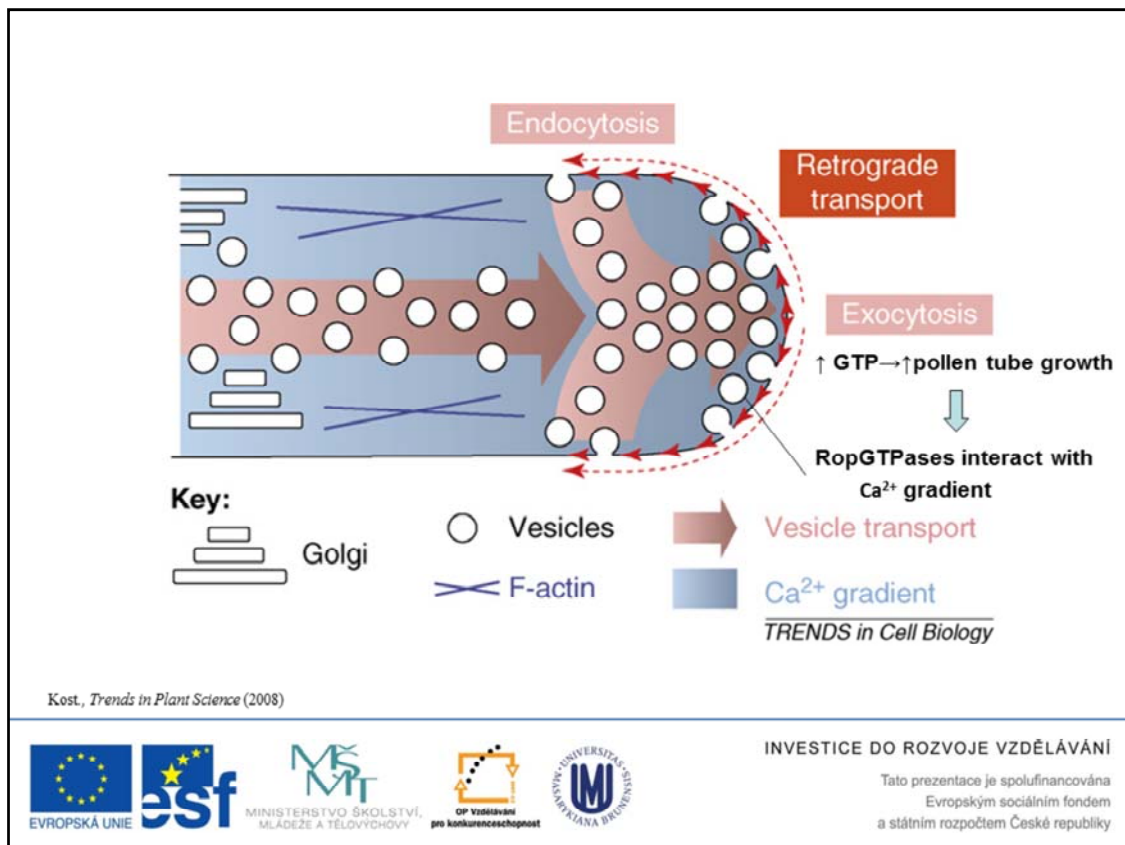
Pollen tube growth is a result of complicated molecular events, allowing single-cell structure of the pollen tube to elongate.

Retrograde transport via exocytosis and endocytosis of the intracellularly delivered material, changing of the hydrodynamic equilibrium leading to changes in the cell turgor and Ca^{2+} gradient-based signalling take place in the complex process.

Pollen tubes are among the most rapidly extending cells. These growth rates are possible due to a highly polarized apical fusion of vesicles, which transports cell wall components to the growing tip.

A tip-focused cytoplasmic Ca^{2+} gradient is known to play a central role in the regulation of pollen tube growth. Modulation of the cytoplasmic Ca^{2+} concentration results in changes in the rate and direction of pollen tube growth.

It was found that increases in GTP levels could promote pollen tube growth. Rop GTPases have been implicated in the regulation of pollen tube growth probably by interaction with the tip-focused Ca^{2+} gradient.
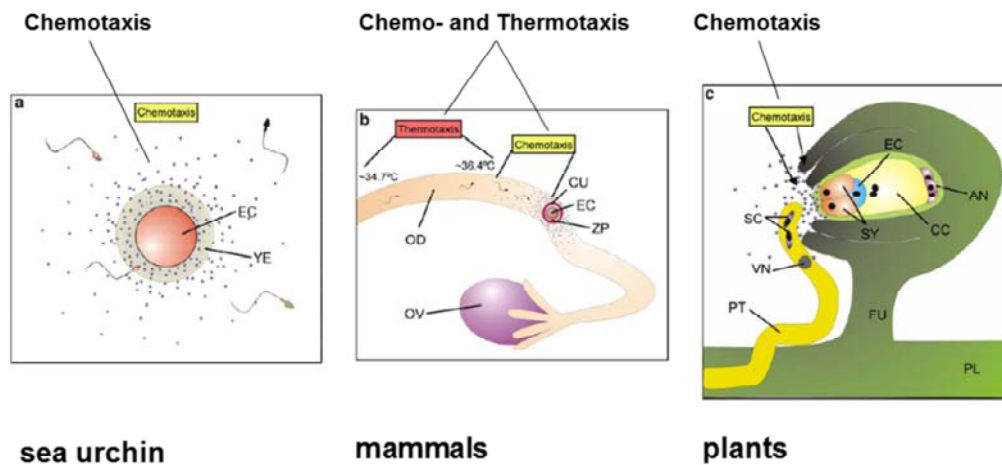
Marton and Dresselhaus., *Plant Sex Reprod* (2007)

Similar to animals, in plants there is chemotaxis and cell surface interaction also involved in the fertilization.

In sea urchin (panel A), sperm cells are attracted to the egg cell in a species-specific way via secretion of peptides.

Chemotaxis and probably also thermotaxis takes place inside the mammalian oviduct once sperm have passed the uterus. Dots indicate a concentration gradient of signaling ligand molecules secreted by the ovulated egg and/or surrounding cumulus cells.

Pollen tube guidance precedes double fertilization in flowering plants. A pollen tube harboring two sperm cells and a large vegetative nucleus has left the placenta to grow along the placental surface and the funiculus into the micropyle following gradients generated by the maternal tissues of the ovule as well as by the female gametophyte.

AN antipodal cells, CC central cell, CU cumulus cells, EC egg cell or ovum, FU funiculus, OD oviduct, OV ovary, PL placenta, PT pollen tube, SC sperm cell, SY synergid, VN vegetative nucleus, YE egg jelly, ZP zona pellucida.

Okuda et al., *Natture* (2009)
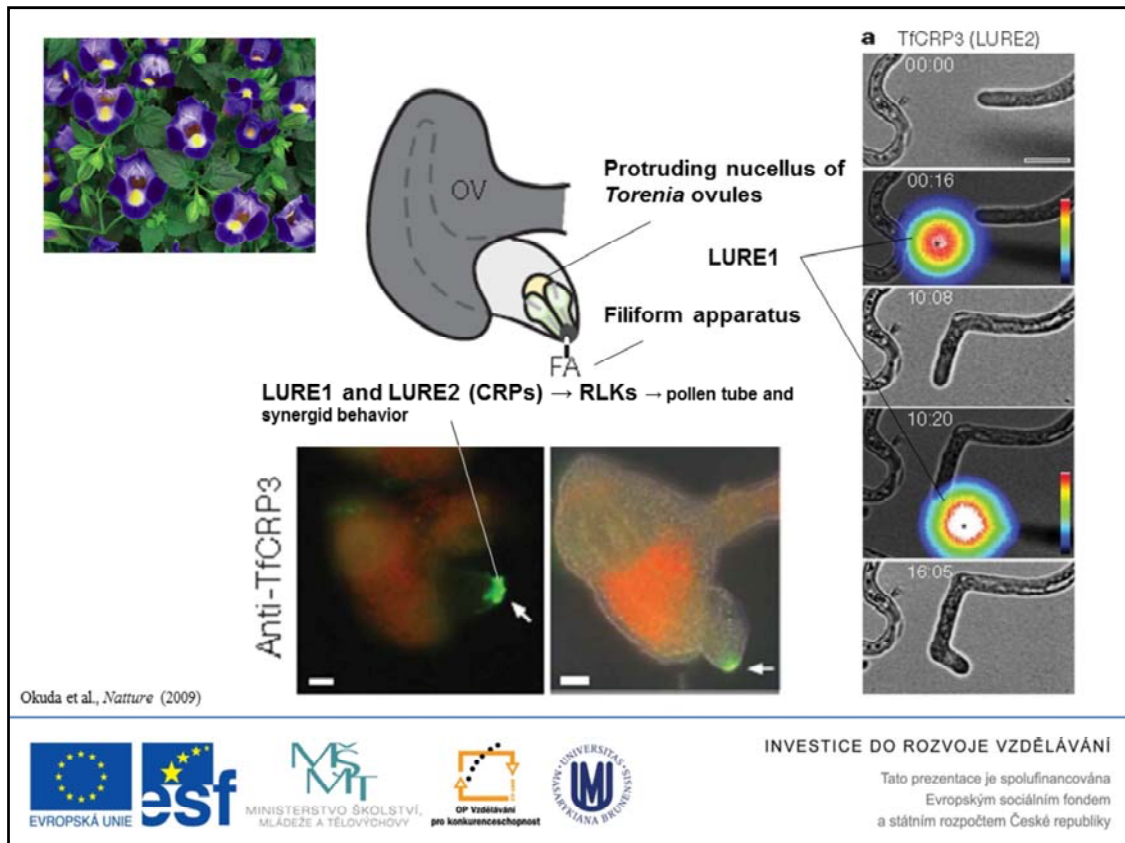
Pollen tube growth via stigma and style that is a relatively huge distance in range of mm. Thus, one of the important question is the factor involved in the pollen tube guidance.

Experiments using the *in vitro* fertilization system of *Torenia ,* it has been recently found that the synergid cell is necessary for the short-range pollen tube guidance before fertilization.

Torenia forms an embryo sac protruding at the microplyar pole (in the figure left), with a filiform apparatus (FA) at the mycropylar pole formed by two synergid cells (green).

Filiform apparatus is a structure formed at the micropylar (distal) pole of the synergid cell wall that is thickened, forming finger-like projections into the synergid cell cytoplasm.

Recently, the signaling molecules were identified as **LURE1** and **LURE2**, small cysteine-rich polypeptides (CRPs). These CRPs are very probably recognized by receptor-like kinases (RLKs) that affect both synergid cells and pollen tube behavior. Thus, very probably, mutual interactions occur among male and female gametophytes just before fertilization.

The figure in the middle demonstrates results of immunostaining showing CRP peptides secreted to the surface of the micropylar end of the embryo sac (green Alexa Fluor fluorescence, arrows).

Finally, on the right-hand panel, the experiments showing the ability of LURE1 to attract pollen growth *in vitro* are shown. The fluorescently-labelled LURE1 was added at time points of 00:16 (mm:ss) and 10:20. Spectral colours correspond to the intensity of fluorescence (concentration of the Alexa Fluor dye), with white representing the highest level (see colour scales).

Marton etal., *Science* (2005)
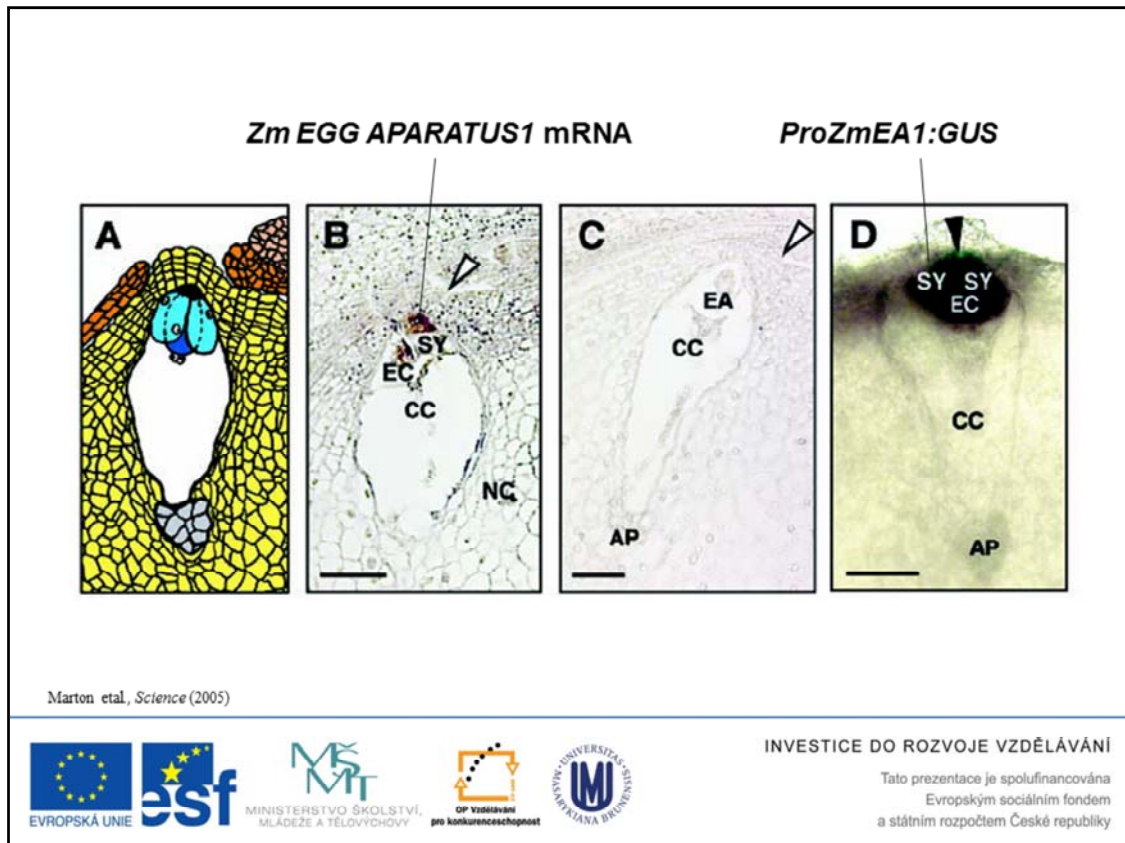
In maize, the protein Zea mays EGG APPARATUS1(ZmEA1) consisting of 94 aa was shown to be involved in the attraction of the pollen tube growth.

As shown on in the figure above, *ZmEA1* is expressed in what is called *egg apparatus*, i.e. synergid cells and egg cell of maize.

On the schematic drawing (A), there is shown the female gametophyte of maize with two synergids (light blue), egg cell (blue), central cell (white) and antipodal cells (grey).

In maize, similar to *Arabidopsis*, the embryo sac has similar, *Polygonum* type of organization. In contrast to *Arabidopsis*, however, in maize the antipodal cells do not degrade. Instead, they proliferate and form approximately 40 cells at the time of fertilization.

On the figure B, there is shown the result of *in situ* hybridization of maize unfertilized embryo sac with anti-sense probe, while in the figure C, there is control, using sense-probe.

Panel D shows GUS activity (staining) in the ovule of unfertilized transgenic line carrying transcriptional fusion of the *ZmEA1* promoter with β-glucuronidase from *E. coli* (*ProZmEA1:GUS*).

Marton etal., *Science* (2005)
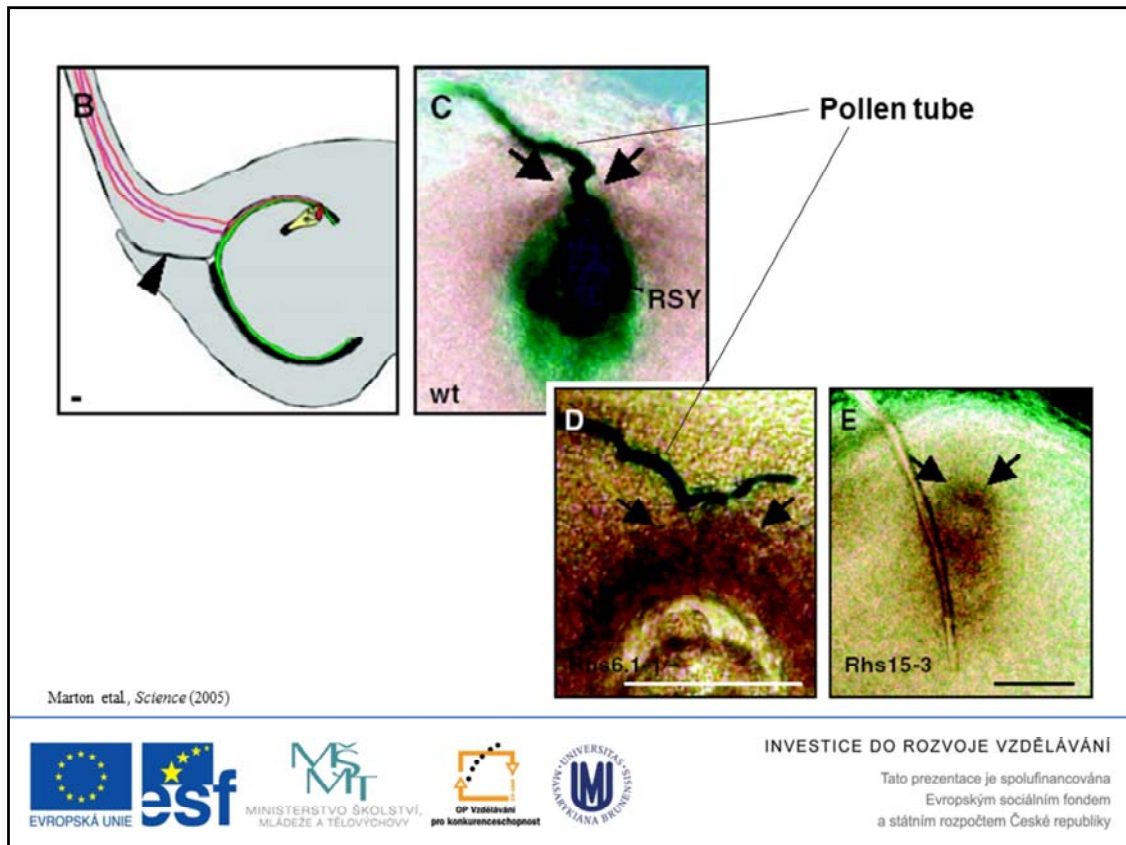
In lines with downregulated ZmEA1 via RNAi (will be discussed later), the pollen tubes are misleading and do not enter the micropyle (arrows).

Berger etal., *Trends in Plant Science* (2008)
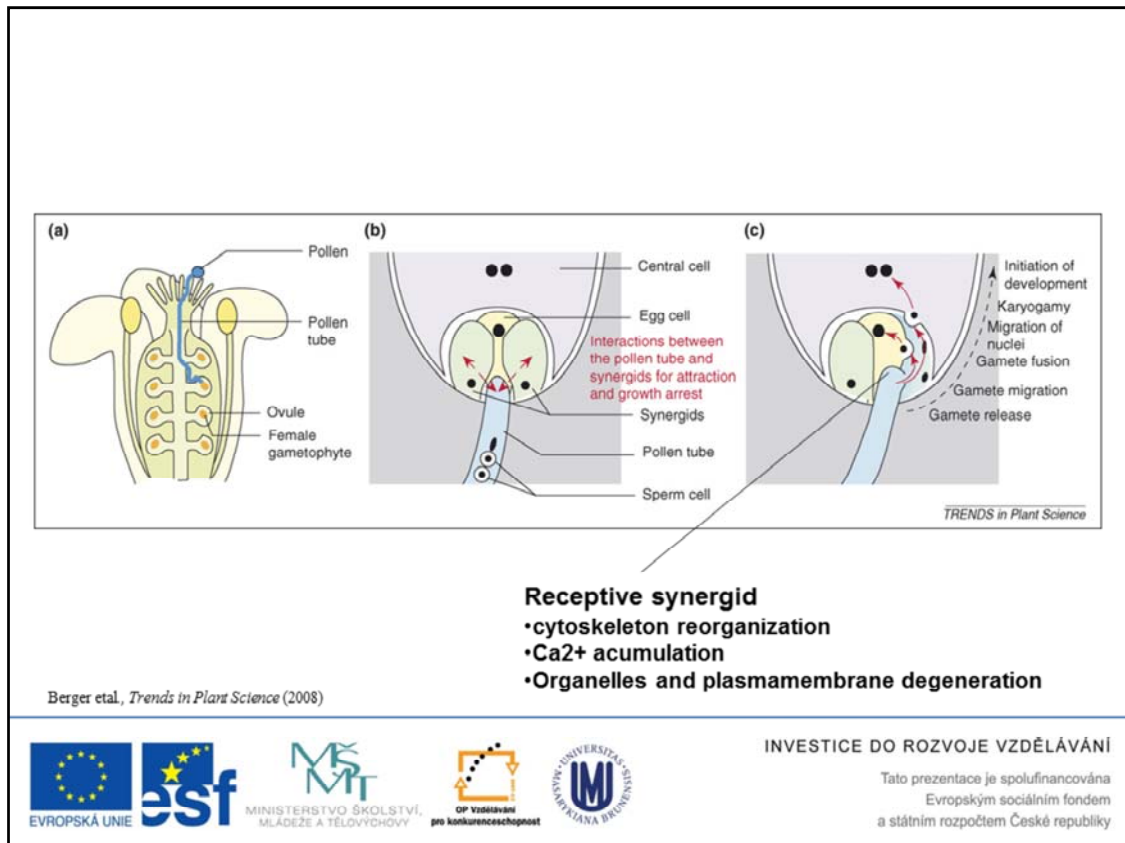
Pollen tube enters the embryo sac through the **receptive synergid.**

The signs of the synergid receptivity are: (1) cytoskeleton reorganization, (2) Ca^{2+} accumulation, and (3) degeneration of organelles and plasma membrane.

The pollen tube develops an aperture on the tip and discharges its contents into the receptive synergid or in the space previously occupied by this synergid (depending on the time of the synergid degeneration). Pollination initiates disintegration of one of two synergids, which were identical before pollination.

The mechanism of this process initiation at the molecular level is still unclear. In some species, synergid cell death occurs even in the absence of the pollination, suggesting that the process might be an intrinsic feature of megagametogenesis.

Dumas and Rogowsky., *C. R. Biologies* (2008)
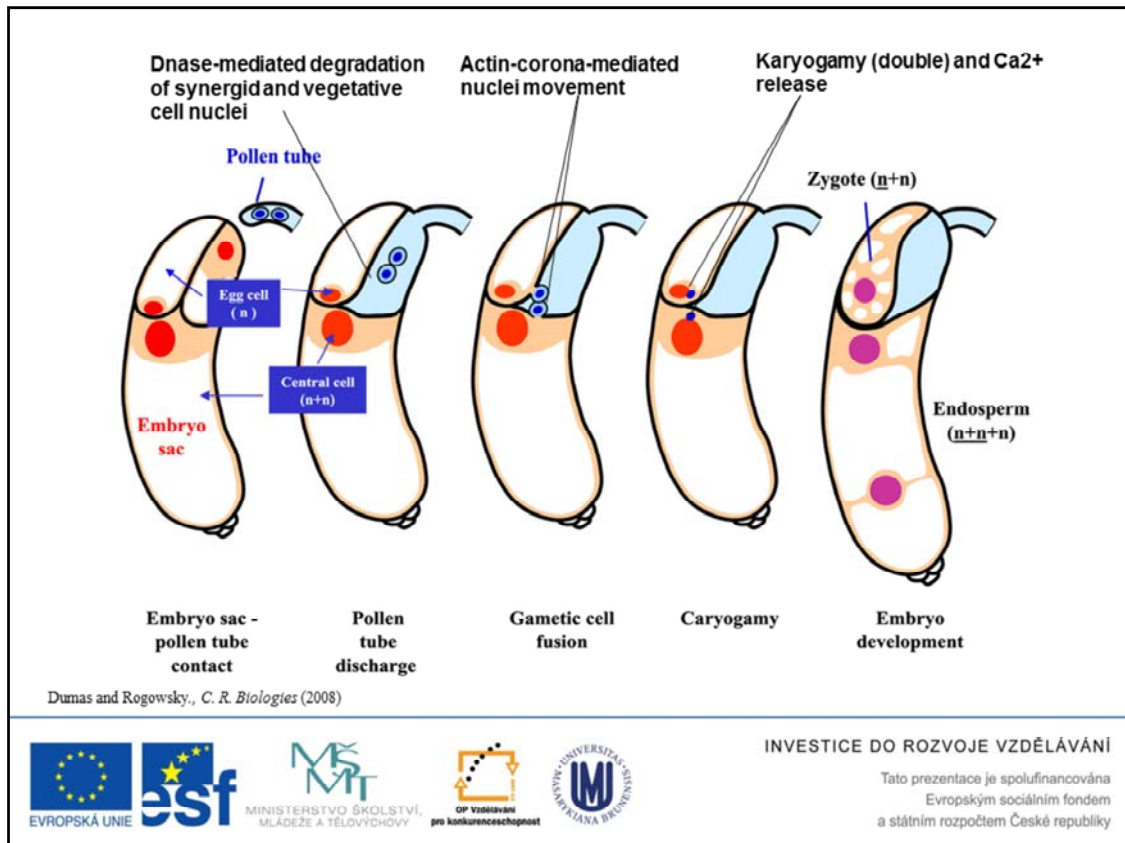
DNase degrades both the vegetative cell nucleus and the nucleus of the synergid and the sperm cells are transferred to the egg and central cells. Bundles of actin filaments form two corona-shaped structures; one is located near the egg nucleus and the other near the central cell.

Coordinated actions of actin and myosin on the sperm cell surface mediate sperm cell transport to the position near the egg cell and the central cell nuclei. Regulation of the actin-corona formation is not yet fully known. The cell walls of the egg cell and the central cell are modified and surrounded only by the cell membrane that facilitate delivery of the sperm nuclei with the male cytoplasm. This delivery is initiated by the apposition of plasma membranes of the sperm and the egg and those of the second sperm cell and the central cell.

After pollination, two sperm cells, delivered by a pollen tube, fuse with the **egg** and **central cell**, which results in the formation of the **embryo** and **endosperm**, respectively.

This is called **double fertilization** in plants and includes both two nuclear fusions (**karyogamy**) and the coalescence of male and female cytoplasm (**plasmagamy**).

The first karyogamy (syngamy) is the fusion of the egg and sperm cell nuclei, which produces the diploid nucleus of the **zygote**. Measurements of calcium concentration during *in vitro* fertilization of maize egg demonstrated that fusion of gametes triggers a calcium influx, which is then followed by an increase of cytoplasmic calcium (similarly as in animal systems).

The second karyogamy, occurring only in angiosperms, is the fusion of the (usually) diploid nucleus of the central cell with the second sperm cell nucleus forming the triploid **primary endosperm nucleus**. Plasmagamy can be conditioned by the female gametophyte as, in some cases, male cytoplasm is excluded from the egg cell. It seems that despite morphologic uniformity of the two sperm cells some differences on their surface may allow specific trafficking either to the egg or to the central cell.
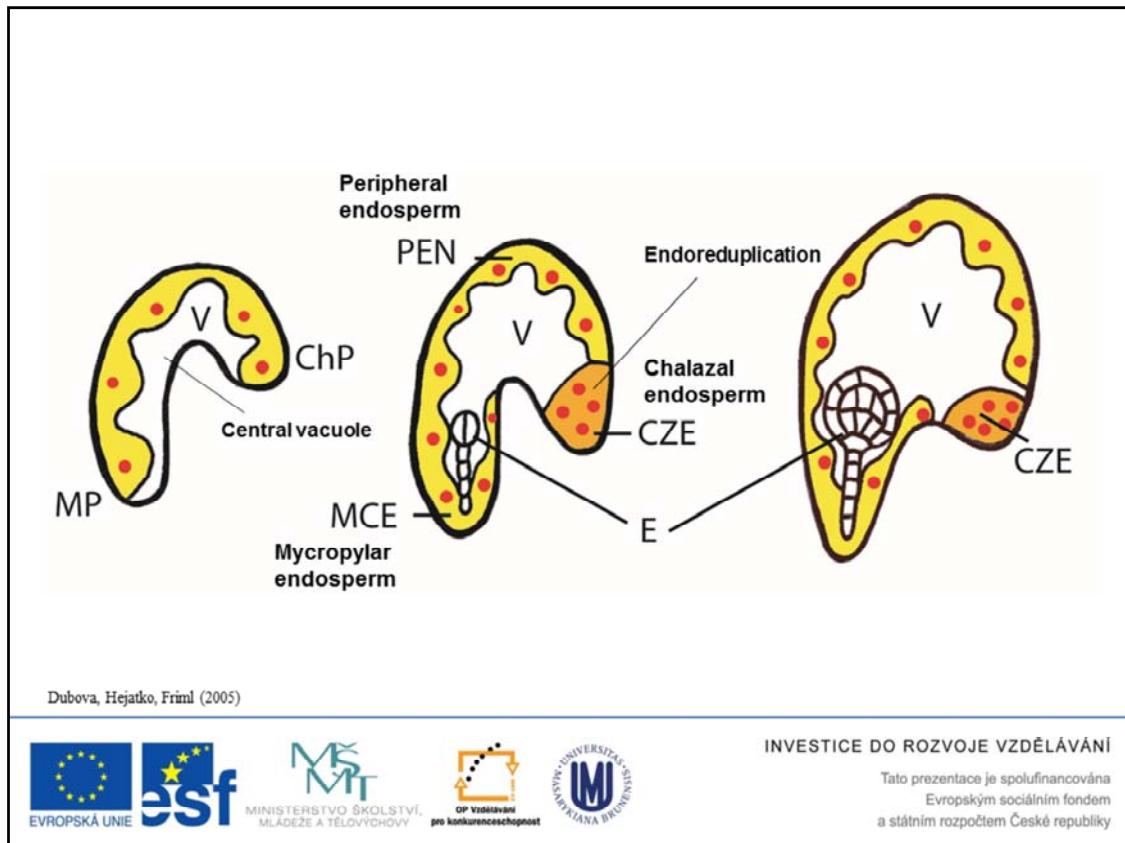
Outline of Lesson 6

Plant Reproduction

Dubova, Hejatko, Friml (2005)

Early after fertilization, zygote is formed and endosperm starts to develop.

The embryo and endosperm both develop within the confines of the maternal tissue (seed coat), but each follows a different developmental program.

Within the embryo, the basic body plan of the mature plant is laid down, whereas the 'life-history' of the endosperm is far shorter and limited to the seed development stage.

Nevertheless, the endosperm fulfills essential functions in nourishing the developing embryo and controlling the whole seed development process. It is also important to note that the endosperm of plants, including cereal species such as rice, maize, wheat, and barley represents one of the most important renewable sources of food and raw materials.

Endosperm development is initiated by the union of the haploid sperm cell and the diploid central cell. Three main types of triploid endosperm can be distinguished depending on coupling between nuclear and cell divisions.

The most common form of endosperm is so called **nuclear-type**, which involves a phase of free-nuclear (syncytial) division that is not accompanied by cytokinesis. The nuclear-type endosperm is the most common type in angiosperms including dicot *Arabidopsis* and monocot cereals.

The young *Arabidopsis* endosperm can be divided into three **domains**: the **micropylar endosperm** (MCE), which surrounds the embryo, the **peripheral endosperm** (PEN), which fills most of the seed volume, and the **chalazal endosperm** (CZE) in the nucelar region.

Nuclear endosperm development is characterized by stages of **syncitial nuclear divisions** (see above) followed by **cellularization** (see the next slide).

The whole syncytial endosperm stage can be divided into nine substages, each representing one of eight rounds of mitotic divisions.

After the initial three synchronous division cycles, the mitotic activity of MCE, PEN, and CZE occurs independently, with nuclei dividing synchronously within domains. Nuclear divisions have never been observed directly in the CZE after the three synchronous rounds of division. Increased intensity of the YTP labeled histone in the CZE nuclei together with the absence of nuclear divisions in CZE suggests that these nuclei undergo endoreduplication.

At the final stage, the syncytial endosperm contains 200 nuclei.

Dubova, Hejatko, Friml (2005)
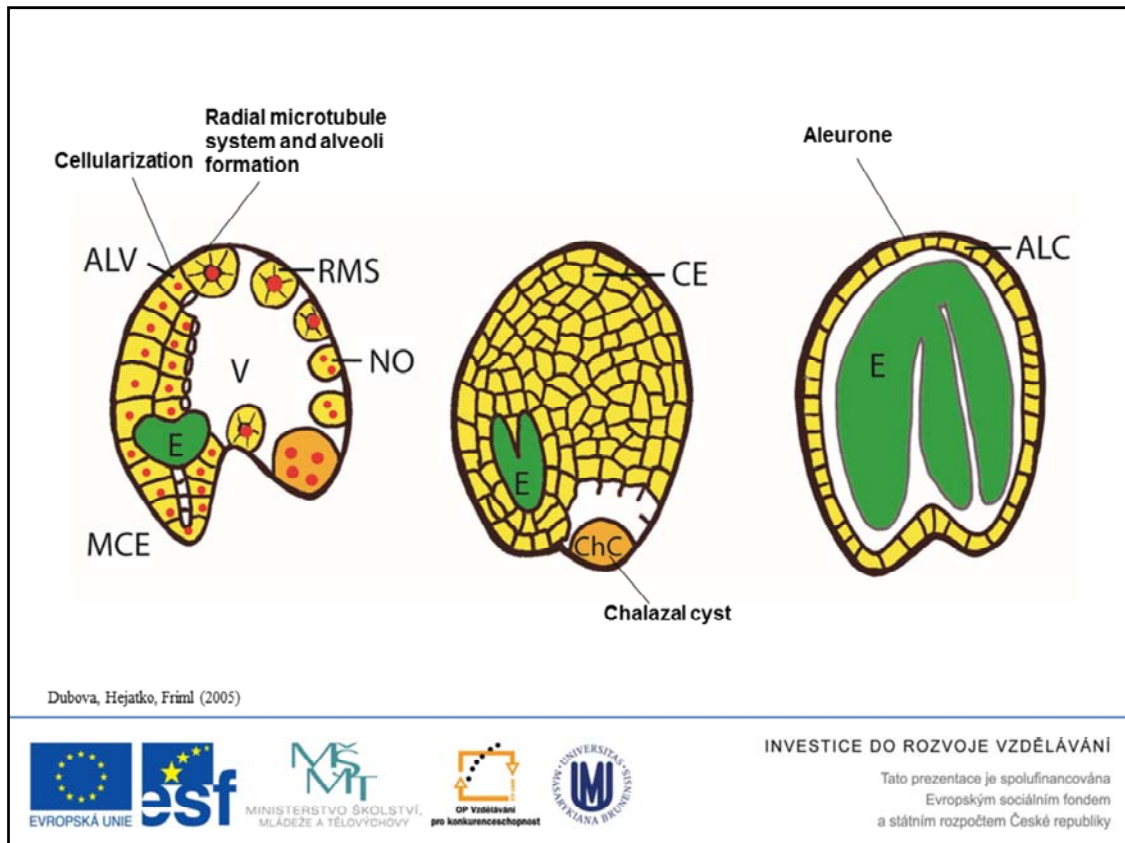
The cellularization process starts as a wave in MCE, progressing through PEN and CZE at different rates and with significant variations between the different parts. When the cellularization around the embryo in MCE is completed, the PEN is still syncytial, whereas CZE remains syncytial until late stages of seed maturation.

Cellularization in *Arabidopsis* as well as cereals involves repeated rounds of formation of a **radial microtubule system** (RMS) emanating from nuclei and enclosure of nuclei with RMS in a tubular cell wall structure (**alveolus**).

In PEN, the process of cellularization starts with the occurrence of RMS at the nuclear surface and subsequent formation of cytoplasmic **phragmoplasts** in interzones between RMS. The phragmoplasts mediate formation of tubular alveolus, which encloses the nucleus with its RMS. Nuclei within the alveoli undergo synchronous periclinal division leading to formation of peripheral cells and internal alveoli.

The cellularization process in MCE also occurs via RMS and cytoplasmic phragmoplasts, but because of spatial constraints, alveoli typically do not form. The cellularization process for *Arabidopsis* results in a completely cellular endosperm except for a small area in the CZE adjacent to the chalazal cyst.

As the *Arabidopsis* embryo grows, the cellular endosperm is gradually depleted, which contrasts with the persistent endosperm of the cereals.

In the mature *Arabidopsis* seed, a massive embryo fills the ovule and only a single peripheral layer (the **aleurone layer**) with unknown function persists. It is assumed that the non-persistent endosperm supports the developing embryo and this support function is later, taken over by the cotyledons during germination.

♀ wt x ♂ *CKI1prom::uidA*

12 HAP | 24 HAP | 48 HAP | 72 HAP

Hejátko et al., *Mol Genet Genomics* (2003)
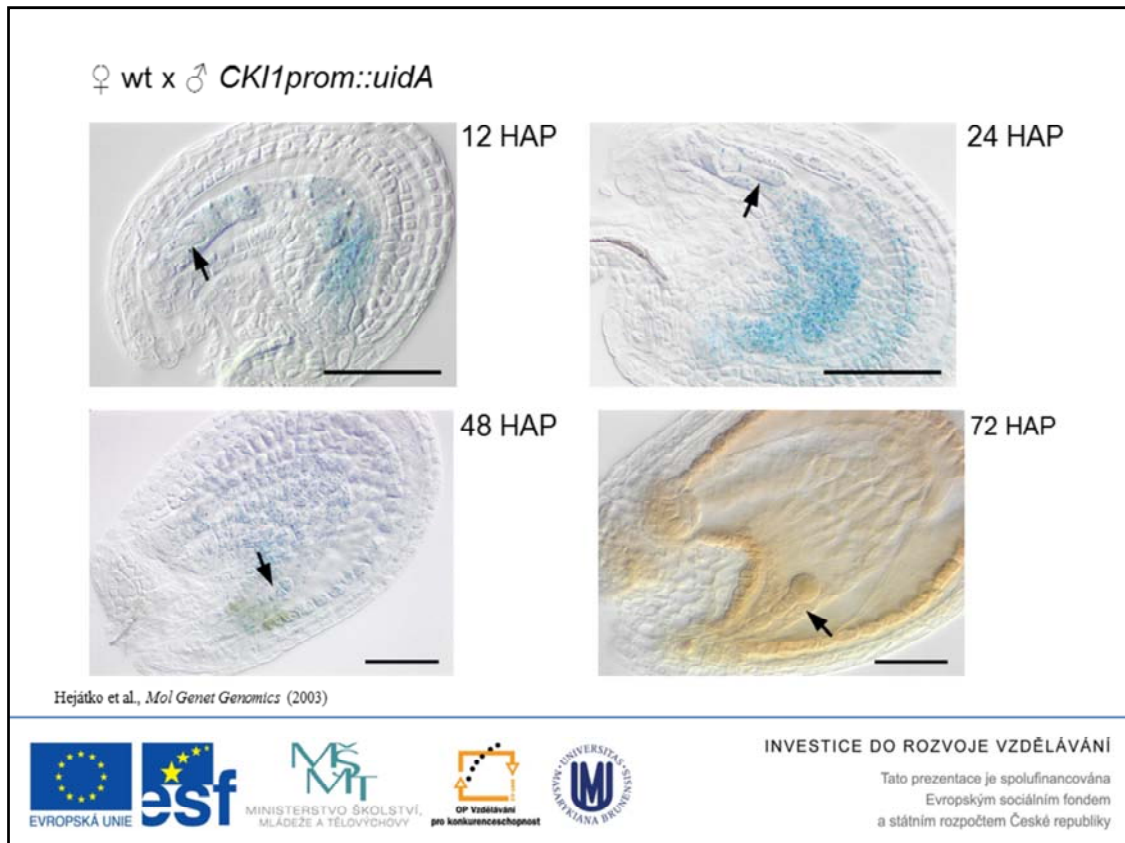
In the early stages after fertilization (approx 6 hours after pollination in *Arabidopsis*), it was supposed that only maternal genome is active and that the paternal genome-wide silencing of expression occurs.

However, together with others, we have clearly shown that this is not the case.

We have used pollen from stable transgenic line carrying *CKI1* promoter (the *CKI1* gene was discussed before) in a transcriptional fusion with *GUS* reporter gene (*ProCKI1:GUS*) and pollinated the pistills of WT (thus, without any *GUS* expression).

As you can see on the figures, the GUS activity was detectable very early after pollination (12 hours after pollination [HAP]).

The activity was reaching its maximum at 24 HAP. At 36 HAP only residual GUS activity was detectable and 72 HAP, no detectable GUS activity was apparent.

Note the developing zygote and later embryo (arrow).

Key Concepts
Plant Reproduction

- In plants, **gametophytic** and **sporophytic portion** ("generation") of the life cycle could be distinguished

- **Initiation of flowering integrates multiple inputs** (light quality/photoperiod, vernalization, autonomous and hormonal signals)

- **Several developmental switches** resulting into **acquisition of floral meristem** and **floral organ identity** take place during onset of flowering. These **switches** are under control of **specific genes** that mutually interact.

- In angiosperms, the gametophyte is reduced to **three-celled pollen tube** (male gametophyte) and mostly **seven-celled female gametophyte** (embryo sac). **Auxin gradient** determines **acquisition of cell identity** during embryo sac development.

- Pollen tube growth is complex process associated with large amount of cell material deposition. **Pollen tube guidance is mediated by specific molecules** allowing **synergid-cell mediated chemotaxis.**

- **Expression of maternal** and **paternal genes** is **tightly regulated** during seed development.