PLANT REPRODUCTION

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Keywords

Auxin gradients
Differential (graded) distribution of the plant signaling molecule indole-3-acetic acid, IAA, which mediates various patterning processes.

Amixis
Asexual propagation of plants by fragmentation, cuttings from stems, leaves and roots, by rhizomes or bulbils. Offspring have identical copies of the parental genome.

Amphimixis
Sexual reproduction including formation of haploid gametes and their fusion.

Apomixis
The asexual formation of a seed from the maternal tissues of the ovule, avoiding the processes of meiosis and fertilization.

Double Fertilization
Fertilization in angiosperms when one sperm fuses with the egg cell and the second sperm fuses with the two polar nuclei. These two events usually result in the formation of the diploid zygote and the triploid primary endosperm nucleus. Ploidy level of the endosperm is determined by the ploidy level of the fusion nucleus, which depends on the type of embryo sac formation.

Embryogenesis
Process that transforms a single-celled zygote into the embryo comprising all the basic pattern elements of the future plant.

Endosperm
Special tissue in angiosperms originating from the triploid primary endosperm cell. The roles of the endosperm in the embryo development are both nutritive and regulatory. Developmental types of endosperm (nuclear, cellular, helobial) are distinguished by differences in nuclear division and cell wall formation. Endosperm can function as a storage tissue of the mature seed (albuminous seeds) or as nutritive tissue for the developing embryo (exalbuminous seeds).

Female Gamete
Egg cell

Gametophyte

Haploid generation producing male and/or female gametes that form a zygote upon fertilization.

Incompatibility
Inability of a fertile hermaphrodite flowering plant to produce a zygote after pollination. Various types of the self-incompatibility prevent inbreeding; cross-incompatibility presents a barrier for remote pollination.

Incongruity
Incompleteness of space and time coordination in the plant reproduction process caused by evolutionary divergence.

Male Gamete
Sperm cell

Somatic Embryogenesis
Formation of embryo-like structures originating from somatic cells. Very rarely spontaneous *in planta*, more often induced in plant tissue cultures.

Sporophyte
Diploid generation producing haploid spores.

Seedling
Juvenile form of plant, which consists of a shoot apical meristem flanked by cotyledons, hypocotyl, and root with root apical meristem. Seedling is a product of seed germination.

Zygote
Product of gamete (egg cell and sperm cell) fusion, the first cell of a new sporophyte generation.

Plant reproduction involves both **asexual propagation** (with various types of vegetative reproduction) and **sexual reproduction**. Alternation of haploid and diploid phases is typical for sexual reproduction. Processes leading to the formation of haploid male and female gamete and their subsequent fusion restoring the diploid state are parts of one plant life cycle. Various types of **apomixis** represent modified forms of asexual reproduction by which seeds are produced without fertilization.

1 Asexual (Vegetative) Reproduction – Amixis

Asexual (vegetative) propagation of plants by fragmentation, bulbils, rhizomes or underground organs like roots and tubers produce offspring that have identical copies of the parental genes. Such propagation allows rapid colonization of a new site and even isolated individuals can reproduce. This ability of vegetative plant reproduction is exploited in

horticultural practice for multiplication of desired clones for a long time. Nevertheless, the development of plant tissue cultures amplified the rate of multiplication and extended the list of organs and tissues, in which induction of organogenesis can be initiated. Furthermore, this approach permitted also the development of structures resembling various stages of regular zygotic embryogenesis from somatic cells and/or pollen grains. ***SOMATIC EMBRYO RECEPTOR KINASE1*** *(SERK1)* is expressed transiently in a small population of embryo competent cells up to the globular stage and seems to be a highly specific signal transduction system of somatic embryogenesis. Most of other genes cloned from somatic embryos of carrot (*DC8, DC59, and EMB1*) are homologous to ***LATE EMBRYOGENESIS ABUNDANT*** (*LEA)* genes. Pollen embryogenesis *in vitro* can be induced by various signals, such as heat-shock treatment or starvation, which can affect organization of cytoskeletal elements and lead to a symmetrical cell division.

2 Sexual Reproduction – Amphimixis

Almost all eukaryotic organisms have evolved a mode of sexual reproduction, permitting the maintenance of the species, but also to increase genetic diversity, which is the basis for adaptation and evolutionary potential. Sexual reproduction is brought about by specialized structures in which the haploid gametes are produced as well as structures and /or mechanisms that ensure the fusion of these gametes. Haploid **male** or **female gametes** are formed in special flower tissues, called **sporangia**, during the processes of **sporogenesis** (formation of haploid spores by meiotic reduction division) and subsequent **gametogenesis** (differentiation of the gametes) (see section 2.3). Somatic chromosome number is restored after gamete fusion – **fertilization -** that results in **zygote** formation. The zygote is the first cell of the next sporophytic generation. Mitotic activity of the zygote and differentiation of these cells leads to **embryo** formation. The mature embryo enclosed within the seed is the structural link between the completed gametophytic generation and the future sporophytic generation of the plant.

2.1 Plant Life Cycle, Alternation of Sporophyte/Gametophyte Generations in Plant Evolution

The eukaryotic life cycle includes events leading to the formation of gametes, as well as the development ensuring their fusion. 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 **sporophytic 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 (self-feeding) gametophyte and a nutritionally dependent sporophyte to a dependent gametophyte and a dominant autotrophic sporophyte. In mosses (Fig. 2.1.1), 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 in the sporangium that remains attached to the gametophyte. Both the gametophyte and the sporophyte of ferns (Fig. 2.1.2) photosynthesize and are thus autotrophic; the shift to a dominant sporophyte generation is apparent. Further reduction of the gametophyte generation during evolution led to the highly reduced gametophyte of angiosperms (Fig. 2.1.3 and the section 2.3), where 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**.

2.2 Structure and Development of Plant Sexual Organs

Molecular mechanisms of flower development have recently been extensively investigated in the model plant *Arabidopsis thaliana*. Although other model plants have been used to study the molecular genetics of flower development (e.g. *Petunia* and *Antirrhinum*), this brief overview will focus on recent models of flower development in *Arabidopsis*.

During vegetative growth, the aerial portion of the vegetative plant organs (**shoot**) is a product of the **shoot apical meristem** (SAM). 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 apical meristem** and (2) formation of individual flowers from **floral meristems**, growing out from the inflorescence. In determinate species, the inflorescence meristem differentiates into a single floral meristem, leading to a formation of a single terminal flower.

Multiple endogenous and environmental stimuli lead to the switch from vegetative to reproductive growth (see below). After bolting is induced, secondary inflorescence meristems, **axillary meristems** are formed giving rise to lateral inflorescences (coflorescences) on the axils of 2-5 **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. The first model predicts that cauline leaves and the axillary meristems are produced from the inflorescence apical meristem simultaneously with the first floral meristems. The later one proposes that cauline leaves and the axillary meristems are products of the "interphase" apical meristem. Recent evidence suggests both genetic and environmental factors control the process and favor the latter possibility under normal conditions, suggesting the existence of **inflorescence**, coflorescence-producing **interphase** (I phase) between the **vegetative**, leaf-producing **phase** (V phase) and the **reproductive**, flower-producing **phase** (F phase). According to this model, not two but three developmental switches would occur during flowering induction in *Arabidopsis*.

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 non-floral**.** 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 (Fig.2.2.1). The floral organ identity genes promote differentiation of floral organ primordia and induce formation of floral organs by activation of organ building genes. Floral organs in *Arabidopsis* (i.e. **sepals, petals, stamens and pistil**) are organized in four concentric whorls (see Fig. 2.2.2). Formation of different organs in each of the whorls reflects interactions of spatially specific gene activities according to the so-called **ABCE model** (Fig. 2.2.3) as described below (section 2.2.4.1).

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.

2.2.1 Transition from Vegetative to Reproductive Growth

The switch from vegetative to reproductive growth is regulated by flowering time genes via four major signaling pathways: **long-day photoperiod/light quality** and **vernalization pathways,** which mediate the influence of environmental factors and the intrinsic **autonomous** and **hormone-dependent pathways**.

2.2.1.1 Long-Day Period and Light Quality

Arabidopsis is a facultative long-day period flowering plant, which means that *Arabidopsis* comes into flower more rapidly when the light period reaches 14-16 hours. However, even under short day conditions (light 8-10 hours) flowering is induced, suggesting the existence of flowering promotion pathways that are independent of the length of the day period (see below). Molecules involved in the perception of the day length are light receptors (e.g. **PHYTOCHROME A** (PHYA) and **CRYPTOCHROME2** (CRY2)), and components of the circadian clock (e.g. **GIGANTEA** (GI) and **EARLY FLOWERING3** (ELF3)). Both of these groups of molecules cooperate by the detection of the day length period and the information is transmitted via cyclic expression of ***CONSTANS*** (*CO*), encoding a putative component of a transcription activation complex that regulates the activity of downstream target genes.

Apart from day length, quality of light also influences initiation of flowering. Under shading, a series of responses known as "shade-avoidance syndrome", including stem elongation and accelerated flowering can be observed. In crowded plant populations, the light is depleted in red wavelengths by chlorophyll of neighboring plants and consequently the ratio of red to far-red light is reduced. In general, red light represses flowering in *Arabidopsis*, whereas far-red light has a flowering promotion effect. The change in light quality is perceived by red/far-red receptors, so-called phytochromes. **PHYTOCHROME B** (PHYB)

acts predominantly in red light perception and mediates inhibition of flowering via repression of ***PHYTOCHOME AND FLOWERING TIME1*** *(PFT1)*, encoding a nuclear protein and putative transcriptional factor (see also 2.2.1.5). PFT1 might promote flowering by indirect activation of both *CO* and ***FLOWERING LOCUS T*** (*FT*, see Fig. 2.2.1). **PHYA** acts primarily in perception of far-red light and together with the blue/ultraviolet A light receptor CRY2, activates flowering. Both PHYA and CRY2 repress *PHYB* and activate *CO* via independent pathways.

2.2.1.2 Vernalization

Vernalization, i.e. extended cold treatment, mimics over wintering under natural conditions and induces flowering in most of the *Arabidopsis* accessions in a quantitative manner. It was found that vernalization leads to changes in chromatin structure or DNA methylation state and subsequent transcriptional inactivation of one of the major floral pathway integrators ***FLOWERING LOCUS C*** *(FLC,* see below). After cold treatment, *FLC* repression persists even at subsequent higher growing temperatures. This "memory of vernalization" involves the participation of **VERNALIZATION2** (VRN2), a putative Polycomb-group protein. Another molecule necessary for the negative regulation of *FLC* by vernalization is **VERNALIZATION-INSENSITIVE3** (VIN3), a plant homeodomain protein, which seems to be involved in the measurement of the cold period.

2.2.1.3 Autonomous Flowering Promotion Pathway

The autonomous pathway controls flowering time independently of environmental factors, i.e. vernalization and photoperiod/light quality. The central role in the integration of different flowering promotion pathways (see below) is played by *FLC*. *FLC* encodes a MADS-box transcription factor and the level of *FLC* product is negatively correlated with the time of the flowering induction. Members of the autonomous pathway induce flowering by repression of *FLC*. ***FLOWERING LOCUS D*** *(FLD)* encodes a protein similar to the component of human histone deacetylase complex and it is supposed to act in the transcriptional control of *FLC* by histone H4 deacetylation. Another member of the autonomous pathway is **FVE**, a putative retinoblastoma-associated protein, and like FLD regulates *FLC* activity by histone H4 deacetylation. **FCA**, a protein with two RNA binding domains and a protein binding domain is supposed to interact with **FY**, a protein with

similarity to yeast RNA processing proteins. FCA/FY complex might be involved in posttranscriptional repression of *FLC*. Similarly, ***FPA***, another gene with a negative effect on the accumulation of FLC, encodes an RNA binding protein. However, the role of RNA processing in the regulation of *FLC* remains unclear.

2.2.1.4 Plant Hormones

The plant hormone **gibberellic acid (GA)** promotes flowering in *Arabidopsis* under short day conditions. In the absence of GA, *Arabidopsis* is an obligate long-day flowering plant. Genes involved in the biosynthesis (e.g. ***GA REQUIRING1*** (***GA1)***) and perception of GA (e.g. ***FLOWERING PROMOTIVE FACTOR1*** *(FPF1)*) are known to be involved in the gibberellin-dependent regulation of flowering time in *Arabidopsis*. **Abscisic acid**, another plant hormone, plays a role opposite to that of GA in flowering promotion, i.e. it delays flowering. Mutants with lowered biosynthesis of abscisic acid and mutants in genes involved in abscisic acid signaling (e.g. ***ABA INSENSITIVE1*** (***ABI1)*** and ***ABI2***) flower earlier under non-inductive conditions. Other plant hormones (e.g. cytokinins and ethylene) are also implicated in the regulation of flowering induction, however, the molecular mechanism of their action in flowering induction and interaction with other flowering promoting factors is largely unclear.

2.2.1.5 Integration of Flowering Promotion Signals

All of the above-mentioned signals are integrated in a complex network (Fig. 2.2.1), leading to the final regulation of downstream targets, the meristem identity genes (e.g. LFY, AP1, TFL1). There is quite a small group of factors in *Arabidopsis*, integrating multiple pathway outputs, so-called **floral pathway integrators** (e.g. *FLC*, ***SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*** *(SOC1), FT* and *LFY*, see above and Fig. 2.2.1). *LFY* plays a special role as one of the flower pathway integrators and together with *AP1* belongs to the floral meristem identity genes (see below). *FLC* integrates outputs mediated by both flowering repressing factors (e.g. ***FRIGIDA*** *(FRI)*, ***PHOTOPERIOD INDEPENDENT EARLY FLOWERING1*** *(PIE1)*) as well as flowering promoting factors (e.g. ***FLD, FCA***, see above and Fig. 2.2.1). *Arabidopsis* accessions with functional *FRI* allele overwinter in the vegetative state and after vernalization they flower in spring. Allelic variations found in *FRI* correspond to the variations in flowering time found among different accessions of

Arabidopsis. *FRI* encodes a protein, which is suggested to be involved in the interactions with DNA or other proteins and FRI inhibits flowering by upregulating *FLC*. This effect cannot be overcome even by the strong flowering promoting effect of a long-day photoperiod. PIE1, a protein with similarity to ATP-dependent chromatin remodeling proteins, also delays flowering by positive regulation of *FLC*. *SOC1*, encoding a MADS-box protein (for more details on MADS-box proteins see below) and a flowering activator, is directly repressed by FLC via a MADS binding sequence in the *SOC1* promoter. *SOC1* is also activated by CO, and gibberellic acid. The antagonistic effects of FLC and CO on *SOC1* are mediated by the same 351-bp promoter sequence of *SOC1*. *LFY* integrates the outputs from long-day period and GA pathways by separated *LFY* promoter elements. The effects of long-day period on *LFY* might be mediated by SOC1 or another MADS-box protein, **AGAMOUS-LIKE24** (AGL24). GA might influence *LFY* via activation of ***AtMYB33***, encoding a MYB-like transcription factor that binds to the 8-bp sequence identified in the *LFY* promoter. Finally, *FT* integrates positive control from the long-day photoperiod pathway mediated by *CO* and negative control from multiple pathways integrated by *FLC*. The inhibition of flowering under shading by the PHYB pathway mentioned in section 2.2.2.1 is also integrated by *FT*, as PHYB downregulates *FT* via repression of *PFT1*, encoding a nuclear activator of *FT* expression. Expression of *FT* could also be affected by GA; that process might be repressed by **EARLY BOLTING IN SHORT DAYS** (EBS), a protein suggested to be involved in chromatin remodeling. For a scheme summarizing the above-mentioned interactions see Fig.2.2.1.

2.2.2 Floral Meristem Identity Acquisition

The final downstream targets of the above-mentioned interactions of flowering time genes are *LFY* and *AP1*, the floral meristem identity genes. The regulation of *TFL1*, a member of the shoot meristem identity group of genes, is still unclear. *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. That 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.

2.2.3 Flower Formation

2.2.3.1 Acquisition of Floral Organ Identity

Individual flowers are formed by floral meristems growing out from the lateral parts of the inflorescence meristem. Once flower meristem identity is acquired; the prerequisite for proper flower development is spatially delimited expression of flower organ identity genes. This finding led to the postulation of the so-called **ABCE model**, which explains the proper formation of *Arabidopsis* floral organs as a result of interactions among ABCE gene classes (see Fig. 2.2.3). *Arabidopsis* floral organs are organized in four concentric whorls. There are four sepals in the most outer whorl 1, four petals in whorl 2, six stamens (four longer medial and two shorter lateral) in whorl 3 and two carpels in whorl 4; the two carpels fuse to form a bilaterally symmetrical gynoecium (pistil, see Fig. 2.2.2). The ABCE model postulates, that activity of class A genes alone specifies sepals in a flower whorl 1, A+B specifies petals in whorl 2, B+C specifies stamens in whorl 3 and C alone specifies carpels in whorl 4. Genes of class E were additionally found to be necessary for the proper identity of petals, stamens and carpels (see Fig. 2.2.3). Function of ABC genes is specific to the flower meristem, however in combination with class E genes, they are able to transform even vegetative organs (e.g. leaves) into floral organ-like structures. With the exception of **APETALA2** (AP2), a member of a small plant-specific family of transcription factors, products of ABCE genes (see Tab. 2.2.1) are MADS transcription factors. The MADS transcription factors are analogs of animal homeiotic genes, which, instead of homeodomain, contain the so-called **MADS-box**, found in **M**CM1 (yeast), **A**GAMOUS (*Arabidopsis*), **D**EFICIENS (*Antirrhinum*) and **S**ERUM 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 (see section 2.3.2.1.1).

2.2.3.2 Activation of Floral Organ Identity Genes

The major activators of floral organ identity genes are *LFY* and *AP1*. As mentioned above, *LFY* and *AP1* are flower 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 **UNUSUAL FLOWER ORGANS** (UFO) co-activate *AP3*: 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 (see Fig. 2.2.1). Proper function of B-class genes in petal formation is also dependent on *AP1*, probably via AP1 activation of *UFO* by promoting *UFO* mRNA accumulation at the base of petals in whorl 2 late during flower development (stage 5, see section 2.2.3.4). The central role of LFY in the activation of floral organ identity genes is further supported by its role in co-activation of 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 (see Fig. 2.2.1). After *AG* is activated by LFY/WUS, AG in turns represses *WUS* in whorls 3 and 4. WUS promotes the proliferative state of meristem cells and absence of the feedback in *ag* mutants leads to the indeterminate flower phenotype.

2.2.3.3 Interactions Among ABCE Genes

There are two major types of interactions among ABCE genes during flower formation. First, they co-operate in the specification of precursor cells to form individual flower organs as mentioned in previous sections. Second, after initial activation of the floral organ identity genes, the fine tuning of their expressional patterns is achieved by mutual repressive interactions between A and C class genes; the territorial competition is also-called **cadasteral activity**. The molecular nature of those two types of interactions will be briefly described in following sections.

2.2.3.3.1 Multifactorial MADS-box Protein Complexes

The vast majority of ABCE genes encode type II MADS-box proteins. Besides an N-terminal MADS-box, a domain with DNA binding, nuclear targeting and dimerization functions, there are two additional conserved domains in most of the ABCE proteins: a K-domain, responsible for protein-protein interactions and a C-domain, involved in transcriptional activation. The recent **quartet model** suggests formation of tetramers, composed of two pairs of MADS-box proteins, homo- or heterodimers, each of which binds to a single MADS binding site. There are two possible models, explaining the molecular

mechanisms of functionality of those complexes. One suggests synergistic effects of individual subunits on the affinity of the entire complex to individual MADS binding sites, the other considers functional complementation of MADS proteins lacking transcription activating domains (e.g. AP3) by the transcription activating domain of interacting partner(s) (e.g. AP1). The quartet model implies formation of tetramers consisting of AP3/**PISTILLATA** (PI) and **SEPALLATA** (SEP)/AP1 heterodimers in petals whorl 2, (there are four members of the *SEP* gene family in *Arabidopsis*, SEP1-4), AP3/PI and SEP/AG heterodimers in stamens whorl 3 and AG/AG and SEP/SEP homodimers in whorl 4 (see Fig. 2.2.4). Whether AP1 alone (possibly as a homotetramer) or in combination with any other product of a class A gene is responsible for sepal specification in whorl 1 remains unclear. Similar interactions among floral organ identity genes were found in *Petunia* and *Antirrhinum*. It could suggest that the above-mentioned principles might be a unifying dogma of flower organ development, with some species-specific modifications, e.g. only class B and C genes were identified in *Antirrhinum*. However, it should be noted here that the *in planta* nature of MADS protein complexes remains to be characterized.

2.2.3.3.2 Cadasteral Activity of A and C Genes

AP1 and *AP2* are the earliest class A genes expressed in the floral meristem. *AP1* is expressed throughout the meristem to specify the meristem as floral. Later on (stage 3), the expression of the class C gene *AG* is activated by LFY and WUS (see 2.2.3.2) in whorls 3 and 4, where the expression of *AG* suppresses transcription of *AP1*. Vice versa, *AP2*, another class A gene, inhibits expression of *AG* in whorls 1 and 2. Interestingly, in the case of *AP2*, posttranscriptional regulation via micro RNA (miRNA) leads to the localization of AP2 protein to whorls 1 and 2 (*AP2* mRNA is detectable throughout the flower). Besides class A genes, there are also other, non-MADS genes, working as repressors of *AG* (e.g. ***CURLY LEAF*** (*CLF)*, a homologue of the *Drosophila* Polycomb group protein *Enhancer of Zeste*, ***LEUNIG*** (*LUG)*, which encodes protein with multiple WD-repeats, ***SEUSS*** (*SEU)*, encoding a protein with a glycine-rich domain and showing similarity to animal LIM domain binding coregulators, ***STERILE APETALA*** *(SAP)* and ***AINTEGUMENTA*** (*ANT*), encoding DNA binding protein related to AP2, see also 2.3.2.1.2). Like *AP2*, *LUG, SEU* and *ANT* repress *AG* in whorls 1 and 2.

2.2.3.4 Development of Floral Organs

Development of the *Arabidopsis* flower was classified into 12 stages, as based on the successive appearance of individual landmarks. Stages 1-3 are schematically depicted in Fig. 2.2.1. Flower development starts when a flower buttress arises (stage1) and proceeds to the next stage when the floral buttress is separated by grooves (stage 2). In stage 3, sepal primordia arise that overlie the primordium at stage 4. During stage 5, the petal and stamen primordia become apparent and are enclosed by the sepals at stage 6. During stage 6 the gynoecium starts growth as an open-ended tube. At stage 7, primordia of middle stamens become constricted towards their base, thus defining the future stamen stalks (filaments) and develop locules (stage 8). At stage 9 petal primordia become stalked and all organs undergo rapid elongation. When the petals reach the length of lateral stamens, stage 10 begins. Papillae of stigma appear at stage 11 and the stage 12 starts when petals reach the height of medial stamens. Stage 12 ends with the opening of the flower.

Recently, the molecular factors regulating floral organ development have been identified using expressional studies and mutant analysis. Expression of ***TAP1*** and ***FILAMENTOUS FLOWER1*** *(FIL1)* encoding a secreted protein and a candidate cell wall protein respectively were identified in anthers of *Antirrhinum*. In *Arabidopsis*, ***NAC-LIKE ACTIVATED BY AP3/PI*** *(NAP)* represents the downstream target of B class genes in petals and stamens. ***SHOOT MERISTEMLESS*** *(STM),* ***CUP-SHAPED COTYLEDONS1*** *(CUC1)* and ***CUC2*** are expressed in the so-called medial domain, located at the interface of the fused lateral carpel margins in the pistil. These genes are supposed to be important for maintaining the meristematic state of the medial domain, where later on the **placenta** tissue differentiates and from which the ovule primordia arise. ***CRABS CLAW*** *(CRC)*, ***SPATULA*** *(SPT)* and ***TOUSLED*** *(TSL*, see also section 2.3.2.1.3) are expressed in the medial domain of the pistil and might affect the production of ovules.

2.3 Structure and Formation of Male and Female Gametes

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** (Fig.2.1.3), 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.

2.3.1 Male Gamete Formation

2.3.1.1 Microsporogenesis

2.3.1.1.1 Archesporial Cell Number and Differentiation

The formation of pollen in *Arabidopsis thaliana* is schematically shown in Fig. 2.3.1. In each of four developing locules (microspororangia) formed in the anther, one **archesporial cell** differentiates. 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 AG (see also section 2.2.3.2). The number of archesporial cells in *Arabidopsis* seems to be determined by the activity of ***EXTRA SPOROGENOUS CELLS/EXCESS MICROSPOROCYTES1*** *(EXS/EMS1),* encoding a putative Leu-rich receptor (LRR) kinase; in *exs/ems1* plants multiple archesporial cells per microsporangium are formed. A similar phenotype was found in mutants in ***TAPETAL DETERMINANT1*** *(TPD1)*, encoding a putative secreted protein. Whether TPD1 is involved in the same signaling pathway, perhaps as a ligand of EXS/EMS1, remains to be clarified. The ortholog of *EXS/EMS1* ***MULTIPLE SPOROCYTE1*** (*MSP1*) was identified in rice and mutants in maize ***MULTIPLE ARCHESPORIAL CELLS1*** *(MAC1)* also resemble *exs/ems1* plants. The archesporial cell divides to form a **primary parietal cell** and a **primary sporogenous cell**. The primary parietal cells divides periclinally to give rise to the **tapetum**, **endothecium** and the **middle layer** of the anther. The primary sporogenous cell divides and the progeny differentiate into **microsporocytes - pollen mother cells** (PMCs) that undergo meiosis, leading to the formation of tetrads of haploid **microspores**.

2.3.1.1.2 Meiosis

The basic processes during meiotic division I and II in plants do not seem to differ from other eukaryotes. However, in contrast to animals, there are differences in some of the cellular processes accompanying meiosis. In plant microsporocytes, the reduction of rRNA and mRNA together with plastid and mitochondria dedifferentiation and division were observed. The reason for that "purging of sporophytic information" is unclear. It might facilitate later gametophytic development or free the developing microspores of detrimental RNA like viruses and silencing elements.

There are many genes found to affect plant meiosis, but only a few of them have been cloned and characterized in detail. Mutants in ***SWITCH (SWI1)/DYAD*** are defective in both male and female meiosis. Interestingly, male meiosis is unaffected in *swi1-1* and *dyad* mutant alleles, which might suggest distinct processes involved in the male and female germ lines during meiosis in *Arabidopsis*. *SWI1* was cloned, but the putative amino acid sequence of SWI1 does not reveal similarity to any known protein. Based on the phenotype of *swi1* male meiocytes (absence of bivalents during meiosis), the protein is suggested to be involved in establishment of chromatid cohesion and chromosome structure during meiosis. ***AtDMC1***, the *Arabidopsis* homologue of the yeast *DMC1* gene was identified and its expression was found to be specifically associated with meiosis in both pollen mother cells in anthers and megaspore mother cells in ovules. DMC1 in yeast, the homologue of *E. coli* recA, plays a role in homology-dependent paring of chromosomes and chiasmata formation during meiosis. The *Arabidopsis Atdmc1* mutant shows defects in meiosis, however, in contrast to mouse and yeast, meiosis is not completely arrested; ten univalents instead of five bivalents were observed in *Atdmc1* mutant meiocytes. The absence of bivalents results in a formation of non-viable gametes with an aberrant number of chromosomes. The occurrence of double strand breaks (DSB) is a consequence of the *dmc1* mutation in yeast. However, the presence of DSB was not observed in *Arabidopsis dmc1* mutants. That might be explained by either efficient DNA repair systems or that DSBs do not occur in *Arabidopsis* mutant meiocytes. ***arabidopsis homologue pairing2*** (*ahp2)* represents another recently identified *Arabidopsis* meiosis-defective mutant. The *ahp2* mutant plants are sterile, with defects in both male and female gametophyte development. AHP2 is a homologue of the fission yeast MEU 13^{+}. MEU 13^{+} is supposed to be involved in chromosome pairing during early prophase I and monitoring of the sequence homology of the paired chromosomes to either promote homologue pairing or destabilize pairing between non-homologues ones. In good concert with that, the *ahp2* male meiocytes are characterized by the absence of bivalents during prophase I and by the presence

of chromosome fragments, chromatin bridges and unbalanced segregation in anaphase I and II.

2.3.1.1.3 Microspore Cell Wall Formation

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). In *Arabidopsis*, ***TETRASPORE/STUD*** (*TES/STD*) gene was identified, encoding a putative kinesin. In *tes/std* mutants formation of radial microtubule arrays at the end of meiosis is hampered and all the microspores develop in a common cytoplasm. In *tes/std* plants few functional pollen grains are produced revealing different aberrations, e.g. polyploid sperm cells. Interestingly, the recessive nature of *tes/std* mutation, together with the expression of *TES/STD* throughout the pre-meiotic anther, suggests sporophytic control of meiotic cytokinesis in *Arabidopsis*. Recently, the *Arabidopsis* gene ***GLUCANSYNTHASE-LIKE5*** (*AtGSL5*) with similarity to yeast β-1,3-glucan synthase was identified that is supposed to be involved in callose wall formation.

Individual microspores are released by the action of the enzyme **callase,** comprising endo- and exoglucanase activities, which is produced by the tapetum. This step was shown to be essential for male fertility in some species (e.g. *Petunia*). *Arabidopsis* gene ***A6*** is expressed in tapetum and encodes a protein similar to yeast β-1,3-glucanase and represents a candidate that may function in microspore release. Nevertheless, callose degradation was shown to be necessary but not sufficient to release the microspores in *Arabidopsis*. During microsporogenesis in ***quartet*** mutants in spite of normal callose wall formation and dissolution, permanent tetrads are formed, probably as a result of persistent pectic components in the parent cell wall of the tetrad. However, in contrast to *Arabidopsis*, other species (e.g. members of *Juncaceae* and *Oenotheraceae*) produce permanent tetrads that are fully functional.

2.3.1.2 Microgametogenesis

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 (see Fig. 2.3.1). 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**. In species with bicellular pollen (e.g. tobacco), pollen mitosis II occurs after pollen grain germination, within the pollen tube (see section 2.4). In the *Arabidopsis* mutant ***gemini pollen1*** (*gem1*), microgametogenesis is arrested after the first mitotic division. The initial microspore polarization and phragmoplast formation seems to be affected, leading to the formation of two cells that are both able to express vegetative cell-specific markers. *GEM1* encodes a microtubule-associated protein, which was shown to bind microtubules *in vivo* and is possibly involved in the correct positioning of the phragmoplast during pollen mitosis I. The vegetative nucleus and two sperm cells after pollen mitosis II are physically connected, forming a functional assemblage, the male germ unit (MGU). The connection of vegetative nucleus is disrupted in male gametophytic mutants of two classes, ***germ unit malformed*** (*gum*) and ***germ unit displaced*** (*gud*). The penetrance of both of these male-specific gametophytic mutations is complete, suggesting functional importance of the MGU.

2.3.1.2.1 Pollen Grain Wall Formation

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**. The exine is composed of sporopollenin comprising long-chain fatty acids and a minor component of phenolic compounds. Sporopollenin is polymerized from precursors secreted by the microspore and (after

microspore is released from tetrad) by the tapetum. The exine forms a rigid, 3D-coat structure, providing protection against physical, chemical and biological attacks and that one allows attachment to animal pollinators and stigma surface. The exine does not develop over the entire surface of the pollen grain, creating precisely positioned germination apertures or lacunae. The number and positions of apertures could be used as taxonomic characters, e.g. in fossil records. During pollen wall development, the individual layers of the pollen wall are added from the outermost layer inwards, so that the ephemeral callose wall is first deposited on the surface of the developing pollen, followed by the primexine (a precursor of the sexine), the nexine and finally the intine. There is some evidence that genes involved in pollen wall patterning are transcribed premeiotically. However, there are very few mutations identified up to now that affect the patterning of pollen wall formation. In *Arabidopsis* the ***DEFECTIVE IN EXINE FORMATION1*** (*DEX1)* gene was identified, encoding a putative membrane-associated calcium binding protein that is proposed to act as a nucleation site for sporopollenin polymerization.

2.3.1.3 Interactions of Mitochondrial and Nuclear Genes During Male Gametophyte Development

The mitochondria seem to play a key role in male gametophyte development. There are many loci identified in the mitochondrial genome that are associated with so-called **cytoplasmic male sterility** (CMS). The defects co-segregating with CMS loci occur in different stages of the male gametophyte development and range from anther degeneration and a lack of fertile pollen (e.g. *Petunia*) to absence of exerted anthers (e.g. maize) and homeotic transformation of anthers into petal- or bract-like structures (e.g. carrot). Interesting feature of the CMS phenomenon in plants is existence of nuclear located genes, so-called **fertility restorers** (referred to as Rf of Fr). Specific alleles of these genes are able to restore male fertility in CMS lines, suggesting importance of nuclear to mitochondrial genome communication during male gametophyte development. Molecular nature of this communication remains to be elucidated. However, in the last few years substantial progress has been achieved in the understanding of the CMS phenomenon in plants.

2.3.1.3.1 Mitochondrial Loci Associated with CMS and Mechanism of Their Action

Most of the loci identified in CMS are the result of (often multiple) recombination events in the mitochondrial genome, leading to the formation of new open reading frames (ORFs) that could lose regulatory association with their original regulatory sequences. In CMS loci identified up to now, genes for ATP synthase subunits were often found to be involved in such recombination events. As an example, one of the best understood CMS systems in maize could be mentioned in detail. In mitochondrial genome of maize CMS-T (Texas) male sterile line, the existence of *urf13* gene was identified, encoding a 13 kD protein. The locus is a result of multiple recombination events, leading to the fusion of primarily non-coding sequences normally found 3' to 23S rRNA gene that now come under the control of a regulatory sequence of the gene encoding 6-th subunit of ATP-synthase (*atp6*). At the 3' proximity to *urf13,* cotranscribed *orf221* gene was identified, encoding a protein similar to *Arabidopsis* F_0 subunit of ATP synthase. In addition to ATP synthase subunits 4, 6, 8 and 9 coding sequences, other known sequences (e.g. cytochrome oxidase subunit coding regions) and sequences with unknown function were identified in various CMS-associated loci. The molecular nature of the defects caused by CMS associated loci is mostly unknown. It has been found that many of the CMS associated loci encode putative transmembrane domains. A recent hypothesis suggests that mitochondrial membrane could be a potential target for those proteins, as it has been shown in case of URF13, which forms a pore in the mitochondrial inner membrane in the presence of T-toxin produced by fungus *Cochliobolus heterostrophus*. Whether there is a naturally occurring biosynthetic product analogous to fungal T-toxin in young anthers of maize is not known.

2.3.1.3.2 Fertility Restorers, Nature and Mechanisms of Action

The nuclear genes found to act as fertility restorers are mostly loci found to be involved in regulation of mitochondrial gene expression; however there are also genes that seem to work through metabolic effects. The regulation of mtDNA expression could be achieved at both transcriptional and post-transcriptional levels, either by regulation of the mitochondrial transcription profile or protein accumulation. Good examples are *Rf1/Rf2* genes found in the restoration of the above-mentioned CMS-T in maize. *Rf1* acts as a primary CMS-T restorer and affects processing of *urf13* transcripts. *Rf2* is unable by itself to restore fertility in CMS-T lines but it is supposed to remove residual sterility effect of *urf13* in the presence of *Rf1*. *Rf2* encodes a protein with aldehyde dehydrogenase activity and the role of RF2 could be to reduce toxic levels of aldehyde, potentially present as a result of residual *urf13*

expression. In the sequence of other cloned fertility restorer genes, a pentatricopeptide (PPR) motif was often identified (e.g. *Rf* genes from *Petunia*, *Brassica*, *Raphanus* and *Oryza*). PPR proteins were implicated in organelle gene expression in both mitochondria and chloroplasts and they seem to have different functions, e.g. in RNA processing and translation or accumulation of plastid ribosomes.

2.3.2 Female Gamete Formation

There are more then 15 different patterns in the development of female gametophyte in angiosperms based on the variations in cytokinesis during meiosis, the various number of functional megaspores and the number and pattern of subsequent mitotic divisions and cellularization patterns. In more then 70% of flowering plant species the **monosporic, *Polygonum* type** of female gametophyte occurs. That type of female gametophyte development can be found in many biologically and economically important species like *Zea* (maize), *Triticum* (wheat), *Gossypium* (cotton), *Brassica* (e.g. cabbage, kohlrabi, cauliflower, rape, mustard) and *Arabidopsis*. The basics of the *Polygonum* type of the female gametophyte development will be summarized here with an emphasis on the understanding of the entire process at the molecular level.

2.3.2.1 Ovule Development

In angiosperms, the key structure where the pre- and post-zygotic 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. 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**. During ovule development, three anatomically distinguishable and functionally distinct parts of the ovule are formed (see Fig. 2.3.2). 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 (Fig. 2.3.2). 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**.

2.3.2.1.1 Ovule Identity

It seems that there are parallels between ovule and flower patterning and that those two processes share common factors (see also section 2.2). Similarly to flower organ identity acquisition, the identity of ovule primordia and ovule structures (e.g. integuments) is genetically determined and it seems to be a result of complex interactions of factors that might resemble both cadasteral and cooperative interactions found among ABCE genes during flower patterning (see section 2.2.3.3). For example, the balanced activity of *AG*, the potent promoter of carpel identity in *Arabidopsis* and its homologs in *Brassica* and *Nicotiana,* was found to be necessary for proper identity acquisition of ovule primordia. AG was also shown to control *NZZ/SPL,* involved in ovule primordia patterning (see below). In *Arabidopsis*, activity of ***BELL1*** *(BEL1)*, encoding a homeodomain protein is crucial for the determination of ovule integument identity, possibly via negative regulation of *AG*. In this model, the AG-dependent, carpel-promoting pathway overrides effects of integument identity genes in the absence of BEL1. BEL1, together with members of a monophyletic clade of *AG*, the MADS box genes *SHP1*, *SHP2* and *STK,* is necessary for integument identity acquisition and in the absence of these factors integuments are transformed into carpelloid structures, possibly as a result of above-mentioned dominance of *AG*. *SHP1, SHP2* and *STK* have apparently redundant functions in the promotion of integument identity and *SHP1* and *SHP2* can also substitute for *AG* in carpel specification. In *Petunia*, homologs of *Arabidopsis STK* ***FLORAL BINDING PROTEIN7*** *(FBP7)* and ***FBP11*** share a similar expression pattern with *STK, SHP1* and *SHP2* and in plants with lowered expression of both *FBP7* and *FBP11*, the conversion of ovules into carpel-like structures was observed. In *Arabidopsis* the E genes ***SEP1-3*** collaborate with B and C genes in the acquisition of floral organ identity probably via formation of multimeric complexes (see section 2.2.3.3.1). Similar interactions might be involved in the ovule identity acquisition. Expression of *SEP* genes was identified in developing ovules and *SEP1*/*sep1, sep2, sep3* mutants show phenotype alterations similar to phenotype of *stk, shp, shp2* triple mutants (e.g. homeiotic transformation of integuments into carpeloid structures). STK, AG, SHP1 and SHP2 were shown to interact with SEP proteins in

multimeric complexes, suggesting the existence of a molecular mechanism resembling those identified in acquisition of flower organ identity. In line with that, a similar situation was found in *Petunia*, where STK orthologs FBP7 and FBP11 interact with FBP2 and FBP5, putative orthologs of SEP proteins in *Arabidopsis*.

2.3.2.1.2 Ovule Patterning

The developing ovule primordium differentiates into three zones: the most distal nucellus, the central chalaza, where the integuments initiate and the most proximal funiculus. These three pattern units are distinguished very early in ovule development after the non-differentiated protrusion of the mother placental tissue appears. Recently, the first molecular evidence has become available that might explain how the supposed positional information distributed along the proximal-distal axis is interpreted into cell fate determination. In *Arabidopsi*s, 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 (see section 2.3.1.1.1). The expression of ***WUSCHEL*** (*WUS)*, encoding a homeodomain protein was also found to be involved in the positional specification of integument outgrowth (see also section 2.2.3.2). 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. However, as *NZZ* is expressed throughout the developing ovule primordium, the spatial specificity mechanism of its negative regulation of *ANT* is so far unclear.

2.3.2.1.3 Integument Initiation and Growth

Multiple factors, involved in outer integument initiation, regulation of differential integument growth and integument extension were identified. ***INNER NO OUTER*** *(INO)* encoding a YABBY protein transcription factor seems to be a specific determinant of the outer integument outgrowth. Spatial specificity of its expression depends on *ANT*, whose role was discussed in inner integument initiation (see previous section). The expression of *INO* not only seems to regulate outer integument outgrowth along the proximal-distal axis but also along the anterior-posterior (or adaxial-abaxial, recently also-called gynoapical-gynobasal)

axis (see Fig. 2.3.2). *INO* is expressed only on the posterior/abaxial/gynobasal side of the ovule, where the outer integuments are initiated and exhibit extended growth, leading to the typical ovule curvature (Fig. 2.3.2). ***SUPERMAN*** *(SUP)* encoding a putative zinc finger transcription factor was identified to be necessary for this posterior specific location of *INO* expression domain, probably via negative regulation of *INO* positive autoregulation. However, the factors involved in the initial induction of the asymmetrical *INO* expression remain unknown. Additionally, *INO* seems to be necessary for the abaxial identity of the outer integument and its growth.

Several other factors were identified in the coordination of the inner and outer integument growth in *Arabidopsis*. For example *TSL*, encoding a Ser/Thr protein kinase (see also 2.2.3.4), represents a good candidate for the molecule mediating signaling between apparently functional and evolutionarily different tissues of inner and outer integuments. Also factors influencing growth of both inner and outer integuments were identified, e.g. ***DICER-LIKE1*** (*DCL1*, former *SHORT INTEGUMENTS1*) encodes RNase III/RNA helicase that might be involved in the production of small RNAs associated with the translational regulation of developmental genes during integument cell elongation.

2.3.2.2 Megasporogenesis

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 ovule is tenuinucellate, which means that the MMC is in direct contact with the nucellar epidermis. 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 (see also previous sections).

Cytokinesis in *Arabidopsis* occurs after meiosis is completed, which results in the formation of a multiplanar or linear tetrad. Molecular control of meiosis that is mostly

common for both male and female meiocytes was already discussed previously (see section 2.3.1.1.2). 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.

2.3.2.3 Megagametogenesis

Megagametogenesis follows megasporogenesis, which ends with the formation of the functional megaspore (see previous section). Recently, megagametogenesis in *Arabidopsis* was described in detail. Based on the occurrence of anatomically distinguishable traits, the entire process was classified into 7 distinct steps FG1-FG7 (see Fig. 2.3.2). 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. 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 (see sections 2.4 and 2.5). 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.

2.3.2.4 Molecular Nature of Female Gametophyte Development

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 in the last decade, 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 (see also section 2.5).

First gametophytic mutation with impact on female gametophyte development, which was characterized at the molecular level, is ***gametophytic factor2*** *(gfa2)*. The *GFA2* gene encodes a J-domain containing protein targeted to mitochondria. *GFA2* was shown to partially complement the yeast mutation *mitochondrial DNAJ homolog 1(mdj1)*, a gene for a chaperone in the mitochondrial matrix and *GFA2* is supposed to be an *Arabidopsis* ortholog of the yeast *Mdj1p*. The *GFA2* gene was found to affect both female and male gametophyte. The penetrance of *gfa2* in the female gametophyte is almost complete and a substantial part of the male gametes is affected (99 and 86%, respectively). Female gametophyte development in

gfa2 embryo sacs is terminated at FG5. Synergids of The mature female gametophyte were suggested to be a source of the diffusive attractants mediating signaling that directs the pollen tube into the micropyle (see also section 2.4.1). In *Arabidopsis*, cell death of one of the synergids occurs as a consequence of pollen tube entry into the micropyle or just before pollen tube arrival (for more details see section 2.4.2). However, pollen tube entry did not induce synergid cell death in *gfa2* mutants. In spite of incomplete megagametogenesis that prematurely terminates at FG5, the *gfa2* mutant ovules were still able to attract pollen tubes. That might suggest functional independence of synergid cells in the final stages of megagametogenesis, at least in terms of their functionality in pollen tube attraction. The other female gametophyte mutant well characterized at the molecular level is ***nomega***. The ovules in *nomega* mutants were found to be arrested at the FG2 phase of megagametogenesis. The *NOMEGA* gene was found to encode a homolog of the APC6/Cell Division Cycle (CDC) 16 subunit of the Anaphase Promoting Complex/Cyclosome (APC/C) that is a part of the APC/C complex, which together with the SCF pathway represents a key regulatory component in cell cycle regulation by ubiquitin-dependent proteolysis in yeast. The APC/C complex was found to be involved in the degradation of cyclins A and B in clam and *Xenopus*. In the *nomega* mutant embryo sacs, the absence of cyclin B degradation was shown. The APC/C complex was also found to be involved in the earlier checkpoint of the cell cycle, the degradation of the anaphase inhibitor securin, thus facilitating the separation of chromatids. Therefore a functional APC/C complex seems to be necessary for cell exit from mitosis. The ability of the *nomega* mutant embryo sacs to accomplish the first mitosis is explained by the residual activity of the APC/C originating from the MMC, which is sufficient for metaphase to anaphase progression. The later checkpoint allowing exit from first mitosis and leading to the formation of the two-nuclear embryo sac might be overcome by inactivation of the CDK1 mitotic complex by phosphorylation. The absence of central vacuole formation in the *nomega* mutants remains unclear.

2.3.2.5 Sporophyte to Gametophyte Communication During Female Gametophyte Development

In contrast to fucoid algae, where the polarity of the naked zygote is dependent on external environmental signals, embryo sac polarity in *Arabidopsis* is fully position dependent. Based on current genetic data, an interregional interaction between the developing female gametophyte and surrounding sporophytic tissue representing two consecutive

generations seems to be crucial for proper embryo sac assembly and development. Presence of strong mutant alleles of *bel1*, *sin 1* and *ant* genes, involved in the formation of the ovule sporophyte (see also 2.3.2.1.2), results in the early arrest of embryo sac development in *Arabidopsis*. Further, despite the fact that the ovule sporophyte and female gametophyte tissue development has been genetically dissected, the presence of at least inner integument seems to be a prerequisite for successful embryo sac development. That might suggest either functional dependency and/or common check point, respectively, linking female gametophyte and ovule sporophyte development. A role for positional cues transmitted by sporophytic tissue adjacent to the developing embryo sac was suggested in the regulation of female gametophyte development.

Mutations in ***CYTOKININ INDEPENDENT1*** (*CKI1)* result in the abortion of megagametogenesis at transition from FG5/FG6. Degeneration of chalazal nuclei at FG4 and distortion and later degradation of the central vacuole, coupled with mispositioning of some of the nuclei at FG5 *III* represents one of the first anatomically distinguishable traits in the *cki1* mutant embryo sacs. In terminal *cki1* mutant phenotypes, pleiotropic effects were observed, including different degrees of embryo sac degradation and increased number of nuclei. Thus the *cki1* mutant phenotypes might imply importance of the central vacuole for the timing and spatial distribution of specific cellular events, e.g. mitosis during megagametogenesis. CKI1 is a putative receptor histidine kinase that belongs to the two-component signaling system and it is presumed to act in communication between ovule sporophyte and female gametophyte generations in *Arabidopsis*. However, despite various experimental efforts, the signaling molecule recognized by CKI1 remains unknown.

2.4 Angiosperm Fertilization

Double fertilization in flowering plants involves a complex series of interactions between gametophytes and sporophyte, which results in the fusion of sexual cells and nuclei.

2.4.1 Progamic Phase

2.4.1.1 Sporophyte – Gametophytes Interactions

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. In some species, the style remains hollow, with only one layer of secretory tissue lining the inner surface of the cylinder. 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 coevolved 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**.

2.4.1.2 Stigma Types and Their Role in Pollen Attachment and Hydration

Appropriate temporal coordination of stigma development is crucial for receptivity, which is defined as the ability to attach pollen by adhesion, to facilitate pollen hydration and subsequent germination, leading to the formation a pollen tube. Two basic types of the stigma influence the mode and place of the first sporophytic cell - gametophytic cell interaction. **Dry stigmas** are characteristic with surface cells protruding as papillae, which are covered by a primary cell wall, a waxy cuticle and proteinaceous pellicle. During the receptive stage the cuticle is broken either by visiting insects, due to the activity of some enzymes such as esterases or cutinases or by increasing stigma cell turgidity. The surface of **wet stigmas** is covered by a viscous layer of exudates containing lipids, proteins, polysaccharides and pigments produced by the surface cells or by cells of the secretory zone of the stigma. A thin layer of water is found beneath this viscous layer.

Pollen grains are shed either in a bicellular or in a tricellular condition and are transferred to stigmas by pollinators either biotic (such as insects, birds or animals) or abiotic (mainly wind, and less frequently water). The first interactions of the proteins and lipids of the pollen coat and proteins of the stigma surface follow pollen capture mediated by the structure of exine. A cysteine-rich protein with similarity to lipid transfer proteins called **STIGMA/STYLAR Cys-RICH ADHESIN** (SCA) and pectic polysaccharide are required for pollen tube adhesion in lily. In *Brassica* with a dry stigma, ***S-LOCUS-RELATED PROTEIN*** *(SLR1*) is a stigma-expressed gene related to the S-glycoprotein, which is involved in self-incompatibility (see section 2.4.3).

Recently, general similarities were found in the pollination of dry and wet stigmas; the presence of lipids modulates water transfer to the desiccated pollen and tube penetration, while highly diverse proteins and peptides mediate self and foreign pollen recognition as well as pollen tube growth. Hydration of pollen grains, which are dehydrated and metabolically quiescent when they are released from anthers, seems to be actively regulated on stigmas. Hydration induces pollen grain activation, subsequent germination and pollen tube formation. Ultrastructural rearrangements during pollen activation are mainly connected with the endomembrane systems, such as expansion of ER, appearance of dictyosomes and secretory vesicles. Polarized migration of organelles to the domain of pollen aperture is observed just before pollen tube emergence. The cytoplasm of the vegetative cell exhibits a rotational or streaming movement and passes into the tip of pollen tube followed by the movement of the MGU (see section 2.3.1.1). Pollen tube penetration of the stigma surface is controlled by means of various enzymes. Activities of numerous hydrolases, such as acid phosphatase, esterases (e.g. cutinase), pectate lyase, ribonuclease and protease, were localized to the walls of pollen grains and tubes; while cutinase inhibitors that can significantly reduce the ability of the pollen tube to penetrate the stigma were detected in stigma cells. Enzymatic penetration of the stigma is strictly controlled, which demands communication between pollen tube and the stigma. Receptor kinases are supposed to be mediators of such communication [e.g. ***Lycopersicum esculentum*** **PROTEIN RECEPTOR KINASE1** (LePRK1**)** and (LePRK2)]. The pollen specific cysteine-rich protein **LATE ANTHER TOMATO52** (LAT52), which is essential for pollen hydration and germination *in vitro* and for normal tube growth, interacts *in vivo* with LePRK2. Other proteins like **PISTIL POLLEN ALLERGEN-LIKE** (PPAL) protein with similarity to β-expansin, **LIPID TRANSFER PROTEIN** (LTP) or SCA were

detected in stigma exudates. These proteins are members of a signaling system that regulates pollen adhesion, germination and pollen tube growth.

2.4.1.3 Pollen Tube Growth

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.

The growth of pollen tubes through the style is promoted by products of the secretory cells in the transmitting tissue of the central core of solid (closed) styles or those of the canal lining in hollow (open) styles. The cells of the transmitting tissue form a continuous connection with the cells of the stigma sectretory zone. Longitudinal walls of the transmitting tissue cells are separated by extracellular matrix that contains a mixture of sugars, proteins and phenolic compounds. Hyp-rich glycoproteins (HRGPs) like arabinogalactan proteins, **PISTIL-SPECIFIC EXTENSION-LIKE PROTEIN** (PELP), **TRANSMITTING TISSUE-SPECIFIC** (TTS) and **GALACTOSE-RICH STYLE GLYCOPROTEIN** (GaRSGP) form an intercellular matrix that serve as a source of nutritive and regulative compounds for tobacco pollen tube growth. A gradient of TTS protein glycosylation in the styles of tobacco or a gradient of γ-aminobutyric acid (GABA) in *Arabidopsis* pistil could function as possible mechanisms for the control of pollen tube growth. Pollination of the stigma also triggers a cascade of responses in distal flower organs. It was found that ethylene and its biosynthetic precursor 1-aminocyclopropane-1-carboxylate (ACC) play an important role in the regulation of these responses. Analysis of ACC metabolism showed that pollination induces high ACC-oxidase transcript levels in all cells of the transmitting tissue, while the ACC-synthase transcripts accumulate in a wave-like pattern in which the peak coincides with the front of the ingrowing pollen tube tips. This wave of ACC-synthase expression can be also induced by incongruous pollination and partially by wounding indicating that pollen tube invasion displays similar features to wounding and such effects

might be recruited for evoking the post-pollination response. Barriers preventing incorrect pollination negatively affect pollen tube growth in the pistil (see section 2.4.3.).

Within the ovary, the pollen tube is guided on the surface of the placenta and the funiculus toward the micropyle of the **receptive ovule**. The most common way of pollen tube entry to the embryo sac located inside the ovule is through the opening in the ovule integuments, the micropyle**.** Attraction signals for pollen tube growth could include calcium accumulation in the synergid during embryo sac maturation, as well as other chemotropic signals created near micropylar region by various ovule structures (e.g. micropylar cells of the nucellus, the inner integument or the filliform apparatus of synergids). Presence of proteins, RNA and carbohydrates has been demonstrated in the micropylar region. Thus, it can be concluded that numerous checkpoints exist along the whole pollen tube pathway, which provide active signaling that guides the pollen tube to its destination, the embryo sac.

2.4.2 Syngamic Phase: Karyogamy and Plasmagamy, Double Fertilization

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.

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.

Double fertilization 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. Formation of MGU could significantly influence sperm cell traffic.

2.4.3 Pre-Zygotic and Post-Zygotic Barriers to Fertilization

Various physiological or genetic barriers can preclude successful fertilization. Male or female sterility, which arrest normal development of gametes, is one example of such a genetic barrier. Furthermore, other mechanisms called **incompatibility** exist, which are defined as the inability of functional gamete fusion. Many types of intraspecific and interspecific incompatibility have developed in plants to prevent inbreeding on the one hand (self-incompatibility) and on the other to maintain stability of species preventing gene flow between unrelated species (cross-incompatibility, incongruity). These reproductive strategies can function either as pre-zygotic barriers that inhibit pollen tube growth or as post-zygotic barriers causing embryo abortion.

2.4.3.1 Self-Incompatibility

The widely distributed mechanism of the pre-zygotic barrier named **self-incompatibility** (SI) is an inherited phenomenon, by which plants can recognize and reject their own pollen by inhibiting pollen hydration, germination and/or tube invasion and thus block the delivery of sperm cells to the ovary and prevent inbreeding. Variants of self-incompatible systems were classified according to different aspects. Based on flower morphological differences, heteromorphic, and homomorphic types of SI, are distinguished. **Heteromorphic SI** is characteristic by floral polymorphism. Flowers of species with

heteromorphic SI vary in position, size or shape of the reproductive floral organs. Nevertheless, most of the self-incompatible plants are of a **homomorphic type**. Plants with this type of SI produce morphologically identical flowers. Homomorphic SI is further divided into sporophytic and gametophytic types according to determination of successful pollen phenotype. In **sporophytic SI**, the pollen phenotype is determined by the genotype of parent; while in **gametophytic SI**, the pollen phenotype is determined by its own genotype. A single locus with multiple alleles named the S-gene (***STERILITY)*** represents the genetic basis of self-incompatibility. Later, two separate genes at the S-locus were distinguished that control male and female specificities. In sporophytic SI, pollen rejection occurs on the stigma surface when the pollen parent has one or both S-haplotypes in common with the pistil, independently of the S-haplotype of the pollen grain. Inhibition of pollen tube growth in case of more frequent gametophytic self-incompatibility occurs mainly in the upper part of the style in case when S-haplotype of the pollen matches either one of the two S-haplotypes in the pistil. Gametophytic SI in the *Poaceae* family is genetically controlled by two unlinked multi-allelic loci S and Z. Alleles of both S and Z need to match in the pollen and pistil to reject pollen.

2.4.3.2 Mechanisms of Self-Incompatibility

It is now known that the common phenomenon of self-incompatibility is not realized uniformly by all flowering plants, since several biochemically distinct mechanisms of S-locus expression were revealed among different families. (1) Gametophytic SI mechanism in *Solanaceae*, *Rosaceae* and *Scrophulariaceae* involves **S-RNase**-mediated degradation of RNA in own pollen tubes. Several variants of a receptor or inhibitor models explaining this mechanism were proposed. (2) The *Papaveraceae* gametophytic SI mechanism represents **S-protein**-mediated signal transduction cascade *via* Ca^{2+}, phosphoinositides, protein kinase and phosphatases in pollen tubes. Actin cytoskeleton was found to be a target for signaling pathways involved in this SI response. (3) Mechanism of the sporophytic SI in *Brassicaceae* is based on the different genes of S-locus that control male and female specificity. Stigma specific proteins **S-LOCUS-SPECIFIC GLYCOPROTEIN** (SLG) and **S-LOCUS RECEPTOR KINASE** (SRK) are associated with self-incompatibility in the pistil, while **S-LOCUS CYSTEIN-RICH / S-LOCUS PROTEIN-11/** (SCR/SP11) represents the pollen specific S protein.

2.4.3.3 Incongruity

Interspecific incompatibility caused by evolutionary divergence is based on the absence of information that leads to an incomplete of spatial and temporal coordination in the plant reproduction process.

2.4.4 In vitro Fertilization

Experimental approaches for the study of fertilization or overcoming incompatibility involve methods of *in vitro* pollination/fertilization such as intraovarian pollination, test tube pollination of segments of the placenta with ovules, and *in vitro* fusion of isolated, single gametes.

2.5 Embryo and Endosperm Development

Fertilization of the female gametophyte in angiosperm plants initiates a process of coordinated development of embryo, endosperm, and seed coat that ensures the production of a viable seed. It is more than a century since **double fertilization** was discovered. 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. 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.

2.5.1 Endosperm Development

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. On the other hand, the **cellular-type** of endosperm develops via a series of mitotic divisions always coupled with cytokinesis. A third possibility is provided by **helobial-type**, in which the proendosperm divides to give rise to two daughter cells, one of which undergoes cellular type of development, whereas the other nuclear type of development.

2.5.1.1 Evolutionary Aspects of Endosperm Development

Interestingly, gymnosperms instead of developing true endosperms, posses characteristically enlarged female gametophytes that accumulate nutrients before fertilization. Thus from evolutionary point of view, the endosperm can be viewed as a functional homologue of the gymnosperm female gametophyte. Many aspects of nuclear-type endosperm development appear to have been repeatedly sequestered from pre-existing mechanisms found in female gametophytes of land plants. Both female gametophytes and nuclear endosperms undergo multiple rounds of free-nuclear divisions forming a multinucleate **syncytium** and later arrangement of nuclei into nucleoplasmic domains via the establishment of radial microtubule arrays. Finally, **cellularization** (formation of cell walls around the nuclei and surrounding regions) is achieved through the formation of tube-like structures termed **alveoli**. Also genetic evidence supports a common mechanism in the regulation of endosperm and female gametophyte since a number of mutants have been isolated, which are affected in both these processes.

2.5.1.2 Development of Nuclear Endosperm

The nuclear-type endosperm is the most common type in angiosperms including dicot *Arabidopsis* and monocot cereals. Due to availability of molecular markers for different endosperm compartments and developmental stages, most of the information on nuclear endosperm development has been obtained from studies in *Arabidopsis*. 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** (Fig. 2.5.1) followed by **cellularization** (Fig. 2.5.2). 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.

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**) (Fig. 2.5.2). 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.

Despite obvious differences in some aspects of endosperm development between *Arabidopsis* and many monocots including cereals, the cellularization processes reveal striking similarities. Importantly, the process of cellularization by RMS formation also occurs during the cellularization process in a number of other systems, including sporogenesis in lower plants, male and female gametophyte development in gymnosperms and angiosperms, and embryogenesis in gymnosperms. Thus, the findings obtained from studies on *Arabidopsis*

and cereals endosperm have more general validity for our understanding of plant development.

2.5.2 Embryo Development

Embryogenesis is a process, which transforms a single-celled zygote into the embryo containing all the basic pattern elements of the future plant. Unlike in higher animals, where the embryo is a miniature variant of the adult, the juvenile form of the plant (**seedling**) show only basic pattern elements, which can be strongly modified by elaborated postembryonic development. The seedlings of different higher plant species are remarkably uniform. They display a main **apical-basal axis** of polarity, with the shoot meristem at the apical end and root meristem at the basal end and the **radial pattern** of tissue layers, which are arranged concentrically. This basic embryonic pattern is then postembryonically elaborated mainly by the activity of the **meristems** (permanent stem cell populations) and *de novo* organogenesis, which can lead to a great increase in architectural complexity and which generates most species-specific features of the adult plant.

2.5.2.1 Pattern Formation in *Arabidopsis* Embryogenesis

In the progression of plant embryogenesis, three main patterning events can be distinguished: the generation of the apical-basal axis, formation of radial pattern and establishment of bilateral symmetry. In different higher plant species, the mature embryos show the same basic patterning elements, however, the early pattern of cell division during embryogenesis is not uniform beween species. Therefore, it is unclear how important the early patterning events are. By far, most of the information on mechanisms of embryonic patterning come from the studies in *Arabidopsis* and other *Brassicaceae*. These species display an exceptionally regular and reproducible pattern of cell divisions during embryo development.

The apical-basal pattern in *Arabidopsis* has been traced back to the earliest stages of embryogenesis. The zygote has an asymmetrically positioned nucleus with a vacuole, which elongates in a polar manner at the apical end. The zygote divides asymmetrically and produces a small **apical cell** and a larger **basal cell**. The apical cell divides vertically and generates the '**proembryo**', which later gives rise to most regions of the seedling including the shoot meristem. The basal cell continues to divide horizontally and produces the

suspensor - a single file of cells that attaches the proembryo to maternal tissue. At the early globular stage, the uppermost cell is specified to become the **hypophysis** – the founder of the root meristem. Thus both apical and basal ends of the future seedling are established. This initial apical-basal subdivision is also reflected molecularly: for example genes encoding the transcription factors **AtML1** or WUS are expressed exclusively in the apical cell lineage whereas specific arabinogalactan epitopes or a regulator of auxin transport **PIN-FORMED7** (PIN7) were detected in the basal cell lineage of early embryos.

The formation of the radial pattern initiates at the eight-cell stage of the proembryo. These cells divide periclinally and set apart the primordial epidermis (**protoderm**). At the next stage, the inner cells divide vertically producing outer ground tissue and inner provascular cells. Thus the globular embryo is composed of a radial pattern consisting of three concentric cell layers that later develop into epidermis, ground tissue (endodermis and cortex) and vascular tissue of the seedling. The early marker of embryonic ground tissue is the transcription factor **SCARECROW** (SCR), which has been shown in roots to be required for endodermis specification. Interestingly, SCR transcription is regulated by a homologous factor **SHORTROOT** (SHR), which moves into the endodermis from the cells of vascular bundle, where it is expressed.

At the early heart (triangular) stage, early patterning is completed with the establishment of bilateral symmetry, which is manifested by the initiation of two symmetrically positioned embryonic leaves - **cotyledons**. The initiation of cotyledon primordia is preceded by production of transcription factor AINTEGUMENTA (ANT) that is required for maintaining the proliferative cell state during various organogenesis processes.

The early patterning is followed by a phase of growth and elongation of organs that merely elaborate already pre-laid architecture. The next and last phase of embryo development is characterized by accumulation of storage reserves that include lipid, starch and protein bodies within embryo cells. Hereafter, the seed dries and become separated from the mother plant. After seed dormancy is overcome, germination occurs and the embryo gives rise to a seedling.

2.5.2.2 Lessons from Embryo Mutant Screens

The mechanisms, which govern patterning processes in *Arabidopsis* embryogenesis, have been addressed mainly using genetic approaches. In mutant screens for genes encoding

the regulators of embryo development, mutants were categorized by seedling phenotypes and by early embryonic division patterns.

2.5.2.2.1 Morphogenetic Embryo Mutants

Many of the mutants recovered from these screens were found to be involved in basic cellular processes that only subsequently affect pattern formation. Cytokinesis mutants such as those in the ***KNOLLE*** *(KN)* gene (encodes a cytokinesis-specific syntaxin) or its interactor ***KEULE*** *(KEU)* (the yeast Sec1 homologue) fall into this category. Both these mutants are affected in vesicle fusion in the plane of cell division, which results in incomplete cell walls and multinucleate cells.

Other mutants are defective in the proper orientation of the plane of division rather than in cell division per se. These are, for example, *fass* mutant embryos, which are made up of irregularly shaped and enlarged cells. ***FASS***/***TONNEAU2*** *(FASS/TON2)* encodes a type 2a protein phosphatase and seems to be required for the formation of ordered microtubular arrays that make up the plant cytoskeleton. Also members of the ***pilz*** and ***titan*** (*ttn*) groups of mutants have abnormally formed embryos as a result of cytoskeletal defects that interfere with mitosis and cytokinesis. The *pilz* mutant embryos contain actin but lack microtubules, which results in a mushroom-shaped embryo with one or a few large cells. *TTN5* encodes a small G protein Arl2 with a possible role in regulation of vesicle trafficking, whereas other genes of the *pilz* group encode orthologs of mammalian tubulin-folding cofactors.

Another class of mutants, which shows more pleiotropic defects during embryogenesis, is defective in genes involved in sterol biosynthesis such as ***STEROL METHYLTRANSFERASE1/ CEPHALOPOD*** *(SMT1/CPH)*, ***FACKEL/HYDRA2*** *(FK/HYD2)*, and ***HYDRA1*** *(HYD1)*. Early embryo defects in cell division and expansion in these mutants result in embryos with abnormally shaped cotyledons and reduced central and basal regions, however the exact role of sterols in embryogenesis is unclear.

In summary, molecular analysis of morphogenetic mutants did not provide much insight into plant patterning mechanisms but provided important entry points into molecular studies of basic cellular functions such as vesicle trafficking and cytoskeleton arrangement.

2.5.2.2.2 Pattern Formation Embryo Mutants

Most of the mutants specifically affected in embryo patterning processes were identified by loss or alterations in apical-basal or radial pattern elements at the seedling stage. In contrast to the many transcription factors revealed by similar screens in animal systems such as *Drosophila*, only a few transcriptional regulators were found in *Arabidopsis*.

Several mutants with apical-basal patterning defects, such as ***monopteros*** (*mp*), ***bodenlos*** (*bdl*), ***auxin resistant6*** (*axr6*) or ***gnom*** (*gn*), have been isolated. All these mutants show deletion of basal structures (root and occasionally hypocotyl) and variable defects in formation of apically positioned cotyledons. ***MP*** encodes the transcriptional activator Auxin Response Factor 5 (**ARF5**) and ***BDL*** the transcriptional repressor from Aux/IAA family (**IAA12**), both of which are involved in auxin response. Consistent with the similar early embryo phenotypes, *mp* and *bdl* interact genetically, and yeast two-hybrid studies also indicate that a physical protein interaction can occur. ***AXR6*** encodes the *Arabidopsis* **CULLIN1** (AtCUL1), a component of the SCF (**SKIP, CDC53/CULLIN, and F-box protein**) TIR1 complex. The $\mathbf{SCF^{TIR1}}$ is a complex with ubiquitin ligase function, which by binding and **ubiquitination** of Aux/IAA proteins (e.g., BDL) mediates their degradation. The involvement of BDL, MP, and AXR6 in the same patterning processes suggests that BDL and MP form a complex *in vivo* that prevents MP from activating target genes. In this scenario, auxin promotes interaction between BDL and SCF^{TIR1} complex, which ultimately leads to BDL degradation. Consequently, derepressed MP is able to mediate auxin-dependent gene expression and thereby initiate root formation.

On the other hand, the *gn* mutant, which also displays severe aberrations in apical-basal pattern, is defective in the guanine-nucleotide exchange factor on ADP-ribosylation factor G proteins (ARF-GEF). Endosomal membrane-associated **GNOM ARF-GEF** acts as an activator of ARF G proteins, which play a role in the recruitment of vesicle coats required for their budding and cargo selection. Here, the mechanistic connection to auxin signaling and transport was established through a role for GN in vesicle trafficking that mediates proper subcellular targeting of **PIN-FORMED** (PIN) auxin transport components.

Thus, the molecular analysis of embryo patterning mutants suggested a prominent role for the phytohormone **auxin** (indole-3-acetic acid, **IAA**) in the regulation of this process.

2.5.2.3 Differential Auxin Distribution in Embryonic Axis Formation

Complementary evidence for the involvement of auxin in embryo development has been obtained through chemical manipulation of auxin homeostasis in excised *Brassica* embryos. The main breakthrough in the understanding of the role of auxin in embryo patterning was the discovery of dynamic **auxin gradients** during embryogenesis. Cellular distribution of auxin and its response was indirectly visualized using anti-IAA antibodies and an auxin-responsive reporter ***DR5::GFP***. Immediately after the division of the zygote, auxin accumulates in the apical cell, which is being specified. During subsequent development the maximum of auxin activity persists in the proembryo. Approximately at the 32 cell stage, when the basal embryo pole is being specified, the gradient of auxin accumulation suddenly reverses and forms a new maximum in the uppermost suspensor cells, including the hypophysis. At later stages of embryogenesis, additional auxin accumulation foci appear in the tips of the developing cotyledons. The next important question was, by what mechanism are these auxin gradients established and maintained. Conceptually, cellular auxin levels could be regulated by different levels of synthesis/degradation and conjugation/deconjugation or by intercellular transport.

Auxin is unique among plant hormones, being actively distributed within the plant by cell-to-cell movement. This movement is strictly directional (polar) and involves auxin influx and efflux carrier proteins. The direction of auxin flow is supposed to be determined by the asymmetric cellular localization of efflux carriers. The plant-specific PIN proteins are so far the best characterized candidates for facilitators of auxin efflux. Since both chemical and genetic (*gn* and *pin* mutants) inhibition of auxin transport interferes with the embryonic auxin gradients, it seems that polar auxin transport is a major determinant for establishing and maintaining auxin gradients. Furthermore, inhibition of auxin transport leads to severe embryo developmental defects, ranging from cup-shaped embryos with misspecified apical structures and a non-functional root pole, to ball-shaped embryos without any discernible apical–basal axis. These findings indicate that auxin transport-dependent auxin gradients are required for proper embryo development. Analysis of expression and localization of PIN auxin transport components finally completed the model describing the role of PIN-dependent auxin distribution in the establishment of the embryonic apical-basal axis.

In the early stages, auxin is actively provided to the apical cell from the adjacent basal cell by the action of basal cell-specific, apical-plasma membrane-localized PIN7. In subsequent stages, the cells of the suspensor continue to localize PIN7 on their apical side, while in the proembryo another protein, **PIN1**, is produced without apparent polarity. But

after the 32-cell stage, PIN1 becomes localized to the basal membranes of the provascular cells and PIN7 polarity in suspensor cells reverses suggesting downward transport towards the region of the future root pole and out of the embryo. *PIN4* expression then starts at the basal pole of the embryo, supporting the action of both PIN1 and PIN7. As a result of these changes in auxin flow, the auxin gradient reverses, displaying its new maximum in the uppermost suspensor cell, which in response to auxin is specified to become the **hypophysis** - the founder of the future root meristem.

Thus developmentally regulated changes in polarity of PIN proteins result in the redirection of auxin fluxes for local auxin accumulation, which is required first for specification of the apical and later the basal pole of the embryonic apical-basal axis. Interestingly it seems that a similar mechanism utilizing PIN-dependent auxin distribution also operates in postembryonic development such as organogenesis. Regardless of the type of organ primordia, the new direction of PIN-mediated auxin transport determines the growth axis of the developing organ.

2.5.3 Interregional Communication during Seed Development

It seems obvious that seed development requires the coordination of embryo and endosperm development and therefore requires communication between these two regions. The available evidence for this communication relies mostly on the analysis of mutant phenotypes in *Arabidopsis*. For example, mutations in two genes, *FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)* and *FERTILIZATION INDEPENDENT SEED2 (FIS2)* have similar phenotypes to the *Arabidopsis mea* (*MEDEA* mutant). In the absence of double fertilization, continual divisions of polar nuclei are followed by cellularization and overproliferation of the endosperm is accompanied by aberrant embryo development. Specifically, analysis of the ***haiku*** mutant confirmed that endosperm development plays a role in late embryo development. Further elegant studies using conditional expression of diphtheria toxin in different regions of developing seed allowed to selective ablation of the endosperm. This leads to clear embryo and seed development defects demonstrating an important role for the endosperm in embryo development and confirming the communication between these seed regions. However, the molecular nature and mechanism of this communication remains unknown.

2.6 Seed and Fruit Formation

Double fertilization activates, in parallel with embryo and endosperm development inside the embryo sac, transformation of an ovule into a **seed** and transformation of the ovary into a **fruit**. In case where pollination and fertilization do not occur, flower abscission follows.

2.6.1 Seed and Fruit Maturation

Both seed and fruit formation include processes of growth and maturation (ripening). General changes during fruit ripening include (1) changes of color through chlorophyll degradation and carotenoid or flavonoid accumulation; (2) textural modifications caused by changes of cell wall structure and cell turgor; (3) qualitative as well as quantitative changes in sugars, acids and other substances that affect flavor, aroma and nutritional quality. (4) Increase of respiration and ethylene synthesis during ripening. Such an increase is typical only for the so-called climacteric fruits such as the fleshy fruits of tomato, apple, and banana. Ethylene production is not involved in ripening of nonclimacteric fruits, in which exogenous ethylene can cause increased respiration while natural ripening is not promoted. Multigene families of ACC synthase and ACC oxidase control regulation of ethylene synthesis during climacteric ripening. Genes for both synthesis and degradation of proteins are activated during the ripening process.

2.6.2 Dormancy and Seed Germination

The period of seed maturation is rounded off by transition to a stage of reduced growth, which can be either **quiescence** or **dormancy**. Quiescent seeds can germinate in appropriate physiological conditions, while dormant seeds require additional hormonal and environmental regulation mechanisms for initiation of germination. Seed dormancy can represent a benefit either during embryo formation, when it prevents precocious germination, or as a regulator of seed germination in unfavorable conditions; otherwise, seed dormancy can be an inconvenient factor in agricultural crops, where rapid germination is required. Processes leading to seed dormancy are a part of the normal developmental pathway of seed formation. They include (1) gradual desiccation; (2) differentiation of the seed coat; (3) changes in gene transcription; and (4) reduction of cellular metabolism. Regulation of these processes involves activation of the transcription of new genes. Some of them can shut off transcription of genes regulating cellular metabolism, while others may be involved in regulation of seed formation.

The phytohormone abscisic acid (ABA) is involved in the regulation of the onset and in maintenance of dormancy. ABA accumulation in seeds is low during early embryogenesis and then increases during storage-reserve biosynthesis and declines when the seed undergoes maturation. Lowering of water potential is the signal for ABA accumulation. The course of dormancy is regulated by differential gene activation. *LEA* genes encode proteins more abundant in mature embryos then in young or germinated embryos. Expression of *LEA* genes can be induced by ABA.

Overcoming seed dormancy can be achieved by various pretreatments like scarification, prolonged washing, and storage at low or high temperature enabling additional ripening of seeds, or exposure to light or dark conditions. Seed **germination** begins with events such as water uptake, resumption of metabolic activity, degradation of LEA proteins, production of new proteins and mobilization of stored food reserves. Termination of seed germination is manifested by the elongation of the embryonic axis, which is completed by penetration of a root pole through the seed envelope. Information for synthesis of the first proteins that are essential for germination and root cell elongation comes from mRNA stored in the embryo during embryo development.

3. Apomixis

Apomixis is a naturally occurring process of asexual reproduction through seeds. It involves elimination of key developmental processes including meiosis and double fertilization. In nature, apomixis is widespread but infrequent: it occurs in 40 families of flowering plants, but only in about 400 species, which represents 1% of the species that make up those families. Apomixis is most frequent in *Poaceae*, *Asteraceae* and *Rosaceae* families. Occurrence of various forms of apomixis in different angiosperm taxa can suggest that it has evolved independently many times. Classification of numerous mechanisms of apomixis into two main subgroups is based on the origin and further development of the unreduced cells (Fig.3.). **Sporophytic apomixis**, called also **adventitious embryony**, is a process in which the embryo arises spontaneously directly from the cells of the nucellus or the integuments of the ovule. More common **gametophytic apomixis** occurs when the apomictic embryo arises from the cells of the unreduced megagametophyte. Gametophytic mechanisms are further divided according to the origin of the cells that give rise to the apomictic embryo. In **diplospory**, the megaspore mother cell that failed in meiosis is the progenitor of unreduced

embryo sac. **Apospory** is the most common mechanism of apomixis; in which the unreduced embryo sac arises from the somatic cells of the ovule. Pollen of apomictic plants is often viable. Sometimes, development of both sexual and asexual seeds occurs in the same flower, although precocious apomictic embryo formation before flower opening or synthesis of complete cell wall around the unreduced egg cell can prevent fertilization in apomictic species. Apomixis as a mechanism for rapid generation and multiplication of desired genotypes through seeds is a very attractive idea for plant breeders. Artificial introduction of apomixis into crops as a reproductive alternative is the aim of many attempts as it could represent a unique opportunity to simplify breeding schemes and genetically perpetuate any desired heterozygous genotype.

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Figure captions

Figure 2.1. Schematic representation of the alternation of sporophyte/gametophyte generations in plant evolution

Figure 2.1.1 Life cycle in mosses

The sporophyte generation in mosses is reduced to only a sporangium within which haploid spores are formed during meiosis. Germinating spores 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 the sperm cell in a drop of water to the egg cell within the archegonium. The next sporophyte generation develops in the archegonium and remains attached to the gametophyte.

Figure 2.1.2 Life cycle in a fern

Most (but not all) ferns are homosporous and produce only one type of spore within a structure called the sporangium. The gametophyte develops from the spores and can produce both male and female sex organs. Water facilitates fertilization like in mosses. The biggest contrast between the mosses and the ferns is that both the gametophyte and the sporophyte of the fern are autotrophic (according to Fosket 1994).

Figure 2.1.3 Life cycle in the angiosperms

The gametophyte generation of the angiosperms is reduced to just a few cells. The male gametophyte is formed after meiosis in microsporangia located in anthers and is represented

by the pollen grains, which are complexes of two or three cells. The female gametophyte - the embryo sac - containing the egg cell, synergids, antipodal and the central cell, is formed within the megasporangium (ovule). Angiosperm fertilization no longer relies on water like in mosses or ferns. Production of a seed coat, which forms a layer of protection around the embryo, and a further protective layer, the fruit, are unique features of angiosperms. PMCs, pollen mother cells, MMC, megaspore mother cell.

Figure 2.2.1 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 take 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*. 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 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).

Figure 2.2.2 Scheme of the *Arabidopsis* flower

Arabidopsis flower organs are organized in four concentric whorls. Whorl 1 contains four **sepals**, whorl 2 four **petals**, whorl 3 six **stamens** (four medial and two lateral) and whorl 4 two fused **carpels,** which form the female reproductive organ, the **gynoecium** (pistil).

Figure 2.2.3 ABCE model

The overlapping spatial specificity of individual gene classes in the flower whorls 1-4 in *Arabidopsis* is depicted. Below there are putative complexes of the flower organ identity genes, whose interactions are supposed to be involved in the acquisition of floral organ identity according to the quartet model (according to Jack, 2004).

Figure 2.3.1 Male gametophyte development

Formation of mature pollen grain from pollen mother cell in *Arabidopsis* is schematically depicted. Cytoplasm is in yellow, nuclei are in red, and vacuoles are in white. For details see text (section 2.3.1).

Figure 2.3.2 Female gametophyte development

Ovule development (a-d) and individual stages of female gametogenesis (FG1-FG7) in *Arabidopsis* are schematically depicted. **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. **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. **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 and Schneitz 1998). **FG1-FG7** Megagametogenesis in *Arabidopsis*. Cytoplasm is in yellow, nuclei in red and vacuoles in white. EC, egg cell; SC, synergid cell; CCN, central cell nucleus; AC, antipodal cells. Only one of the two synergid cells is shown. For details of female gametophyte development see section 2.3.2.

Figure 2.5.1 Development of the endosperm coenocyte in the *Arabidopsis* embryo sac
Endosperm nuclei migrate from the micropylar pole (MP) toward the chalazal pole (ChP) of the embryo sac. A large central vacuole (V) fills up most of the volume and is surrounded by cytoplasm. Cytoplasm is in yellow, nuclei in red and vacuole in white. As development progresses, the coenocyte forms three distinct domains: (1) the region surrounding the embryo (MCE), (2) the peripheral endosperm (PEN), and (3) the region of the chalazal endosperm (CZE), which contains the chalazal cyst (ChC). At the end of the globular embryo stage, the

embryo (E) becomes completely surrounded by cytoplasm (modified according to Olsen 2004).

Figure 2.5.2 Endosperm cellularization in *Arabidopsis*

The endosperm at the late globular embryo stage shows a cellular endosperm in MP, a gradient of stages in the alveolation process in PEN, and endosperm nodules (NO) as well as chalazal cyst (ChC) formation in ChP. During alveolation, radial microtubule system (RMS) emanates from the nuclear membranes, cell walls form tubes or alveoli (ALV) around each nucleus with open ends toward the central vacuole (V). Later, endosperm becomes completely cellular (CE). The endosperm is consumed during seed maturation, leaving only the peripheral aleurone-like cell (ALC) layer in a mature seed (modified according to Olsen 2004).

Figure 2.5.3 Model for a role of auxin distribution in embryo patterning

Two-cell stage embryo – specification of the apical cell. Auxin accumulates (depicted in green) in the proembryo by PIN7-dependent transport (red arrows) through the suspensor. Auxin response (*mp*, *bdl*) and transport (*gn*) mutants show defects in the establishment of the apical cell. Young globular embryo – specification of the root pole. Auxin production is established in the apical part (purple) and auxin transport routes reverse. Auxin accumulates, in a PIN1- and PIN4-dependent manner (blue and purple arrows) in the hypophysis and is further transported through the suspensor. *mp*, *bdl* and *gn* show defects in root pole establishment. Early heart (triangular) stage – establishment of cotyledons. Most auxin is still transported to the basal part of the embryo and then further through the suspensor. New sites of auxin accumulation emerge at the tips of forming cotyledons. MP and GN are also involved in cotyledon establishment.

Figure 3. Comparison of apomixis and sexual life cycle of flowering plants

Apomictic reproduction by seed occurs when the sexual life cycle is shortened. Sporophytic apomixis (adventitious embryony) occurs when the unreduced cells of the ovule directly give rise to an embryo. Gametophytic apomixis occurs by the formation of an unreduced megaspore either due to failure of megaspore mother cell reduction (diplospory) or by the formation of an unreduced megaspore from the somatic cells of a diploid ovule (apospory).