Charles University

Faculty of Science

Study programme: Biology

Branch of study: Biology

Oliver Pitoňak

Parent-of-origin contributions to gene expression during sexual plant reproduction

Příspěvek rodičovských genomů ke genové expresi během pohlavního rozmnožování rostlin

Bachelor´s thesis

Supervisor: doc. RNDr. David Honys, Ph.D.

Consultant: Said S. Hafidh, Ph.D.

Prague, 2020

Acknowledgments

I wish to express thanks to my supervisor David Honys, for giving me an opportunity to approach plant science in Pollen Biology Lab and for his advice on this thesis. I am grateful to my consultant Said Hafidh, for his valuable comments and inspiration I got during time that I spent in lab. I would like to thank my friends Vinod Kumar and Antonietta Saccomanno for beautiful memories I have from both inside and outside of the lab. Last (but not least) I want to thank my friends from school and my parents for their support.

Declaration

I hereby declare that I have written this thesis on my own and that I have cited all used publications and other sources. This thesis or any part of it has not been used to acquire other or the same academic title.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získaní jiného nebo stejného akademického titulu.

Prague, 1st June 2020

.....

Oliver Pitoňak

Abstract

Sexual reproduction in flowering plants is fundamental to seed formation. After fertilization, the embryo is enclosed and develops in a seed together with a triploid tissue – the endosperm. In animals, early embryogenesis is well-known to be controlled by maternal RNA and proteins deposited in the ovule before fertilization. Even after the activation of zygotic genome, paternal and maternal genome do not play interchangeable roles. A few genes affecting embryo growth and development are transcribed either from maternally or paternally inherited alleles only. Such genes are example of the well-known phenomenon of gene imprinting. In plants, imprinting is well documented in the endosperm. The role of parent-of-origin contributions to gene expression has been studied less extensively in embryo. The aim of this work is to critically assess current knowledge of parent-of-origin contributions to embryo development in different plant species.

Keywords

sexual plant reproduction, seed development, embryogenesis, zygotic genome activation, imprinting

Abstrakt

Pohlavní rozmnožování je důležitým procesem vzniku semen u krytosemenných rostlin. Embryo se vyvíjí v semeni spolu s triploidním pletivem – endospermem. U živočichů je časný vývoj embrya řízen mateřskou RNA a proteiny uloženými ve vajíčku před oplozením. Mateřský a otcovský genom nemají zaměnitelné role ani po aktivaci genomu zygoty. Některé geny ovlivňující růst a vývoj embrya jsou přepisovány jenom z maternálně nebo paternálně zděděné alely. To je příkladem fenoménu genového imprintingu. U rostlin je imprinting dobře znám z endospermu. Rodičovský příspěvek ke genové expresi v embryu byl studován méně intenzivně. Tato práce si klade za cíl kriticky zhodnotit aktuálně dostupné informace o rodičovském příspěvku ve vývoji embrya u různých druhů rostlin.

Klíčová slova

pohlavní rozmnožování rostlin, vývoj semen, embryogeneze, aktivace zygotického genomu, imprinting

List of frequently used abbreviations

Col	Columbia
Cvi	Cape Verde Islands
DAP	days after pollination
EST	expressed sequence tag
GFP	green fluorescent protein
GUS	β-glucuronidase
Ler	Landsberg *erecta*
RdDM	RNA dependent DNA methylation
RNAi	RNA interference
TF	transcription factor
Tsu	Tsushima
ZGA	zygotic genome activation

Table of contents

Introduction

Plants have colonized places as diverse as the shores of Antarctica and the Sahara Desert. Their life cycles have adapted to different environmental conditions. A diploid sporophytic and a haploid gametophytic phase alternate in plant life cycle.

Life of a sporophyte begins as a single cell – the zygote. Its divisions give rise to an embryo – a structure with both root and shoot primordia. Even at this stage, remarkable structural diversity is found in various species. Maternal plant provides energy to the embryo in its first days of development. Embryo is a part of a seed along with a triploid tissue, the endosperm. When a seed matures, it leaves maternal plant. It may be carried by wind or animals to a spot where it germinates. Seeds of various species germinate in different conditions. For example, orchid seeds depend on infection by mycorrhizal fungi. Throughout its life, a plant cannot leave the place where it germinated and is challenged by diverse stress conditions. Finally, it undergoes a vegetative-to-reproductive transition and forms flowers that will eventually bear seeds to restart the life cycle as a new generation.

Reproductive organs in flowers produce spores, which develop into the haploid gametophyte phase. Male spore gives rise to a pollen grain. Attached to a pollinator or brought by wind, as it lands on stigma, it is rehydrated and forms a pollen tube. The pollen tube carries male gametes, sperm cells, to the female gametophyte, called embryo sac. Even though structurally diverse in various species, it commonly consists of 4 cell types: an egg cell, a diploid central cell, synergids and antipodes. Thanks to an intense communication between male and female gametophytes, a pollen tube reaches an embryo sac. After pollen tube burst, sperm cells are released and freed to fuse with female gametes. Fusion of a sperm cell with the egg cell produces the zygote. The second sperm cell fuses with the central cell and gives rise to the endosperm.

It is known that parental genomes do not contribute equally to gene expression in the endosperm. Genomic imprinting is widespread in the endosperm. For many genes, only paternally or maternally inherited alleles are expressed. However, the phenomenon of parent-of-origin contribution to gene expression has been studied less extensively in the embryo.

This thesis reviews current knowledge on parent-of-origin contributions to gene expression in the embryo in different plant species.

1. From gamete to embryo

Double fertilization is a unique characteristic of angiosperms. Female gametes – the egg cell and the central cell – reside in an embryo sac in ovules. Synergids surrounding the egg cell release molecules that mediate communication and attraction of pollen tubes. Pollen tubes carry two male gametes – sperm cells. As pollen tubes burst, sperm cells are freed to adhere and fuse with the egg cell and the central cell. After fusion of male and female membrane, male and female nuclear fusion – karyogamy – takes place in both zygote and central cell, which develop into embryo and endosperm, respectively.

1.1 Gamete adhesion and fusion

In *Arabidopsis thaliana*, molecular details of gamete fusion are still not completely understood. After release from the pollen tube, sperm cells are connected and need to be separated before fusion. Separation can be direct, followed by an adhesion to the egg cell and the central cell. Alternatively, sperm cells can adhere to a female gamete as a pair and one of them undergoes repositioning and separation after stagnation (Huang et al., 2015). A transmembrane protein GEX2 localized on sperm plasma membrane is crucial to gamete adhesion (Mori et al., 2014). In response to pollen tube burst, the egg cell releases EC1 proteins by exocytosis (Sprunck et al., 2012). These proteins may be involved in separation and activation of sperm cell pair (Cyprys et al., 2019).

EC1 proteins cause HAP2/GCS1 to relocalize from the endomembrane system to sperm plasma membrane (Sprunck et al., 2012). HAP2/GCS1 is a conserved single-pass membrane protein that is involved in fusion of male and female gametes (von Besser et al., 2006). *At*HAP2/GCS1 induces plasmogamy of mammalian cells which normally do not fuse (Valansi et al., 2017). This indicates its direct role in the fusion process as a fusogen. Interestingly, it is structurally homologous to class II viral fusogenic glycoproteins (Valansi et al., 2017). Their extracellular part is composed of three domains – a central DI domain surrounded by DII and DIII domains. DII tip is inserted into target membrane prior to fusion. Class II viral glycoprotein insertion induces membrane curvature and formation of a fusion pore (Kielian, 2006). Evolutionary analysis revealed unexpected diversity in membrane-inserted segment (Fedry et al., 2018). In flowering plants, an amphipatic α-helix interacts with target (female gamete) membrane. Recently, DMP8 and DMP9 were shown to facilitate HAP2-mediated gamete fusion, with greater effect on the sperm-egg cell fusion over sperm-central cell fusion (Cyprys et al., 2019). Fusion of sperm cells with the egg cell and the central cell seem to be coordinated, which implies communication between gametes (Huang et al., 2015). A novel method of mutant screening based on vanillin staining is promising to discover more genes involved in the process of gamete fusion and fertilization (Liu et al., 2020).

Gamete adhesion and fusion remain largely unexplored in other plant species, mainly as a result of gamete inaccessibility and limitations in microscope technology. Study of post-fusion processes in other plant species is mostly based on in vitro fertilization experiments.

1.2 Karyogamy

Fusion of sperm cell and egg cell induces a transient increase in calcium concentration in *Arabidopsis thaliana* (Denninger et al., 2014) and *Oryza sativa* (Ohnishi and Okamoto, 2017), which possibly activates the egg cell. Sperm cell nucleus moves towards egg cell nucleus and fuses. Actin cytoskeleton has been discovered to be crucial for nuclear migration in *A. thaliana* (Kawashima et al., 2014), *O. sativa* (Ohnishi and Okamoto, 2015), *Nicotiana tabacum* and *Zea mays* (Peng et al., 2017). In contrast to most animal species, microtubules are dispensable for karyogamy in zygotes of both *O. sativa* (Ohnishi and Okamoto, 2015) and *A. thaliana* (Kawashima et al., 2014). In rice egg cell, actin filaments continuously move from cell cortex toward the nucleus (Ohnishi and Okamoto, 2017). This movement – convergence – is thought to mediate sperm nuclear migration. Upon fusion with sperm cell, actin network keeps migrating toward the nucleus but with 1,4-fold higher velocity (Ohnishi and Okamoto, 2017). This could explain the fact that the fusion of egg nucleus with sperm nucleus is faster than an induced fusion with another egg cell nucleus or leaf protoplast nucleus (Ohnishi et al., 2019). Karyogamy is accompanied by male chromatin decondensation, reported in maize (Scholten et al., 2002) and rice zygotes (Ohnishi et al., 2014).

Fusion of the second sperm cell with central cell does not induce a change in calcium concentration (Denninger et al., 2014). Sperm nuclear migration towards central cell nucleus is dependent on actin cytoskeleton in *A. thaliana* (Denninger et al., 2014), *N. tabacum* and *Z. mays* (Peng et al., 2017). Arp2/3 complex is involved in this nuclear migration in *Z. mays* and *N. tabacum*. Surprisingly, *A. thaliana* Arp2/3 complex mutants have no phenotype defects. Instead, Rho-GTPase Rop8 that controls actin assembly has been implicated in the process (Denninger et al., 2014). Rop8 expression has not been detected in egg cell, which could suggest different regulation of nuclear migration between female gametes.

1.3 Zygote polarization and division

Zygote divides asymmetrically into a smaller apical and a larger basal cell in *A. thaliana*, *N. tabacum* (Zhao et al., 2011), *O. sativa* (Sato et al., 2010), *Z. mays* (Chen et al., 2018) and *T. aestivum* (Bakos et al., 2009). Even though the outcome is similar in various plant species, microscopically observable changes prior to division differ.

In eudicots, the egg cell is polarized with nucleus at the chalazal (apical) and vacuoles at the micropylar (basal) pole. Shortly after fertilization, the zygote transiently loses polarity in *A. thaliana* (Kimata et al.,

2016) and *N. tabacum* (Zhao et al., 2011). It shrinks and vacuole volume is reduced. It is essential that zygote polarity is re-established before division. Zygote elongates and the nucleus migrates back to the apical pole. Vacuoles in apical part remain small but vacuoles in basal part of the zygote enlarge (Kimata et al., 2019).

Following fertilization, cytoskeleton is disorganized in *A. thaliana* zygote. Novel cytoskeleton patterns arise (Kimata et al., 2016). Microtubules form a subapical transverse ring, creating an apical bulge that grows out. Treatment with microtubule polymerization inhibitor oryzalin results in a shorter zygote in comparison to non-treated wild type. In tobacco, a dynamin-related protein NtDRP associates with microtubules and participates in zygote elongation (Zhao et al., 2016). Actin is arranged longitudinally in *Arabidopsis* zygote and forms an actin cap at the top of growing apical part – a structure associated with tip growth in plants (Kimata et al., 2016). Actin contributes to nuclear migration. Polar distribution of vacuoles is also crucial to the process (Kimata et al., 2019). Molecular basis governing zygote polarization in *A. thaliana* is a fascinating example of influence of parent-of-origin gene expression and will be elaborated in section 2.1.2.

In rice egg cell, the nucleus is localized at the micropylar pole and vacuoles are gathered at the chalazal pole. In contrast to eudicot species, zygote does not elongate prior to division in rice (Uchiumi et al., 2007), maize (Chen et al., 2018) or wheat (Bakos et al., 2009). Zygote undergoes polarization in some monocot species. In rice, nucleus moves to the apical pole and is surrounded by denser cytoplasm (Sato et al., 2010). In contrast, wheat zygote polarization has remained enigmatic. Apical and basal cell are similar in respect to organellar organization even though they differ in shape (Bakos et al., 2009).

1.4 Embryo patterning

Arabidopsis thaliana is a valuable model in the study of cell fate specification and embryo pattern formation. Divisions of the apical cell give rise to embryo proper and divisions of the basal cell produce a transient structure called suspensor. Only its uppermost cell – the hypophysis – is incorporated into embryo and gives rise to root apical meristem and columella. Remaining suspensor cells undergo programmed cell death. Suspensor is important for nutrients and hormone transfer to the embryo.

Cell divisions up to 16-cell stage are invariant in *A. thaliana* (Moukhtar et al., 2019). They are not synchronized (Gooh et al., 2015). Two rounds of vertical division of the apical cell followed by a horizontal division produce 8-celled embryo, the octant stage (Mansfield and Briarty, 1991). These cells form the upper and the lower tier precursors of apical and basal parts of a seedling, respectively. In octant stage, cells divide asymmetrically and tangentially, giving rise to the outer protoderm tissue and the inner tissue. Up to late globular stage, asymmetrical divisions give rise to precursors of ground tissue and vascular tissue (Yoshida et al., 2014). Cotyledon primordia grow out in heart stage.

Differentiation and growth continue in torpedo and bent-cotyledon stages. Embryogenesis results in a mature embryo with precursors of both shoot and root apical meristem, epidermis, ground and vascular tissues.

Plant cells possess a rigid cell wall. Patterning is thus dependent on cell plate orientation during division, which has been a subject of intensive research. Symmetrical cell division with minimal interface area between daughter cells is thought to be default. It has been proposed that auxin can override this rule and enable asymmetrical division (Yoshida et al., 2014). Interestingly, a recent study highlights the role of mother cell geometry in choosing division plane in both asymmetrical and symmetrical division (Moukhtar et al., 2019). It is well-known that auxin is involved in embryo patterning. Initially, auxin is transported from the integuments to the apical cell where it activates downstream response and contributes to cell division (Robert et al., 2018). Later in embryogenesis, auxin flow is reversed, and auxin is transported to uppermost cells of the suspensor, including hypophysis (Friml et al., 2003). Auxin participates in ground tissue (Möller et al., 2017) and vascular tissue patterning (De Rybel et al., 2014).

It is interesting that even the first divisions in tobacco differ from *A. thaliana*. The apical cell first divides vertically and then twice horizontally (He et al., 2007). However, the overall pattern established in embryogenesis is similar to *A. thaliana*. The embryo passes globular, heart, torpedo and bent-cotyledon stages. Auxin participates in patterning in *N. tabacum*. Surprisingly, it was detected in tobacco zygote, which was the first reported case of auxin presence in land plant zygote (Chen et al., 2010). Auxin signal is rather weak and uniform in early embryo. Later, auxin flows from the cotyledons to the root apical meristem, as in *A. thaliana* (Chen et al., 2010).

Embryogenesis in model monocot species differs significantly from described stereotyped process in eudicots (Itoh et al., 2005). Embryos are polarized not only along apical-basal axis but also along dorsal-ventral axis. The apical cell divides in a variable pattern to produce a globular embryo. In late globular stage, shoot apical meristem and radicle begin to differentiate. The globular stage is followed by organogenesis. A coleoptile forms on the ventral side above differentiating shoot apical meristem. On the dorsal side, a structure called scutellum – described as a monocot cotyledon – enlarges. Leaf primordia that would be initiated only after germination in eudicots appear under the coleoptile during organogenetic phase in monocots. If present, epiblast formed on the basal ventral side enlarges. Embryogenesis results in a mature embryo with the scutellum, coleoptile, leaf primordia, shoot apical meristem and radicle.

Auxin is involved in embryo development in maize (Chen et al., 2014). Genes involved in auxin signalling are upregulated in the zygote but show downregulation in both apical and basal cell (Chen

et al., 2017). Auxin is present in early endosperm and may participate in apical pole and shoot apical meristem specification (Chen et al., 2014). Only during organogenesis, auxin seems to flow from the endosperm to the embryo and influence differentiation. Auxin participates in later stages of embryo development in wheat (Fischer-Iglesias et al., 2001). Spatiotemporal analysis of gene expression in rice revealed biased transcription of genes involved in auxin signalling along apical-basal and dorsal-ventral axis in different developmental stages (Itoh et al., 2016).

Embryo developmental stages of plant species discussed in this thesis are summarized in Figure 1.

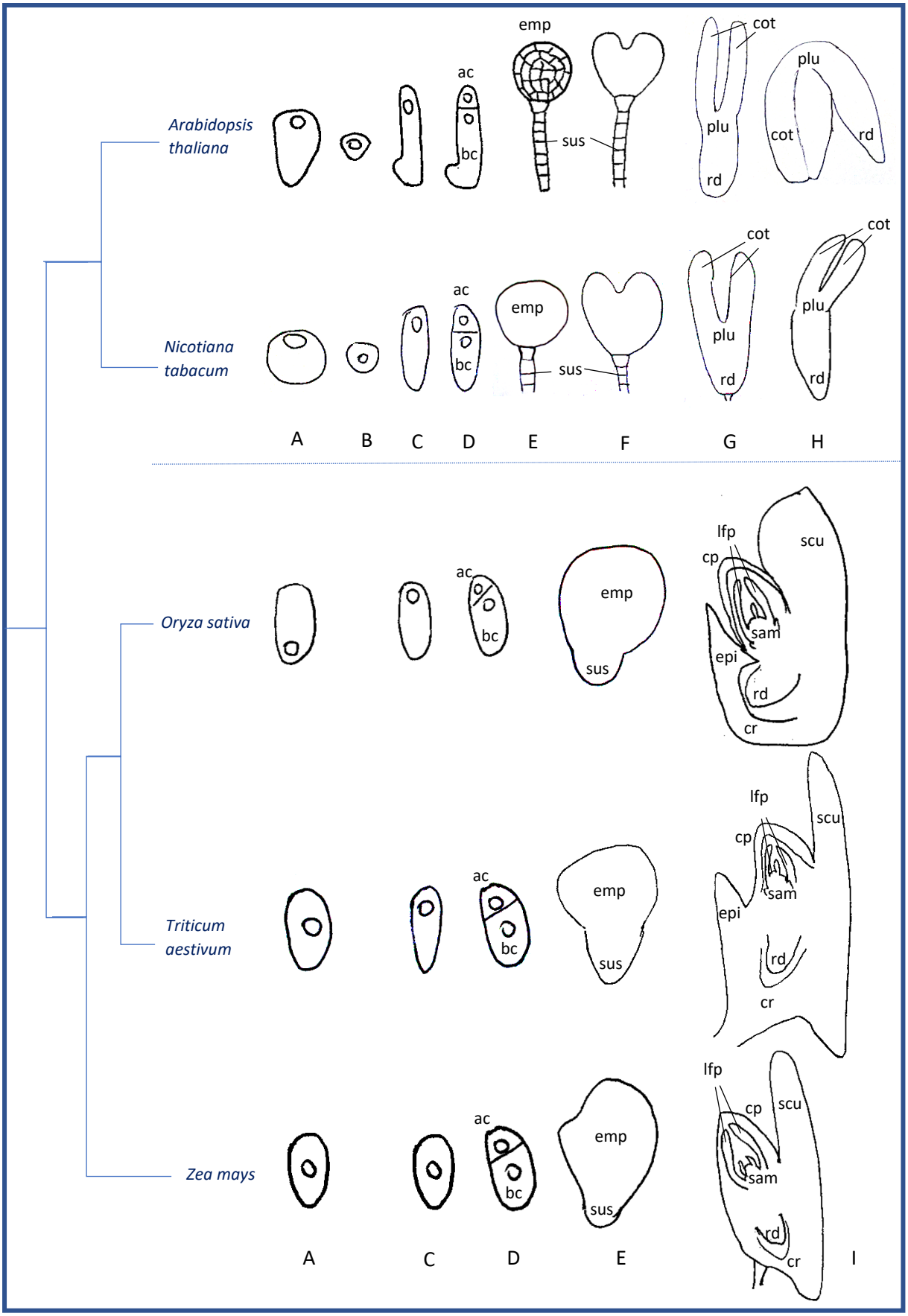

Figure 1. Stages of embryo development in different plant species. *A – egg cell, B – zygote shrinkage after fertilization, C – zygote shortly before cell division, D – 2-cell embryo, E – globular stage, F – heart stage, G – torpedo, H – bent cotyledon, I – mature monocot embryo, ac – apical cell, bc – basal cell, cot – cotyledon, cp – coleoptile, cr – coleorhiza, emp – embryo proper, epi – epiblast, lfp – leaf primordia, plu – plumule, sam – shoot apical meristem, scu – scutellum, sus – suspensor, rd – radicule. See text for details. Position of nuclei indicated in A, B, C and D. Size changes depicted in A, B, C and D. Later stages not depicted proportionally to A, B, C, D in respect to size. Redrawn according to Kimata et al., 2016; Zhao et al., 2011; Sato et al., 2010; Chen et al., 2018; Bakos et al., 2009; Mansfield and Briarty, 1991; Chen et al., 2010; Itoh et al., 2005; Itoh et al., 2016; Chen et al., 2014. Scanned and processed in ImageJ.*

2. Parent-of-origin contributions to gene expression in plant embryogenesis

It is well known that maternal RNA and proteins deposited in the oocyte control early embryo development in animals. Paternal RNA contribution has been demonstrated recently as well (Stoeckius et al., 2014; Yuan et al., 2016; Guo et al., 2017). There is no transcription in the embryo until the so called maternal-to-zygotic transition (Jukam et al., 2017). This event is associated not only with zygotic genome activation (ZGA) and increase in transcription but also with degradation of maternal transcripts. The timing of ZGA is specific for different animal species (Figure 2).

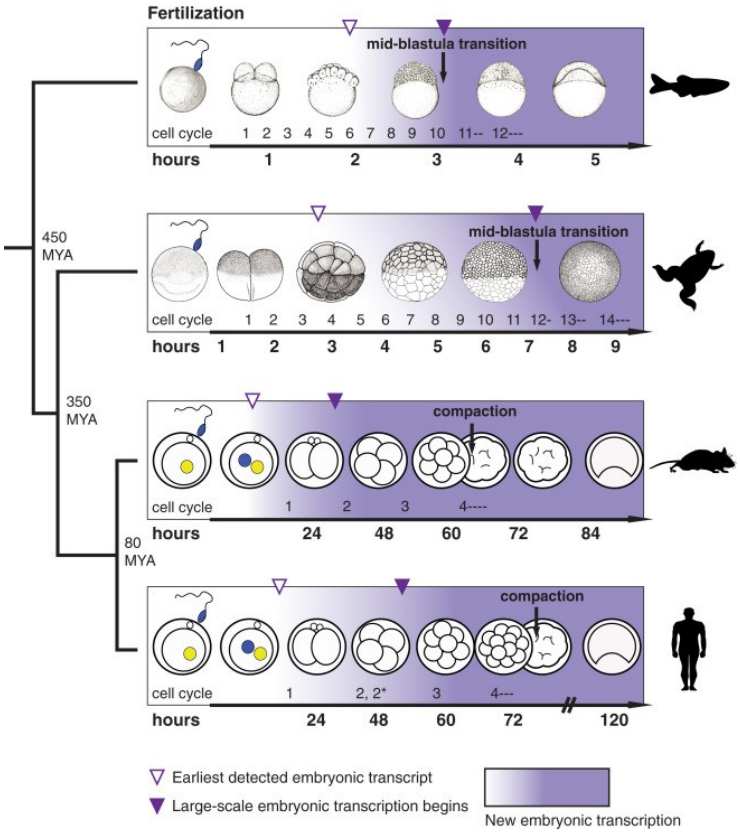

Figure 2. Timing of zygotic genome activation in different animal species.. *Zygotic genome is activated in different stages of embryo development in different species. Adapted from Jukam et al., 2017.*

After the activation of zygotic genome, zygotic transcripts guide embryogenesis. However, parent-of-origin gene expression from either paternal or maternal allele only can occur. An example is the phenomenon of gene imprinting. Imprinting is crucial to embryo growth and development in animals (Cassidy and Charalambous, 2018). In plants, gene imprinting is well documented in endosperm, even though its molecular basis remains unclear for many imprinted genes (Hornslien et al., 2019). Furthermore, it is suggested that male chromatin is transiently silenced upon ZGA and transcription from paternal alleles is initiated later than from maternal alleles (Vielle-Calzada et al., 2000). This phenomenon is known as asymmetric genome activation.

In plants, research in this area began only at the beginning of the 21st century. Currently, plant scientists hope to answer three basic questions. 1. To what extent is early embryo development in plants controlled by parentally inherited RNA and proteins and when does ZGA occur? 2. Is paternal and maternal genome activated symmetrically? 3. Is there evidence for genomic imprinting in the embryo? In remaining sub-chapters, methods that help to answer these questions are presented along with current understanding of the topic in *Arabidopsis thaliana, Nicotiana tabacum, Oryza sativa, Zea mays* and *Triticum aestivum*.

2.1 Inheritance of parental RNA and proteins in embryo development and zygotic genome activation

In animals, proteins and RNA in gametes guide early embryo development before zygotic genome is activated. How can ZGA be studied in plants and what is known about this process in different plant species?

2.1.1 Research methods

Genes that encode RNA or proteins to be stored in gametes and participate in embryogenesis can be identified by a combination of genetic and molecular biological methods. Heterozygotes with a knock-out allele of such gene segregate their progeny in a 1:1 ratio, even though gametophytic transmission is not affected (Bayer et al., 2009). Reciprocal crosses show that the phenotype of progeny is dependent on either maternal or paternal genotype only. This result could also be explained by gene imprinting or asymmetric activation of parental genomes.

If early plant embryo development is controlled by parental RNA and proteins deposited in the egg cell like in animals, transcription is not supposed to occur until zygotic genome activation.

A possibility to investigate the role of transcription in early embryo development is to supress or inhibit transcription. Triptolide, produced by a Chinese plant *Tripterygium wilfordii* Hook F, binds to XPB subunit of human TFIIH (Titov et al., 2011). TFIIH is a general transcription factor essential for transcription initiation. *Arabidopsis thaliana* genome encodes two functionally redundant XPB proteins with conserved structure and function (Ribeiro et al., 1998; Morgante et al., 2005). α-amanitine is a peptide synthesized by fungus *Amanita phalloides*. It binds to RNA polymerase II, an enzyme catalysing mRNA synthesis in eukaryotes, and blocks transcription elongation (Brueckner and Cramer, 2008). Actinomycin D is produced by bacterium *Streptomyces parvullus* and thought to bind to the boundary between double-stranded DNA and single-stranded DNA to block transcription elongation (Sobell, 1985). The fungus *Cordyceps militaris* synthesizes another transcription inhibitor – cordycepin. It is an adenosine analog that lacks 3´-OH hydroxyl group. Its incorporation into a growing RNA chain results in termination. (Horowitz et al., 1976). An alternative strategy to supress transcription is inhibiting RNA polymerase II activity using RNA interference under promoter specific for mature embryo sac (Pillot et al., 2010).

Another way to determine if transcription takes place in early embryo is to monitor RNA polymerase II activity. Residues of the C terminal domain (CTD) are phosphorylated during transcription elongation (Komarnitsky et al., 2000). Detection of this phosphorylation is indicative of active transcription.

Comparison of RNA profiles of the gametes and early embryo stages provides further clues to zygotic genome activation. If parental transcripts control early embryo development, little difference between transcriptional profiles of gametes and early embryo is expected. Thanks to the development of transcriptomic methods, it is possible to identify differentially expressed genes in different developmental stages.

Transcriptomic analysis of different stages in male gametophyte development has been performed in various species (summarized in Fíla et al., 2017). For some of them, sperm cell transcriptome data is available: *A. thaliana* (Borges et al., 2008; Misra et al., 2019), *N. tabacum* (Xin et al., 2011), *Plumbago zeylanica* (Gou et al., 2009), *Solanum lycopersicum* (Liu et al., 2018), *O. sativa* (Abiko et al., 2013; Anderson et al., 2013) and *Z. mays* (Chen et al., 2017). Study of female gametophyte transcriptome is challenging because of surrounding sporophytic tissues. *A. thaliana* mutants *sporocyteless* and *coatlique* do not form an embryo sac. This enables female gametophyte transcriptome analysis by genetic subtraction (Johnston et al., 2007; Yu et al., 2005). Transcriptional profile of egg cells has been analysed in various species: *A. thaliana* (Zhao et al., 2019), *N. tabacum* (Zhao et al., 2011), *O. sativa* (Anderson et al., 2013), *Z. mays* (Chen et al., 2017), *T. aestivum* (Domoki et al., 2013; Sprunck et al., 2005) and *A. trichopoda* (Flores-Tornero et al., 2019), often alongside zygotic transcriptome to assess ZGA.

Every transcriptome analysis begins with the collection of plant material and subsequent RNA isolation. Sperm cells are isolated from the pollen tubes. Osmotic shock induces pollen tube burst and sperm cells are released. Afterwards, sperm cells can be collected manually (e.g. Li et al., 2019), using centrifugation (e.g. Lu et al., 2015) or fluorescence-activated cell sorting (FACS) if marker lines are available to distinguish sperm cells and vegetative nuclei (Borges et al., 2012). In most studies cited in this thesis, egg cells are isolated manually. The zygote and early embryo can be isolated manually, using laser capture microdissection (LCM) or FACS. In LCM, a fixed sample is embedded, sectioned and a laser beam separates cells or tissues desired for RNA isolation from surrounding tissues (Kerk et al., 2003).

The zygote and developing embryo are surrounded by maternal tissues and endosperm. Contamination by transcripts from these tissues is a major concern and should be avoided. However, most embryo transcriptomic data prior to 2017 turned out to be contaminated, especially early developmental stages isolated by hand dissection or FACS (Schon and Nodine, 2017). Embryo samples isolated by LCM show contamination from micropylar endosperm. Washing is essential to minimize contamination. Contaminants are major issues when discussing parental genome contributions to

early embryogenesis and will be further discussed in section 2.2. A software to assess contamination from maternal tissues or endosperm is available (Schon and Nodine, 2017).

Transcriptome of gametes and early embryos in different plant species is studied with help of three major technologies – expressed sequence tag (EST) analysis, microarray and RNA-seq. Principles of these methods are outlined in Figure 3.

Transcriptomic technologies differ in respect to sensitivity, labour intensity, input and prior knowledge needed (Lowe et al., 2017). RNA-seq is laborious but is considered very sensitive. It requires low input, which is an advantage when analysing samples like gametes and zygotes. Single-cell RNA-seq protocol for analysis of *Arabidopsis* sperm cell transcriptome was introduced recently (Misra et al., 2019). Microarray analysis is limited to probes present on the plate. In contrast, RNA-seq and EST can detect novel transcripts. Microarray and RNA-seq enable direct quantification of transcript levels, which is important for estimating differential expression between cell types. A study of *A. thaliana* embryo transcriptome compared different RNA-seq methods and showed that not all of them are equally useful (Hofmann et al., 2019).

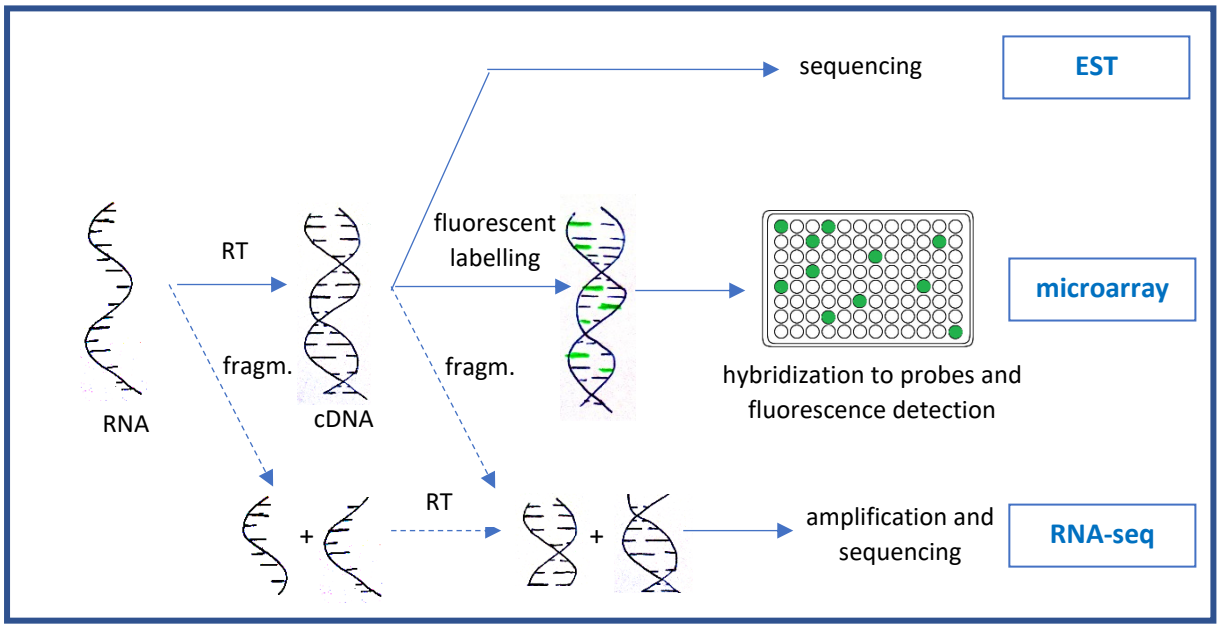

Figure 3. Transcriptomic technologies used in the study of plant gametes and early embryos *– expressed-sequence tag analysis, microarray analysis and RNA-seq. Principles of protocol are outlined, separate steps indicated by arrows. Dashed arrows indicate alternative steps. Fluorescent labelling marked green. fragm. – fragmentation, RT – reverse transcription. Drawn according to Lowe et al., 2017. Scanned and processed in ImageJ.*

2.1.2 *Arabidopsis thaliana*

A. thaliana sperm cells have a unique transcriptional profile (Borges et al., 2008). Microarray analysis revealed 74 out of more than 5,000 detected genes to be expressed specifically in sperm cells. They may be involved in fertilization (such as *HAP2/GCS1*) and their potential role in early embryo development is suggested. A recent RNA-seq study found more than 7,000 genes to be expressed in sperm cells (Misra et al., 2019).

One gene (annotated as *At2g17090*) found specifically expressed in the sperm cell microarray study became a sensation just a year later. *SHORT SUSPENSOR* (*SSP*) belongs to *BSK* (*BRASSINOSTEROID SIGNAL KINASE*) gene family. *SSP* mRNA is deposited in sperm cells but is translated only after fertilization (Bayer et al., 2009). *SSP* originated in the *Brassicaceae* family after a whole-genome duplication (Liu and Adams, 2010). It is a *BSK1* paralog. BSK1 is a component of brassinosteroid signal pathway. Whereas *SSP1* is expressed in sperm cells only, *BSK1* is expressed in most plant tissues. BSK1 and SSP share two domains – a kinase domain and a tetratricopeptide (TPR) domain. In inactive state, these two domains are thought to interact in BSK1 (Neu et al., 2019). Upon activation, intramolecular interaction is supposed to be lost and BSK1 can further transmit the signal. It is interesting that a similar intramolecular interaction is not detected between TPR and kinase domains of SSP (Neu et al., 2019). After translation in the zygote, SSP is translocated to the plasma membrane. As a constitutively active protein, it can interact and activate its target YODA (YDA). BSK1 and BSK2 can activate the same target but only after their own activation. BSK1 and BSK2 are functionally redundant proteins. *ssp* phenotype is not as serious as *yda* phenotype, which suggests existence of factors with additive effect. BSK1 and BSK2 contribute to YDA activation later in embryo development, as *bsk1 bsk2 ssp* triple mutant phenotype is comparable to *yda* phenotype (Neu et al., 2019). Their homologs were identified in the basal angiosperm *Amborella trichopoda* (Neu et al., 2019). Future research is needed to discover their role in zygote development in other plant species. There is no report of brassinosteroid signalling in the zygote.

Both BSK1 and SSP directly interact with YDA (Neu et al., 2019). Zygotes in *yda* mutants do not elongate (Lukowitz et al., 2004). Zygotic division gives rise to an apical and a basal cell of comparable size. Suspensor development is disturbed in the mutants. YDA is a ubiquitously expressed MAP kinase kinase kinase (MAP3K) (Lukowitz et al., 2004). It participates not only in zygote elongation but also in stomatal patterning (Bergmann et al., 2004). A MAP3K phosphorylates a MAP kinase kinase (MAPKK). In turn, a MAPKK phosphorylates a MAP kinase (MPK), which finally transfers a phosphoryl group onto a target protein. MPK in YDA pathway in the zygote are functionally redundant proteins MPK3 and MPK6. Components of MAP kinase pathways tend to assemble on scaffold proteins. AGB1 - β subunit of heterotrimeric G protein – is suggested to contribute to YDA pathway as a scaffold (Yuan et al., 2017).

In 2014, a unique type of parent-of origin gene expression was discovered. Cysteine-rich peptides EMBRYO SURROUNDING FACTOR 1-3 (ESF1-3) deposited in the central cell before fertilization turned out to participate in embryo patterning by contribution to YDA pathway (Costa et al., 2014). Molecular basis of their action is unknown.

MPK3 and MPK6 phosphorylate the transcription factor WRKY2 (Ueda et al., 2017). Phosphorylation is essential for WRKY2 activation. WRKY2 is detected in both egg cell and pollen (Ueda et al., 2011). This protein binds to *cisB* promoter element of *WUSCHEL HOMEOBOX 8 (WOX8)* and promotes its transcription (Ueda et al., 2011). *WOX8* expression is crucial to the establishment of polarity and asymmetric division in *A. thaliana* zygote. Cytoskeleton plays an indispensable role in zygote elongation and re-polarization. It will be interesting to investigate if there is a link between *WOX8* expression and changes in cytoskeleton dynamics in the zygote. It is noteworthy that *WOX8* homolog has not been identified in any plant except *A. thaliana* (Zhou et al., 2018).

Another maternal parent-of-origin contribution to YDA pathway are transcription factors HOMEODOMAIN GLABROUS 11 (HDG11) and HDG12. HDG11/HDG12 bind to *cisC* promoter element of *WOX8* and contribute to the activation of its transcription (Ueda et al., 2017). *HDG11* and *HDG12* are expressed in egg cells and asymmetric expression from maternal allele only is detected early in the zygote (Ueda et al., 2017). Paternal inherited allele is probably activated later in zygote development but cannot complement elongation and division defects anymore. Observed maternal parent-of-origin effect is thus possibly a combination of gamete deposited proteins and asymmetric zygotic genome contribution, introduced in section 2.2 (Ueda et al., 2017).

WOX8 acts redundantly with WOX9 to activate *WOX2* expression (Breuninger et al., 2008). After zygotic division, *WOX2* expression becomes restricted to the apical cell and *WOX8/9* expression to the basal cell. This pathway contributes to the establishment of auxin flow in early embryo. This could explain disruptions of auxin flow in *esf1*-3 RNAi lines (Costa et al., 2014). Recently, a protein homologous to a spliceosome subunit, JANUS, was discovered to affect transcriptional initiation of *WOX2* and *PIN7* (Xiong et al., 2019). *WOX8* transcription is unchanged in *janus* mutant. It will be interesting to investigate if there is a link between WOX8 and JANUS. The role of parent-of-origin contributions in early embryo development in *A. thaliana* is summarized in Figure 4.

Transcription of *WOX8* in the zygote gives a clue that maternal-to-zygotic transition occurs before the first zygotic division. This idea is supported by studies of RNA polymerase II activity in the zygote and global transcriptome. RNA polymerase II activity was thought to be dispensable for the first divisions according to observations in RNAi knockdown of *POLII* (Pillot et al., 2010). However, recent studies pointed to the activation of transcription very early in embryogenesis before zygotic division (Kao and Nodine, 2019; Zhao et al., 2019). Transcriptional profiles of egg cells and zygotes in symmetric and elongated stages show significant differences (Zhao et al., 2019). 3,251 genes expressed in the egg cell are downregulated after fertilization. At least some of them encode proteins involved in gamete fusion, e.g. *EC1*. On the other hand, 4,436 genes are upregulated in the zygote in comparison to the egg cell,

corresponding to nearly 14% of all annotated genes in *A. thaliana* genome. Transcriptional profile of the symmetric zygote differs markedly from the elongated zygote. Firstly, genes associated with transcription and its regulation are upregulated. In the polarized stage, transcripts encoding products involved in cell cycle and its regulation, organization of cytoskeleton and chromosome organization predominate.

De novo transcription in the zygote is essential for zygote elongation and division. This is supported by studies with transcription inhibitors α-amanitine (Kao and Nodine, 2019; Zhao et al., 2019) and triptolide (Kao and Nodine, 2019). Another evidence for the activity of RNA polymerase II shortly after fertilization is phosphorylation of Ser2 residue in its CTD (Kao and Nodine, 2019).

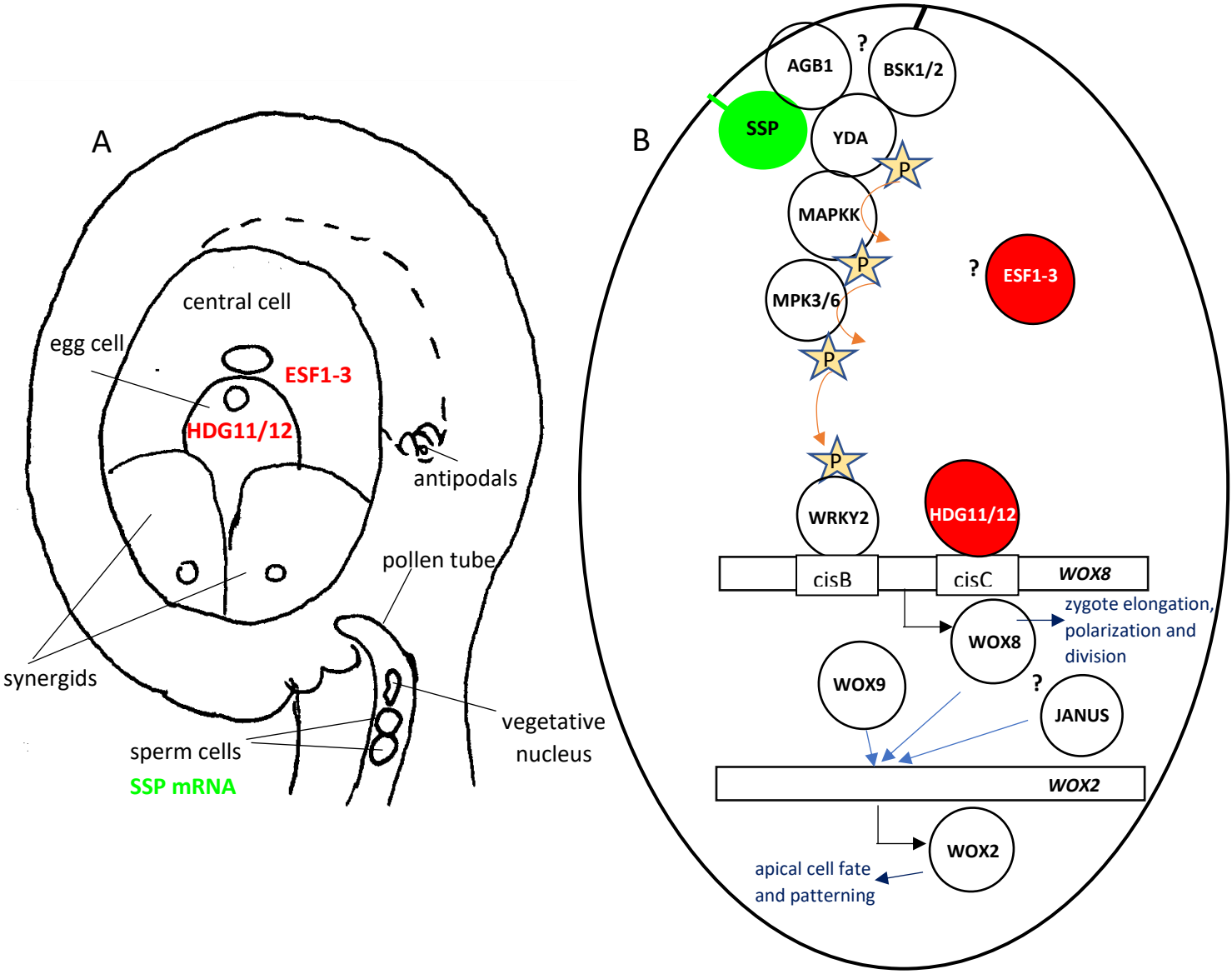

Figure 4. Parent-of-origin contributions in YDA MAP kinase pathway in A. thaliana zygote. *Maternal contribution depicted as red, paternal contribution depicted as green. A) mRNA and proteins deposited in male and female gametes before double fertilization and B) their involvement in YDA pathway. Proposed interactions marked with a question mark. See text for further details. Picture in A redrawn and modified from Maruyama and Higashiyama, 2016.*

2.1.3 *Nicotiana tabacum*

EST sequencing of tobacco sperm cells identified 1,050 genes to be expressed in sperm cells (Xin et al., 2011). 37,2% of these transcripts were not characterized before. Some transcripts are sperm cell specific and probably involved in sperm cell differentiation or fertilization. Whereas some transcripts cannot be identified in the zygote shortly after fertilization, a few of them persist and are potentially involved in zygote and early embryo development. Such a transcript has not been identified and characterized yet.

However, spatiotemporal analysis of *WOX* gene family expression in embryo development uncovered *WOX11* and *WOX13b* to be expressed at high level in the egg cell. Their transcription decreases slightly in the zygote. This could be explained by maternal parent-of-origin effect (Zhou et al., 2018).

In tobacco, zygotic genome is thought to be activated before first zygotic division. Transcription is crucial to zygote elongation and division. EST sequencing of egg cells, zygotes and two-cell embryos revealed substantial differences between each of these cells´ transcriptomes (Zhao et al., 2011). Some transcripts present in high level in the egg cell are not present in the zygote, indicating their degradation. On the other hand, gene upregulation occurs in both zygote and two-cell embryos of *Nicotiana tabacum*. Transcription of ribosome biosynthetic genes is activated in the zygote. The role of transcription in zygote elongation and division is supported by experiments with transcription inhibitors actinomycin D and cordycepin. Treated zygotes neither elongate nor divide (Zhao et al., 2011).

2.1.4 *Oryza sativa*

A microarray study in 2013 compared sperm cell, egg cell and zygote transcriptomes in rice (Abiko et al., 2013a). Egg cell and zygote RNA profiles are more similar than sperm cell and zygote RNA profiles but still show differences. This result indicates maternal-to-zygotic transition early in the zygote stage. Proteomic analysis of rice gametes identified proteins enriched specifically in both sperm cells and egg cells (Abiko et al., 2013b). If these genes are mutated in rice or if their homologs are mutated in *A. thaliana*, they show fertility defects. This could indicate their role in either gametophyte development, fertilization or zygote/early embryo development and deserves further research.

A recent RNA-seq study supports zygotic genome activation before first zygotic division (Anderson et al., 2017). A substantial downregulation of gene expression is observed in the zygote in comparison to the egg cell as soon as nuclear fusion. On the other hand, 181 genes are probably transcribed *de novo* in the zygote, including genes encoding WOX transcription factors. Genes associated with cell cycle and DNA metabolism are upregulated earlier than genes involved in translation.

Chromatin structure reorganization possibly participates in maternal-to-zygotic transition in rice. A dynamic structure called compact silent centre (CSC) is detected in both egg cells and zygotes but is absent in sperm cells, depicted in Figure 5 (Zhou et al., 2019). This structure is observed in the centre of nucleus. Chromatin fibers repeatedly fold in and out of it. Chromatin in this region is enriched in H3K9me2 modification, which indicates low expression. CSC is dynamic and its reorganization could play a role in developmental transitions. It is interesting that 46 genes upregulated in the zygote are detected in CSC of the egg cell but not in the zygote. On the other hand, 59 genes downregulated in the zygotes are not part of CSC in the egg cell but become associated with CSC in the zygote. Sperm cell genes supressed in the zygote are incorporated into CSC as well.

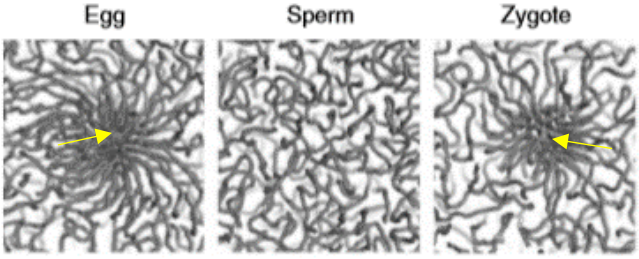

Figure 5. Structure of compact silent centre in rice egg cell and zygote*. CSC is absent in the sperm cell. CSC is thought to play a role in ZGA. CSC indicated with a yellow arrow. Adapted and modified from Zhou et al., 2019.*

2.1.5 *Zea mays*

A microarray study in *Tripsacum-Zea* hybrids in 2005 suggested that maternal-to-zygotic transition does not occur early in embryo development (Grimanelli et al., 2005). However, a recent RNA-seq comparison of gamete and zygote transcriptional profiles supports zygotic genome activation before zygotic division (Chen et al., 2017). More than 1700 genes are downregulated in the zygote. Even though some transcription factors seem to be parentally inherited, zygotic transcriptome differs significantly from both sperm and egg cell transcriptomes. More than 9% genes in annotated maize genome show upregulation in the zygote in comparison to gametes, including 428 genes encoding transcription factors. It is interesting that more than 1500 genes are upregulated only transiently in the zygote, indicative of a major transcriptome reconstruction in the zygote. Shortly after fertilization, genes homologous to *A. thaliana* WOX transcription factors are activated and possibly play a role in embryo patterning.

2.1.6 *Triticum aestivum*

Egg cell and early embryo transcriptome has been compared in wheat as well, using EST analysis and RT-qPCR for quantification of selected transcripts. Studies revealed downregulation and upregulation of genes following fertilization (Sprunck et al., 2005; Domoki et al., 2013). Genes involved in cell cycle, transcription and signalling are upregulated before the first zygotic division (Domoki et al., 2013). Novel transcripts were detected in these studies, some of them differentially expressed in the egg cell and the zygote/2-cell embryo. They could participate in early embryo development in wheat.

2.2 Is zygotic genome activated symmetrically or asymmetrically?

Zygotic genome seems to be activated shortly after fertilization in evolutionary distant plant species. Some scientists propose that paternal genome is activated later, and only maternal alleles are initially transcribed. This would result in parent-of-origin gene expression.

2.2.1 Research methods

To reveal if ZGA is symmetric or asymmetric, gene expression from maternally and paternally inherited alleles must be distinguished. All methods used are based on two types of crosses. Isogenic cross is a cross of plants from the same accession/subspecies and species. If plants from different accession/subspecies or species are crossed, it is called a hybrid cross. Isogenic and hybrid crosses can lead to conflicting results and it is important to differentiate between them.

Even simple microscopic observations can give a clue if paternal genome is activated at the same time as maternal genome. Many embryo defective mutants have been characterized. They show disruptions in embryo development in different stages. Many of these mutations are recessive. If paternal and maternal genome are activated at the same time and to the same extent, a functional allele inherited from either parent and transcribed *de novo* should complement the defect. In contrast, if paternal genome is activated later, mutant phenotype could be observed until gene expression from paternal allele is initiated (e.g. Del Toro-De León et al., 2014).

Molecular methods can broaden the knowledge of ZGA (a)symmetry. A reporter gene (e.g. *GUS/GFP*) can be driven by early embryo specific promoter. In case signal (e.g. blue staining for GUS or green fluorescence for GFP) is detected at the same time for both maternally and paternally inherited copy, symmetric ZGA can be assumed (e.g. Weijers et al., 2001). Expression of the transgene might be influenced by the site of integration (Baroux et al., 2001). Reporter expression should thus be examined in multiple transgenic lines. Furthermore, result can be affected by different sensitivity of reporters. In case expression from paternal alleles is weak, it might be above detection limit of one reporter and under the limit of another (Baroux et al., 2001).

A gene active shortly after ZGA can be identified by observable disruptions in mutant development. However, gene redundancy and potential RNA/protein inheritance from gametes prevents detection of many genes using this approach. Alternatively, early gene activity can be uncovered with help of gene trap transposable elements (Vielle-Calzada et al., 2000). Transposition of a construct carrying reporter gene downstream of the promoter of early transcribed gene leads to signal. If transposant is crossed with wild type and signal is detected at the same time in reciprocal crosses, symmetric genome activation can be deduced.

An alternative strategy is a transactivation system. One parental genome encodes a transcription factor under the control of early embryo specific promoter. The transcription factor activates reporter gene expression from the genome of the other parent. If maternal and paternal genome are activated simultaneously, signal appears at the same time in reciprocal crosses. Methods that enable gene specific investigation of ZGA symmetry are summarized in Figure 6.

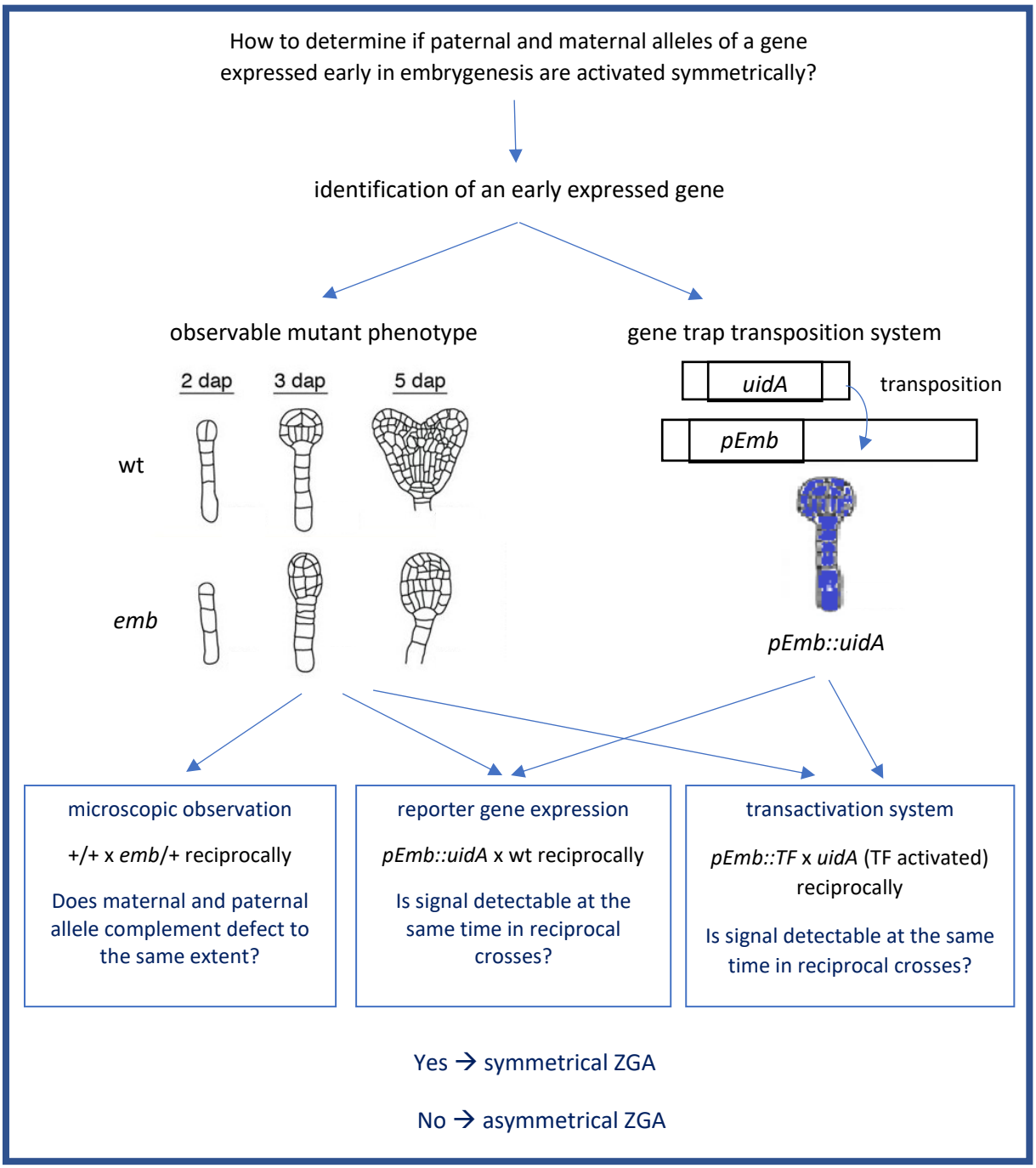

Figure 6. Experimental methods used to identify genes expressed early in embryo development and investigate symmetry of their expression from paternal and maternal alleles in plants*. uidA encoding GUS as an example of a reporter gene. emb – early expressed gene mutant, pEmb – promoter of an early expressed gene, wt – wild-type, TF – transcription factor, dap – days after pollination. See text for further details. Sketch adapted and modified from Alaniz-Fabián et al., 2020.*

On global scale, ZGA can be assessed by transcriptomic methods introduced in section 2.1.1. Isogenic crosses will not be informative as of parental contribution to the transcriptome because transcripts of paternal and maternal alleles cannot be distinguished. Only hybrid crosses enable differentiation of paternal and maternal transcripts thanks to difference in nucleotide sequences of different accessions, subspecies or species. In case paternal genome is activated later in embryogenesis, RNA transcribed from paternally inherited allele should not be detected in early stages.

It is essential that crosses between different accessions/subspecies/species are performed reciprocally. Some genes are not maternally or paternally biased but show accession bias instead (e.g. Meng et al., 2018). Reciprocal crossing enables differentiation from accession bias.

Contamination by maternal tissues and endosperm is a major concern. It could cause apparent maternal transcript dominance. Most studies prior to 2017 used contaminated samples for RNA-seq experiments and are not considered reliable in this thesis (Schon and Nodine, 2017).

2.2.2 *Arabidopsis thaliana*

A. thaliana has more than 1,100 accessions with sequenced genomes (Alonso-Blanco et al., 2016) and many embryo-defective mutants have been characterized. A well-known example is *emb30/gnom* mutant. *EMB30/GNOM* encodes a protein involved in intracellular trafficking of auxin transporters. One of the first studies investigating ZGA symmetry revealed that paternal *EMB30/GNOM* allele complements the defect only later in embryo development in an isogenic cross (Vielle-Calzada et al., 2000). Paternal *emb30/gnom* transcripts were not detected in hybrid Landsberg *erecta* (Ler) x Columbia (Col) crosses either, supporting asymmetric ZGA. Surprisingly, early paternal genome activity was detected only a year later for different genes (Weijers et al., 2001). The authors suggest locus-specific paternal activation. A study based on microscopic observations confirms this assumption (Del Toro-De León et al., 2014). 40 of 49 examined genes show gradual complementation by paternal alleles. A recent study uncovered epigenetic changes in sperm cells that could contribute to transcription of paternal alleles in early embryo (Borg et al., 2020). Loss of a repressive H3K27me3 mark is accompanied by *de novo* deposition of an activating H3K4me3 mark in some loci. Genes with paternally but not maternally biased expression and a few genes with unbiased expression (*WOX2, WOX8, WOX9*) active early in embryogenesis were detected among reprogrammed loci.

The first attempt to compare paternal and maternal contributions to global transcription in early embryo came in 2011 (Autran et al., 2011). The EST-based study concludes maternal dominance in gene expression up to globular stage in Ler x Col cross. However, reciprocal cross was not performed and substantial contamination by maternal tissues has been revealed (Schon and Nodine, 2017). An RNA-seq study based on Col x Cape Verde Islands (Cvi) cross published in 2012 shows equal

contributions of maternal and paternal genome from as soon as 1-cell stage, with only a few genes transiently showing parent-of-origin expression (Nodine and Bartel, 2012). This study is reported to be free of maternal contamination (Schon and Nodine, 2017). A recent study based on Ler x Col cross further supports equal maternal and paternal contribution from as early as elongated zygote (Zhao et al., 2019).

Hybrid and isogenic crosses may produce different results, however (Del Toro-De León et al., 2014). According to this study, paternal allele activation is not only locus specific, but also depends on accessions crossed. Paternal alleles are reported to be activated earlier in particular hybrid crosses than in an isogenic cross. Results produced by Col x Tsushima (Tsu) cross are the most comparable to the results of isogenic cross. More than 42% of genes in the zygote and 29% of genes in the octant stage are maternally biased in Col x Tsu cross according to a pre-print article (Alaniz-Fabián et al., 2020). Even though contamination by maternal tissues was detected, authors argue that it does not significantly affect their results.

Early paternal activity in hybrid embryos could contribute to hybrid vigour (Del Toro-De León et al., 2014). However, size of early hybrid Ler x C24 embryo is comparable to size of maternal accession embryo in the same developmental stage (Alonso-Peral et al., 2017). The transcriptome of early globular embryo shows higher similarity to the transcriptome of maternal accession than reciprocal hybrid. This indicates existence of maternal factors that may influence transcription in the hybrid. RNA dependent DNA methylation (RdDM) pathway has been suggested to play a role in maternal control of paternal allele activity (Autran et al., 2011). Since then, the role of RdDM has not been examined in early embryo development in *A. thaliana*. The epigenome of more than 1,000 accessions has been investigated. It is well-known that DNA methylation on cytosine can influence gene expression. Among *A. thaliana* accessions, 78% of methylated cytosines are methylated differentially (Kawakatsu et al., 2016). Ler x C24 hybrids show increased methylation in comparison to Ler and C24 parents (Shen et al., 2012), especially in differentially methylated regions. 96% of this increase is in regions targeted by sRNAs, which could indicate RdDM pathway involvement. Transcriptomes of reciprocal hybrids after germination show higher similarity than to either parent, which contrasts observations from early embryo development. It could be interesting to investigate DNA methylation in early embryogenesis.

To conclude, research of ZGA (a)symmetry in *A. thaliana* has produced less answers and generated more questions. Up to globular stage, ZGA seems to be locus specific and differs for various hybrid crosses and isogenic cross. The basis of this variability is unclear. RdDM pathway and epigenomic marks have a proposed role in the process. Further research in this topic is thus needed.

2.2.3 *Nicotiana tabacum*

Nicotiana is one of the largest genera in *Solanaceae* family. To investigate parent-of-origin gene expression, researchers performed an interspecific cross of *N. tabacum* with *N. rustica*, another allotetraploid species from *Nicotiana* genus.

A study in 2011 revealed the possibility of asymmetric ZGA in tobacco. In the interspecific cross between *N. tabacum* and *N. rustica*, paternal expression was detected for 4 genes, maternal expression for 1 gene and biparental expression for 3 genes (Zhang et al., 2011). Transcripts of some of these genes were not detected in gametes. These results indicate locus specific asymmetry of ZGA, as suggested in *A. thaliana*. However, reciprocal cross was not successful. Parentally biased gene expression was thus indistinguishable from species bias.

Hybridization protocol has been optimized to produce reciprocal hybrid since (Luo et al., 2016). Embryo development in reciprocal hybrids of *N. tabacum* var. SR1 and *N. rustica* var. Hamayan differs. The basal cell gives rise to a transient structure – suspensor. Suspensor morphology shortly before programmed cell death (PCD) is different in SR1 and Hamayan. It is interesting that reciprocal hybrids also differ in this trait. Suspensor development of each hybrid is similar its maternal parent. This could be caused by parent-of-origin gene expression (Luo et al., 2016). Suspensor PCD is regulated by a cystatin (CYS) expressed specifically in the basal cell. It binds to protease CP14 and inactivates it (Zhao et al., 2013). *CYS* expression decrease releases CP14 from its inactivity and it triggers suspensor PCD. Hamayan genome encodes *CYS* homolog (Luo et al., 2016). In SR1 x Hamayan hybrids, *CYS* is expressed biparentally but a decrease in expression is detected only for maternal copy. Suspensor PCD is thus proposed to be regulated maternally. A recent study shows another maternal control of the process. Gibberellin produced in maternal tissues is transported into the suspensor (Shi et al., 2019). There, it binds to GID1 – gibberellin receptor. This interaction induces binding of DELLA proteins and mediates their degradation. CRF1 is a DELLA protein that binds *CYS* promoter and stimulates its expression. As gibberellin from maternal tissues reaches the suspensor, CRF1 is degraded and *CYS* expression falls. Maternal control of suspensor PCD could thus be a combination of maternally derived gibberellin and a decrease of maternal *CYS* expression. It could be interesting to investigate if *CRF1* expression is maternally biased as well. Another question is whether decrease of maternal *CYS* only would be observed in an isogenic background.

2.2.4 *Oryza sativa*

Rice has traditionally been divided into two subspecies – *O. sativa subsp. indica* and *O. sativa subsp. japonica*. Genomes of more than 3,000 rice accessions have been sequenced. Sequence analysis revealed vast genetic diversity in *O. sativa* species and divided it into nine subgroups (Wang et al.,

2018). In studies cited in this thesis, crosses between cultivars of *indica* and *japonica* were performed to investigate ZGA asymmetry.

Parent-of-origin expression after ZGA is now well-documented in rice. Parental genomes do not play an interchangeable role in early embryogenesis. Rice has a developed system for in vitro fertilization based on gamete electrofusion (Uchiumi et al., 2007). It is thus possible to observe early embryo development in polyploid zygotes. Surprisingly, zygote division is sensitive to the excess of paternal genome only (Toda et al., 2018). Even though study in 2017 revealed high maternal bias in gene expression in the zygote, genes expressed exclusively from paternal allele were identified as well, including *WOX8/9* homolog (Anderson et al., 2017). A recent study identified only paternal alleles to be expressed in a highly monoallelic fashion in early embryogenesis (Rahman et al., 2019). Such expression was observed for *BABY BOOM-LIKE 1* (*OsBBML1*), a gene encoding a well-known transcription factor. If *BBML1* expression is induced in the egg cell in rice, embryo development starts without the need of fertilization (Khanday et al., 2019). Male-expressed BBML1 is thought to activate expression from the maternal allele later. *BBML1* expression switches into a biparental expression.

BBML1 in rice functionally reminds of SSP in *A. thaliana*. From evolutionary viewpoint, it could be beneficial for some species to avoid asexual reproduction thanks to parent-of-origin gene expression.

2.2.5 *Zea mays*

Zea mays is a diverse crop with more than 135 000 accessions worldwide. In maize, paternal transcripts have been identified in the zygote stage for more than 20 genes in UH005 x UH031 inbred line cross (Meyer and Scholten, 2007). Early activation of paternal alleles is suggested to contribute to hybrid vigour. A recent study confirms that parent-of-origin gene expression in early embryo is not global (Meng et al., 2018).

2.2.6 *Triticum aestivum*

To date, there is no study investigating activation of paternal genome or parent-of-origin expression in embryo in wheat.

2.3 Genomic imprinting in plant embryogenesis

As shown in previous section, a few genes display parent-of-origin gene expression only in early embryo development. Such genes are later expressed biparentally. However, there is also evidence that a few genes maintain parent-of-origin gene expression longer. Genomic imprinting is well known in the endosperm. Endosperm is a transient structure which does not genetically contribute to next generation. There is thus no need to erase and reset epigenetic marks in endosperm chromatin. On the other hand, if there is imprinted gene expression in the embryo, it is necessary to do it before gametes are produced. As plants do not set aside the germ cell line like animals do, epigenetic marks

could be erased any time during vegetative development. Genomic imprinting in plant embryo is considered rather controversial in the scientific community.

The first imprinted gene – *maternally expressed in embryo1 (mee1)* – in embryo was discovered in maize (Jahnke and Scholten, 2009). It is maternally expressed in both embryo and endosperm. *mee1* is detected neither in egg cells nor in sperm cells. In embryo, it is expressed between 3 days after pollination (DAP) and 8 DAP. Differential methylation of maternal and paternal alleles is believed to explain parent-of-origin gene expression. Interestingly, maternal allele is thought to be demethylated shortly after fertilization and remethylated later in embryo development. Genes with specific parent-of-origin expression in embryo have been identified since (Meng et al., 2018). Most of strongly imprinted genes identified in the study show parent-of-origin expression from early stages of embryo development. Methylation of paternal and maternal alleles of these genes has not been assessed.

Genes with continuous parent-of-origin expression in embryo are known in *A. thaliana* as well (Raissig et al., 2013). Interestingly, histone modification – differential methylation of H3K27 – and not DNA methylation is suggested to cause parent-of-origin gene expression. Epigenetic marks are thought to be erased in late embryogenesis or early in vegetative development, as biparental expression is detected in the seedlings. The role of imprinted genes is unclear. T-DNA insertion lines of identified imprinted genes do not have a phenotype. This could be caused by redundancy. An alternative explanation would be that these genes play a minor role in embryo development.

Conclusion

In contrast to the animal model, zygotic genome is activated before the first zygotic division in different plant species. However, transcripts and proteins present in the gametes can participate in embryogenesis, as demonstrated by *SSP*, *ESF1-3* and *HDG11/12* in *Arabidopsis thaliana*. Due to conflicting results, it is not clear whether paternal and maternal genomes are activated symmetrically. Current evidence points to locus-specific activation symmetry. In different plant species, both paternally and maternally biased gene expression has been detected in early embryogenesis. Most genes are expressed biparentally at later stages of embryogenesis. Only a few genes have been identified to maintain parent-of-origin gene expression. These are considered imprinted. Genomic imprinting remains controversial in embryo. It might be based on different mechanisms in different plant species. Undoubtedly, parent-of-origin gene expression in plant embryogenesis and its evolutionary significance deserve further scientific attention.

References

Primary references:

Abiko, M., H. Maeda, K. Tamura, I. Hara-Nishimura, and T. Okamoto. **2013a**. Gene expression profiles in rice gametes and zygotes: identification of gamete-enriched genes and up- or down-regulated genes in zygotes after fertilization. Journal of Experimental Botany 64: 1927–1940.

Abiko, M., K. Furuta, Y. Yamauchi, C. Fujita, M. Taoka, T. Isobe, and T. Okamoto. **2013b**. Identification of Proteins Enriched in Rice Egg or Sperm Cells by Single-Cell Proteomics. PLOS ONE 8: e69578.

Alaniz-Fabián, J., D. Xiang, G. D. T.-D. León, A. Orozco-Nieto, P. Gao, A. Sharpe, L. V. Kochian, et al. **2020**. Maternal genome dominance in early plant embryogenesis. bioRxiv: 2020.01.14.905992.

Alonso-Blanco, C., J. Andrade, C. Becker, F. Bemm, J. Bergelson, K. M. Borgwardt, J. Cao, et al. **2016**. 1,135 Genomes Reveal the Global Pattern of Polymorphism in *Arabidopsis thaliana*. Cell 166: 481–491.

Alonso-Peral, M. M., M. Trigueros, B. Sherman, H. Ying, J. M. Taylor, W. J. Peacock, and E. S. Dennis. **2017**. Patterns of gene expression in developing embryos of *Arabidopsis* hybrids. The Plant Journal 89: 927–939.

Anderson, S. N., C. S. Johnson, D. S. Jones, L. J. Conrad, X. Gou, S. D. Russell, and V. Sundaresan. 2013. Transcriptomes of isolated *Oryza sativa* gametes characterized by deep sequencing: evidence for distinct sex-dependent chromatin and epigenetic states before fertilization. The Plant Journal 76: 729–741.

Anderson, S. N., C. S. Johnson, J. Chesnut, D. S. Jones, I. Khanday, M. Woodhouse, C. Li, et al. **2017**. The Zygotic Transition Is Initiated in Unicellular Plant Zygotes with Asymmetric Activation of Parental Genomes. Developmental Cell 43: 349-358.e4.

Autran, D., C. Baroux, M. T. Raissig, T. Lenormand, M. Wittig, S. Grob, A. Steimer, et al. **2011**. Maternal Epigenetic Pathways Control Parental Contributions to *Arabidopsis* Early Embryogenesis. Cell 145: 707–719.

Bakos, F., L. Szabó, A. Olmedilla, and B. Barnabás. **2009**. Histological comparison between wheat embryos developing in vitro from isolated zygotes and those developing in vivo. Sexual Plant Reproduction 22: 15–25.

Baroux, C., R. Blanvillain, and P. Gallois. **2001**. Paternally inherited transgenes are down-regulated but retain low activity during early embryogenesis in *Arabidopsis*. FEBS Letters 509: 11–16.

Bayer, M., T. Nawy, C. Giglione, M. Galli, T. Meinnel, and W. Lukowitz. **2009**. Paternal control of embryonic patterning in *Arabidopsis thaliana*. Science (New York, N.Y.) 323: 1485–1488.

Bergmann, D. C., W. Lukowitz, and C. R. Somerville. **2004**. Stomatal Development and Pattern Controlled by a MAPKK Kinase. Science 304: 1494.

Borg, M., Y. Jacob, D. Susaki, C. LeBlanc, D. Buendía, E. Axelsson, T. Kawashima, et al. 2020. Targeted reprogramming of H3K27me3 resets epigenetic memory in plant paternal chromatin. Nature Cell Biology.

Borges, F., G. Gomes, R. Gardner, N. Moreno, S. McCormick, J. A. Feijó, and J. D. Becker. **2008**. Comparative Transcriptomics of *Arabidopsis* Sperm Cells. Plant Physiology 148: 1168–1181.

Borges, F., R. Gardner, T. Lopes, J. P. Calarco, L. C. Boavida, R. K. Slotkin, R. A. Martienssen, and J. D. Becker. **2012**. FACS-based purification of *Arabidopsis* microspores, sperm cells and vegetative nuclei. Plant Methods 8: 44.

Breuninger, H., E. Rikirsch, M. Hermann, M. Ueda, and T. Laux. **2008**. Differential Expression of *WOX* Genes Mediates Apical-Basal Axis Formation in the *Arabidopsis* Embryo. Developmental Cell 14: 867–876.

Brueckner, F., and P. Cramer. **2008**. Structural basis of transcription inhibition by α-amanitin and implications for RNA polymerase II translocation. Nature Structural & Molecular Biology 15: 811–818.

Chen, D., Y. Ren, Y. Deng, and J. Zhao. **2010**. Auxin polar transport is essential for the development of zygote and embryo in *Nicotiana tabacum* L. and correlated with ABP1 and PM H+-ATPase activities. Journal of Experimental Botany 61: 1853–1867.

Chen, J., A. Lausser, and T. Dresselhaus. **2014**. Hormonal responses during early embryogenesis in maize. Biochemical Society Transactions 42: 325–331.

Chen, J., K. Kalinowska, B. Müller, J. Mergner, R. Deutzmann, C. Schwechheimer, U. Z. Hammes, and T. Dresselhaus. **2018**. DiSUMO-LIKE Interacts with RNA-Binding Proteins and Affects Cell-Cycle Progression during Maize Embryogenesis. Current Biology 28: 1548-1560.e5.

Chen, J., N. Strieder, N. G. Krohn, P. Cyprys, S. Sprunck, J. C. Engelmann, and T. Dresselhaus. **2017**. Zygotic Genome Activation Occurs Shortly after Fertilization in Maize. Plant Cell 29: 2106–2125.

Costa, L. M., E. Marshall, M. Tesfaye, K. A. T. Silverstein, M. Mori, Y. Umetsu, S. L. Otterbach, et al. **2014**. Central cell-derived peptides regulate early embryo patterning in flowering plants. Science (New York, N.Y.) 344: 168–172.

Cyprys, P., M. Lindemeier, and S. Sprunck. **2019**. Gamete fusion is facilitated by two sperm cell-expressed DUF679 membrane proteins. Nature Plants 5: 253–257.

De Rybel, B., M. Adibi, A. S. Breda, J. R. Wendrich, M. E. Smit, O. Novák, N. Yamaguchi, et al. **2014**. Integration of growth and patterning during vascular tissue formation in *Arabidopsis.* Science 345: 1255215.

Del Toro-De León, G., M. García-Aguilar, and C. S. Gillmor. **2014**. Non-equivalent contributions of maternal and paternal genomes to early plant embryogenesis. Nature 514: 624–627.

Denninger, P., A. Bleckmann, A. Lausser, F. Vogler, T. Ott, D. W. Ehrhardt, W. B. Frommer, et al. **2014**. Male–female communication triggers calcium signatures during fertilization in *Arabidopsis*. Nature Communications 5: 1–12.

Domoki, M., A. Szűcs, K. Jäger, S. Bottka, B. Barnabás, and A. Fehér. **2013**. Identification of genes preferentially expressed in wheat egg cells and zygotes. Plant Cell Reports 32: 339–348.

Fedry, J., J. Forcina, P. Legrand, G. Péhau-Arnaudet, A. Haouz, M. Johnson, F. A. Rey, and T. Krey. 2018. Evolutionary diversification of the HAP2 membrane insertion motifs to drive gamete fusion across eukaryotes. PLOS Biology 16: e2006357.

Fischer-Iglesias, C., B. Sundberg, G. Neuhaus, and A. M. Jones. **2001**. Auxin distribution and transport during embryonic pattern formation in wheat. The Plant Journal 26: 115–129.

Flores-Tornero, M., S. Proost, M. Mutwil, C. P. Scutt, T. Dresselhaus, and S. Sprunck. **2019**. Transcriptomics of manually isolated *Amborella trichopoda* egg apparatus cells. Plant Reproduction 32: 15–27.

Friml, J., A. Vieten, M. Sauer, D. Weijers, H. Schwarz, T. Hamann, R. Offringa, and G. Jürgens. 2003. Efflux-dependent auxin gradients establish the apical–basal axis of *Arabidopsis*. Nature 426: 147–153.

Gooh, K., M. Ueda, K. Aruga, J. Park, H. Arata, T. Higashiyama, and D. Kurihara. **2015**. Live-Cell Imaging and Optical Manipulation of *Arabidopsis* Early Embryogenesis. Developmental Cell 34: 242–251.

Gou, X., T. Yuan, X. Wei, and S. D. Russell. 2009. Gene expression in the dimorphic sperm cells of *Plumbago zeylanica*: transcript profiling, diversity, and relationship to cell type. The Plant Journal 60: 33–47.

Grimanelli, D., E. Perotti, J. Ramirez, and O. Leblanc. **2005**. Timing of the Maternal-to-Zygotic Transition during Early Seed Development in Maize. The Plant Cell 17: 1061–1072.

Guo, L., S.-B. Chao, L. Xiao, Z.-B. Wang, T.-G. Meng, Y.-Y. Li, Z.-M. Han, et al. **2017**. Sperm-carried RNAs play critical roles in mouse embryonic development. Oncotarget 8, 67394-67405.

He, Y.-C., Y.-Q. He, L.-H. Qu, M.-X. Sun, and H.-Y. Yang. **2007**. Tobacco zygotic embryogenesis in vitro: the original cell wall of the zygote is essential for maintenance of cell polarity, the apical–basal axis and typical suspensor formation. The Plant Journal 49: 515–527.

Hofmann, F., M. A. Schon, and M. D. Nodine. **2019**. The embryonic transcriptome of *Arabidopsis thaliana*. Plant Reproduction 32: 77–91.

Hornslien, K. S., J. R. Miller, and P. E. Grini. **2019**. Regulation of Parent-of-Origin Allelic Expression in the Endosperm. Plant Physiology 180: 1498–1519.

Horowitz, B., B. A. Goldfinger, and J. Marmur. **1976**. Effect of cordycepin triphosphate on the nuclear DNA-dependent RNA polymerases and poly(A) polymerase from the yeast, *Saccharomyces cerevisiae*. Archives of Biochemistry and Biophysics 172: 143–148.

Huang, J., Y. Ju, X. Wang, Q. Zhang, and Sodmergen. **2015**. A one-step rectification of sperm cell targeting ensures the success of double fertilization. Journal of Integrative Plant Biology 57: 496–503.

Itoh, J.-I., Y. Sato, Y. Sato, K.-I. Hibara, S. Shimizu-Sato, H. Kobayashi, H. Takehisa, et al. **2016**. Genome-wide analysis of spatiotemporal gene expression patterns during early embryogenesis in rice. Development 143: 1217–1227.

Jahnke, S., and S. Scholten. **2009**. Epigenetic Resetting of a Gene Imprinted in Plant Embryos. Current Biology 19: 1677–1681.

Johnston, A. J., P. Meier, J. Gheyselinck, S. E. Wuest, M. Federer, E. Schlagenhauf, J. D. Becker, and U. Grossniklaus. 2007. Genetic subtraction profiling identifies genes essential for *Arabidopsis* reproduction and reveals interaction between the female gametophyte and the maternal sporophyte. Genome Biology 8: R204.

Kao, P., and M. D. Nodine. **2019**. Transcriptional Activation of *Arabidopsis* Zygotes Is Required for Initial Cell Divisions. Scientific Reports 9: 1–11.

Kawakatsu, T., S. C. Huang, F. Jupe, E. Sasaki, R. J. Schmitz, M. A. Urich, R. Castanon, et al. **2016**. Epigenomic Diversity in a Global Collection of *Arabidopsis thaliana* Accessions. Cell 166: 492–505.

Kawashima, T., D. Maruyama, M. Shagirov, J. Li, Y. Hamamura, R. Yelagandula, Y. Toyama, and F. Berger. **2014**. Dynamic F-actin movement is essential for fertilization in *Arabidopsis thaliana*. eLife 3: e04501.

Kerk, N. M., T. Ceserani, S. L. Tausta, I. M. Sussex, and T. M. Nelson. **2003**. Laser Capture Microdissection of Cells from Plant Tissues. Plant Physiology 132: 27–35.

Khanday, I., D. Skinner, B. Yang, R. Mercier, and V. Sundaresan. **2019**. A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. Nature 565: 91–95.

Kimata, Y., T. Higaki, T. Kawashima, D. Kurihara, Y. Sato, T. Yamada, S. Hasezawa, et al. **2016**. Cytoskeleton dynamics control the first asymmetric cell division in *Arabidopsis* zygote. Proceedings of the National Academy of Sciences 113: 14157–14162.

Kimata, Y., T. Kato, T. Higaki, D. Kurihara, T. Yamada, S. Segami, M. T. Morita, et al. **2019**. Polar vacuolar distribution is essential for accurate asymmetric division of *Arabidopsis* zygotes. Proceedings of the National Academy of Sciences 116: 2338-2343.

Komarnitsky, P., E.-J. Cho, and S. Buratowski. **2000**. Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes & Development 14: 2452–2460.

Li, C., H. Xu, S. D. Russell, and V. Sundaresan. **2019**. Step-by-step protocols for rice gamete isolation. Plant Reproduction 32: 5–13.

Liu, L., Y. Lu, L. Wei, H. Yu, Y. Cao, Y. Li, N. Yang, et al. 2018. Transcriptomics analyses reveal the molecular roadmap and long non-coding RNA landscape of sperm cell lineage development. The Plant Journal 96: 421–437.

Liu, S.-L., and K. L. Adams. **2010**. Dramatic Change in Function and Expression Pattern of a Gene Duplicated by Polyploidy Created a Paternal Effect Gene in the *Brassicaceae*. Molecular Biology and Evolution 27: 2817–2828.

Liu, X., X. Wu, P. B. Adhikari, S. Zhu, Y. Kinoshita, F. Berger, T. Higashiyama, and R. D. Kasahara. **2020**. Establishment of a novel method for the identification of fertilization defective mutants in *Arabidopsis thaliana*. Biochemical and Biophysical Research Communications 521: 928–932.

Lu, Y., L. Wei, and T. Wang. **2015**. Methods to isolate a large amount of generative cells, sperm cells and vegetative nuclei from tomato pollen for “omics” analysis. Frontiers in Plant Science 6: 391.

Lukowitz, W., A. Roeder, D. Parmenter, and C. Somerville. **2004**. A MAPKK Kinase Gene Regulates Extra-Embryonic Cell Fate in *Arabidopsis*. Cell 116: 109–119.

Luo, A., P. Zhao, L.-Y. Zhang, and M.-X. Sun. **2016**. Initiation of programmed cell death in the suspensor is predominantly regulated maternally in a tobacco hybrid. Scientific Reports 6: 1–9.

Mansfield, S. G., and L. G. Briarty. **1991**. Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. Canadian Journal of Botany 69: 461–476.

Meng, D., J. Zhao, C. Zhao, H. Luo, M. Xie, R. Liu, J. Lai, et al. **2018**. Sequential gene activation and gene imprinting during early embryo development in maize. The Plant Journal 93: 445–459.

Meyer, S., and S. Scholten. **2007**. Equivalent Parental Contribution to Early Plant Zygotic Development. Current Biology 17: 1686–1691.

Misra, C. S., M. R. Santos, M. Rafael-Fernandes, N. P. Martins, M. Monteiro, and J. D. Becker. **2019**. Transcriptomics of *Arabidopsis* sperm cells at single-cell resolution. Plant Reproduction 32: 29–38.

Möller, B. K., C. A. Ten Hove, D. Xiang, N. Williams, L. G. López, S. Yoshida, M. Smit, et al. **2017**. Auxin response cell-autonomously controls ground tissue initiation in the early *Arabidopsis* embryo. Proceedings of the National Academy of Sciences of the United States of America 114: E2533–E2539.

Morgante, P. G., C. M. Berra, M. Nakabashi, R. M. A. Costa, C. F. M. Menck, and M.-A. Van Sluys. 2005. Functional *XPB/RAD25* redundancy in *Arabidopsis* genome: characterization of *AtXPB2* and expression analysis. Gene 344: 93–103.

Mori, T., T. Igawa, G. Tamiya, S. Miyagishima, and F. Berger. 2014. Gamete Attachment Requires GEX2 for Successful Fertilization in *Arabidopsis*. Current Biology 24: 170–175.

Moukhtar, J., A. Trubuil, K. Belcram, D. Legland, Z. Khadir, A. Urbain, J.-C. Palauqui, and P. Andrey. **2019**. Cell geometry determines symmetric and asymmetric division plane selection in *Arabidopsis* early embryos. PLOS Computational Biology 15: e1006771.

Neu, A., E. Eilbert, L. Y. Asseck, D. Slane, A. Henschen, K. Wang, P. Buergel, et al. **2019**. Constitutive signaling activity of a receptor-associated protein links fertilization with embryonic patterning in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences 116: 5795–5804.

Nodine, M. D., and D. P. Bartel. **2012**. Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos. Nature 482: 94–97.

Ohnishi, Y., and T. Okamoto. **2015**. Karyogamy in rice zygotes: Actin filament-dependent migration of sperm nucleus, chromatin dynamics, and de novo gene expression. Plant Signaling & Behavior 10: e989021.

Ohnishi, Y., and T. Okamoto. **2017**. Nuclear migration during karyogamy in rice zygotes is mediated by continuous convergence of actin meshwork toward the egg nucleus. Journal of Plant Research 130: 339–348.

Ohnishi, Y., I. Kokubu, T. Kinoshita, and T. Okamoto. **2019**. Sperm Entry into the Egg Cell Induces the Progression of Karyogamy in Rice Zygotes. Plant and Cell Physiology 60: 1656–1665.

Ohnishi, Y., R. Hoshino, and T. Okamoto. **2014**. Dynamics of Male and Female Chromatin during Karyogamy in Rice Zygotes. Plant Physiology 165: 1533–1543.

Peng, X., T. Yan, and M. Sun. **2017**. The WASP-Arp2/3 complex signal cascade is involved in actin-dependent sperm nuclei migration during double fertilization in tobacco and maize. Scientific Reports 7: 43161.

Pillot, M., C. Baroux, M. A. Vazquez, D. Autran, O. Leblanc, J. P. Vielle-Calzada, U. Grossniklaus, and D. Grimanelli. **2010**. Embryo and Endosperm Inherit Distinct Chromatin and Transcriptional States from the Female Gametes in *Arabidopsis*. The Plant Cell 22: 307–320.

Rahman, M. H., E. Toda, M. Kobayashi, T. Kudo, S. Koshimizu, M. Takahara, M. Iwami, et al. **2019**. Expression of Genes from Paternal Alleles in Rice Zygotes and Involvement of *OsASGR-BBML1* in Initiation of Zygotic Development. Plant and Cell Physiology 60: 725–737.

Raissig, M. T., M. Bemer, C. Baroux, and U. Grossniklaus. **2013**. Genomic Imprinting in the *Arabidopsis* Embryo Is Partly Regulated by PRC2. PLOS Genetics 9: e1003862.

Ribeiro, D. T., C. R. Machado, R. M. A. Costa, U. M. Praekelt, M.-A. Van Sluys, and C. F. M. Menck. **1998**. Cloning of a cDNA from *Arabidopsis thaliana* homologous to the human *XPB* gene. Gene 208: 207–213.

Robert, H. S., C. Park, C. L. Gutierrez, B. Wojcikowska, A. Pencik, O. Novak, J. Chen, et al. **2018**. Maternal auxin supply contributes to early embryo patterning in *Arabidopsis*. Nature Plants 4: 548–553.

Sato, A., K. Toyooka, and T. Okamoto. **2010**. Asymmetric cell division of rice zygotes located in embryo sac and produced by in vitro fertilization. Sexual Plant Reproduction 23: 211–217.

Scholten, S., H. Lörz, and E. Kranz. **2002**. Paternal mRNA and protein synthesis coincides with male chromatin decondensation in maize zygotes. The Plant Journal 32: 221–231.

Schon, M. A., and M. D. Nodine. **2017**. Widespread Contamination of *Arabidopsis* Embryo and Endosperm Transcriptome Data Sets. The Plant Cell 29: 608–617.

Shen, H., H. He, J. Li, W. Chen, X. Wang, L. Guo, Z. Peng, et al. **2012**. Genome-Wide Analysis of DNA Methylation and Gene Expression Changes in Two *Arabidopsis* Ecotypes and Their Reciprocal Hybrids. The Plant Cell 24: 875–892.

Shi, C., P. Luo, Y.-T. Du, H. Chen, X. Huang, T.-H. Cheng, A. Luo, et al. **2019**. Maternal control of suspensor programmed cell death via gibberellin signaling. Nature Communications 10: 1–12.

Sobell, H. M. **1985**. Actinomycin and DNA transcription. Proceedings of the National Academy of Sciences of the United States of America 82: 5328–5331.

Sprunck, S., S. Rademacher, F. Vogler, J. Gheyselinck, U. Grossniklaus, and T. Dresselhaus. **2012**. Egg Cell-Secreted EC1 Triggers Sperm Cell Activation During Double Fertilization. Science 338: 1093–1097.

Sprunck, S., U. Baumann, K. Edwards, P. Langridge, and T. Dresselhaus. **2005**. The transcript composition of egg cells changes significantly following fertilization in wheat (*Triticum aestivum* L.). The Plant Journal 41: 660–672.

Stoeckius, M., D. Grün, and N. Rajewsky. **2014**. Paternal RNA contributions in the *Caenorhabditis elegans* zygote. The EMBO Journal 33: 1740–1750.

Titov, D. V., B. Gilman, Q.-L. He, S. Bhat, W.-K. Low, Y. Dang, M. Smeaton, et al. **2011**. XPB, a subunit of TFIIH, is a target of the natural product triptolide. Nature Chemical Biology 7: 182–188.

Toda, E., Y. Ohnishi, and T. Okamoto. **2018**. An imbalanced parental genome ratio affects the development of rice zygotes. Journal of Experimental Botany 69: 2609–2619.

Uchiumi, T., I. Uemura, and T. Okamoto. **2007**. Establishment of an in vitro fertilization system in rice (*Oryza sativa* L.). Planta 226: 581–589.

Ueda, M., E. Aichinger, W. Gong, E. Groot, I. Verstraeten, L. D. Vu, I. De Smet, et al. **2017**. Transcriptional integration of paternal and maternal factors in the *Arabidopsis* zygote. Genes & Development 31: 617–627.

Ueda, M., Z. Zhang, and T. Laux. **2011**. Transcriptional Activation of *Arabidopsis* Axis Patterning Genes *WOX8/9* Links Zygote Polarity to Embryo Development. Developmental Cell 20: 264–270.

Valansi, C., D. Moi, E. Leikina, E. Matveev, M. Graña, L. V. Chernomordik, H. Romero, et al. 2017. *Arabidopsis* HAP2/GCS1 is a gamete fusion protein homologous to somatic and viral fusogens. Journal of Cell Biology 216: 571–581.

Vielle-Calzada, J.-P., R. Baskar, and U. Grossniklaus. **2000**. Delayed activation of the paternal genome during seed development. Nature 404: 91–94.

von Besser, K., A. C. Frank, M. A. Johnson, and D. Preuss. **2006**. *Arabidopsis HAP2 (GCS1)* is a sperm-specific gene required for pollen tube guidance and fertilization. Development 133: 4761-4769.

Wang, W., R. Mauleon, Z. Hu, D. Chebotarov, S. Tai, Z. Wu, M. Li, et al. **2018**. Genomic variation in 3,010 diverse accessions of Asian cultivated rice. Nature 557: 43–49.

Weijers, D., N. Geldner, R. Offringa, and G. Jürgens. **2001**. Early paternal gene activity in *Arabidopsis*. Nature 414: 709–710.

Xin, H.-P., X.-B. Peng, J. Ning, T.-T. Yan, L.-G. Ma, and M.-X. Sun. **2011**. Expressed sequence-tag analysis of tobacco sperm cells reveals a unique transcriptional profile and selective persistence of paternal transcripts after fertilization. Sexual Plant Reproduction 24: 37–46.

Xiong, F., H.-H. Liu, C.-Y. Duan, B.-K. Zhang, G. Wei, Y. Zhang, and S. Li. **2019**. *Arabidopsis* JANUS Regulates Embryonic Pattern Formation through Pol II-Mediated Transcription of *WOX2* and *PIN7*. iScience 19: 1179–1188.

Yoshida, S., P. Barbier de Reuille, B. Lane, G. W. Bassel, P. Prusinkiewicz, R. S. Smith, and D. Weijers. **2014**. Genetic Control of Plant Development by Overriding a Geometric Division Rule. Developmental Cell 29: 75–87.

Yu, H.-J., P. Hogan, and V. Sundaresan. 2005. Analysis of the Female Gametophyte Transcriptome of Arabidopsis by Comparative Expression Profiling. Plant Physiology 139: 1853–1869.

Yuan, G.-L., H.-J. Li, and W.-C. Yang. **2017**. The integration of Gβ and MAPK signaling cascade in zygote development. Scientific Reports 7: 1–11.

Yuan, S., A. Schuster, C. Tang, T. Yu, N. Ortogero, J. Bao, H. Zheng, and W. Yan. **2016**. Sperm-borne miRNAs and endo-siRNAs are important for fertilization and preimplantation embryonic development. Development 143: 635–647.

Zhang, J.-E., A. Luo, H.-P. Xin, J. Zhao, S.-S. Li, L.-H. Qu, L.-G. Ma, et al. **2011**. Genes of Both Parental Origins Are Differentially Involved in Early Embryogenesis of a Tobacco Interspecies Hybrid. PLOS ONE 6: e23153.

Zhao, J., H. Xin, L. Cao, X. Huang, C. Shi, P. Zhao, Y. Fu, and M.-X. Sun. **2016**. NtDRP is necessary for accurate zygotic division orientation and differentiation of basal cell lineage toward suspensor formation. New Phytologist 212: 598–612.

Zhao, J., H. Xin, L. Qu, J. Ning, X. Peng, T. Yan, L. Ma, et al. **2011**. Dynamic changes of transcript profiles after fertilization are associated with de novo transcription and maternal elimination in tobacco zygote, and mark the onset of the maternal-to-zygotic transition. The Plant Journal 65: 131–145.

Zhao, P., X. Zhou, K. Shen, Z. Liu, T. Cheng, D. Liu, Y. Cheng, et al. **2019**. Two-Step Maternal-to-Zygotic Transition with Two-Phase Parental Genome Contributions. Developmental Cell 49: 882-893.e5.

Zhao, P., X. Zhou, L. Zhang, W. Wang, L. Ma, L. Yang, X. Peng, et al. **2013**. A Bipartite Molecular Module Controls Cell Death Activation in the Basal Cell Lineage of Plant Embryos. PLOS Biology 11: e1001655.

Zhou, S., W. Jiang, Y. Zhao, and D.-X. Zhou. **2019**. Single-cell three-dimensional genome structures of rice gametes and unicellular zygotes. Nature Plants 5: 795–800.

Zhou, X., Y. Guo, P. Zhao, and M. Sun. 2018. Comparative Analysis of WUSCHEL-Related Homeobox Genes Revealed Their Parent-of-Origin and Cell Type-Specific Expression Pattern During Early Embryogenesis in Tobacco. Frontiers in Plant Science 9: 311.

Secondary references:

Cassidy, F. C., and M. Charalambous. **2018**. Genomic imprinting, growth and maternal–fetal interactions. The Journal of Experimental Biology 221: jeb164517.

Fíla, J., L. Záveská Drábková, A. Gibalová, and D. Honys. 2017. When Simple Meets Complex: Pollen and the -Omics. Pollen Tip Growth: From Biophysical Aspects to Systems Biology, 247–292.

Itoh, J.-I., K.-I. Nonomura, K. Ikeda, S. Yamaki, Y. Inukai, H. Yamagishi, H. Kitano, and Y. Nagato. **2005**. Rice Plant Development: from Zygote to Spikelet. Plant and Cell Physiology 46: 23–47.

Jukam, D., S. A. M. Shariati, and J. M. Skotheim. **2017**. Zygotic Genome Activation in Vertebrates. Developmental Cell 42: 316–332.

Kielian, M. 2006. Class II virus membrane fusion proteins. Virology 50th Anniversary Issue 344: 38–47.

Lowe, R., N. Shirley, M. Bleackley, S. Dolan, and T. Shafee. **2017**. Transcriptomics technologies. PLOS Computational Biology 13: e1005457.

Maruyama, D., and T. Higashiyama. **2016**. The end of temptation: the elimination of persistent synergid cell identity. Current Opinion in Plant Biology 34: 122–126.