Zygote

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Advances in the study of zygote activation in higher plants

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Review

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Summary

In higher plants, fertilization induces many structural and physiological changes in the fertilized egg that reflect the transition from the haploid female gamete to the diploid zygote – the first cell of the sporophyte. After fusion of the egg nucleus with the sperm nucleus, many molecular changes occur in the zygote during the process of zygote activation during embryogenesis. The zygote originates from the egg, from which some pre-stored translation initiation factors transfer into the zygote and function during zygote activation. This indicates that the control of zygote activation is pre-set in the egg. After the egg and sperm nuclei fuse, gene expression is activated in the zygote, and paternal and maternal gene expression patterns are displayed. This highlights the diversity of zygotic genome activation in higher plants. In addition to new gene expression in the zygote, some genes show quantitative changes in expression. The asymmetrical division of the zygote produces an apical cell and a basal cell that have different destinies during plant reconstruction; these destinies are determined in the zygote. This review describes significant advances in research on the mechanisms controlling zygote activation in higher plants.

Introduction

In some animals and lower plants, egg cells are discharged from the body and fertilization occurs externally (for example, in fish and toads). Zygote activation has been researched more thoroughly in these organisms compared with in organisms with internal fertilization. The female gametophyte of angiosperms is embedded deeply in the ovule inside the ovary, where the process of double fertilization occurs and the zygote is activated. The hidden location of the female gametophyte makes research on the regulation of fertilization difficult. At this time, using a combination of *in vitro* fertilization techniques and molecular biology methods, it is possible to study the process of fertilization and the initiation of zygotic development in higher plants, and to follow the activation process over time as in some animals and lower plants.

Fertilization is the process in which the egg cell fuses with a sperm cell. Fertilization comprises two sequential fusion processes: plasmogamy and karyogamy. Karyogamy is completed after the migration and fusion of the male and female nuclei in the fused egg cell Ohnishi and Okamoto (2015). After the plasma membranes of both the egg and sperm have fused, but before their nuclei have fused, some structural and physiological changes occur in the fertilized egg cell. The earliest event during plasma membrane fusion of the egg and sperm is a rapid increase in the calcium concentration, followed by a decrease several minutes after fusion. This change is induced by the sperm entering the egg cell and occurs before the fusion of the egg and sperm nuclei, which begins the process of egg activation during embryogenesis (Peng *et al.*, 2018) before zygote activation. After the two nuclei fuse to produce a true zygote, DNA demethylation, chromatin remodelling, genome spatial reorganization and substantial transcriptional changes occur in the zygote. These events trigger zygote activation and the beginning of embryogenesis. Therefore, egg activation and zygote activation are two programmed activation processes that occur after fertilization: the fusion of the plasma membranes of the egg cell and sperm cell induces egg activation, and then the fusion of the egg and sperm nuclei induces zygote activation. Developmental turnover in the zygote has been extensively studied in model animals, but rarely in plants because this is technically very difficult. Therefore, the mechanisms that control the zygote activation process in plants are still unclear. However, recent research using new methods to isolate male and female gametes from higher plants and to analyze gene expression in single cells or in a few cells has contributed to our understanding of zygote activation, and especially genome activation in the zygotes of higher plants (Anderson *et al.*, 2017; Chen *et al.*, 2017; Armenta-Medina and Gillmor, 2019). This review discusses recent advances in research on the molecular changes that occur during zygote activation in higher plants, the earliest process of ontogenesis.

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Characteristics of zygote activation

During zygote activation, the most important process is programmed gene expression in the zygote, which triggers zygote activation and controls development in accordance with a special pattern. The spatial and temporal patterns of gene expression in the zygotes of higher plants are complex and diverse. Fertilization of higher plants consists of one sperm cell fusing with one egg cell to produce a zygote. The parents' genetic information is combined to form the diploid zygotic genome, in which parental alleles are expressed with spatial and temporal regulation. There are three main types of parental gene expression in zygotes: (i) activation of intrinsic gene transcription that originates from the egg; (ii) activation of new gene transcription from the zygotic genome before it divides; and (iii) activation of new gene transcription from the zygotic genome after the first division of the zygote.

Intrinsic maternal transcript maturation and activation

In the fertilization process of higher plants, a large egg fuses with a small sperm and produces a zygote. Although the egg and sperm both contribute genetic material to the zygote, the egg cell contributes most of the cytoplasm and organelles. These unequal maternal and paternal contributions differently affect the offspring. The cytoplasmic male sterility of crops is mainly controlled by plastids or mitochondria from the egg cytoplasm. Parthenogenesis phenomena in some plants also confirm that the egg cell can directly divide and develop into an embryo without fusing with a sperm cell, indicating a strong maternal effect on embryogenesis.

In animals, the egg cell stores abundant mRNAs and proteins before fertilization, and these materials support and regulate early embryogenesis. The activated mRNAs in the zygote regulate cell differentiation, polarity formation and morphological changes during early embryogenesis. During this time, gene expression from the zygote genome is inhibited. Only after the maternal transcripts are consumed does gene expression from the zygotic genome begin (Schier, 2007; Tadros and Lipshitz, 2009; Autran *et al.*, 2011). In embryogenesis in mammals, the time when the paternal genome is expressed differs among species. In mouse, maternal factors play a role in embryonic development because the zygotic genome is not active before the two-cell stage (Schultz, 1993), therefore the first cell division depends on maternal gene products (Dosch *et al.*, 2004). However, the transcriptional activation of mouse begins during the S/G2 phase of the first cell cycle when paternal and maternal chromatins are still in separate nuclear entities within the same cytoplasm. After fertilization, hyperacetylated H4 is associated with paternal but not maternal chromatin, suggesting that the male pronucleus exhibits earlier transcriptional activity compared with the female pronucleus (Adenot *et al.*, 1997). Earlier studies have indicated that embryonic transcription begins at the late four- or eight-cell stage of the bovine proembryo, but later studies suggested that bovine zygotes and two-celled embryos are both transcriptionally and translationally activated (Memili and First, 2000). All of these results indicated that the timing of the switch from maternal to zygotic control in animals is very complex and varies among species.

Advances in methods to isolate the egg and sperm from higher plants and to analyze gene expression in single cells or a few cells have allowed for in-depth analyses of the activation of the zygotic genome. In particular, microarray analyses, which provide data for more than 10,000 signals, have shed light on gene expression during zygote development in higher plants. Dresselhaus *et al.* (1999) constructed cDNA libraries of unfertilized egg cells and *in vitro* fertilized zygotes of maize, and identified more than 50 differentially expressed genes, including one encoding the eukaryotic translation initiation factor eIF-5A. This highly conserved factor is necessary for selective mRNA stabilization and translation. Large amounts of *eIF-5A* transcripts were found to be stored in the unfertilized egg cell, which is relatively metabolically inactive. Based on those results, it was speculated that the unfertilized egg cell of maize is prepared for selective mRNA translation that is triggered after fertilization (Dresselhaus *et al.*, 1999). In a study on *Hyacinthus orientalis*, Niedojadło *et al.* (2012) determined the amount of 5′-bromouracil, the size of the total RNA polymerase II pool, and the proportions of hypophosphorylated (initiation) and hyperphosphorylated (elongation) forms of RNA Pol II as indicators of transcriptional activity. They also analyzed rRNA metabolism by monitoring the accumulation of ribosomal ribonucleoprotein complexes in the cytoplasm and immature transcripts in the nucleolus. The results indicated that fertilization was required to initiate the maturation of maternal pre-rRNAs and the expression of zygotic rDNA (Niedojadło *et al.*, 2012). Abiko *et al.* (2013b) studied the gene expression profiles of the egg and zygote of rice using microarray techniques and found that 81% of positive probes in egg cells were also positive in zygotes. This suggested that abundant maternal transcripts in the egg are transferred to the zygote. The transcription and translation initiation factors in the egg cytoplasm are mature and activated in the zygote, and may trigger zygotic genomic expression during zygotic activation. Guo *et al.* (2016) conducted genetic and cell biology analyses of the zygote arrest 1 (zyg1) mutant in Arabidopsis, which displayed a zygote-lethal phenotype and over-accumulated cyclin B1 D-box–GUS in ovules. Map-based cloning showed that ZYG1 encoded the anaphase-promoting complex/cyclosome (APC/C) subunit 11 (APC11), which is expressed in both egg and sperm cells, and in zygotes and during early embryogenesis. Expression of non-degradable cyclin B1 in the zygote, or mutations of either APC1 or APC4, also led to a zyg1-like phenotype. Biochemical studies showed that APC11 had self-ubiquitination activity and was able to ubiquitinate cyclin B1 and promote degradation of cyclin B1 (Guo *et al.*, 2016).

Zygote genome activation

During zygote activation, the most important event is zygotic genome activation (ZGA), when new gene transcription occurs to regulate embryogenesis. Before zygote division, in addition to maturation and activation of maternal transcription, some genes related to zygote division and embryogenesis begin to be expressed. The timing and scale of the elimination of intrinsic maternal genomic mRNAs and proteins and the activation of zygotic gene transcription differ among species, as do the cellular and morphogenetic processes that sculpt their embryos (Schier, 2007; Tadros and Lipshitz, 2009). Data related to the elimination of maternal mRNA and proteins are very limited, but the expression of non-degradable cyclin B1 in the zygote has been shown to lead to developmental arrest (Guo *et al.*, 2016). Most studies on zygotic activation have focused on ZGA, which is an essential event that initiates zygote development.

After the egg and sperm nuclei fuse, the zygotic genome consists of paternal and maternal genomes. During ZGA, the alleles from paternal and maternal genomes may be expressed differently in the zygote. Parent-of-origin-dependent gene expression refers to the differential activity of alleles inherited from the egg and sperm. Based on García-Aguilar and Gillmor (2015), there are two opposite processes for selected parent gene expression: ZGA and gene imprinting. ZGA is a genome-wide process that promotes the expression of selected genes

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from the maternal genome, paternal genome, or both genomes in the zygote after fertilization. Gene imprinting leads to a bias in gene expression by arresting maternal or paternal alleles to allow expression of the other allele. Hundreds of imprinted genes have been discovered in the endosperm, but few have been found in the zygote and proembryo (García-Aguilar and Gillmor, 2015). The timing of ZGA may vary among species, and among different gene types. Therefore, parent-of-origin-dependent gene expression in the zygote may or may not occur: most studies have detected maternal bias in mRNA transcripts and gene activity in early embryogenesis, others have detected a paternal bias, and others have detected equal contributions of maternal and paternal genomes.

Maternal contribution during ZGA

Vielle-Calzada *et al.* (2000) found that none of the paternally inherited alleles at 20 loci was expressed during early seed development in Arabidopsis (Vielle-Calzada *et al.*, 2000). The genes were involved in various processes and were distributed throughout the genome, indicating that most, if not all, of the paternal genome may be initially silenced. These results implied that early embryo and endosperm development was mainly under maternal control. However, that study did not determine whether transcription of the maternal genome started in the egg or the zygote. Autran *et al.* (2011) identified two antagonistic maternal pathways that controlled parental contributions during Arabidopsis embryogenesis. Their findings showed that paternal alleles were initially downregulated by the chromatin siRNA pathway, which is linked to DNA and histone methylation, whereas transcriptional activation required maternal activity of the histone chaperone complex CAF1. Therefore, their results defined the maternal epigenetic pathways controlling the parental contributions in plant embryos (Autran *et al.*, 2011). Del Toro-De León *et al.* (2014) studied paternal gene activation by performing a large-scale genetic analysis of 49 embryo defective genes and tested the ability of wild-type paternal alleles to complement phenotypes conditioned by mutant maternal alleles. They found that wild-type paternal alleles for nine of these genes were completely functional at 2 days after pollination, while the remaining 40 genes showed partial activity beginning at 2, 3 or 5 days after pollination. Different hybrid combinations exhibited significant variations in paternal allele activation. Together, the results confirmed that the maternal genome had transcriptional superiority (Del Toro-De León *et al.*, 2014). Anderson *et al.* (2017) observed large-scale transcriptomic changes in unicellular zygotes in rice, including upregulation of S phase genes. Their results showed that the parental contributions during ZGA were highly asymmetrical, and zygotic transcription was primarily from the maternal genome and included genes related to basic cellular processes, especially S phase. Their results also showed that transcription of the paternal genome was highly restricted but included genes encoding putative pluripotency factors expressed at the onset of ZGA.

Paternal contributions during ZGA

Baroux *et al.* (2001) invested the timing of transgene activation after fertilization in Arabidopsis following crosses and using two transgenic promoters (from the *AtCYCB1* and *AtLTP1* genes). Their results revealed a lack of expression of the paternal components. However, transactivation experiments using the *BARNASE* gene provided evidence that at least some paternal loci retained transcriptional activity, albeit at a low level, during early embryogenesis in *A. thaliana* (Baroux *et al.*, 2001). Bayer *et al.* (2009) reported that the interleukin-1 receptor-associated kinase (*IRAK*)/Pelle-like kinase gene SHORT SUSPENSOR (*SSP*) regulated the YODA (YDA) mitogen-activated protein kinase (MAPK) pathway through a previously unknown parent-of-origin effect. They found that SSP transcripts were produced in mature pollen but did not appear to be translated. Instead, they were delivered via the sperm cells to the zygote and the endosperm, where SSP transiently accumulated. It was proposed that SSP produced from paternal transcripts upon fertilization triggered zygotic YDA activity, providing an essential temporal cue for the regulation of the asymmetrical first division (Bayer *et al.*, 2009). Ueda *et al.* (2017) found that sperm-delivered *SSP* mRNA can potentiate MAPK signalling. Their results showed that paternal SSP/YDA signalling directly led to the phosphorylation of WRKY2, which in turn led to WOX8 upregulation in the zygote. These results indicated that there was some paternal control of maternal gene expression in Arabidopsis during early embryogenesis. Rahman *et al.* (2019) identified 23 genes with paternal allele-specific expression in rice zygotes. Their allele dependencies in the globular-like embryo tended to be biallelic, suggesting that the paternal-dependent expression of these genes was temporary, and only occurred during the early stages of zygote development. They proposed that monoallelic or preferential gene expression from the paternal genome in the zygote might be a safety mechanism allowing egg cells to suppress the gene expression cascade leading to early embryogenesis that was normally triggered by fusion with a sperm cell (Rahman *et al.*, 2019). Toda *et al.* (2018) produced polyploid zygotes with an imbalanced parental genome ratio by fusion of isolated rice gametes, and observed their developmental profiles. The polyploid zygotes with an excess maternal gamete/genome (two eggs fused with one sperm) developed normally, whereas approximately 50–75% of polyploid zygotes with a paternal excess (an egg fused with two sperms) were arrested (Toda *et al.*, 2018). Although these results did not show how the excess male gamete affected zygote development (e.g. the quantity of cytoplasm, nucleus or genome), they showed that genes with monoallelic expression played important roles during zygote activation and embryogenesis, that excess paternal genome negatively affected zygote development, and that both parental genomes needed to be moderately expressed for zygote development.

Equal parental contributions during ZGA

To determine the maternal and paternal contributions to the early embryonic transcriptome, Nodine and Bartel (2012) sequenced the transcripts of hybrid embryos from crosses between two polymorphic inbred lines of *A. thaliana* and used single-nucleotide polymorphism diagnosis of each parental line to quantify parental contributions. Their results showed that, although some transcripts are either inherited from primarily one parent or transcribed from an imprinted loci, the vast majority of transcripts were produced in near-equal amounts from both maternal and paternal alleles during the initial stages of embryogenesis (Nodine and Bartel, 2012). Embryonic factor 19 (FAC19) in *A. thaliana* encodes a putative mitochondrial protein, and its mutant (fac19) was found to be zygote lethal. A genetic analysis showed that fac19 is caused by a single recessive mutation, suggesting that there are equal maternal and paternal contributions of FAC19 towards zygotic embryogenesis (Yu *et al.*, 2012). Del Toro-De León *et al.* (2016) found that isogenic embryos showed delayed activation for many, but not all, paternal alleles. However, in certain combinations of ecotypes, hybrid embryos showed equal parental contributions to early embryogenesis (Del Toro-De León *et al.*, 2016). These results suggested that altered paternal gene activation in hybrids may have an epigenetic basis.

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The results of these studies indicated that ZGA is a complex process. In the diverse species and developmental stages analyzed, different genes in the zygote were expressed in accordance with programmed spatial and temporal models, making it difficult to draw general conclusions. The observed differences reflected the diversity of transcription levels of paternal and maternal genomes in zygotes of different species.

Quantity changes of gene expression of zygotic genome

As well as new gene expression in the zygote, some genes showed quantitative changes in expression in the fertilized egg. Egg cells are developmentally quiescent, but quiescence is broken after fertilization and egg activation. The genes expressed in the egg cell to maintain egg cell quiescence should be suppressed in zygotes, so comparative analyses of gene expression profiles between egg cells and zygotes were able to reveal genes that are downregulated after fertilization. Rahman *et al.* (2019) found that ectopic expression of OsASGR-BBML1 in rice egg cells induced nuclear and cell divisions, indicating that exogenously expressed OsASGR-BBML1 could convert the proliferation status of the egg cell from quiescent to active. Suppression of the function of OsASGR-BBML1 and its homologues in zygotes resulted in developmental arrest, suggesting that OsASGR-BBML1 has an important role in initiating zygotic development. Abiko *et al.* (2013b) used microarray analysis technology to analyze the gene expression profiles of rice gametes and zygotes, and to detect changes in gene expression from the pre-fertilization to post-fertilization phases. They identified 94 genes that had three-fold lower expression levels in zygotes compared with in egg cells. The expression profiles of the 10 most strongly suppressed genes in zygotes were confirmed. Several receptor kinase-related genes appeared to be upregulated in zygotes, suggesting that signal transduction pathways are activated via fertilization. They identified 325 genes whose expression levels were three-fold higher in zygotes compared with in egg cells, including genes related to chromatin/DNA organization and assembly. Among the highly upregulated genes was Os07g0182900, which encodes DNA methyltransferase 1 (MET1), an enzyme that functions in maintaining CG DNA methylation. A specific inhibitor of MET1 partly affected polarity or asymmetrical division in rice zygotes (Abiko *et al.*, 2013a).

Other quantitative changes in gene expression in the zygote take place during the process of zygote division to form the two-celled proembryo. Okamoto *et al.* (2005) identified genes that were upregulated or downregulated in the apical or basal cell of maize two-celled embryos. The genes were categorized into six groups: (i) upregulated only in the apical cell, (ii) upregulated only in the basal cell, (iii) upregulated in both the apical and basal cells after gamete fusion, (iv) downregulated only in the apical cell, (v) downregulated only in basal cell, and (vi) constitutively expressed in the egg cell and embryo. The genes upregulated in the apical or basal cell were already expressed in the early zygote (Okamoto *et al.*, 2005). Hu *et al.* (2011) conducted analyses of gene transcription in tobacco zygotes and asymmetrical two-celled proembryos *in vivo* (via micromanipulation) and in symmetrical two-celled proembryos (in *in vitro* cell cultures) using suppression-subtractive hybridization (SSH) and macroarray analysis. Differentially expressed genes among the zygote, the asymmetrical two-celled embryo, and the symmetrical two-celled proembryo were detected. In total, 1610 expressed sequence tag clones representing 685 non-redundant transcripts were obtained (Hu *et al.*, 2011). A homology search against known genes from Arabidopsis indicated that some transcripts were related to asymmetric cell division and embryogenesis. Quantitative real-time PCR confirmed the upregulation or downregulation of the selected candidate transcripts during zygotic division. Some transcripts were found to be expressed exclusively in the zygote, or in either type of the two-celled proembryos.

Proteins in egg and activated zygote

Some genes encoding homeobox proteins or transcription factors are induced in the egg and the zygote. Changes in protein composition between the egg and zygote are thought to reflect the change in biological function during development. Therefore, the proteins that are enriched in the egg and sperm cells should have a role in reproductive and/or developmental processes such as gamete differentiation, gamete fusion, egg activation and early zygotic development (zygote activation). Methods to isolate the egg and the zygote have made it possible to analyze the proteins in these cells. Okamoto *et al.* (2004) found that the major protein components in maize egg cells included three cytosolic enzymes: two mitochondrial proteins and annexin p35. Uchiumi *et al.* (2007) analyzed rice egg cell lysates and identified the major proteins as glyceraldehyde-3-phosphate dehydrogenase, ascorbate peroxidase and heat shock protein 90. In rice, Os01g0840300 encodes a Wuschel-related homeobox (WOX) protein, which is the key regulator in determining cell fate in plants (Zhao *et al.*, 2009). Os01g0840300 is the rice orthologue of Arabidopsis WOX2 (Deveaux *et al.*, 2008), whose transcripts accumulate in Arabidopsis zygotes and are restricted to the apical cell of two-celled proembryos (Haecker *et al.*, 2004). Establishment of apical and basal fates depends on the YDA MAPK cascade and WOX homeodomain transcription factors. WOX2 has been proposed as the main regulator of apical patterning (Jeong *et al.*, 2011), suggesting that the WOX protein encoded by Os01g0840300 may have a role in determining cell fate during early embryogenesis in rice. Okamoto *et al.* (2004) identified major protein components expressed in the maize egg cell. The major proteins included three cytosolic enzymes in the glycolytic pathway, two mitochondrial proteins, the ATP synthase β-subunit, an adenine nucleotide transporter, and annexin p35. They found that annexin p35 was highly expressed only in the egg cell, and that glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase and the adenine nucleotide transporter were expressed at higher levels in the egg cell compared with in central and cultured cells. The proteins enriched in egg cells had roles in reproductive and/or developmental processes such as egg activation and early zygotic development (Okamoto, 2017).

Regulation of cell cycle of fused egg

Cell cycle regulation is important for the growth and development of plants. The last stage of development of the fertilized egg is division to begin ontogenesis. The regulation of DNA synthesis in gamete cells and the fused egg cell is an important event in egg activation. In the monocots barley (Mogensen and Holm, 1995), maize (Mogensen *et al.*, 1999) and rice (Sukawa and Okamoto, 2018), the DNA content of egg cells is 1 relative content (1C) and that of zygotes is 2C, indicating that the egg is in the G1 phase of the cell cycle during fertilization. However, the pattern of changes in DNA content differs in dicot egg cells. The two newly formed sperm cells of *A. thaliana* in pollen grains showed an increase in DNA content, indicating that they had entered the S phase of the cell cycle. After pollination, both sperm cells continued to synthesize DNA in the

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pollen tube and attain 1.75C when they arrive at the ovary. Close to the time of fertilization, the DNA content of the sperm cells attained 1.98C (Friedman, 1999). In tobacco, when pollen tubes enter the embryo sac and discharge two sperm cells in the degenerated synergid, the two sperm cells begin to synthesize DNA, eventually attaining 2C before fusion with the egg and central cells. Concomitant with pollen tube arrival, the DNA content of the egg cell also begins to increase and finally attains 2C. The DNA content in newly formed zygotes is 4C and remains at 4C until zygote division (Tian *et al.*, 2005). In *Lycium barbarum*, the two sperm in the pollen tube begin to synthesize DNA and the DNA content continues to increase. Before fertilization, the egg DNA content is 1.83C (Deng *et al.*, 2012). In *Helleborus bocconei*, the egg begins to synthesize DNA and attains 2C DNA before fertilization. The central cell attains 4C DNA before fertilization. Interesting, the two sperm cells showed a large difference in their DNA content: one synthesized DNA and attained 2C DNA content before fusion with the central cell to form a sextuploid endosperm, and the other sperm did not synthesize DNA before fusion with the egg and showed 3C DNA (Bartoli *et al.*, 2016). These results indicated that, in these four dicotyledonous plants, the egg cells were in the G2 phase of the cell cycle before fertilization. These results also demonstrated the diversity of fertilization in angiosperms from a cell cycle perspective.

Those studies also highlighted some novel characteristics of fertilization:

(i) The sperm of *Arabidopsis* begins to synthesize DNA in the pollen grain, that of *L. barbarum* in the pollen tube, and that of tobacco in the degenerative synergid after release. The differences among these three species suggested that different mechanisms to initiate DNA synthesis operate in different plants. Given the diversity of fertilization types, more research is required on a wider range of higher plant species.

(ii) Egg cells in unpollinated flowers delay DNA synthesis, indicating that the egg cell DNA synthesis is itself an inherent feature, but can be promoted by sperm that synthesize DNA. In tobacco, the sperm cells that synthesize DNA initiate egg DNA synthesis, whereas in *L. barbarum*, DNA-synthesizing sperm cells promote the continuation of egg DNA synthesis. The promotion of egg DNA synthesis by DNA-synthesizing sperm cells may be one strategy to ensure that egg cells do not divide without fertilization.

(iii) Techniques for studying the cell cycle can be used to analyze the events after the two sperm cells of *H. bocconei* separately fuse with the egg cell and the central cell. Such studies can reveal whether DNA synthesis in the egg cell is a precondition for fusion with a sperm cell, and how the DNA content changes in the central cell. These results of sperm cell cycle area in contrast with that of de Graaf and Dewitte (2019), who both thought that male sperm cells have to reach the correct cell cycle phase, and that the egg cell has to remain quiescent until karyogamy, implying that core cell cycle regulators are involved in blocking gamete cells either in G1 or in G2, especially in the waiting or quiescent egg cell and that, in both the male and female gametophytes, key regulatory pathways ensure this coordination (de Graaf and Dewitte, 2019).

The change in DNA content in the cell is regulated by cell cycle genes. Sauter *et al.* (1998) studied the expression of the cell cycle regulatory genes *cdc2ZmA/B*, *Zeama;CycB1;2*, *Zeama;CycA1;1*, *Zeama;CycB2;1* and *histone H3* in maize sperm cells, egg cells, other cells in the embryo sac and zygotes produced by *in vitro* fertilization. They found that *cdc2* and *histone H3* were expressed constitutively in all cells, and that cyclin genes displayed cell-specific expression in the embryo sac and differential expression during zygote development. During zygote development, the three cyclin genes were expressed at different times (Sauter *et al.*, 1998). Sukawa and Okamoto (2018) measured the amount of DNA in the nuclei of eggs, zygotes and cells of early embryos of rice. Their results showed that the egg cell is at the G1 phase of the cell cycle with 1C DNA. The DNA content in the zygote nuclei increased and DNA replication occurred during the progression of the cell cycle. Analyses of the expression profiles of cell cycle-related genes in egg cells and zygotes showed that the rice egg was arrested at G1 phase, probably by OsKRP2. After fusion with sperm, OsWEE1 mediated parental DNA integrity in the zygote nucleus, and the zygote progressed through the cell cycle to produce a two-celled embryo (Sukawa and Okamoto, 2018). However, the pattern in maize and rice (two monocots) was not the same as that in dicots, which displayed diverse patterns of zygote activation.

Cellular determination of zygotic asymmetric division

Following a predetermined pattern of development, the zygote gives rise to an embryo with the potential to form a complete plant. Embryogenesis in angiosperms is diverse, and each plant has its own special pattern of division. In most plants, the first division of the zygote decides the fate of two daughter cells; the terminal cell will form the stem of the plant, and the basal cell will form the suspensor that nurses the embryo or forms the root. The direction of cell division of the two-celled proembryo also has an exact pattern. In accordance with first two divisions of the zygote, there are seven types of embryogenesis. Within the four-celled proembryo division category, more types of embryogenesis can be categorized (Maheshwari, 1950; Bhojwani and Bhatnagar, 1974).

The separate identities of the two daughter cells begin with the asymmetrical division of the zygote, which is preceded by zygotic polarization. In this process, the nucleus is positioned in the upper part (chalazal end) and a large vacuole occupies the lower part (micropylar end). Therefore, the fate of the two daughter cells is determined by zygote polarization. Haecker *et al.* (2004) found that *A. thaliana WOX2* and *WOX8* genes were co-expressed in the egg cell and zygote, but were limited to the apical and basal daughter cells, respectively, after asymmetrical zygotic division. *WOX2* not only marked apical descendants of the zygote, but was also functionally required for correct development, indicating that the asymmetrical division of the zygote separately determined apical and basal cell fate. On the basis of their results, it was inferred that, during plant embryogenesis, region-specific transcription programmes are initiated very early in single precursor cells (Haecker *et al.*, 2004). Okamoto *et al.* (2005) isolated maize mRNA from egg cells, two-celled embryos, apical cells, basal cells and multicellular embryos, and used mRNA to synthesize the corresponding cDNA. A comparative analysis of the five materials revealed a DNA band that was present in samples from the apical cell, basal cell, two-celled embryo and multicellular embryo, but not in the egg cell sample, suggesting that new gene transcription occurred in both the apical and basal cells after fertilization. Two other bands were also present in apical and basal cell samples, respectively, indicating that new gene transcription occurred in both of the daughter cells. The newly synthesized transcripts may control further development of apical and basal cells. Because cDNA was not produced

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for the zygote in that study, it was inferred that the transcripts from these genes were localized to the putative apical or basal region of the zygote, or that the transcripts were rapidly degraded in one of the daughter cells after zygotic cell division (Okamoto *et al.*, 2005). Ueda *et al.* (2011) found that the transcription factor WRKY DNA binding protein 2 (WRKY2) was required for asymmetric zygote division of *A. thaliana*. Before division, the zygote nucleus moved towards the cell centre, displaying cell depolarization, and then towards the chalazal part (i.e. repolarization). Analyses of wild-type and wrky2 mutants revealed that WRKY2 affected the second process (repolarization), suggesting that WRKY2 may control zygotic repolarization (Ueda *et al.*, 2011). In Arabidopsis, YODA and its downstream MAPKs, MPK3/6, are required for zygote elongation and asymmetrical division. Activation of YDA/MPK signalling in the zygote required the membrane-associated receptor-like kinase SHORT SUSPENSOR (SSP). The *SSP* mRNA was transferred from the sperm cell to the egg during fertilization and was then translated in the zygote [25]. Activated MPK3/6 directly phosphorylated WRKY2, which, in turn, led to the upregulation of *WOX8* transcription in the zygote to activate zygote elongation and asymmetrical division (Ueda *et al.*, 2011, 2017). Yu *et al.* (2016) analyzed a mutant of zygote arrest 1 (ZAR1), a member of the RLK/Pelle family in Arabidopsis, to determine the contributions of zygote asymmetrical division and elongation to daughter cell fate determination. The *zar1* mutant showed normal zygote elongation, but impaired zygote asymmetric division (Yu *et al.*, 2016). This result indicated that zygote elongation and zygote asymmetrical division were two separate events. However, movement of the zygotic nucleus has only been observed in *A. thaliana* so far. Further research is required to determine whether the phenomenon of zygotic depolarization and repolarization also occurs in other plants. The results of all of these studies confirmed that some genes participate in cellular determination in zygotes, but it is still unknown which genes control the formation of the two daughter cells.

More recently, Kimata *et al.* (2019) provided another perspective on zygote polarity. Zygote polarity is usually represented by the nucleus at the apical tip and the large vacuole at the basal end, with most emphasis on the nucleus position. They screened various vacuole mutants of Arabidopsis zygotes, and identified the mutant shoot gravitropism2 (sgr2). This mutant showed impaired structural changes in the vacuole and could not form a tubular vacuole for polar distribution. In sgr2, large vacuoles occupied the apical tip and therefore nuclear migration was blocked, resulting in more symmetrical zygotic division. These results showed that tubular vacuole formation and asymmetrical vacuolar distribution both depended on the longitudinal array of actin filaments. It was concluded that vacuolar dynamics were crucial not only for the polar distribution along actin filaments but also for adequate nuclear positioning, and consequently, the asymmetry of zygote division (Kimata *et al.*, 2019). Unfortunately, very few studies have explored the dynamics of vacuoles in the zygote. To date, nucleus movement in the zygote (zygotic depolarization and repolarization) has been reported only for Arabidopsis. There is still much to learn about how these events unfold in other plant species.

Conclusion and prospects

The zygote is the first cell of ontogenesis in plants. Because eggs and zygotes are buried deep in the ovule in the ovary, few studies have analyzed zygote development in detail. New methods have made it possible to isolate eggs and zygotes from higher plants, so research has entered a new phase in which molecular biology analyses can address many questions about the nature and timing of fertilization mechanisms. Using such new methods and based on the results reported for animals, studies on zygote activation and cellular determination in higher plants have obtained excellent results on the events involved in zygote activation. The results of these studies will shed light on the early ontogeny of higher plants.

Fertilization induces two processes: egg activation and zygote activation. The former represents structural changes before fusion of the egg and sperm nuclei (Peng *et al.*, 2018), and the latter represents molecular biological changes that occur after egg and sperm nuclear fusion, including intrinsic maternal transcript maturation, activation and elimination after fertilization, and activation of selected parental loci. During ZGA after fusion of male and female gametes, there are differences in gene expression between the maternal and paternal genomes. However, different studies have reported different results for paternal and maternal contributions during ZGA because of differences in the species and developmental stages analyzed. As well as parent gene activation, genomic imprinting of eggs and zygotes is another important process in ZGA. Alleles from each parent have different methylation patterns prior to fertilization, and the methylation patterns change during fertilization. Unfortunately, there are still insufficient results to draw general conclusions. The result of zygote activation is division to form a bicellular proembryo, which consists of an apical cell and a basal cell. Interestingly, studies on cell cycle regulation in the zygote, eggs of maize (Sauter *et al.*, 1998) and rice (Sukawa and Okamoto, 2018) were found to be G1 type (eggs were arrested at the G1 phase). How the cell cycle is regulated in plants with G2-type eggs (eggs arrested at the G2 phase) has not been reported. Zygote polarity is the basis of zygotic asymmetrical division and fate determination of daughter cells. Zygotic polarity comes from the egg, but the origin of egg polarity is unclear. Metabolic processes in the vacuole and positional regulation of the zygote contribute to zygote polarity. Zygote activation is the process of gene expression with different spatiotemporal patterns induced by fertilization. Although there are few published studies on zygote activation, their results have begun to uncover the regulation mechanism of the beginning of ontogeny, and will be advanced by further studies using new and sensitive analytical methods.

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