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Current Opinion in Plant Biology

The origin of the plant body axis

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During embryogenesis, the basic body plan of an organism develops from a unicellular zygote. In most flowering plants, the polar zygote divides asymmetrically, making visible the apical–basal axis in the early embryo. The molecular mechanisms governing how the zygote polarizes and how this polarity is linked to embryo axis formation have been obscure, mainly owing to the difficulties to access the zygote that is deeply embedded in the maternal tissue. In this review, we summarize recent findings identifying key regulators in *Arabidopsis* and developing novel approaches in various plant species, which altogether set the stage for unraveling embryo axis formation.

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Current Opinion in Plant Biology 2012, **15**:578–584

This review comes from a themed issue on **Cell biology**

Edited by **Keiko U Torii** and **Masao Tasaka**

For a complete overview see the Issue and the Editorial

Available online 22nd August 2012

1369-5266/$ – see front matter,

http://dx.doi.org/10.1016/j.pbi.2012.08.001

Introduction

Initiation of the main body axis is one of the first patterning steps in the development of an organism from a single-celled zygote. In animal and plant model organisms, the zygote undergoes an asymmetric division, converting its unicellular polarity into the main body axis of the early embryo [1,2]. Significant insight into the mechanisms controlling this event in animals has been obtained from studies in *Caenorhabditis elegans*, *Xenopus laevis*, *Drosophila melanogaster*, and mammals [3]. In most flowering plants, a first apical–basal axis is morphologically evident in the egg cell, where the nucleus is localized in the apical half and a large vacuole at the basal end [4,5]. This axis aligns with the micropylar–chalazal axis of the surrounding maternal tissue, raising the question whether maternal factors provide cues for egg cell polarity. A long held view was that the polar localization of the nucleus and vacuoles in the egg cell is contiguous with that in the zygote. However, this appears not to be the case. Upon fertilization, the zygote rapidly undergoes a number of changes, called 'egg cell activation', including those affecting the numbers and shapes of mitochondria, ribosomes, and Golgi vesicles [6,7]. Furthermore, recent studies in *Arabidopsis* and *Nicotiana tabacum* (tobacco) report a symmetrical organization of the zygote after fertilization, with the nucleus in the center and the vacuoles spread throughout the cell (Figure 1) [8,9,10••,11•]. During subsequent zygote elongation, the nucleus relocalizes to the apical part, and a large vacuole again forms at the basal end of the zygote (Figure 1). In parallel, the microtubule orientation gradually changes from a random orientation in the young *Arabidopsis* zygote into a transverse pattern of cortical microtubules [12]. The regulatory mechanisms that drive these changes in the zygote are unknown. Despite the transient symmetric morphology of the zygote, it is nevertheless possible that polar marks are laid down in the egg cell to provide spatial cues for the re-polarization of the zygote. Investigation of species displaying an opposite polarity in the egg cell (i.e. the nucleus at the basal pole and the vacuole in the apical region), such as *Oryza sativa* (rice) and *Papaver nudicaule* (Iceland poppy), can provide insight into whether transient symmetry and a subsequent symmetry breaking step in the zygote are common in plants [13,14].

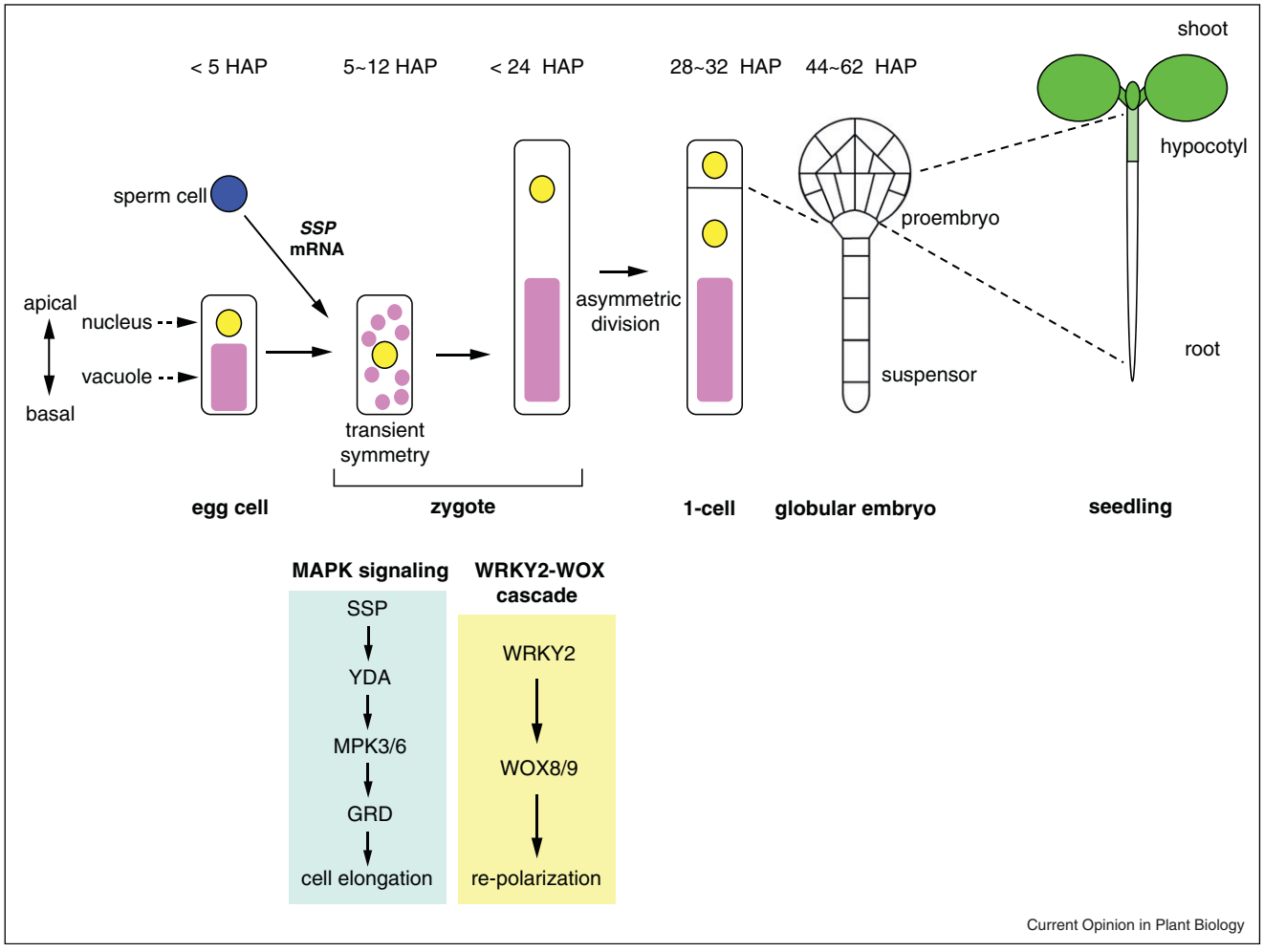
In most plant species, including *Arabidopsis*, the zygote elongates after fertilization and divides asymmetrically into a small cytoplasmic apical daughter cell and a large vacuolated basal daughter cell (Figure 1) [6]. The apical daughter cell gives rise to a spherical proembryo and eventually to most of the plant body (Figure 1) [5]. The initial steps occur by a precise pattern of cell divisions in *Arabidopsis*, whereas divisions are more random in larger embryos such as in maize. The vacuolated basal daughter generates the suspensor and, from its uppermost descendant, the hypophyseal cell, a part of the root meristem. The suspensor shapes may vary between different plant species, ranging from a filamentous suspensor in *Arabidopsis* (Figure 1) to the more elaborated branched haustoria as in *Sedum* [15]. It is noteworthy, that in some species, such as *Pelargonium zonale*, the zygotic division generates two cells of similar size [16]. Nevertheless, these species display conspicuous differences between apical and basal cells; for example, small, and circular mitochondria are abundant in the apical cell, whereas the basal cell mainly contains long rod-shaped mitochondria [16].

In summary, the intracellular polarity of the zygote correlates with the apical–basal axis of the embryo.

The *Arabidopsis* sperm triggers zygote elongation and suspensor fate

In plants mutant for the MAPKK kinase YODA (YDA) or its downstream MAP kinases MPK3/MPK6, the zygote

Figure 1



Model for the role of YDA MAPK signaling and the WRKY2-WOX genetic network in *Arabidopsis* zygote polarization. Upon fertilization, the subcellular positions of the nucleus (yellow) and the vacuole (pink) are apolar, and become asymmetrically localized during zygote elongation. The sperm cell provides *SSP* mRNA that, after translation in the zygote, activates YDA MAP kinase signaling. This indirectly promotes cell elongation of the zygote through activation of the GRD transcription factor. By contrast, zygote re-polarization requires WRKY2, partly via *de novo* transcription of *WOX8/9* in the zygote. The asymmetric division of the zygote produces the small apical cell, which generates the proembryo, and the large basal cell, which gives rise to a filamentous suspensor. Subsequently, the proembryo constitutes the entirety of the plant, except for part of the root tip, which is specified by one cell of the suspensor. An approximate time scale in HAP (hours after pollination) based on published reports is indicated [6,9].

fails to elongate and subsequently divides symmetrically, producing a normally sized apical and an abnormally short basal cell [17,18]. Whether these two processes, zygote elongation and asymmetric division are independently affected in *yda* mutants, or whether cell length is a prerequisite for the asymmetry of the zygote division remains an interesting question to be answered. The requirement of MAP kinase signaling for zygote elongation in *Arabidopsis* is reminiscent of the role of the MAP kinase PAR-1 of *C. elegans*, which organizes microtubule dynamics in the zygote [19]. The possibility of a similar link between YODA function and the reorganization of the microtubular network during zygote development can now be addressed.

The direct target of the YDA phosphorylation cascade has not yet been identified. However, Jeong *et al.* show that the RWP-RK-type transcription factor GROUNDED (GRD)/RKD4 is an indirect, but crucial effector of YDA signaling [20••]. The *grd* mutant resembles *yda* and *mpk3 mpk6* mutants at all embryo stages and suppresses the effects of a hyperactive form of YDA. Because all of these mutants fail to generate the suspensor properly, YDA signaling plays also an important role to specify suspensor fate. In a parallel study, Waki *et al.* demonstrate that overexpression of GRD in seedlings induces the expression of several embryo-specific genes and the formation of somatic embryos, suggesting that GRD has a potential activity to initiate embryogenesis [21•].

In an elegant study, Bayer *et al.* reveal that YDA is activated by the receptor-like cytoplasmic kinase SHORT SUSPENSOR (SSP), whose mRNA is delivered from the sperm cell to be translated in the zygote (Figure 1) [22]. Thus, sperm-triggered activation of YDA MAPK signaling by a paternally provided component connects the fertilization event to the elongation of the zygote and the initiation of embryogenesis. The role of *SSP* mRNA reveals a different type paternal contribution to axis formation compared to model animal species, where symmetry breaking in the zygote is triggered by sperm-derived proteins [23].

A WRKY2-WOX transcriptional cascade activates zygote re-polarization

Members of the plant-specific WUSCHEL RELATED HOMEOBOX (WOX) transcription factors are expressed asymmetrically along the apical–basal axis throughout embryogenesis [24]. Among them, *WOX2* and *WOX8* are co-expressed in the egg cell and in the zygote, but their expression becomes predominant to the apical (*WOX2*) or basal embryo lineage (*WOX8*) after the zygotic division [24]. A recent study detected weak *WOX8* expression also in the apical daughter cell of the zygote [25]. *WOX2* expression is further confined within the apical lineage to the upper half of the globular embryo from which the shoot is derived. There it regulates apical patterning and shoot development, partly through promoting polar transport and asymmetric distribution of a plant hormone auxin [26]. Expression of the closely related WOX8 and WOX9 affects not only basal lineage development, but also through *WOX2* the development of the embryo proper [26,27]. Subsequently, expression of the stem-cell regulating *WOX* genes, *WUS* and *WOX5*, is induced in the shoot and root apical meristems, respectively [28,29]. Finally, expression of *WOX1*, *PRESSED FLOWER* (*WOX3*), and *WOX5* in the cotyledons contributes to organ patterning and outgrowth [24,26]. This cascade of *WOX* functions resembles animal *HOX* genes, the key axis regulatory homeobox genes that are sequentially expressed in specific domains of the embryo [30].

The co-expression of *WOX2* and *WOX8/9* implies that the zygote contains a mixture of basal and apical regulators that becomes separated through the asymmetric division. In a study to reveal how the asymmetric expression patterns are linked to the structural polarity of the zygote, Ueda *et al.* show that the plant-specific zinc-finger transcription factor WRKY2 is a direct activator of *WOX8/WOX9* transcription [10••]. In the *wrky2* mutant, egg cell polarity and the transiently symmetric zygote are normal, but re-polarization of the zygote does not take place and the zygote divides symmetrically [10••]. This reveals the different genetic requirements between the polarization processes of the egg cell and the zygote. Forcing WOX8 expression partially restores all defects in the *wrky2* zygote, indicating that WRKY2 polarizes the zygote partly through activating *WOX8* (Figure 1) [10••]. One important observation from this study is that *WRKY2* provided from the pollen is sufficient to activate *WOX8* transcription and to establish zygote polarity. This indicates that transcription after fertilization functions in zygote polarity, in contrast to animal models where early pattern information is derived from maternally supplied products [31]. Several expression profiling experiments corroborate this difference and indicate transcription in the plant zygote on a large scale (see below).

In contrast to the *yda* mutant, *wrky2* does not severely affect zygote elongation, indicating that cell elongation is not sufficient to establish zygote polarity and asymmetric division, and the specific requirement of WRKY2 in the mechanism to establish zygote polarity [10••]. Furthermore, when *yda* or *grd* mutation are combined with the *wox8 wox9* double mutant, the triple mutant embryos display synergistic phenotypes and arrest the development at the zygote stage or shortly thereafter [20••,26]. Therefore in the early embryo patterning, a WRKY2-WOX cascade and YDA MAPK signaling seem to regulate independently zygote polarity and elongation, respectively (Figure 1).

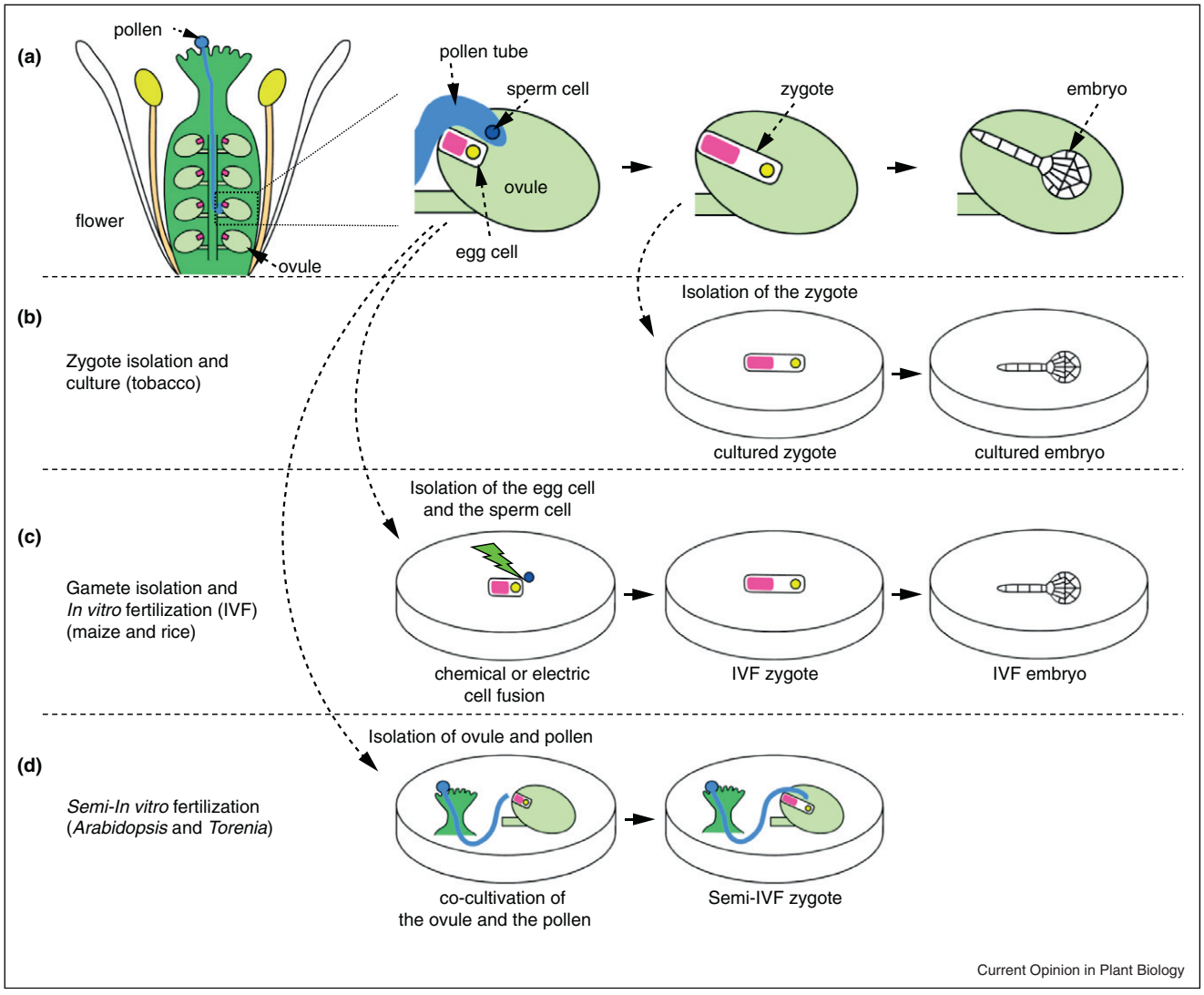
Transcriptome analysis of the zygote

When transcription is activated after fertilization and whether parental imprinting plays an important role in plants has been a matter of debate. Xiang *et al.* collected *Arabidopsis* zygotes and young embryos from various developmental stages and studied the expression profiles [32•]. They find that mRNA for 58% of all genes in the *Arabidopsis* genome are present in the zygote, with an over-representation of transcription factor transcripts compared to subsequent embryo stages [32•]. Autran *et al.* report that maternal transcripts dominate early stages in plant embryogenesis, similar to the situation in animals [33••]. Initially, paternal alleles are repressed via DNA-methylation and histone-methylation and only become activated at later stages through the maternally supplied histone chaperone complex CAF1. In contrast to that study, the transcriptome analysis by Nodine and Bartel of 1-cell or 2-cell embryos provides evidence that paternal and maternal derived mRNA are equally represented, with only very few transcripts preferentially derived from a specific parent [34••]. Widespread transcription in the zygote is also inferred by studies in maize and tobacco [11•,35,36]. Altogether, these results indicate that plants have a different transcriptional strategy in early embryo development compared to model animals, where *de novo* transcription is largely repressed after fertilization until the late embryo stage [31].

Cellular analysis and *in vitro* culture of the zygote

Because it is deeply embedded in the ovule (Figure 2A), the development of *in vitro* culture protocols is of great

Figure 2



Methods of zygote studies. Schematic representations of: fertilization and embryogenesis in a flower **(A)**, development of a cultured zygote **(B)**, *in vitro* fertilization (IVF) of an egg cell **(C)**, and *semi*-IVF of an ovule **(D)**. Currently available species for each protocol are designated. In the flower, the pollen grain germinates on the stigmatic surface of the pistil, and the pollen tube delivers the two sperm cells to the ovule. Only one of the two sperm cells is shown to simplify the drawing. The pollen tube that grows through a cultured pistil section is shown in **(D)**.

importance for studying the zygote at the cellular level. The *in vitro* protocol of He *et al.* supports development from a dissected, elongated tobacco zygote to a fertile plant (Figure 2B) [37]. However, if the zygote is not yet elongated, developmental arrest takes place [37]. This could be owing to the requirement of maternal tissue for the very first steps of embryogenesis, or alternatively, a higher sensitivity of the young zygote to the dissection procedure. Interestingly, an elongated zygote lacking the cell wall also fails to develop although a new wall is regenerated on culture media [37]. This finding is reminiscent to pioneering studies in zygotes of the brown algae *Fucus*, where attachment of the cell wall has been shown to be instrumental for specifying cell fate [38]. In a new study analyzing the transcriptome of zygotes after inhibition of Arabinogalactan proteins (AGPs), Hu *et al.* report that AGPs are required for normal expression of genes involved in various cellular processes including vesicle transport and tip growth [39•]. AGPs were originally found to localize preferentially at the apical cell surface of the tobacco zygote, and AGP inhibition forced the zygote to divide symmetrically [40]. Together with the finding that AGPs promote cell elongation in the tip-growing cells in the moss *Physcomitrella patens* [41], a role of AGPs in promoting zygote elongation appears plausible. It will be of interest to understand how this function intersects with the role of the YDA pathway in zygote elongation.

Live imaging of fertilization

In order to study processes during or immediately after fertilization, *in vitro* fertilization (IVF) protocols have been established in grass species (Figure 2C). Here, the isolated gametes are fused electrically or in the presence of polyethylene glycol (PEG) or bovine serum albumin (BSA), and the resulting cell can be cultured into a mature, fertile plant [42–44]. These studies reveal that sperm entry causes a calcium spike to promote cell wall synthesis in the maize zygote [45], and that the entry site is not crucial to set the cell division plane in the rice zygote [46]. In dicots, however, *in vitro* fertilization has not been successful yet. Chemical fusion of tobacco gametes results in a zygote that arrests without any division [43]. What could cause the differential effectiveness of IVF in grasses and dicotyledons? In grasses, the sperm cell formed in the pollen grains is at G1 and is ready to fuse with the egg cell that is also in G1 [43]. Therefore, sperm isolated from grass pollen is at the right stage for fertilization. The sperm of *Arabidopsis* and tobacco is also at G1 in the pollen grain, but completes S phase after the pollen tube enters the ovule and before fusion with the egg cell [47]. Thus, fertilization-competent sperm may have to be isolated from the discharged pollen tube in the ovule, which seems technically challenging.

As an alternative to IVF, *semi*-IVF has been established in *Torenia fournieri* and *Arabidopsis* (Figure 2D) [48••,49,50]. In this procedure, ovules are co-cultured with germinating pollen from a cut pistil, and fertilization occurs in almost intact ovules [48••]. Hamamura *et al.* report live-imaging of ovules fertilized by this approach revealing that the sperm cell invariantly fuses with apical tip of the egg cell [48••]. Using a similar approach, Ingouff *et al.* show that sperm-specific and egg-specific HISTONE 3 variants are degraded shortly after fertilization and are rapidly replaced by newly synthesized variants in the zygote [51]. Because HISTONE 3 variants carry epigenetic information, this finding suggests that significant epigenetic restructuring of both parental genomes takes place in the zygote [51].

Conclusions

The mechanism of plant zygote polarization has long been obscure, but recent studies identify principal components of this process. Furthermore, experimental advances set the stage for significant and new discoveries at the cellular level of the zygote. Fundamental questions can now be addressed. What are the cues that polarize the zygote? If the sperm entry site is not crucial for zygote polarity, does the egg cell establish stable polarity marks that are used in the zygote? How is zygote polarity connected to embryo patterning? Specifically, does the zygotic division distribute cell fate determinants in an unequal manner to its daughter cells?

Acknowledgements

We thank Masaaki Umeda for helpful discussion. We gratefully acknowledge financial support by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 24113514) and a Grant-in-Aid for Young Scientists (B, No. 24770045) to M.U., and support to T.L. from the Deutsche Forschungsgemeinschaft including the SFB592 and AFGN programs, and by the European Commission (FP7-PEOPLE-2007-1-1-ITN Contract no. 214788-2; SIREN program).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Gonczy P, Rose LS: **Asymmetric cell division and axis formation in the embryo**. *WormBook* 2005:1-20.

2. Vroemen C, de Vries S, Quatrano R: **Signalling in plant embryos during the establishment of the polar axis**. *Semin Cell Dev Biol* 1999, **10**:157-164.

3. Munro E, Bowerman B: **Cellular symmetry breaking during *Caenorhabditis elegans* development**. *Cold Spring Harb Perspect Biol* 2009, **1**:a003400.

4. Lau S, Slane D, Herud O, Kong J, Jurgens G: **Early embryogenesis in flowering plants: setting up the basic body pattern**. *Annu Rev Plant Biol* 2012, **63**:483-506.

5. Zhang Z, Laux T: **The asymmetric division of the *Arabidopsis* zygote: from cell polarity to an embryo axis**. *Sex Plant Reprod* 2011, **24**:161-169.

6. Mansfield SG, Briarty LG: **Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo**. *Can J Bot* 1991, **69**:461-476.

7. Mansfield SG, Briarty LG, Erni S: **Early embryogenesis in *Arabidopsis thaliana*. I. The mature embryo sac**. *Can J Bot* 1991, **69**:447-460.

8. Christensen CA, Gorsich SW, Brown RH, Jones LG, Brown J, Shaw JM, Drews GN: **Mitochondrial GFA2 is required for synergid cell death in *Arabidopsis***. *Plant Cell* 2002, **14**:2215-2232.

9. Faure JE, Rotman N, Fortune P, Dumas C: **Fertilization in *Arabidopsis thaliana* wild type: developmental stages and time course**. *Plant J* 2002, **30**:481-488.

10. •• Ueda M, Zhang Z, Laux T: **Transcriptional activation of *Arabidopsis* axis patterning genes WOX8/9 links zygote polarity to embryo development**. *Dev Cell* 2011, **20**:264-270.
The authors show a transient, symmetric localization of the nucleus and vacuoles in the zygote, which repolarize independently of zygote elongation. This study provides also evidence that zygote repolarization involves direct transcriptional activation of the *WOX8* gene in the zygote by the WRKY2 transcription factor.

11. • Zhao J, Xin H, Qu L, Ning J, Peng X, Yan T, Ma L, Li S, Sun MX: **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**. *Plant J* 2011, **65**:131-145.
Gene expression profiles (from cDNA libraries) of isolated tobacco egg cells, zygotes and the divided zygote are markedly different. Additionally, the authors show that the zygote fails to divide in the presence of a general transcription inhibitor, adding further evidence to other studies that transcription is important in the plant zygote.

12. Webb MC, Gunning BES: **The microtubular cytoskeleton during development of the zygote, proembryo and free-nuclear endosperm in *Arabidopsis thaliana* (L.) *Heynh***. *Planta* 1991, **184**:187-195.

13. Olson AR, Cass DD: **Changes in megagametophyte structure in *Papaver nudicaule L.* (*Papaveraceae*) following in vitro placental pollination**. *Am J Bot* 1981, **68**:1333-1341.

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

15. Yeung EC, Meinke DW: **Embryogenesis in angiosperms: development of the suspensor**. *Plant Cell* 1993, **5**:1371-1381.

16. Kuroiwa H, Nishimura Y, Higashiyama T, Kuroiwa T: **Pelargonium embryogenesis: cytological investigations of organelles in early embryogenesis from the egg to the two-celled embryo**. *Sex Plant Reprod* 2002, **15**:1-12.

17. Lukowitz W, Roeder A, Parmenter D, Somerville C: **A MAPKK kinase gene regulates extra-embryonic cell fate in *Arabidopsis***. *Cell* 2004, **116**:109-119.

18. Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S: **Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis***. *Plant Cell* 2007, **19**:63-73.

19. Hayashi K, Suzuki A, Ohno S: **A novel function of the cell polarity-regulating kinase PAR-1/MARK in dendritic spines**. *Bioarchitecture* 2011, **1**:261-266.

20. •• Jeong S, Palmer TM, Lukowitz W: **The RWP-RK factor GROUNDED promotes embryonic polarity by facilitating YODA MAP kinase signaling**. *Curr Biol* 2011, **21**:1268-1276.
Based on an elegant genetic analysis in *Arabidopsis*, the authors reveal that the putative transcription factor GRD acts downstream of YDA MAPK signaling. However, GRD is probably not the direct target because mutating a putative phosphorylation site of GRD didn't affect its activity.

21. • Waki T, Hiki T, Watanabe R, Hashimoto T, Nakajima K: **The *Arabidopsis* RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis**. *Curr Biol* 2011, **21**:1277-1281.
An overexpression of GRD induces expression of several embryonic genes and finally generates embryo-like structures on the surface of seedlings. Therefore the authors propose that GRD is a global activator of the embryonic program.

22. Bayer M, Nawy T, Giglione C, Galli M, Meinnel T, Lukowitz W: **Paternal control of embryonic patterning in *Arabidopsis thaliana***. *Science* 2009, **323**:1485-1488.

23. Bastock R, St Johnston D: **Going with the flow: an elegant model for symmetry breaking**. *Dev Cell* 2011, **21**:981-982.

24. Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T: **Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana***. *Development* 2004, **131**:657-668.

25. Lie C, Kelsom C, Wu X: **WOX2 and *STIMPY-LIKE*/*WOX8* promote cotyledon boundary formation in *Arabidopsis***. *Plant J* 2012, http://dx.doi.org/10.1111/j.1365-313X.2012.05113.x, in press.

26. Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T: **Differential expression of WOX genes mediates apical–basal axis formation in the *Arabidopsis* embryo**. *Dev Cell* 2008, **14**:867-876.

27. Wu X, Dabi T, Weigel D: **Requirement of homeobox gene STIMPY/WOX9 for *Arabidopsis* meristem growth and maintenance**. *Curr Biol* 2005, **15**:436-440.

28. Mayer KF, Schoof H, Haecker A, Lenhard M, Jurgens G, Laux T: **Role of WUSCHEL in regulating stem cell fate in the *Arabidopsis* shoot meristem**. *Cell* 1998, **95**:805-815.

29. Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, Scheres B, Heidstra R, Laux T: **Conserved factors regulate signalling in *Arabidopsis* thaliana shoot and root stem cell organizers**. *Nature* 2007, **446**:811-814.

30. Krumlauf R: **Hox genes in vertebrate development**. *Cell* 1994, **78**:191-201.

31. Schier AF: **The maternal–zygotic transition: death and birth of RNAs**. *Science* 2007, **316**:406-407.

32. • Xiang D, Venglat P, Tibiche C, Yang H, Risseeuw E, Cao Y, Babic V, Cloutier M, Keller W, Wang E *et al.*: **Genome-wide analysis reveals gene expression and metabolic network dynamics during embryo development in *Arabidopsis***. *Plant Physiol* 2011, **156**:346-356.
The authors develop a dissection protocol to collect embryos from fertilized *Arabidopsis* ovules that range in stage from the elongated zygote to the mature embryo. Based on gene-network analysis of expression profiles, the authors reveal metabolic changes occurring during embryogenesis. For example, fatty acid biosynthesis is initiated later than carbohydrate metabolism.

33. •• Autran D, Baroux C, Raissig MT, Lenormand T, Wittig M, Grob S, Steimer A, Barann M, Klostermeier UC, Leblanc O *et al.*: **Maternal epigenetic pathways control parental contributions to *Arabidopsis* early embryogenesis**. *Cell* 2011, **145**:707-719.
In a similar manner to Nodine and Bartel, 2012, the authors analyze gene expression profiles in hybrid embryos of two *Arabidopsis* accessions, Col-0 and L*er*. They find that the paternally derived transcripts are underrepresented owing to epigenetic regulation via the maternally supplied CAF1 complex.

34. •• Nodine MD, Bartel DP: **Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos**. *Nature* 2012, **482**:94-97.
The authors isolate hybrid embryos of a cross between accessions Cvi-0 and Col-0 at three stages: one to two cells, 8 cells, and ca. 32 cells. Transcriptomic analysis of the maternal and paternal genomes exhibit a similar representation during these stages, suggesting that the onset of *de novo* transcription is earlier than that in animals. Additionally, there is no significant impact of parental imprinting on the embryonic transcriptome.

35. Meyer S, Scholten S: **Equivalent parental contribution to early plant zygotic development**. *Curr Biol* 2007, **17**:1686-1691.

36. Ning J, Peng XB, Qu LH, Xin HP, Yan TT, Sun M: **Differential gene expression in egg cells and zygotes suggests that the transcriptome is restructed before the first zygotic division in tobacco**. *FEBS Lett* 2006, **580**:1747-1752.

37. He YC, He YQ, Qu LH, Sun MX, Yang HY: **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**. *Plant J* 2007, **49**:515-527.

38. Berger F, Taylor A, Brownlee C: **Cell fate determination by the cell wall in early fucus development**. *Science* 1994, **263**:1421-1423.

39. • Hu TX, Yu M, Zhao J: **Comparative transcriptional analysis reveals differential gene expression between asymmetric and symmetric zygotic divisions in tobacco**. *PLoS ONE* 2011, **6**:e27120.
Using a tobacco zygotic culture system, the authors identify genes whose expression is affected by the AGP inhibitor, beta-glucosyl Yariv reagent (bGlcY), which causes symmetric division of the zygote. Among the 685 genes are predicted homologues of embryonic axis regulators, such as MAPK kinases and ARF GTPases, which supports the importance of AGPs for zygote polarization.

40. Qin Y, Zhao J: **Localization of arabinogalactan proteins in egg cells, zygotes, and two-celled proembryos and effects of beta-D-glucosyl Yariv reagent on egg cell fertilization and zygote division in *Nicotiana tabacum L***. *J Exp Bot* 2006, **57**:2061-2074.

41. Lee KJ, Sakata Y, Mau SL, Pettolino F, Bacic A, Quatrano RS, Knight CD, Knox JP: **Arabinogalactan proteins are required for apical cell extension in the moss *Physcomitrella patens***. *Plant Cell* 2005, **17**:3051-3065.

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

43. Wang YY, Kuang A, Russell SD, Tian HQ: ***In vitro* fertilization as a tool for investigating sexual reproduction of angiosperms**. *Sex Plant Reprod* 2006, **19**:103-115.

44. Kranz E, Lorz H: ***In vitro* fertilization with isolated, single gametes results in zygotic embryogenesis and fertile maize plants**. *Plant Cell* 1993, **5**:739-746.

45. Antoine AF, Faure JE, Cordeiro S, Dumas C, Rougier M, Feijo JA: **A calcium influx is triggered and propagates in the zygote as a wavefront during *in vitro* fertilization of flowering plants**. *Proc Natl Acad Sci USA* 2000, **97**:10643-10648.

46. Nakajima K, Uchiumi T, Okamoto T: **Positional relationship between the gamete fusion site and the first division plane in the rice zygote**. *J Exp Bot* 2010, **61**:3101-3105.

47. Lord EM, Russell SD: **The mechanisms of pollination and fertilization in plants**. *Annu Rev Cell Dev Biol* 2002, **18**:81-105.

48. •• Hamamura Y, Saito C, Awai C, Kurihara D, Miyawaki A, Nakagawa T, Kanaoka MM, Sasaki N, Nakano A, Berger F *et al.*: **Live-cell imaging reveals the dynamics of two sperm cells during double fertilization in *Arabidopsis thaliana***. *Curr Biol* 2011, **21**:497-502.
In this paper, live imaging of semi-IVF elucidates the spatio-temporal regulation of double fertilization in *Arabidopsis*. Within 0.2 s of pollen tube discharge, sperm nuclei move toward the female gametes, the central cell and the egg cell. One of the sperm reaches the apical end of the egg cell within 8–9 s. The authors provide strong evidence that the two sperm cells in a pollen tube can equivalently fuse with either the egg cell or the central cell. This supports a model requiring a rapid block in polyspermy of each female gamete.

49. Palanivelu R, Preuss D: **Distinct short-range ovule signals attract or repel *Arabidopsis thaliana* pollen tubes in vitro**. *BMC Plant Biol* 2006, **6**:7.

50. Higashiyama T, Yabe S, Sasaki N, Nishimura Y, Miyagishima S, Kuroiwa H, Kuroiwa T: **Pollen tube attraction by the synergid cell**. *Science* 2001, **293**:1480-1483.

51. Ingouff M, Rademacher S, Holec S, Soljic L, Xin N, Readshaw A, Foo SH, Lahouze B, Sprunck S, Berger F: **Zygotic resetting of the HISTONE 3 variant repertoire participates in epigenetic reprogramming in *Arabidopsis***. *Curr Biol* 2010, **20**:2137-2143.