Insights into Dynamic Coenocytic Endosperm Development: Unraveling Molecular, Cellular, and Growth Complexity

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Abstract

The endosperm, a product of double fertilization, is one of the keys to the evolution and success of angiosperms in conquering the land. While there are differences in endosperm development among flowering plants, the most common form is coenocytic growth, where the endosperm initially undergoes nuclear division without cytokinesis and eventually becomes cellularized. This complex process requires an interplay among networks of transcription factors such as MADS-box, ARFs, and phytohormones. The role of cytoskeletal elements in shaping the coenocytic endosperm and influencing seed growth also becomes evident. This review offers a recent understanding of the molecular and cellular dynamics in coenocytic endosperm development and their contributions to the final seed size.

Keywords- Endosperm development, Auxin, MADS-box, F-actin, Microtubule, Seed size

Introduction

Seed development in flowering plants begins with a complex process known as double fertilization [1,2]. One sperm cell fertilizes the egg cell, giving rise to the embryo, while the other fertilizes the central cell, initiating endosperm formation. The endosperm is vital for nourishing the embryo; in monocots, the majority of the endosperm persists throughout seed development, also serving as a nutrient supply for germination. On the other hand, the endosperm in dicots diminishes as the embryo becomes mature. The developmental trajectory of the endosperm in most plants unfolds through two distinct phases: the coenocytic phase, marked by divisions of endosperm nuclei without cytokinesis, resulting in a multinucleate single-cell structure, followed by cellularization, transforming into a structured cellular endosperm [3-6,7**] (Figure 1). The duration of the coenocytic endosperm phase has been shown to highly correlate with the final seed size, with a shorter duration resulting in smaller seeds and a longer duration producing larger seeds [8-12]. The supply of more nutrients from the mother plant to the endosperm, which supports embryo development, ultimately leads to larger seeds [13-15].

While the general understanding of endosperm development spans various plant species, the early stages of seed development in *Arabidopsis thaliana*, particularly the formation of coenocytic endosperm, stand out as extensively studied. This model system serves as a cornerstone for unraveling the molecular and cellular dynamics of endosperm development, and this review aims to provide an overview of endosperm development in flowering plants with an emphasis on recent discoveries that illustrate the dynamic nature of Arabidopsis coenocytic endosperm development.

Endosperm development in flowering plants

Distinct types of endosperms are observed across flowering plants; morphologically, three forms of endosperm development are present in flowering plants: cellular, coenocytic, and helobial [16]. In the coenocytic form, the endosperm undergoes nuclear divisions without cytokinesis. In the helobial form, the endosperm consists of both a cellular part and a free nuclear or coenocytic part. In many flowering plants like *Oryza sativa* (rice), *Zea mays* (maize), *Glycine max* (soybean), and Arabidopsis, fertilization of the central cell results in a triploid endosperm, composed of two maternal polar nuclei and one sperm nucleus [17]. In the basal angiosperm Nymphaeaceae, the female gametophyte possesses only one polar nucleus, and double fertilization results in the formation of a diploid cellular endosperm [17,18]. Endosperm development in Nymphaeaceae is minimal, and embryo nourishment is carried out by the sporophyte-derived tissue called perisperm, which surrounds the endosperm (Figure 1a). The perisperm accumulates starch, while the chalazal endosperm (CZE) protrudes into the perisperm and has been speculated to function like haustoria (Figure 1a), providing nourishment to the embryo [19]. Other basal angiosperms, such as *Amborella*, possess two polar nuclei that fuse to form a homo-diploid central cell nucleus, resulting in a triploid cellular endosperm after fertilization [20,21]. Although examining basal angiosperms provides insights, endosperm evolution is dynamic, and it still remains unclear what constitutes the ancient form of endosperm.

Endosperm development in Brassicaceae undergoes three distinct phases (Figure 1b): coenocytic, cellularization, and maturation [4,22,23]. In the coenocytic phase, the endosperm establishes three subregions based on nuclear positioning [24] with a differential gene expression pattern [25**,26**] – Micropylar Endosperm (MCE), confined to the region surrounding the

developing embryo; Peripheral Endosperm (PEN), thought to play a major role in endosperm expansion through rapid nuclear divisions; and Chalazal Endosperm (CZE), forming at the chalazal pole of the endosperm, acting as a link between maternal tissue and filial tissue (seed) (Figure 1b) [4,27]. The large central vacuole residing in PEN pushes the nuclei and cytoplasm to the plasma membrane, whereby these nuclei form an individual nuclear cytoplasmic domain (NCD) (Figure 1b). Similar to the Nymphaeaceae, CZE in Brassicaceae is recognized as the site where nutrients are absorbed from the mother plant and transported into the endosperm [28,29]. Endosperm cellularization causes the central vacuole to shrink, leading the embryo to switch as a sink for all the nutrients [30]. As the embryo begins to expand, it initiates invasion into the endosperm. This embryonic growth is accompanied by both the weakening of the endosperm wall and programmed cell death [31,32*]. The endosperm eventually remains as a thin aleurone-like layer (Figure 1b) [33].

Monocot endosperm development resembles Arabidopsis until cellularization and diverges after cellularization. Differentiation of tissues such as the basal endosperm transfer layer (BETL) for grain filling, aleurone layer, embryo surrounding region (ESR) for nutrient transfer to the embryo [34], and starchy endosperm occur in cereal crops (Figure 1c). These tissues are not only major food sources (e.g., rice, maize, wheat), but also nourish the embryo during embryogenesis and seed germination [35*].

An anomalous case of degenerating endosperm occurs in the family Orchidaceae [36]. The orchid endosperm undergoes a few rounds of nuclear divisions but diminishes as the zygote develops. Some orchid species fail to initiate nuclear division, resulting in no endosperm formation [37]. Despite the lack of endosperm, orchid seeds germinate normally, indicating

modifications in the orchid embryo's developmental or germination program [32*]. Orchid embryos form a protocorm establishing a symbiotic association with mycorrhizal fungi to support germination and survival [36,38]. Orchids without endosperm, thus, still require an alternative system to support the embryo, further highlighting the essential role of nutrient storage and supply in flowering plant endosperm.

Molecular dynamics of endosperm

Transcriptional profiling, coupled with laser capture microdissection, has been conducted in diverse species to elucidate the functions of subregions within the coenocytic and cellularized endosperm [25**,39-49]. A recent advancement involves single-nuclei RNA sequencing (snRNA-seq) in Arabidopsis endosperm, providing a comprehensive map of transcriptomes and unraveling distinct gene imprinting patterns among the endosperm subregions [26**]. The endosperm displays gene imprinting, a phenomenon in which gene expression is biased depending on the parent of origin. Genes that show preferential expression from the maternal allele are referred to as maternally expressed imprinted genes (MEGs), whereas genes preferentially expressed from the paternal allele are referred to as paternally expressed imprinted genes (PEGs) [50,51]. The Arabidopsis endosperm is triploid, with a parental genome contribution ratio of maternal 2n to paternal 1n. Disrupting this parental genome balance, either through interploidy crosses or using mutants that can alter the ploidy levels [52], results in a change in the deregulation of gene imprinting in the endosperm, the mechanisms for which still remains unknown [53]. Disrupting parental genome balance also alters the seed sizes [11,40,54] (Figure 2). The parental conflict

theory for nutrient allocation suggests that paternal genome expression leads to more resource allocation to the progeny from the mother plant, while maternal genome expression restricts the flow of nutrients to the endosperm [55-57], thereby maintaining a balance required for all progeny seeds to survive. Notably, CZE exhibits a very high level of imprinting of the paternal genome, presumably acting as the region of active conflict for resource accumulation from the mother plant [26**]. The impact and mechanism of endosperm gene imprinting have been extensively reviewed [51,58,59], providing valuable insights into the regulatory processes governing endosperm development and its interaction with maternal and paternal genetic contributions.

Manipulation of parental genome balance in the endosperm has also led to the identification of genes with altered expression levels in the endosperm compared to the wild type (Figure 2) [40,60-62]. Many of the genes encode MADS-box transcription factors (TFs), proteins involved in phytohormone pathways, and cell cycle-related proteins [40,60-62]. For example, among the MADS-box TF encoding genes, *AGAMOUS LIKE 62 (AGL62)*, which is not imprinted, exhibits a decrease in expression level in the case of maternal excess cross, while showing an increase in expression level in the case of paternal excess cross (Figure 2) [40]. MADS-box TFs constitute an ancient gene family conserved across kingdoms [63], and plant MADS-box TFs are divided into two classes, type I and type II. Initially, type II MADS-box TFs were identified as regulators of floral development and organization [64]. On the other hand, type I MADS-box TFs remained not well-characterized until transcriptomic studies provided insights into their predominant expression in the endosperm [39,40,65] and the female gametophyte [66]. Molecular and phylogenetic analyses further classified type I MADS-box TFs into four groups –

129 Mα, Mβ, Mγ, and Mδ [67]. Mγ and Mγ-interacting Mα show specific expression in the endosperm,
130 and interestingly, these Mγ and Mα are unique to flowering plants [68*]. Given that the
131 endosperm is also unique to flowering plants, this may suggest a special genome reprogramming
132 in flowering plants involving the significance of MADS-box TFs in the evolution and/or
133 development of the endosperm.

134 In Arabidopsis, mutation of *AGL62,* belonging to the Mα type, causes precocious
135 endosperm cellularization, serving as a negative regulator for endosperm cellularization [8].
136 Additionally, *agl91* (Mγ type) and *agl40* (Mα type) mutants produce smaller seeds, while
137 overexpression of *AGL40* leads to the development of larger seeds [69]. Before the initiation of
138 cellularization, there is a noticeable decrease in the expression of a subset of type I MADS-box
139 genes such as *AGL62, AGL40, PHE1,* and *PHE2* in the endosperm (Figure 2) [40,62,65]. Consistent
140 with the role of AGLs as negative regulators for endosperm cellularization, higher paternal dosage
141 endosperm (resulting in larger seeds with delayed cellularization) shows elevated and prolonged
142 expression of these genes [40,62,65,70], and higher maternal dosage endosperm (resulting in
143 smaller seeds with small endosperm showing early cellularization) exhibits downregulation
144 [40,71,72] .

145 Using the R2D2 auxin sensor [73], it was demonstrated that fertilization triggers auxin
146 production in the fertilized central cell (primary endosperm). Increasing auxin levels in the central
147 cell, achieved by overexpression of auxin biosynthesis genes*,* initiated nuclear divisions in the
148 central cell without fertilization [74]. Mutants associated with auxin biosynthesis and signaling
149 exhibit defects in endosperm proliferation [74]. Additionally, the endosperm-specific expression
150 of the dominant-negative IAA32, which impedes auxin signaling and thus induces auxin deficiency

phenotypes, manifests a similar defect in endosperm proliferation [74]. Conversely, higher paternal dosage endosperm displays auxin overproduction, resulting in a delay in endosperm cellularization, and the overproduction of auxin in the endosperm also shows the same cellularization delay phenotype [61]. Collectively, these findings emphasize the essential role of auxin in endosperm development and highlight its regulatory role in the timing of endosperm cellularization (Figure 2) [61,74].

The Arabidopsis *agl62* mutant reduces the auxin level in the endosperm compared to the wild-type [75*]. Similarly, in the case of *Fragaria vesca* (strawberry), *Fveagl62* showed reduced expression of auxin biosynthesis genes [75*]. The interplay between auxin and *AGL62* post-fertilization becomes evident, playing a crucial role in endosperm proliferation. Auxin response factors (ARFs) govern the expression of auxin-responsive genes both in positive and negative manners [76,77]. A cluster of *ARFs (cARFs)* is expressed in the coenocytic endosperm [61]. Increased paternal dosage reduces and delays *cARFs* expression, while higher maternal dosage increases expression of *cARFs* (Figure 2) [78*]. Furthermore, overexpression of *cARFs* in the endosperm also causes early cellularization [78*], functioning in a dosage-dependent manner, positively regulating endosperm cellularization [78*]. An antagonistic relationship exists between auxin and *cARFs* in regulating endosperm cellularization; higher auxin levels prolong the coenocytic phase, causing a delay in cellularization, while *cARFs* initiate their expression just before cellularization, restricting the auxin signaling and promoting cellularization (Figure 2).

Another phytohormone, cytokinin, which promotes nuclear and cell division in plant cells [79], has also been observed in the Arabidopsis coenocytic endosperm. Cytokinin-synthesizing genes *AtIPT4* and *AtIPT8* are expressed in the coenocytic endosperm [80], and indeed, the

cytokinin reporter *TCS::erGFP* [81] showed the highest activity in the early stage, gradually decreasing as development progresses, and ultimately only remaining in the CZE [80]. To maintain steady-state cytokinin homeostasis, the coenocytic endosperm also sustains the expression of cytokinin oxidase/dehydrogenases (CKX) [82], which serve as negative regulators of cytokinin by catalyzing irreversible catabolizing actions. The expression of *CKXs* goes down with the progression of coenocytic development and they remain active only in MCE at the late globular embryo stage [80]. Mutants of *HAIKU1 (IKU1),* which codes for VQ motif protein [83], and *HAIKU2 (IKU2),* which encodes a leucine-rich repeat kinase [84], show a reduced seed size phenotype with early cellularization [12]. In *iku1* and *iku2* mutants, *CKX2* expression is inhibited compared to the wild type, resulting in higher cytokinin levels in the endosperm [80]. Conversely, mutants that block cytokinin signaling exhibit a larger seed phenotype [85-87]. Although the detailed molecular mechanism remains unclear and further investigation into the timing of endosperm cellularization in these lines is necessary, these results demonstrate the importance of maintaining intricate cytokinin balance in endosperm development; increased cytokinin levels prompt early cellularization, whereas reduced levels and signaling of cytokinin lead to larger seeds.

Brassinosteroid (BR) is broadly present in the developing seed [88] and plays a positive role in gene expressions that promote seed size, such as *SHORT HYPOCOTYL UNDER BLUE1, MINISEED3,* and *IKU2* in the endosperm [89]. Simultaneously, BR represses the expression of negative regulators of seed size, such as *APETAL2* and *ARF2* in the integuments and endosperm [89]. In addition to these extensively studied factors, other factors and pathways have been identified to be involved in endosperm development and seed size control [90]. A complex

interplay and regulation among hormones and TFs likely occur in the coenocytic endosperm, governing its development and ultimately determining the final seed size. Further exploration of the connections among these factors and pathways will contribute to unraveling this unique and essential aspect of development, with implications for both biology and agriculture.

Cellular dynamics of endosperm

In addition to examining gene expressions and their associated phenotypes as described in the previous section, researchers have also intensively investigated the cellular dynamics of the unique coenocytic endosperm to further understand its development and have elucidated its link with seed size determination. In barley (*Hordeum vulgare*) [5], Lesser Swine Cress *(Coronopus didymus)* [91], and Arabidopsis [28,92,93], immunostaining revealed a distinctive microtubule (MT) arrangement known as the radial MT system in the coenocytic endosperm during interphase, forming an aster-shaped pattern around the nucleus. This radial MT system orchestrates cell wall placement during endosperm cellularization by generating phragmoplast at the border of nuclear-cytoplasmic domains (NCDs) [5,91,92,94]. Advances in confocal microscopy and live-cell imaging have allowed a detailed exploration of the dynamics and functions of both MT and actin filament (F-actin) in the entire coenocytic endosperm development [7**]. Similar to the radial MT, F-actin also generates an aster-shaped structure around each nucleus soon after the third nuclear division (Figure 3), with this pattern being more prominent in the PEN subregion [7**]. Perturbation of F-actin, achieved through the expression of the semi-dominant negative *ACTIN* transgene (*DN-ACTIN)* (Figure 3) [95], or treatment with the actin inhibitor latrunculin B (Lat B), caused irregular nuclei positioning and random, bouncing-like movement in the endosperm immediately after nuclear division [7**]. Overexpression of the wild-type *ACTIN* gene

(*OX-ACTIN)* led to an increased number of actin cables around each nucleus (Figure 3), maintaining an overall similarity to wild-type F-actin structures and nuclear movement. Interestingly, the distance between nuclei increased further in *OX-ACTIN* compared to the wild-type, generating a larger endosperm/seed, with *DN-ACTIN* resulting in the shortest distance and smaller endosperm/seed [7**]. Taken together, one of the F-actin functions in the coenocytic endosperm is to retain the newly divided nuclei at proper positions and maintain coenocytic endosperm subregions as well as distinct NCDs. During interphase in the *Arabidopsis* coenocytic endosperm, the radial MTs co-localize with F-actin asters [7**]. During nuclear division, MT forms spindles, and concurrently, the aster structures of F-actin become disorganized. Treatment with the MT inhibitor oryzalin, followed by drug washout, further demonstrated the dependence of F-actin aster organization on radial MT [7**]. On the other hand, Lat B treatment does not exhibit any effect on MT structures, and nuclear divisions proceed normally in *DN-ACTIN*, indicating that MT function is independent of F-actin [7**].

Growth dynamics of endosperm and seed

Changes in the cellular dynamics of endosperm can influence the final seed size, and understanding how the seed translates these cellular changes in the endosperm to impact the ultimate seed size is also crucial. As plant cells undergo dynamic growth, the regulation of differential turgor pressure emerges as a pivotal mediator for maintaining plant cell shape and promoting cell expansion during growth and development [96-98]. The rapid expansion of a developing seed raises critical questions about the role of turgor pressure in this process. To measure turgor pressure in the coenocytic endosperm, a strategy was developed utilizing the tissue indentation technique generating force versus displacement curves of the developing seed

to determine the seed stiffness [99,100]. The slope of these curves is shown to be correlated to the turgor pressure [100]. Seeds containing the early stage of the coenocytic endosperm exhibit high seed stiffness, indicative of high turgor pressure in the endosperm. As the endosperm develops, the stiffness (*i.e.,* coenocytic endosperm turgor pressure) gradually decreases, undergoing a significant reduction at cellularization [99]. The *fis2* mutant, characterized by larger seeds with the prolonged coenocytic endosperm phase, displays higher seed stiffness compared to WT at the early coenocytic stage, indicating higher turgor pressure [99]. By contrast, the *iku2* mutant, which produces smaller seeds with early endosperm cellularization, initially shows no difference in seed stiffness compared to WT; however, the stiffness persists even after endosperm cellularization [101**]. In *iku2*, the walls of the testa have a higher presence of demethylesterified pectins [101**], likely contributing to the persisted seed stiffness observed after endosperm cellularization. Taken together, the results from *fis2 and iku2* mutants suggest that at the early stage of coenocytic endosperm, turgor pressure positively regulates seed growth, while at the later stage, the persisted pressure mediates testa stiffening, thereby restricting seed size [99,101**,102].

Vacuoles actively participate in the control of plant cell turgor pressure and play an important role in turgor pressure-dependent cell elongation [97,103]. Changes in the vacuole structure in actin-dependent manner have been reported in plant cells [104]. Disrupting F-actin in the coenocytic endosperm *(DN-ACTIN)* resulted in smaller seeds and defects in the vacuole morphology (Figure 3). By contrast, *OX-ACTIN* shows no such defects in the vacuole morphology and rather produces larger seeds [7**]. These results suggest that F-actin dynamics during the coenocytic stage influence vacuole structure, potentially altering its function and leading to

changes in turgor pressure in the early stage of the coenocytic endosperm (Figure 3). Alternatively, F-actin may also control the distance between NCDs in the peripheral endosperm and establish the volume of the coenocytic endosperm, potentially contributing to seed size changes (Figure 3). Nevertheless, these works set the stage for exploring how turgor pressure in the coenocytic endosperm is regulated and, in turn, how turgor pressure may govern seed growth and development.

Conclusion and perspectives

The endosperm, a highly complex structure within the seeds of flowering plants, plays an essential role in nourishing the embryo during development and germination as well as the evolution of flowering plants. Further investigations on a genome-scale level regarding the emergence of newly duplicated genes, specific to flowering plants, and their expressions in the endosperm hold promise not only for uncovering the functions of yet unexplored genes in endosperm development but also shedding light on the evolution of flowering plants.

Phytohormones, particularly auxin, have demonstrated a prominent role in endosperm development, with ongoing efforts to decipher the complex regulatory pathways. While the link between the MADS-box TF AGL62 and auxin has been explored, the roles of other AGL genes in phytohormone regulation remain uncharted territory. Cytokinin, another key phytohormone in endosperm development, is connected with the HAIKU pathway, which also involves epigenetics [80,84]. Understanding the spatiotemporal crosstalk among cytokinin, auxin, TFs, and imprinting during endosperm development will pave the way to unraveling additional layers of complexity in seed size regulation.

In contrast to the endosperm, F-actin in the central cell forms a meshwork structure and displays constant inward movement from the plasma membrane to the central cell nucleus [95]. This dynamic F-actin movement aids in the migration of the sperm nucleus towards the central cell nucleus for karyogamy and is independent of MT functions [95]. Collectively, fertilization not only alters the dynamics of cytoskeletons but also influences interactions between F-actin and MT, posing a fundamental question of the transition in fate at the cellular dynamics level within the same cell (central cell to endosperm without cell division).

Very recently, comparative transcriptomics among seeds with single fertilization of either the egg or central cell using the mutant producing single-sperm-cell pollens has revealed a set of endosperm genes that are dependent on embryo development and *vice versa* [105*]. The communications among the embryo, endosperm, and seed coat also orchestrate their development as a seed and influence the final seed size [32*,106-110]. Continued research into the highly complex mechanisms governing seed development, including this unique coenocytic endosperm, promises to unlock new avenues for improving seed traits and, consequently, enhancing yields per capita on a global scale.

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Annotated references

7** Ali MF, Shin JM, Fatema U, Kurihara D, Berger F, Yuan L, Kawashima T: **Cellular dynamics of coenocytic endosperm development in Arabidopsis thaliana**. *Nature Plants* 2023, **9**:330-342.
The authors provide details on the nuclear movement and cytoskeleton dynamics of Arabidopsis coenocytic endosperm. With the help of confocal and live-cell imaging, this study showed that microtubule and actin filaments form aster-shaped structures around the nucleus, and actin filaments are responsible for the nuclear organization and final seed size.

25** Belmonte MF, Kirkbride RC, Stone SL, Pelletier JM, Bui AQ, Yeung EC, Hashimoto M, Fei J, Harada CM, Munoz MD: **Comprehensive developmental profiles of gene activity in regions and subregions of the Arabidopsis seed**. *Proceedings of the National Academy of Sciences* 2013, **110**:E435-E444.
The authors lay foundational work by generating transcriptomic data from Arabidopsis seed. This study utilized laser capture microdissection for precisely differentiating the regions in Arabidopsis seed from stages of fertilization to seed maturity. This study became a fundamental resource for seed biology.

26** Picard CL, Povilus RA, Williams BP, Gehring M: **Transcriptional and imprinting complexity in Arabidopsis seeds at single-nucleus resolution**. *Nature Plants* 2021, **7**:730-738.
This study applies single-nuclei RNA-sequencing and generated a transcriptional atlas of Arabidopsis coenocytic endosperm. The authors show the transcriptional regulation in the entire endosperm at a nucleus level and show the heterogenous imprinting pattern, especially in the chalazal endosperm.

32* Doll NM, Ingram GC: **Embryo–endosperm interactions**. *Annual Review of Plant Biology* 2022, **73**:293-321.
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35* Liu J, Wu M-W, Liu C-M: **Cereal endosperms: development and storage product accumulation**. *Annual Review of Plant Biology* 2022, **73**:255-291.
The authors provide a detailed review of endosperms in cereals focusing on rice, maize, and wheat. The review also discusses the regulation of cell-cycle and hormone signaling during endosperm development.

68* Qiu Y, Köhler C: **Endosperm evolution by duplicated and neofunctionalized Type I MADS-box transcription factors**. *Molecular Biology and Evolution* 2022, **39**:msab355.
The authors show that type I MADS-box have duplicated during evolution and neofunctionalized, and some of the newly acquired type I MADS-box are only expressed in the endosperm of angiosperms, thus owing to the endosperm evolution in angiosperms.

75* Guo L, Luo X, Li M, Joldersma D, Plunkert M, Liu Z: **Mechanism of fertilization-induced auxin synthesis in the endosperm for seed and fruit development**. *Nature communications* 2022, **13**:3985.
Auxin is key for seed growth and development. The authors show that AGL62 is required for auxin synthesis in the endosperm in both Arabidopsis and strawberry. They provide details about the interplay between auxin and AGL62 in endosperm for seed and fruit development.

78* Kohler C, Butel N, Qiu Y, Xu W, Santos-Gonzalez J: **The molecular basis of parental conflict driven regulation of endosperm cellularization**. *bioRxiv* 2023:2023.2006. 2022.546051.
This study reveals the regulation of auxin levels in endosperm by a cluster of ARFs (cARFs) causes the endosperm cellularization. The study further showed that these cARFs function in a parental dosage dependent manner.

101** Creff A, Ali O, Bied C, Bayle V, Ingram G, Landrein B: **Evidence that endosperm turgor pressure both promotes and restricts seed growth and size**. *Nature Communications* 2023, **14**:67.
Turgor pressure generated from the Arabidopsis coenocytic endosperm drives seed growth and development. This study reveals that the turgor pressure from the endosperm works in both increasing and restricting seed growth depending on the timing.

105* Zhang Y, Maruyama D, Toda E, Kinoshita A, Okamoto T, Mitsuda N, Takasaki H, Ohme-Takagi M: **Transcriptome analyses uncover reliance of endosperm gene expression on Arabidopsis embryonic development**. *FEBS letters* 2023, **597**:407-417.
Comparative transcriptomics among seeds with single fertilization of either the egg or central cell have revealed a set of endosperm genes that are dependent on embryo development, and vice versa.

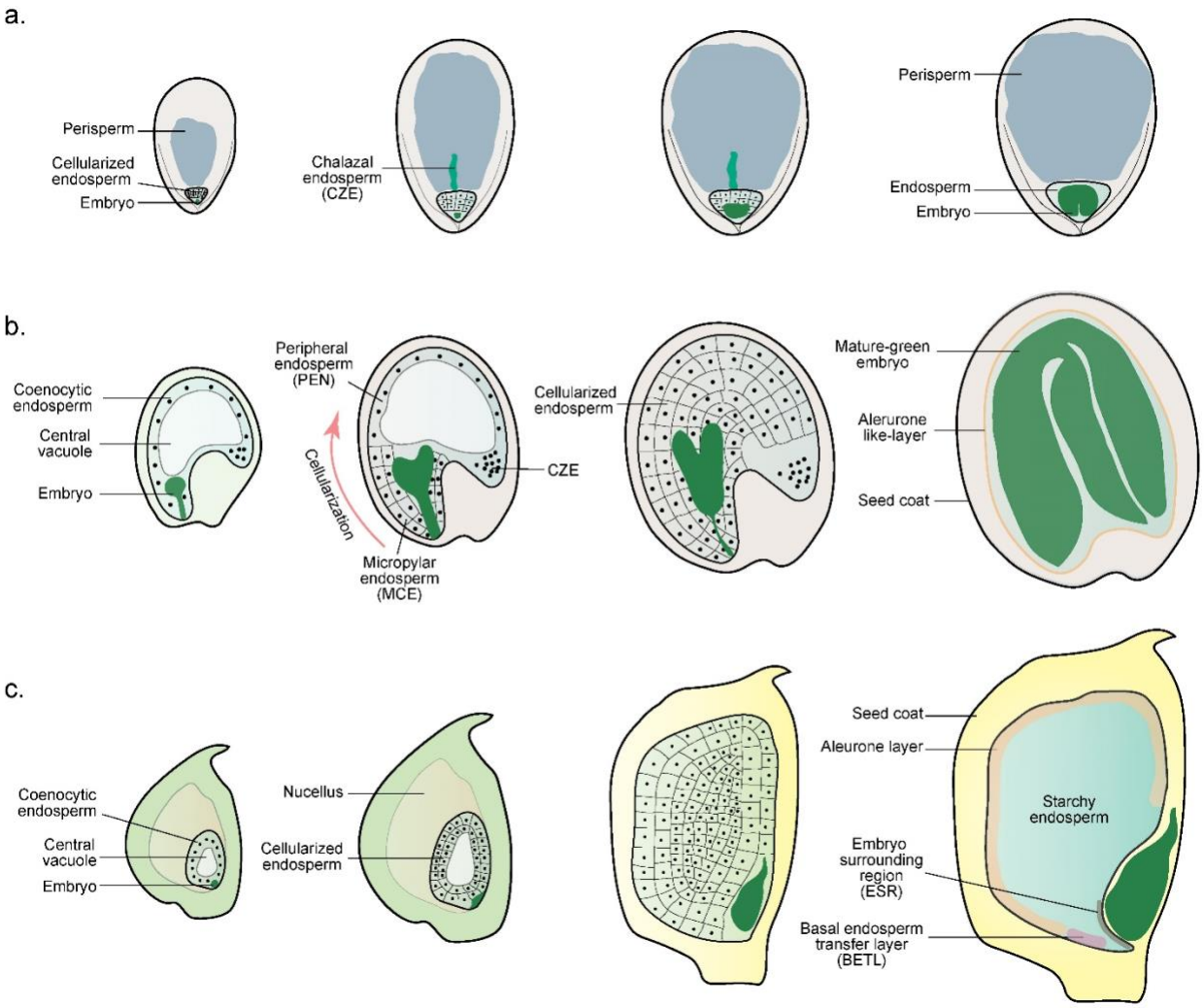

Figure 1. **Seed growth and development in flowering plants. a.** Double fertilization leads to the formation of the embryo and diploid cellular endosperm in Nymphaeaceae. The perisperm (nucellus), a sporophytic tissue, stores starch and provides nourishment to the developing embryo via the endosperm. The chalazal endosperm forms a haustoria-like structure that transfers nutrients to the embryo. At seed maturity, the perisperm persists, possibly to support the embryo for germination. **b.** Double fertilization in Arabidopsis forms an embryo and a triploid nuclear endosperm. Endosperm development initially undergoes nuclear divisions without cytokinesis to form a coenocyte and then it cellularizes starting from the micropylar endosperm. In mature seeds, the endosperm is almost completely absorbed by the embryo and remains as a thin aleurone-like layer. **c.** In maize, the endosperm undergoes coenocytic development at an early stage and then starts to cellularize. After cellularization, the endosperm differentiates into the basal endosperm transfer layer (BETL) which acts as a barrier and supply route, embryo surrounding region (ESR), aleurone layer, and starchy endosperm. The endosperm is not absorbed by the embryo, and it supports the embryo during germination by providing all the necessary nutrients.

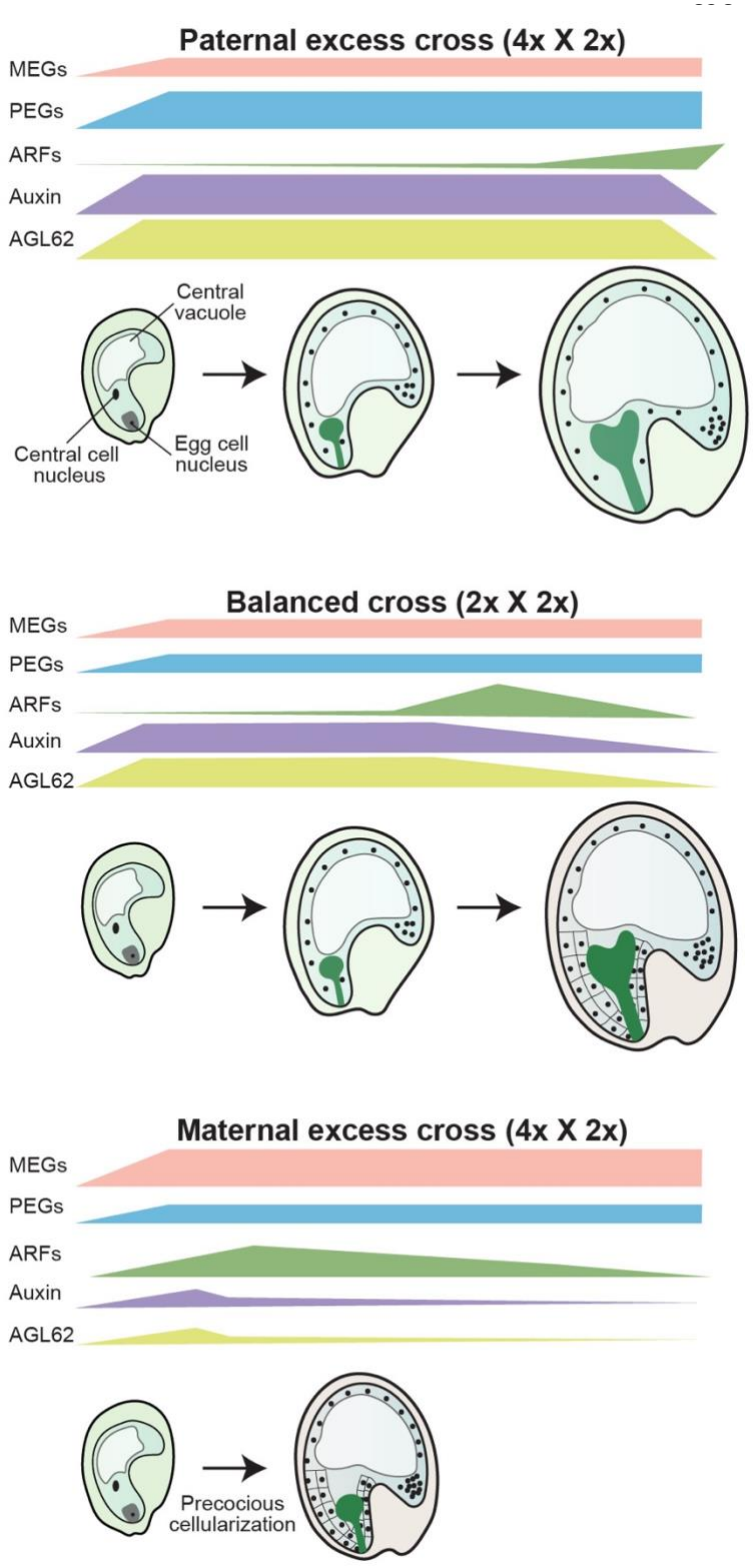

Figure 2. **Molecular Dynamics of Arabidopsis Endosperm Development.** Endosperm development is orchestrated by an interplay between and the balance of transcription factors (TFs) and phytohormones. After double fertilization in the case of a balanced cross (2x X 2x), *AGL62* levels increase, leading to an elevation in auxin levels. This increase in auxin levels induces the primary endosperm to initiate nuclear divisions. *AGL62* and auxin levels remain high, correlating with the maintenance of the coenocytic endosperm phase. Before cellularization, TF *ARFs* come into play, acting as negative regulators for auxin signaling. This action by *ARFs* halt the coenocytic phase, initiating endosperm cellularization. In the case of a paternal excess cross (2x X 4x), both *AGL62* and auxin exhibit elevated levels, resulting in a prolonged coenocytic phase duration. This delays the expression of *ARFs*, causing a subsequent delay in the cellularization of the endosperm. The expression of paternally expressed genes (PEGs) is higher than maternally expressed genes (MEGs) in the paternal excess cross. Conversely, in the case of a maternal excess cross (4x X 2x), *AGL62* and auxin levels remain low from the start of double fertilization. This leads to an early expression of *ARFs*, causing precocious endosperm cellularization. The expression of MEGs is higher than PEGs in the maternal excess cross.

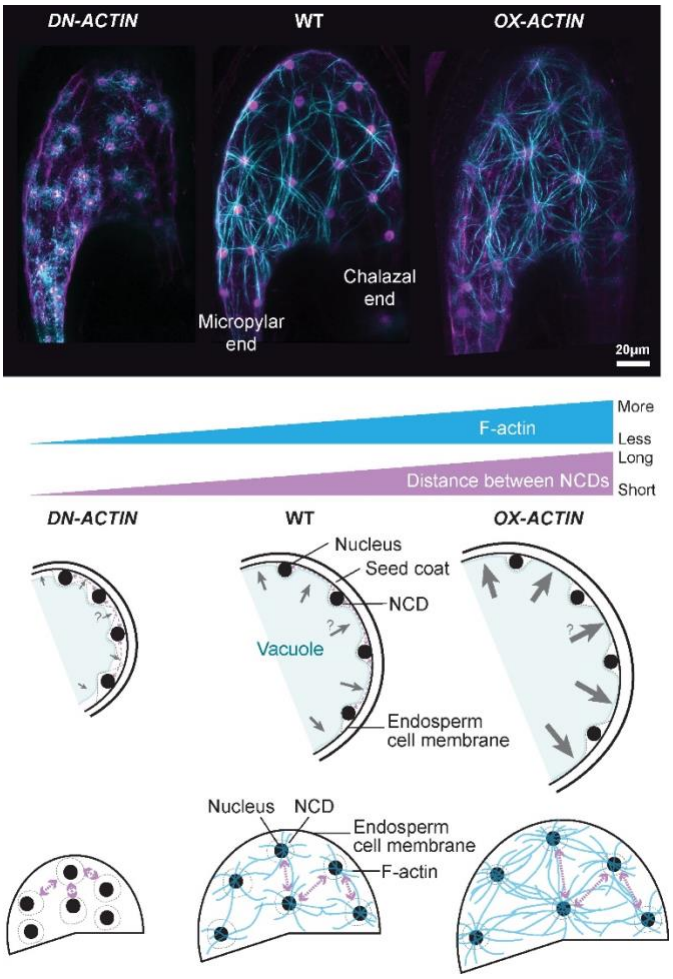

Figure 3. **Cytoskeleton Dynamics in Coenocytic Endosperm.** Z-projected confocal images depict F-actin (cyan, *proFWA::Lifeact-Venus*) and nuclei (magenta, *proFWA::H2B-mRuby2*) in the Arabidopsis coenocytic endosperm. F-actin forms aster-shaped structures around nuclei. In *DN-ACTIN*, the absence of F-actin aster formations disrupts nuclei organization. Conversely, *OX-ACTIN* exhibits a higher F-actin abundance and larger endosperm compared to the wild-type (WT). The central vacuole in the endosperm pushes nuclei to the periphery, forming nuclear cytoplasmic domains (NCDs). In *DN-ACTIN*, the vacuole structure is affected, resulting in less pushing of nuclei to the periphery compared to the WT. In *OX-ACTIN*, NCDs are more spaced, potentially influencing endosperm volume and size. These vacuole morphology and NCD alterations may contribute to variations in endosperm size and turgor pressure, possibly explaining diverse seed sizes among F-actin-manipulated lines