Review

Regulation of the Early Phase of Seed Development: An Illustrative Review

Prakash Babu Adhikari * and Ryushiro Dora Kasahara

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Regulation of the Early Phase of Seed Development: An Illustrative Review

Prakash Babu Adhikari * and Ryushiro Dora Kasahara

Bioscience and Biotechnology Center, Nagoya University, Nagoya, Japan

* Correspondence: pbadhikari@yahoo.com

Abstract

Seeds are the central innovation of angiosperms, earliest developmental steps of which hinge on tightly coordinated fate decisions across sporophytic and gametophytic tissues. This review synthesizes recent advances in the regulation of the early phase of seed development, beginning with ovule primordium growth and the specification of a single megaspore mother cell (MMC), through female gametophyte maturation, double fertilization, and the onset of embryo–endosperm–seed coat programs. Canonical regulators (e.g., SPL/NZZ-centered networks and auxin transport/threshold control) operate alongside repressive safeguards mediated by small-RNA pathways, transposon silencing, and Polycomb/RBR1-linked cell-cycle gates that prevent premature proliferation and autonomous seed formation. After fertilization, parent-of-origin epigenetic asymmetries and dynamic chromatin states reshape transcriptional competence in endosperm and embryo, with auxin emerging as a key mobile coordinator of endosperm growth, integument differentiation, and seed coat initiation. Mechanical inputs, including endosperm turgor and mechanosensitive responses in maternal tissues, are integrated with hormone signaling to tune seed growth and the timing of endosperm cellularization. Together with the accompanying figures, the review provides an illustrative depiction of the earliest regulatory logic underlying successful seed formation.

Keywords: seed; auxin; imprinting; mechanosensing

1. Introduction

Seeds are fundamental to the life cycles of flowering plants, serving as the primary means of reproduction. Beyond their biological significance, they are also the key food sources for human and other animal consumption. Normally, seed development begins with a double fertilization event wherein two sperm cells from pollen fuse with two female gametes in the ovule—one sperm fertilizes the egg cell to form the diploid zygote (future embryo), and the other fertilizes the central cell to form the triploid endosperm (Adhikari *et al.,* 2020b). These two fertilization products, together with the surrounding maternal tissue that forms the seed coat, undergo a coordinated development that results in a mature seed (Baroux and Grossniklaus, 2019). The regulation of early-stage seed development involves multiple checkpoints and signals that integrate maternal and paternal inputs (Adhikari *et al.,* 2020b). Ovule maturation produces a fertilization-ready structure in which growth is arrested awaiting fertilization (Ingram, 2010). In the present context, the term 'developmental block' refers to pre-fertilization and early post-fertilization barriers that prevent autonomous embryo/endosperm development, enforced by cell-cycle gating, epigenetic repression, and constrained signaling. Fertilization alleviates these barriers and initiates synchronized growth of embryo, endosperm, and seed coat.

Several studies have addressed seed development. The present review focuses on the earliest developmental window, from MMC speciation to ovule maturation, double fertilization, and progression of seed development through onset of cellularization and selected early embryo–endosperm interface modules. It summarizes current mechanistic models for sporophyte-gametophyte communication and coordinated growth of the embryo, endosperm, and seed coat.

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Evidence is drawn primarily from Arabidopsis, with selected comparisons to other species where they sharpen or challenge Arabidopsis-based models.

2. Evolutionary Triggers Behind Seed (Plant) Development

Angiosperm success on land is closely linked to innovations in reproductive development, including ovules, seeds, and associated maternal–offspring coordination. Comparative syntheses highlight that evolutionary inference for ovule/seed traits remains constrained by sampling and analytical limitations, and mechanistic generalizations should therefore be treated cautiously (Mathews and Kramer, 2012, Rudall, 2021, Benton *et al.*, 2022, Boyko and Vasconcelos, 2025).

One influential hypothesis proposes that the ovule represents a modified shoot-derived developmental program, motivated by structural similarities between the nucellar apex and shoot apex and by nucellar expression of the shoot stem-cell regulator *WUSCHEL* (*WUS*) (**Figure 1**) (Mathews and Kramer, 2012). This view is compatible with 'nested program' models (synonymously referred to as 'babushka doll' and 'hierarchical shifts') in which inflorescence, flower, and ovule like complex branching structures development emerge through hierarchical redeployment of organogenesis modules. Integument regulators such as YABBY factors (including INNER NO OUTER (*INO*)) further illustrate the reuse of laminar organ-growth regulators in reproductive structures (Yamada *et al.*, 2011, Arnault *et al.*, 2018, Rudall, 2021).

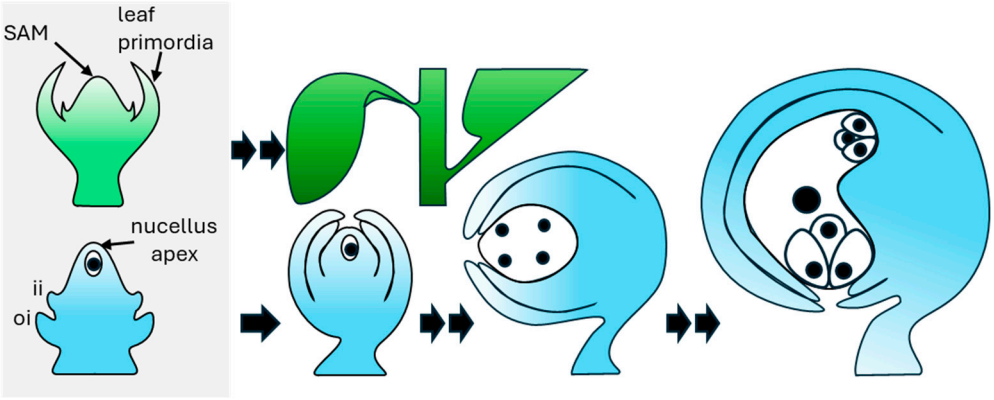

Figure 1. Structural similarities between shoot apical meristem (SAM) and ovule primordium (highlighted panels), which subsequently develop into shoot and ovule respectively. ii = inner integument; oi = outer integument.

Comparative anatomy also supports multiple evolutionary trajectories for the outer integument (OI) in bitegmic ovules. The OI has been proposed to represent a later innovation in angiosperms and, in some lineages, a convergent feature; ovules of Gnetales also possess an outer envelope that is suggested to originate from fusion of lateral bracts, consistent with initiation from two primordia (Rydin *et al.*, 2010, Rudall, 2021). Given that auxin–cytokinin interplay can modulate integument initiation and bitegmic–unitegmic-like features in Arabidopsis, hormonal rewiring is a plausible route by which developmental programs were evolutionarily repurposed (Bencivenga *et al.*, 2012).

At the cellular level, meiosis and double fertilization represent major evolutionary transitions that frame the pre- and post-fertilization 'developmental blocks' discussed below. The origin of meiosis has been argued to reflect selection for recombination in large eukaryotic genomes and avoidance of the long-term mutational accumulation (Muller's ratchet) (**Figure 2**) (Muller, 1964, Takeuchi *et al.*, 2014, Berger, 2024). It is an integral part of angiosperm reproductive success as it plays a fundamental role in genetic and epigenetic reset during the process. Angiosperm double fertilization typically produces zygote and endosperm. Two principal hypotheses for endosperm origin have been advanced primarily based on the inferential evidences: derivation from a supernumerary embryo versus derivation from a sexualized female gametophyte (FM). Conserved

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features such as micropylar–chalazal asymmetry of nuclear division are observed across basal angiosperms (Floyd and Friedman, 2000, Baroux *et al.*, 2002, Becker *et al.*, 2025). These evolutionary perspectives motivate the mechanistic focus of subsequent sections on how fertilization alleviates developmental blocks and coordinates early embryo, endosperm, and seed coat growth.

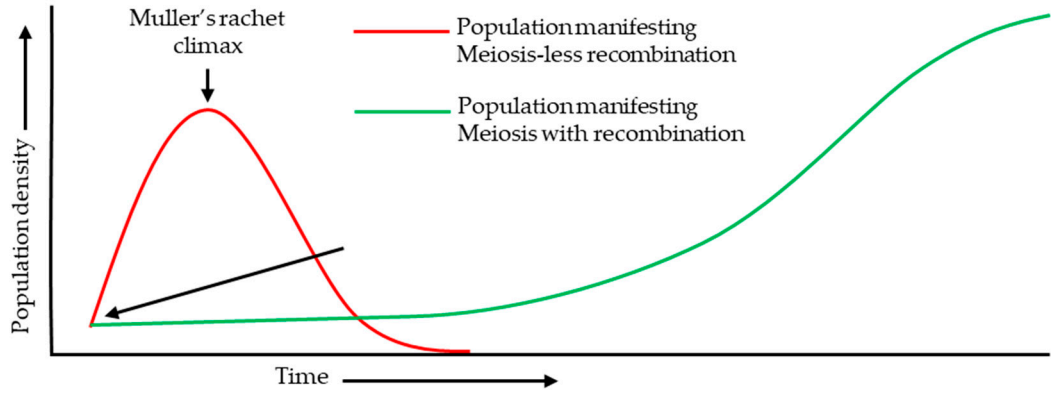

Figure 2. Illustrative depiction of the meiosis-with-recombination led persistence of the eukaryotic population over long period of time (green). Any population lacking functional recombination likely succumbed to the Muller's rachet due to the accumulation of unmanageable deleterious mutation bringing it to its subsequent extinction (red).

3. Ovule Development

At the onset of megasporogenesis, a single L2 cell differentiates into the megaspore mother cell (MMC), committing to meiosis and the sporophytic-to-gametophytic transition. The to-be-MMC cell speciates specifically from a single cell of the layer 2 (L2) in the developing ovule primordium indicating a precisely targeted molecular regulation during the process (**Figure 3**). Some of the key factors involved in the MMC speciation is shown in **Figures 4–6**. One of the key factors behind the speciation process is the PRC1-mediated repression of an RdDM-associated 24-nt miRNA-partner effector protein encoding gene, *ARGONAUTE 9* (*AGO9*). The process is facilitated by the PRC1 core component, E3 ligases, Really Interesting New Gene 1 (RING1) and RING2-mediated histone monoubiquitylation (H2Aub1) of the locus. Mutations in *RING1A/B* cause defects in MMC/FM specification and FM mitoses and coincide with ectopic expression of AGO family genes (including AGO9), resulting in aborted ovules (Lv *et al.*, 2024). PRC1 and PRC2 complexes are known to play pivotal role during developmental transitions. PRC1 typically monoubiquitylates the histone (H2Aub1), which is suggested to be a PRC2 recruitment signal for subsequent histone methylation (H3K27me3). In such case, the PRC2 recruitment process likely requires H2Aub binding protein, which however, has not been identified yet. The PRC2 recruitment independent of ubiquitylation is also a common occurrence in plants (Liu *et al.*, 2025b). As one recent study observed, H2Aub predominantly marks the repressed genes with no H3K27me3 marks. On transposable elements (TEs) however, both marks tend to colocalize. Nevertheless, the ubiquitylation in the former and methylation of the latter case play deterministic regulatory role (**Figure 4**) (Wu *et al.*, 2025).

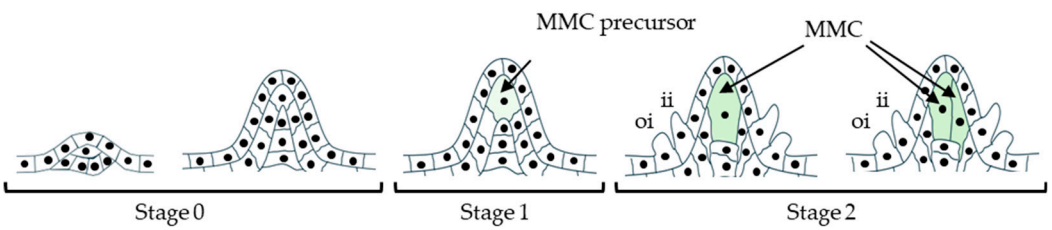

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Figure 3. Typical developmental progression of ovule primordia and MMC speciation. The two images of stage 2 represent for the ovule primordia with normal single MMC speciation (former) and aberrant primordia with more than one L2 cell exhibiting MMC fate.

Typically, the PRC1-regulated *AGO9* is expressed in the nucleus of MMC (Rodríguez-Leal *et al.*, 2015) and cytoplasm of the apical somatic cells (Olmedo-Monfil *et al.*, 2010). Furthermore, the defect in the AGO9-interacting partner miRNA822 leads to the persistence of the more than one post-meiotic cells. The *ago9* mutant or (ovule-specific) miRNA822-target genes defective mutants exhibit similar phenotype (Tovar-Aguilar *et al.*, 2024). The *ring1a/b* mutants exhibit reduced expression of a MADS-box member, *SEEDSTICK* (*STK*) (Chen *et al.*, 2016), a positive regulator of *AGO9* and *RNA-DEPENDENT RNA POLYMERASE 6* (*RDR6*), thereby negatively affecting SPL/NZZ expression and MMC speciation (Mendes *et al.*, 2020). *RDR6* is known to convert single-stranded precursors into double-stranded *RNA*, the process dependent on the function of a *RNA* binding protein *SUPPRESSOR* OF *GENE SILENCING 3* (*SGS3*) during trans-acting siRNA biogenesis (**Figure 4**) (Yoshikawa *et al.*, 2005).

As in *ago9* mutants, sgs3 and rdr6 mutants develop supernumerary MMCs. This supports a model in which tasiRNA-mediated silencing moves between companion somatic cells to restrict MMC fate in a non-cell-autonomous manner (Olmedo-Monfil *et al.*, 2010, Rodríguez-Leal *et al.*, 2015). *AGO9* also promotes DOMAIN REARRANGED METHYLTRANSFERASE 1 (DRM1) and DRM2-mediated silencing (as part of RdDM) at the SPL/NZZ locus and may contribute to SPL/NZZ mRNA degradation in surrounding hypodermal somatic cells, thereby allowing MMC fate acquisition by a single central cell (**Figure 4**) (Matzke and Mosher, 2014, Mendes *et al.*, 2020). Recent work further suggests that single MMC specification depends on balanced methylation and demethylation rather than methylation alone (Jiang *et al.*, 2025).

The study suggested that *DRM2* and *DME* act on largely distinct target sets. Approximately 30% of ovule primordia showed co-expression of *DRM2* and *DME* in surrounding L2 cells, and *dme* mutants produced more than one MMC-like cell. In the normal MMC, *DRM2* and *DME* expression declines, whereas neighboring L2/L3 cells retain *DME* expression with reduced *DRM2* (**Figure 4**) (Jiang *et al.*, 2025).

SPL/NZZ facilitates the recruitment of TOPLESS (TPL) and co-repressor at the CINCINNATA (CIN)-like TEOSINTE BRANCHED1/CYCLOIDEA/PCF (TCP) bound locus to repress the CIN-like TCP-regulated genes via the EAR motif (LXLXL) at its C-terminus. The process is essential for MMC speciation and development (Wei *et al.*, 2015). SPL/NZZ additionally acts as a part of a MADS-box-associated complex to target the canonical CArG motifs in the *ANTEGUMENTA* (*ANT*) promoter and contributes to its repression. When active, *ANT* represses the auxin flux pump encoding member *PIN1*, thereby hindering the auxin-mediated AUX/IAA degradation. Degradation of AUX/IAA is essential for AUXIN RESPONSE FACTOR (ARF) release to act on downstream targets involved in MMC development (**Figure 5**) (Cavalleri *et al.*, 2025). PIN1-driven auxin transport establishes a micropylar auxin maximum and a chalazal minimum in the ovule primordium; SPL/NZZ (with miR160-restricted ARF17) supports the PIN1 expression domain underlying the micropylar maximum, whereas BEL1 restricts PIN1 at the chalaza to maintain the auxin minimum (Bencivenga *et al.*, 2012, Huang *et al.*, 2022, Yu *et al.*, 2025).

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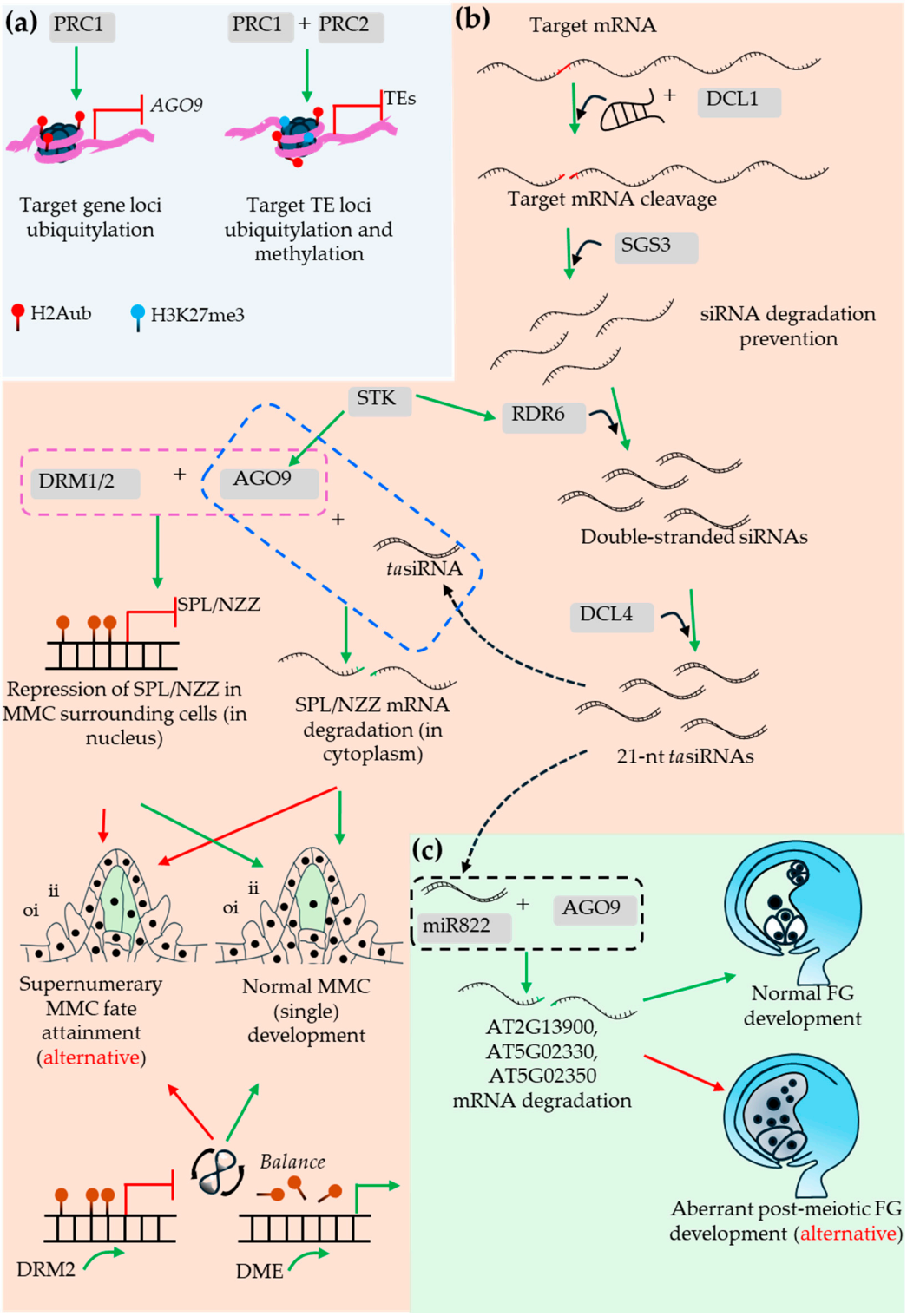

Figure 4. PRC1/PRC2 and small-RNA–directed epigenetic pathways restrict MMC fate to a single L2 cell and support downstream FM development. **(a)** PRC1 represses AGO9 via H2Aub1 at target gene loci; in contrast, at transposable elements (TEs), PRC1 + PRC2 co-marking (H2Aub1 + H3K27me3) is enriched, highlighting locus-type–dependent Polycomb outputs (Lv *et al.*, 2024; Wu *et al.*, 2025; Liu *et al.*, 2025b). **(b)** STK promotes AGO9 and the tasiRNA pathway component RDR6. tasiRNA biogenesis is schematized as precursor processing and stabilization (DCL1, SGS3), dsRNA synthesis (RDR6), and DCL4-dependent production of 21-nt tasiRNAs, which contribute to restricting SPL/NZZ via cytoplasmic RNA decay and/or reinforcing nuclear silencing in surrounding L2 companion cells (Yoshikawa *et al.*, 2005; Matzke and Mosher, 2014; Chen *et al.*, 2016; Mendes *et al.*, 2020).(Bottom left) AGO9-associated RdDM (via DRM1/2) represses SPL/NZZ in MMC-adjacent cells, preventing supernumerary MMC formation; single-MMC outcome is proposed to depend on a balance of

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methylation (DRM2) and demethylation (DME) rather than methylation alone (Matzke and Mosher, 2014; Mendes *et al.*, 2020; Jiang *et al.*, 2025). **(c)** miR822–AGO9-linked mRNA degradation of ovule targets (e.g., AT2G13900/ AT5G02330/AT5G02350) supports normal post-meiotic FM development; disruption yields aberrant FG outcomes (Tovar-Aguilar *et al.*, 2024). Green arrows indicate activation/positive effects; red bars indicate repression; dashed lines indicate indirect/putative links.

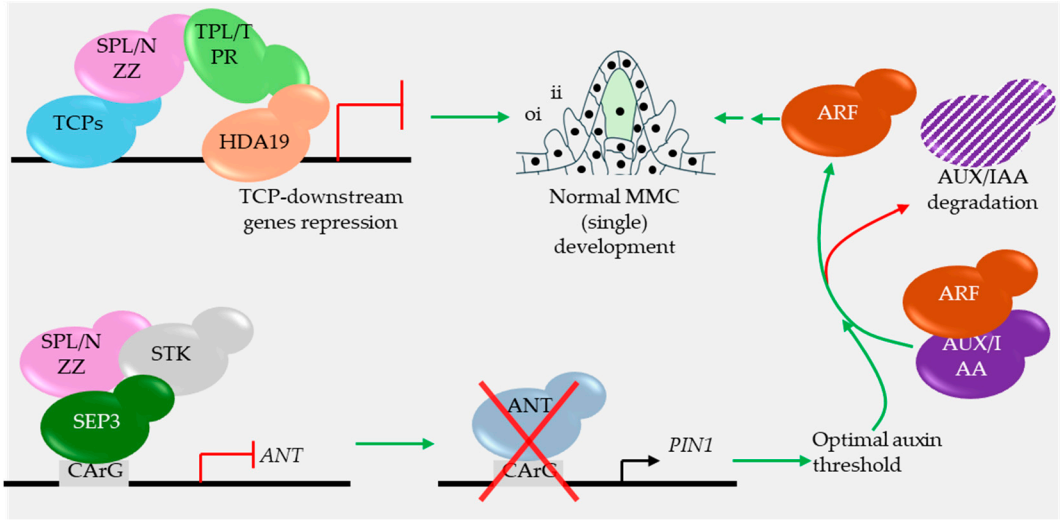

Figure 5. Key SPL/NZZ-mediated positive regulators (primarily) players behind single-MMC speciation. SPL/NZZ recruits the co-repressor TPL/TPR (via its C-terminal EAR motif) to CIN-like TCP–bound loci and, together with HDA19, represses TCP downstream genes, thereby restricting MMC fate and supporting normal single-MMC development (Wei *et al.*, 2015). SPL/NZZ also functions in a STK/SEP3-associated MADS-box complex that targets CArG motifs in the *ANT* promoter to repress its expression; ANT otherwise represses PIN1, so *ANT* repression permits PIN1-dependent auxin flux to reach an optimal auxin threshold that promotes AUX/IAA degradation, releases ARFs, and enables activation of MMC developmental programs (Cavalleri *et al.*, 2025). Green arrows, activation; red bars, repression.

The hypodermal somatic cells surrounding the MMC are repressed from attaining the MMC fate by the SWI/Snf-related group 1 (SWR1), an ATP-dependent chromatin remodeling complex in plants. The SWR1 complex mediates the deposition of histone variant H2A.Z around the transcription start site (TSS) of *WRKY28* locus *via* its component ACTIN RELATED PROTEIN *6* (ARP6) and promotes its expression. The process is non-cell-autonomously enhanced by the inner integument expressed *KLUH* (*KLU*) encoding cytochrome P450 protein. *WRKY28*, then actively represses the hypodermal somatic cells from attaining the MMC fate (**Figure *6***) (Zhao *et al.*, 2018a, Aslam *et al.*, 2019). In the inner integument, KLU contributes to cell proliferation regulates the final seed size (Adamski *et al.*, 2009), which is however counteracted by jasmonate signaling-associated network (**Figure *6***) (Zhang *et al.*, 2025). Regarding gametogenesis, the SWR1 complex further regulates the gametophyte development post-MMC speciation. Its component ARP6 hinders the expression of *DISRUPTED MEIOTIC cDNA1* (*DMC1*) in the megasporocyte and surrounding non-sporangenous ovule cells prior to meiosis. After the initiation of meiosis process, however, ARP6 activates *DMC1* exclusively in the megasporocyte and the process is facilitated by the SWR1 complex-mediated incorporation of the histone variant H2A.Z at the *DMC1* locus (Qin *et al.*, 2014).

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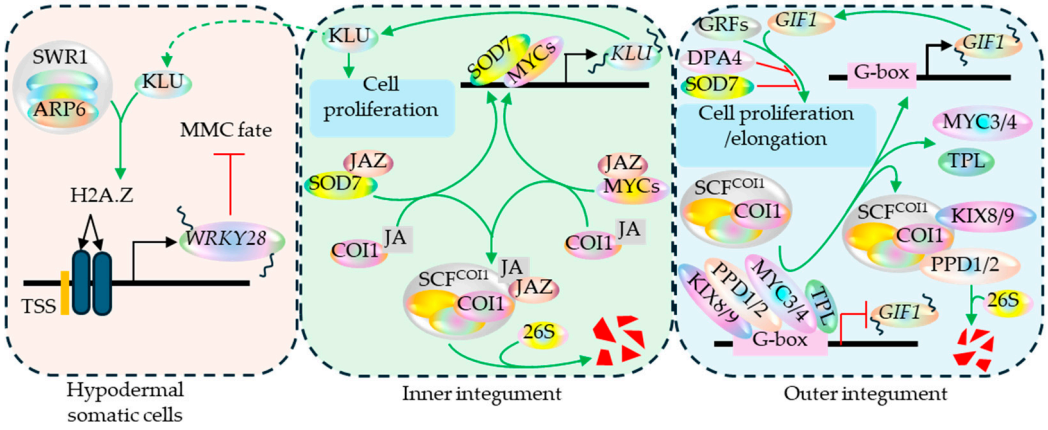

Figure 6. Factors associated with the hypodermal somatic cells, inner integument, and outer integument development. Left (hypodermal somatic cells): the SWR1 chromatin-remodeling complex, via ARP6, promotes H2A.Z deposition around the *WRKY28* transcription start site (TSS), enhancing its expression and thereby repressing ectopic acquisition of MMC fate. The process is non-cell-autonomously promoted by inner-integument KLU activity (Zhao *et al.*, 2018a; Aslam *et al.*, 2019). Middle (inner integument): KLU promotes cell proliferation and contributes to final seed size (Adamski *et al.*, 2009), while jasmonate (JA) signaling through COI1/SCFCOI1 and the 26S proteasome (*via* JAZ–MYC control) counteracts this proliferative program (Zhang *et al.*, 2025). Right (outer integument): a GIF1/GRF growth module is integrated with JA-responsive regulators (including MYC–TPL and PPD1/2–KIX8/9 acting at G-box elements), with proteasome-linked turnover shaping proliferation/elongation outputs (Zhang *et al.*, 2025). Green arrows indicate activation; red bars indicate repression; dashed connectors indicate indirect and/or non-cell-autonomous effects.

4. Ovule Maturity and Growth Progression Halt

Normal ovule maturation culminates in a state of readiness for fertilization (**Figure 1**) (Adhikari *et al.*, 2020b). This pre-fertilization pause is not a passive cessation of activity but rather an actively maintained state. Rhe phase of cell cycle during this quiescent period may vary among different plant species (Liu *et al.*, 2020). This regulatory control guarantees that seed development (and the provisioning of nutrients) proceeds only when a viable embryo is formed, typically after a successful fertilization. Due to such a developmental roadblock, mature ovules typically have a limited lifespan and undergo active programmed cell death afterwards (at 5-6 days after anthesis in Arabidopsis). As the ovules age, plants initiate senescence of the unfertilized ovules through ethylene signaling and activation of cell death-associated genes such as the triad of NAC-family transcription factors, in their outer tissues during the process (Van Durme *et al.*, 2023).

The post-maturity developmental progression halt of the gametes is largely regulated by a plant RETINOBLASTOMA RELATED (RBR) homolog, *RBR1* and its downstream targets in central cell. The whole process can be separated into three distinct modules- a) cell-cycle gate control, b) imprinting setup, and c) FIS-PRC2 execution (**Figure 7**). RBRs are attributed for their positive role in maintaining cellular quiescence at diverse tissues during plant development (Gombos *et al.*, 2023). In the mature ovule, *RBR1* suppresses the E2F-DP complex targets, which include the G1-to-S phase transition-associated genes, by directly interacting with the complex and halts the central cell division (Ebel *et al.*, 2004).

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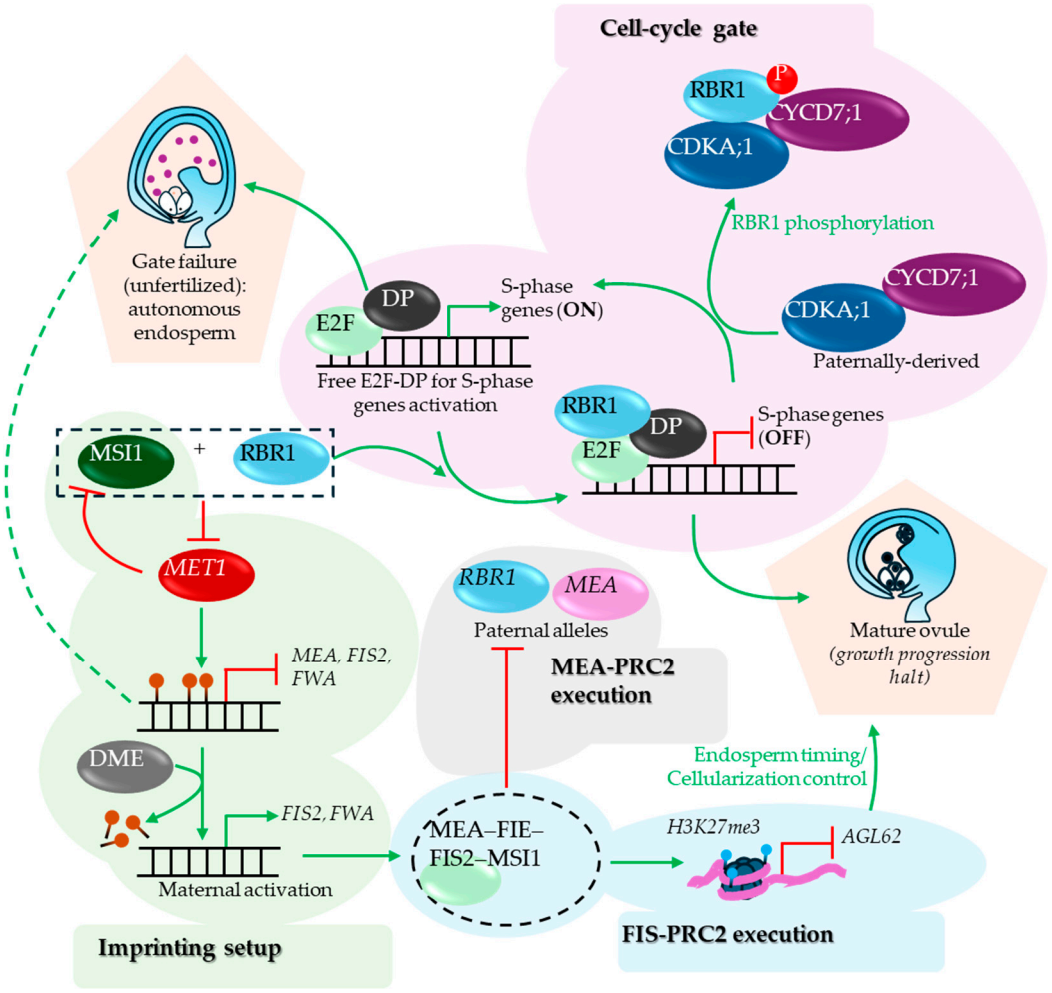

Figure 7. Factors regulating the growth-progression halt and post-fertilization proliferation of central cell (secondary endosperm) in the mature ovule and underlying events in Arabidopsis. In the unfertilized FM, RBR1 restricts G1/S by repressing E2F/DP-driven S-phase genes, enforcing the mature-ovule growth halt; loss of RBR1 causes failure of arrest with ectopic central-cell proliferation and autonomous endosperm-like divisions (Ebel *et al.*, 2004). Central-cell CYCD7;1–CDKA;1 promotes RBR1 phosphorylation/inactivation, thereby sliding it off and releasing E2F/DP to drive extra nuclear proliferation when ectopically expressed (Sornay *et al.*, 2015). Normally, sperm cell-derived (paternal) CYCD7;1 and CDKA facilitates the RBR1 phosphorylation process after fertilization. RBR1–MSI1 represses MET1, enabling DME-linked demethylation and maternal activation of MET1-sensitive imprinted genes (*MEA*, *FIS2*, *FWA*) (Jullien *et al.*, 2008; Johnston *et al.*, 2008; Xiao *et al.*, 2003). After fertilization, maternal MEA-PRC2 contributes to the H3K27me3-mediated repression of the paternal RBR and MEA alleles (Gehring *et al.*, 2006; Johnston *et al.*, 2008). FIS–PRC2 prevents fertilization-independent activation of an endosperm-like program before fertilization; after fertilization, AGL62 supports the coenocytic program and delays cellularization (Figueiredo *et al.*, 2016).

CYCD7;1 phosphorylates the E2F-DP-bound RBR1 thereby sliding it off of its bound loci (Weimer *et al.*, 2018). The *rbr1* mutant and the lines ectopically expressing *CYCD7;1* exhibit autonomous endosperm proliferation as the E2F-DP bound loci are freed of RBR1 in both cases (Ebel *et al.*, 2004, Sornay *et al.*, 2015, Weimer *et al.*, 2018). While the *rbr1* mutant ovules are rarely fertilized, the ovules ectopically expressing *CYCD7;1* exhibits successful fertilization producing seeds relatively larger than that produced by WT (**Figure 7**) (Ebel *et al.*, 2004, Sornay *et al.*, 2015). In addition to its role in cell cycle control, *RBR1*, along with the WD40 domain harboring PRC2 interacting protein, *MSI1*, suppresses *MET1* expression (Johnston *et al.*, 2008), which leads to the gradual decrease in methylation of maternally imprinted genes such as *FIS2* and *FWA* during female gametogenesis after each cycle of DNA replication. The residual methylation in the loci is further removed by *DME*

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thereby activating the FIS2-led developmental block of central cell in a mature ovule (**Figure 7**) (Jullien *et al.*, 2008). Analogous observation was made on a gene encoding an additional PRC2 subunit, *MEA* (Xiao *et al.*, 2003).

The paternal *MEA* and *RBR1* are repressed by PRC2-mediated H3K27me3 in pollens. The paternal *RBR1* repression continues after fertilization as well, which is mediated by MEA-PRC2 (Johnston *et al.*, 2008). The third module governing growth progression halt in mature ovule is FIS-PRC2, which deposits H3K27me3 marks to its target loci. As observed in Arabidopsis a HETEROCHROMATIN PROTEIN 1 family member TFL2/LHP1 broadly co-localizes with H3K27me3-marked genes (>85-90% overlap at the mapped sites), and H3K27me3 profiles remain largely similar in *lhp1* seedlings, indicating that LHP1 likely acts downstream as a reader/effector of Polycomb chromatin (Turck *et al.*, 2007). LHP1 interacts with MSI1 thereby contributing to spread in H3K27me3 deposition toward 3′-ends of the target gene in a context dependent manner and shape local/3D chromatin topology (Veluchamy *et al.*, 2016). A recent Arabidopsis study showed that DEK proteins physically interact with LHP1 and loss of DEK causes genome-wide H3K27me3 increases, consistent with a role in H3K27me3 homeostasis (Nakamura *et al.*, 2026). In mammalian systems, DEK binds nucleosomes (including *via* H2A/H2B-dependent contacts), compacts polynucleosomes, and enhances PRC2 methyltransferase activity *in vitro* (Alexiadis *et al.*, 2000, Kujirai *et al.*, 2025). The different observations made by the two studies on the H3K27me3 change likely reflect differences between reconstituted *in vitro* and *in vivo* regulatory contexts (Kujirai *et al.*, 2025, Nakamura *et al.*, 2026) (**Figure 8**).

Although DEK proteins have been linked to chromatin compaction and Polycomb-associated states in multiple systems, caution is warranted when extrapolating mechanistic conclusions across kingdoms or experimental contexts. Much of the mechanistic detail for DEK derives from *in vitro* or cultured-cell studies where chromatin composition, cell-cycle state, and accessory factors can differ markedly from those operating *in planta* during ovule maturation. In Arabidopsis, genetic and molecular associations between DEK, LHP1, and Polycomb-linked repression are consistent with a role for DEK in shaping repressive chromatin environments, but they do not, on their own, establish that DEK functions as a conserved structural subunit of LHP1/PRC machinery or that it executes an identical biochemical mechanism to that inferred in mammalian systems {Nakamura, 2026 #2367;Alexiadis, 2000 #2368}. Accordingly, the DEK–LHP1–PRC2 relationship is best framed as a working model in which DEK may modulate Polycomb occupancy, chromatin accessibility, or higher-order chromatin organization in a context-dependent manner, pending direct evidence from ovule-stage biochemical interaction tests and locus-resolved chromatin profiling.

Rather than maintaining central-cell quiescence *via* a single downstream target, FIS–PRC2 is best framed as preventing premature activation of an endosperm-like program before fertilization, with evidence that this repression can involve auxin-related outputs and type I MADS-box regulators including *AGL62*. Unlike the lines with defects in the FIS-PRC2 module, ovules with perturbed cell-cycle gating (*rbr1* and ectopic *CYCD7;1* expression) can show autonomous endosperm-like proliferation but do not initiate seed coat development, indicating that fertilization-linked signals are required for full sporophytic responses. A recent study highlighted that FIS-PRC2 also suppresses embryo-nourishing programs, including auxin biosynthesis genes, before fertilization (Heidemann *et al.*, 2025). Consistent with this framework, ovule maturation is linked to repression of auxin biosynthesis in the central cell and to constrained sugar influx, and integument growth ceases unless fertilization-associated auxin export and/or pollen tube reception signaling is perceived.

In contrast to the central cell, the egg cell exhibits a stricter pre-fertilization developmental block, and the underlying mechanisms remain less well resolved. Available evidence supports at least three partially separable modules: (i) a cell-cycle gate, (ii) Polycomb-linked repression involving MEA–PRC2, and (iii) suppression of embryo-program execution (**Figure 9**).

While RBR1 is normally expressed in central cell before fertilization and acts as one of the key fertilization-dependent gate (at S-phase), it is typically not expressed at the egg cell until fertilization (Ingouff *et al.*, 2007, Simonini *et al.*, 2024). Instead, as more recent Arabidopsis studies suggest egg cell

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is typically arrested at the G2 stage of cell division (Simonini *et al.*, 2024, Simonini, 2025). Typically, the anaphase-promoting complex/cyclosome (APC/C) is known to regulate the activation of the genes associated with mitosis - the subsequent stage in the cell cycle – *via* ubiquitylation of the repressive Cyclin B protein (CycB). An Arabidopsis study presented inferential evidence strongly suggesting presence of such mechanism in mature plant egg cell (**Figure 9**). The study highlighted that its APC/C member, APC4 is expressed during the mega-gametogenesis and embryogenesis processes and perturbation of APC4 leads to the accumulation of CycB protein, aberration in gametogenesis, and defect in embryogenesis (Wang *et al.*, 2012).

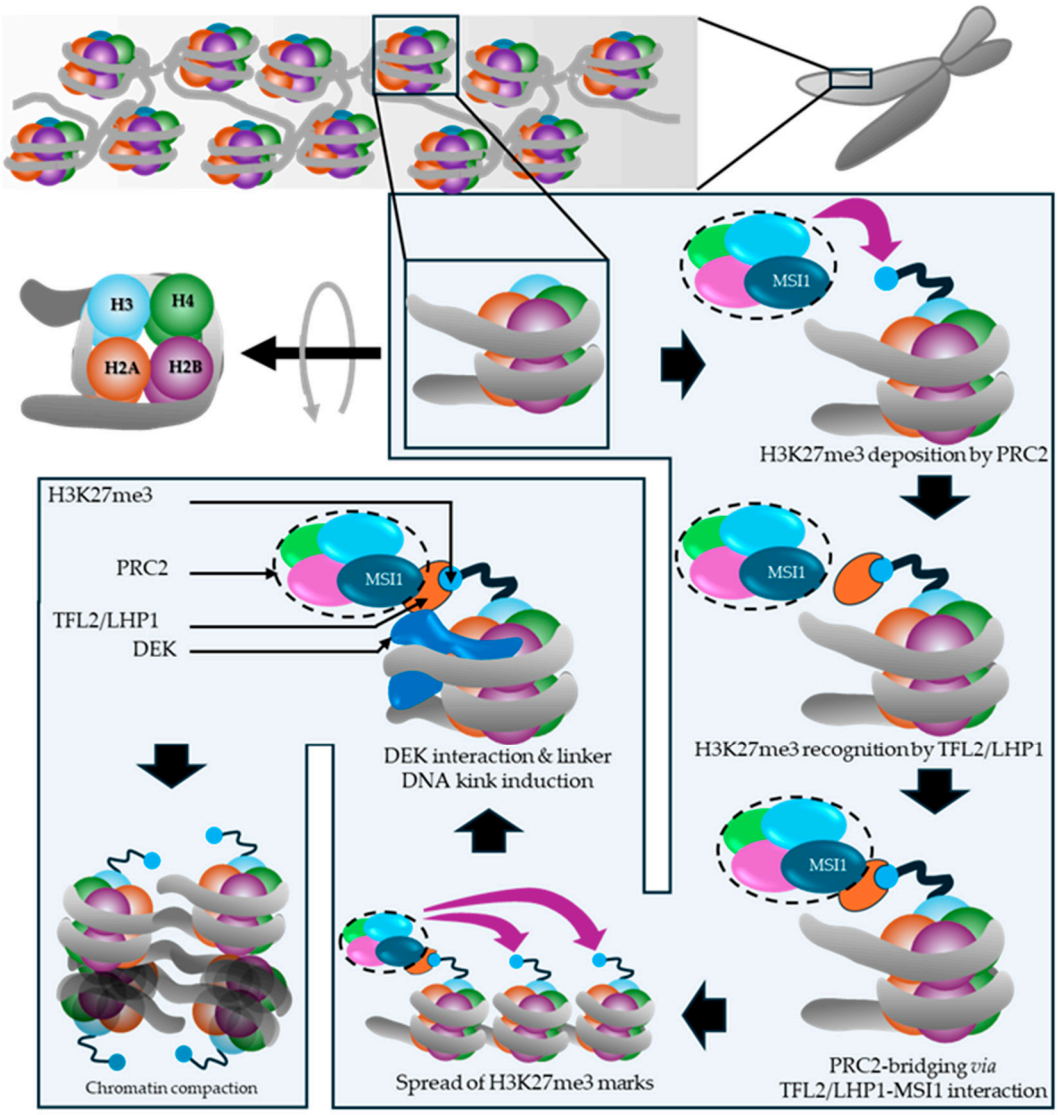

Figure 8. Schematic representation of putative PRC2-mediated H3K27me3 marks deposition and associated mechanism. The histone methylation marks are recognized and bound by TFL2/LHP1 (Turck *et al.*, 2007) and bridges PRC2 to the complex by interacting with MSI1 and facilitating the spread of histone methylation toward the gene's 3'-ends in a context-dependent manner (Veluchamy *et al.*, 2016). TFL2/LHP1 additionally interacts with DEK proteins (Nakamura *et al.*, 2026), which, as suggested by the observations made in the animal reconstituted systems, interacts with H2A/H2B, induces kink in the nucleosome linker DNA and promotes chromatin compaction (Alexiadis *et al.*, 2000, Kujirai *et al.*, 2025).

RBR1 in embryo is attributed for its role in embryo patterning rather than fertilization gating. The MEA-PRC2-mediated controlled regulation of CYCD1.1 by depositing H3K27me3 marks at its locus contributes to the process by affecting the RBR1 phosphorylation rate in the patterning embryo (Figure 9) (Simonini et al., 2021). Additionally, defect in the PRC2 interacting protein, MSI1 brings

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autonomous embryo (n) and endosperm (2n) development as observed in Arabidopsis (Guitton and Berger, 2005). The mutant ovules, however abort at the pre-globular to globular stage (Köhler et al., 2003). It had also been proposed that MSI1 and FIE, subunits of MEA-PRC2 complex, likely contribute to the RBR1-mediated cell cycle gate (Figure 9) (Guitton and Berger, 2005). FIS2 and MEA exhibit detectable exclusive expression at the central cell and endosperm while MSI1 and FIE expression is extended to sporophytic tissues as well . The consequence of MEA defect on embryogenesis however is more severe when it is in egg cell/embryo (hyperproliferative with disorganized development) than when it is in the central cell/endosperm (Simonini et al., 2021).

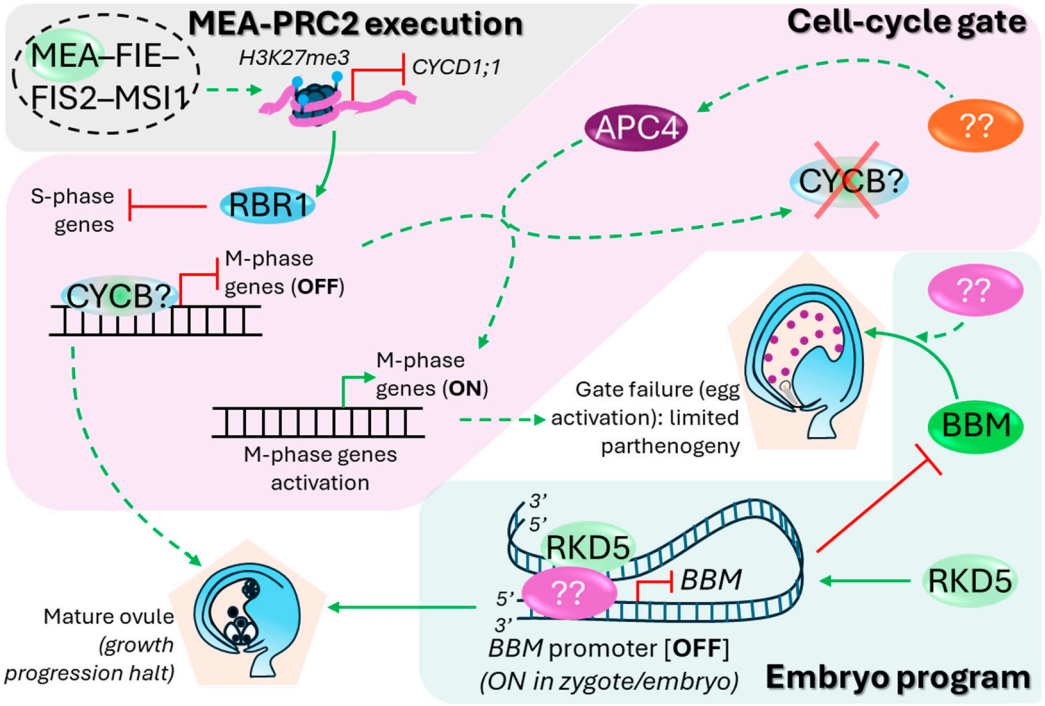

Figure 9. Factors enforcing developmental halt in egg cell. In the mature FM, The Polycomb components MSI1 and FIE are proposed to interface with RBR1-mediated cell-cycle gate during embryo patterning (Guitton and Berger , 2005). Furthermore, the MEA-PRC2 complex deposits histone methylation marks at *CYCD1;1* locus thereby potentially affecting the RBR1 phosphorylation and G1/S-phase transition (Simonini *et al.*, 2021). The key factors associated with the post-maturity developmental halt in the egg cell are those associated with the G2/M-phase transition (Simonini *et al.*, 2021). Likely factors include the Cyclin B member conferring repressive effect to the M-phase progression genes and maternal APC4 at its inactive state awaiting activation via yet unidentified paternal factor (Wang *et al.*, 2012). Additionally, the embryogenesis trigger *BABY BOOM* (*BBM*) is kept OFF in egg cells by egg-cell repression mechanisms including its 3'-bound AtRKD5 (Liu *et al.*, 2024). Green arrows indicate positive regulation; red bars indicate repressive effect; dashed connections indicate proposed/indirect links.

The third module (embryo program) largely depends on a PLETHORA member *BABY BOOM* (*BBM*). In Arabidopsis, an RK domain-containing factor, *RKD5*, contributes to repression of *BBM* in the mature unfertilized egg cell; *rkd5* mutants show a low frequency of parthenogenic seed development, implying that additional repressors constrain *BBM* activity (**Figure 9**) . Native *BBM* expression is silenced in the egg cell before fertilization and is detected in sperm cells, followed by biparental expression shortly after fertilization (Khanday *et al.*, 2019). An aposporous millet accession expresses *BBM*-like genes before fertilization, and promoter-gene combinations from this system can trigger apomixis-like outcomes when introduced into sexual backgrounds (Conner *et al.*, 2015). Ectopic *BBM* expression in the egg cell using heterologous constructs can induce parthenogenesis

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more efficiently, consistent with potential species-specific constraints on embryogenic competence (Chen *et al.*, 2022).

In addition to the independent regulatory networks of the egg and central cells, studies indicate their interaction effect as well. The two component cells have functional plasmodesmatal connections between them facilitating the central cell to egg cell siRNA movement (Schröder *et al.*, 2023). It suggests that the hypomethylation of central cell, mainly by *DEMETER* (and subsequent 24nt-siRNAs biogenesis and their movement to the egg cell is one of the key factors for the hypermethylation of egg cell (Gehring *et al.*, 2009, Bauer and Fischer, 2011, Lafon-Placette and Köhler, 2014).

5. Double Fertilization and Alleviation of the Molecular Block Against Seed Development

Double fertilization coordinates parental-genome union and triggers rapid transcriptional/epigenetic reprogramming. After the release of the sperm cells in the ovule, the independent fertilization of the two female gametes, egg and central cells, occurs almost simultaneously with the event commencing slightly earlier on the former than on the latter. Nevertheless, endosperm proliferation normally precedes post-zygotic proliferation (**Figure 10**) (Lafon-Placette and Köhler, 2014, Doll *et al.*, 2023).

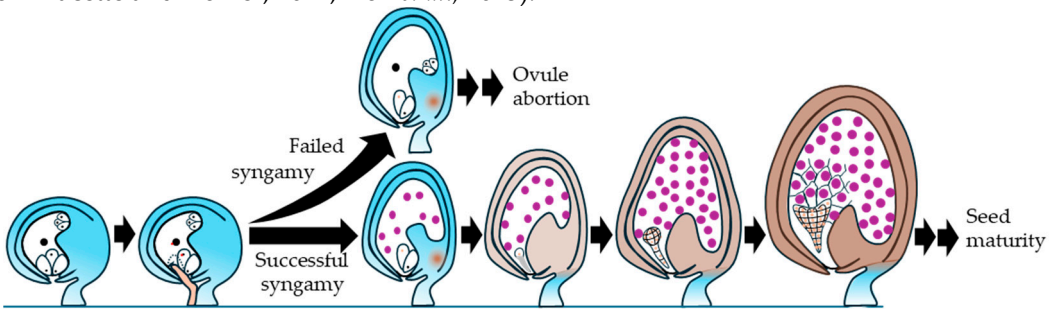

Figure 10. Key developmental stages of early seed development. Mature ovule exhibits burst of the synergid cell receiving the pollen tube. The ovule initiates seed coat initiation (brown region at the chalaza) irrespective of the successful syngamy (Liu *et al.*, 2019). The ovule with failed syngamy enlarges before its subsequent abortion (Kasahara *et al.*, 2016). The successfully fertilized ovule on the other hand, initiates the coenocytic endosperm proliferation. It follows the persistent synergid removal and suspensor-supported embryo development until the embryonic heart stage, when the endosperm cellularization initiates in a wave fashion starting at the micropylar region.

Studies show that erasure of old epigenetic marks (parental) and incorporation of fresh ones (gametophytic) occurs in two successive stages of gametophyte development. Strong evidence on it comes from a Merchantia study, in which the authors observed that meiosis in the species coincides with the overall epigenetic marks erasure, while the subsequent sporulation step deposits fresh epigenetic marks by gamete maturation (Montgomery and Berger, 2023). Such instance also explains how it is possible for each of the FM component cells to exhibit unique epigenetic marks as observed in Arabidopsis (Migicovsky and Kovalchuk, 2012).

Table 1. Developmental timeline from pollen tube reception to early heart stage (Arabidopsis).

Stage / window	Endosperm	Embryo	Maternal integument / testa
Pollen tube reception / pre-syngamy (POEM)	Central cell/endosperm-like responses can be triggered by pollen tube	No zygote formed.	PTC can initiate chalazal seed-coat wall formation; auxin can phenocopy aspects of

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	content release without gamete fusion (Kasahara *et al.*, 2016, Honma *et al.*, 2020).		initiation (Figueiredo *et al.*, 2016, Liu *et al.*, 2019).
Double fertilization	Paternal miR159 helps relieve maternal barriers by targeting *GAMYB* members (*MYB33*/*MYB65*) (Zhao *et al.*, 2018b).	Fertilization initiates embryogenesis and relieves pre-fertilization arrest programs (Adhikari *et al.*, 2020b).	Maternal tissues become responsive to fertilization-derived signals that promote seed coat initiation (Figueiredo *et al.*, 2016).
Early coenocytic endosperm (pre-globular)	*AGL62* supports/maintains the coenocytic program and prevents premature cellularization; an imprinted *PHE1* network supports proliferation and delays cellularization (Hehenberger *et al.*, 2012, Batista *et al.*, 2019).	Early patterning is established as zygotic programs become active (Nodine and Bartel, 2012).	Endosperm expansion generates mechanical load on integuments (turgor) (Beauzamy *et al.*, 2016).
Late coenocytic endosperm / late globular	Endosperm turgor approaches a maximum near the end of the coenocytic stage and then declines before cellularization (Beauzamy *et al.*, 2016).	Embryo reaches globular stage prior to heart-stage organogenesis (Figure 10).	Chalazal seed coat differentiation includes activation of *BAN* and PA deposition (Debeaujon *et al.*, 2003).
Early heart stage (cellularization onset)	Cellularization initiates micropylar-to-chalazal; ABA/*ABI5* represses the *SHB1–MIN3–IKU2* proliferation module (Hehenberger *et al.*, 2012, Cheng *et al.*, 2014, Li *et al.*, 2022).	Heart-stage morphogenesis proceeds alongside increasing zygotic genome activity (Zhao *et al.*, 2019).	As cellularization proceeds, mechanosensitive testa responses are proposed to constrain growth via GA deactivation (*ELA1*/2) and wall stiffening (Creff *et al.*, 2015, Creff *et al.*, 2023).

Stages are presented qualitatively; exact timing varies with accession and growth conditions.

5.1. Pre-Syngamy Changes

The ovules exhibit distinct feature change upon pollen tube reception even in absence of subsequent gamete fusion. As the observations show, the central cell response can simply be triggered by pollen tube content release inside the ovule without subsequent gamete fusion

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(Kasahara *et al.*, 2016, Honma *et al.*, 2020). In rice, such phenomenon facilitates unperturbed sugar influx, which however, cannot be converted into starch - as would normally occur in the fertilized ovule (seed) - thereby developing the sugary grain (Honma *et al.*, 2020). In Arabidopsis, a typical dicot, the ovule exhibits a typical 'pregnant' characteristic – POEM-like ovule enlargement morphology – the feature termed as pollen tube-dependent ovule enlargement morphology (POEM) (**Figure 10**) (Kasahara *et al.*, 2016). Additionally, some studies show that the release of pollen tube content (PTC) alone is sufficient to trigger the cell wall initiation at the chalazal end (Liu *et al.*, 2019). However, earlier study showed that the post-fertilization auxin biosynthesis in the central cell is essential for the cell wall initiation process and demonstrated that exogenous auxin applied ovules phenocopy the trait (Figueiredo *et al.*, 2016). Since the synergid cells are known to constitute auxin albeit at a relatively low level (Sun *et al.*, 2021), whether the seed coat initiation observed by (Liu *et al.*, 2019) is the outcome of the burst of the pollen tube receiving synergid or the PTC itself remains to be determined.

5.2. The Coenocyte

Paternal miR159 copy plays a key role in clearance of maternal barrier of endosperm proliferation. Among others, its targets include GAMYB members, *MYB33* and *MYB65*, which are highly expressed in the pre-fertilized central cell in Arabidopsis (Zhao *et al.*, 2018b). The RBR1-mediated cell-cycle arrest is released upon gamete fusion by putative *RBR1* phosphorylation (and degradation) mediated by the sperm cell derived *CYCD7;1* and CDKA;1 in the central cell/endosperm thereby easing the cell-cycle gate (Figure 7) (Sabelli *et al.*, 2013, Simonini *et al.*, 2024). Fertilization also brings changes to the MET1/DME-mediated methylation status. While *MET1* is expressed at both pre- and post-fertilized endosperms, expression of *DME* is significantly reduced after fertilization and contributes to the suppression of paternal *MEA* allele (Choi *et al.*, 2002, Xiao *et al.*, 2003, Gehring *et al.*, 2006). Arabidopsis *DME* reportedly harbors repressive and enhancing *cis*-elements at its post-TSS region likely contributing to its fertilization event-regulated expression behavior (**Figure 11**) (Park *et al.*, 2017). The maternal MEA-PRC2, still active in the post-fertilized endosperm, contributes to the H3K27me3 marks deposition and silencing of the paternal *MEA* and *RBR1* (**Figure 7**) (Gehring *et al.*, 2006, Johnston *et al.*, 2008).

With the diminished level of functional FIS-PRC2 in the post-fertilized endosperm, expression of *AGL62* increases (Hehenberger *et al.*, 2012). AGL62 supports the coenocytic endosperm program and influences seed growth in a dosage-sensitive manner, with genetic evidence placing it within an early endosperm transcriptional module that includes PHE1 and related type I MADS factors (Kradolfer *et al.*, 2013). In strawberry, AGL62 co-acts with AGL80 in support of early endosperm proliferation programs (Guo *et al.*, 2022).

A mechanistic model for parental asymmetry at PHE1 target loci proposes that PHE1 binds CArG-like motifs and activates AGL62 and other type I MADS-box genes, while imprinting outcomes are shaped by allele-specific chromatin and cis-element configurations. In this model, many paternally expressed PHE1 targets are associated with RC/Helitron transposable-element-derived sequences near the PHE1-binding region and lack gene-body H3K27me3 on the paternal allele, whereas maternally expressed PHE1 targets more frequently exhibit non-CG methylation at the PHE1-binding site on the maternal allele (Batista *et al.*, 2019).

The coenocytic endosperm proliferation is mediated by facilitating the expression of the paternally imprinted crucial auxin biosynthetic genes *YUC10* and *TAR1 via* repression of their repressors, the homeodomain-leucine zipper transcription factors - the *ATHB* members (**Figure 11**) (Figueiredo *et al.*, 2015, Figueiredo *et al.*, 2016, Guo *et al.*, 2022). The persistent synergid is eliminated by merging with the developing endosperm by its second division which is pivotal for blocking polytubey (Maruyama *et al.*, 2015). Additionally, F-actin plays a crucial role in the nuclear positioning within the endosperm at its coenocytic phase. The process is dependent on the microtubules, which prepares the foundation for the F-actin anchorage to the divided nuclei. Perturbation of either

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microtubule or F-actin polymerization negatively affects nuclear division and positioning, reduces coenocytic cavity size, and compromises seed development and final seed size (Ali *et al.*, 2023).

Arabidopsis *fis2* mutant ovules show ectopic expression of *YUC10* and *TAR1* together with *AGL62* and trigger autonomous endosperm proliferation, but do not initiate seed coat development (Roszak and Köhler, 2011, Pankaj *et al.*, 2024a). In addition to failed cellularization, the mutant exhibits increased hexose levels (Hehenberger *et al.*, 2012). *AGL62* defects perturb auxin efflux from the endosperm, consistent with a role for endosperm-derived auxin in coordinating seed coat initiation and growth (Figueiredo *et al.*, 2016).

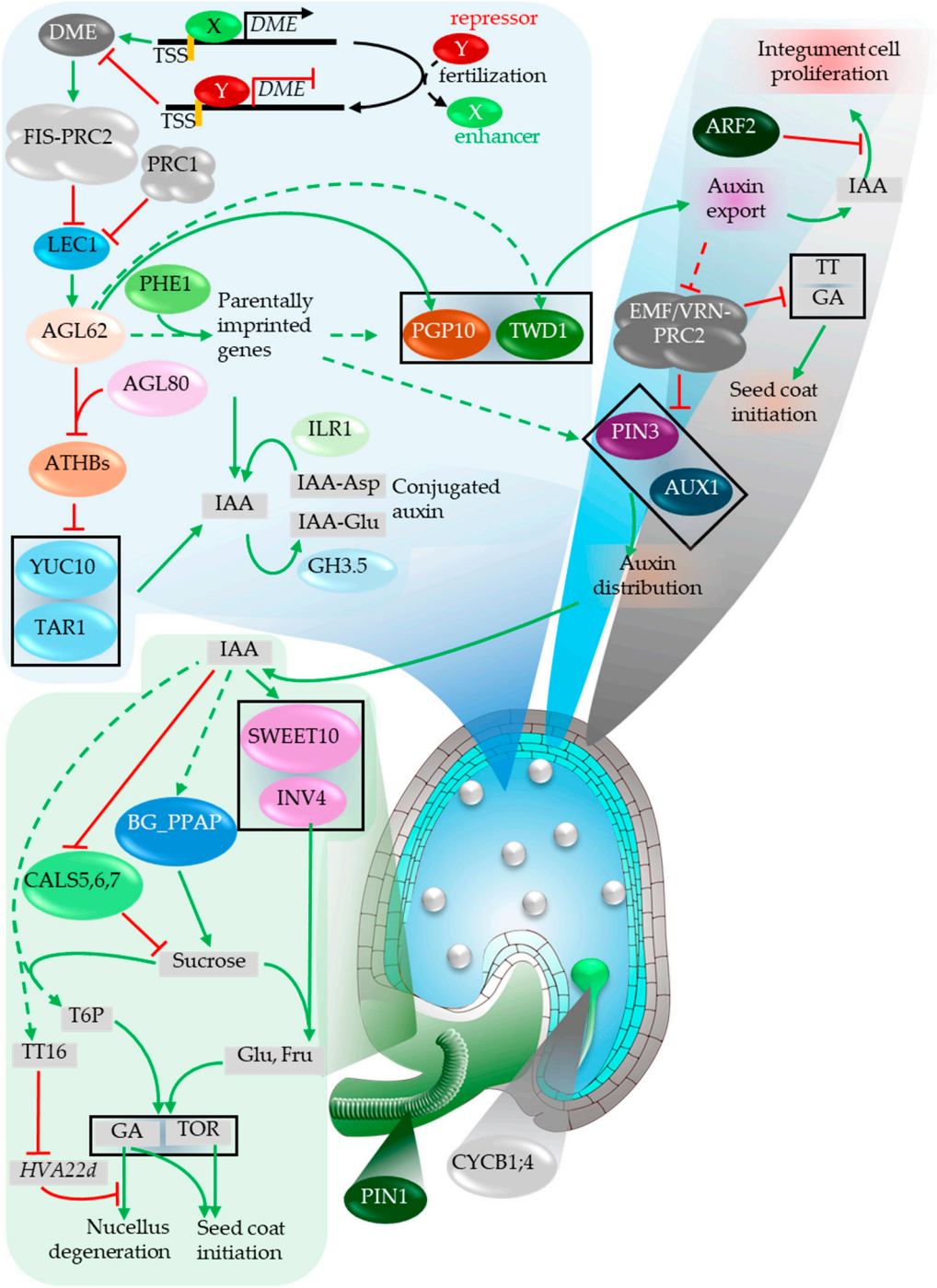

Figure 11. Key factors associated with the post-fertilization seed development. Fertilization reduces DME expression via post-TSS regulatory elements (putative enhancer X and repressor Y are shown) (Park *et al.*, 2017), altering MET1/DME-associated methylation status and contributing to paternal MEA silencing (Choi *et al.*, 2002; Xiao *et al.*, 2003). Reduced FIS-PRC2 constraint permits increased AGL62 (Hehenberger *et al.*, 2012) (positively

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supported by LEC1 (Lee *et al.*, 2003, Song *et al.*, 2025)) which, together with PHE1/AGL80, promotes paternally imprinted programs including auxin biosynthetic genes *YUC10* and *TAR1 via* repression of ATHB factors (Roszak and Köhler, 2011, Kradolfer *et al.*, 2013, Batista *et al.*, 2019, Guo *et al.*, 2022, Pankaj *et al.*, 2024). Auxin homeostasis includes GH3.5-mediated conjugation (prevalent in unfertilized ovule) and ILR1-linked deconjugation (in fertilized ovule) (Aloni *et al.*, 2006; Rampey *et al.*, 2004; Larsson *et al.*, 2017; Vanneste *et al.*, 2025). Endosperm auxin export is proposed *via* PGP10/TWD1 (Wu *et al.*, 2010, Figueiredo *et al.*, 2016, Tsering *et al.*, 2025) and integument distribution *via* PIN3/AUX1 (Larsson *et al.*, 2017, Liu *et al.*, 2023), antagonizing sporophytic EMF/VRN-PRC2 to enable GA and TT pathway-associated genes thereby contributing to the seed coat initiation and seed development (Roszak and Köhler, 2011, Figueiredo and Köhler, 2018). The TT-pathway associated TT16 additionally represses the suppressor of GA-mediated PCD thereby facilitating the nucellus degeneration process (Xu *et al.*, 2016). The integument-exported auxin enhances the sucrose transport and conversion-associated genes, *SWEET10* and *INV4* respectively, in addition to the downregulation and upregulation of the callose biosynthesis (*CALS5/6/7*) and callose degradation (*BG_PPAP*) associated genes respectively at the chalaza. Recent reports propose that endosperm-derived auxin enhances chalazal sucrose unloading and T6P accumulation, potentially linking carbohydrate status to TOR activation and downstream GA-associated responses during early seed development; these connections remain provisional and warrant genetic/temporal validation (Liu *et al.*, 2025a, Xu *et al.*, 2025). Green arrows, activation/transport; red bars, repression; dashed lines, putative/indirect links.

The endosperm-synthesized auxin is quickly exported to the integument potentially *via* the *AGL62*-regulated ABCB transporter, PGP10 in the fertilized ovule. The process may additionally be facilitated by the genes such as *TWD1* by contributing to the membrane localization of PGP10 (Wu *et al.*, 2010, Figueiredo *et al.*, 2016, Tsering *et al.*, 2025). The transported auxin is distributed among the inner and outer integument layers potentially *via* PIN3 and AUX1 respectively (**Figure 11**) (Larsson *et al.*, 2017, Liu *et al.*, 2023).

5.3. Seed Coat Development

This fertilization derived endosperm signal contributes to the suppression of sporophytic PRC2 (EMF/VRN-PRC2). As a mutation study showed defect in either or both of integument expressed PRC2 components *VRN2*, encoding a PRC2 Su(z)12 subunit, and *SWN*, a *MEA* homolog, leads to an increase in the autonomous seed development and seed coat formation (Roszak and Köhler, 2011). The process is mediated by the de-repression of the otherwise EMF/VRN-PRC2-repressed gibberellin (GA) biosynthesis and *TRANSPARENT TESTA* (*TT*) pathway-associated genes (Figueiredo and Köhler, 2018). It is notable to mention that the PRC2-repression-led activation of *TT16* and *GOA* in the endothelium (inner layer of the seed coat) and nucellus redundantly promote nucellus degeneration via repression of *HVA22d*, a suppressor of GA-mediated PCD and autophagy, while the endosperm proliferates. *TT16* is also essential for subsequent seed coat development and serves as a key factor linking the (central cell) fertilization signal to maternal response (**Figure 11**) (Xu *et al.*, 2016).

The unfertilized ovules also constitute auxin but at its inactive (conjugated) form (Aloni *et al.*, 2006). Auxin conjugation has been suggested to be facilitated by GH3.5 based on its synthesis in the unfertilized (at the micropylar end of central cell) and fertilized ovules (at the apical end of funiculus) (Larsson *et al.*, 2017). Post-fertilized ovules exhibit synthesis of an auxin deconjugate, a basic helix-loop-helix leucine zipper protein, ILR1 at its micropylar region (Rampey *et al.*, 2004) suggesting its potential role in activating/freeing the conjugated IAA (Vanneste *et al.*, 2025). *ARF2*, on the other hand, negatively regulates seed size in response to auxin by conferring repressive effect in the integument cell proliferation and organ growth (**Figure 11**) (Schruff *et al.*, 2006).

Endosperm-to-integument signaling is proposed to intersect with carbohydrate gating at the chalaza. Auxin has been suggested to enhance starch accumulation and maternal sucrose responsiveness by promoting SWEET10 and INV4 expression and by influencing callose-gate dynamics at the chalazal phloem end, which could increase sucrose flux and elevate T6P. The sucrose

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transport is additionally facilitated by the downregulation of callose biosynthesis-associated genes, *CALS5/6/7* (Xu *et al.*, 2025). Fertilization event is known to trigger the active degradation of the phloem end callose gate at the chalaza (Kasahara, 2025, Liu *et al.*, 2025a, Nakajima and Kasahara, 2025). The carbohydrate changes in the developing seed may connect to TOR signaling and GA-linked outputs that accompany fertilization responses (seed coat initiation and seed development); however, the TOR-centered branch should be framed as emerging rather than fully established, pending targeted genetic tests and stage-resolved quantification (**Figure 11**).

Studies have shown that very early stage (pre-globular to globular) seeds exhibit high level of GA-biosynthesis and GA-responsive genes in its micropylar region including the embryo tissues such as suspensor and embryo neck (Solfanelli *et al.*, 2005, Ziegler *et al.*, 2019). When observed in a broader developmental time-frame (from globular stage to maturity), in Arabidopsis, a significantly higher expression of GA biosynthesis genes, *GA3ox3* and *GA3ox4*, in addition to those associated with cytokinin and abscisic acid biosynthesis have been detected in the chalazal endosperm (CZE) region (Belmonte *et al.*, 2013). The seed coat (proanthocyanidin (PA) deposition) exclusively initiates at the chalazal integument region adjoining the putative CZE, which is consistent even in the unfertilized ovule when GA or auxin are exogenously applied (Figueiredo *et al.*, 2016). An Arabidopsis study showed that expression of a key gene involved in seed coat PA biosynthesis, *BANYULS* (*BAN*) (**Figure 12**), peaks at late globular to early heart stage (Debeaujon *et al.*, 2003), while bioactive GA peaks at the bent cotyledon stage with a gradual increase in DELLA proteins in subsequent developmental stages peaking at the maturity (Hu *et al.*, 2018).

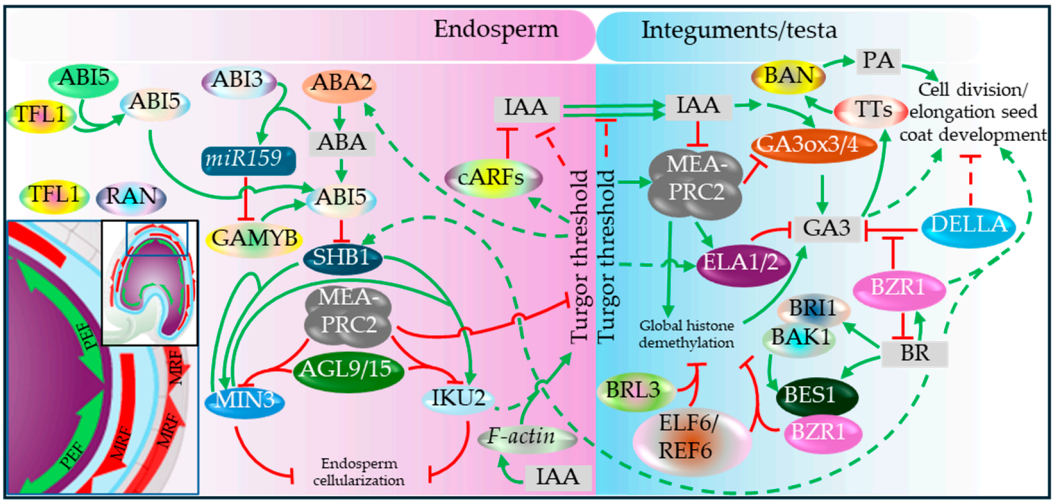

Figure 12. Key factors associated with the endosperm cellularization and seed coat development. During the coenocytic phase, the endosperm generates a paternally biased expansion force (PEF) that promotes cavity growth, while maternal programs impose a maternal restrictive force (MRF) to constrain final seed size (inset). Rising endosperm turgor pressure is proposed to act as a mechanical cue; once a threshold is reached, maternal pathways reduce turgor and trigger cellularization (Weinhofer *et al.*, 2010; Beauzamy *et al.*, 2016; Butel *et al.*, 2024). ABA signaling from the chalazal domain integrates into this transition: TFL1 transport (via RAN) stabilizes ABI5, which represses the pro-proliferative SHB1–MIN3–IKU2 module (Zhang *et al.*, 2020; Cheng *et al.*, 2014; Kang *et al.*, 2013). In the testa, chalazal PA accumulation (via BAN) and GA/BR modules are shown as key outputs and regulators of cell division/elongation and coat differentiation (Debeaujon *et al.*, 2003; Figueiredo *et al.*, 2016; Hu *et al.*, 2018). Mechanosensitive ELA1/2-mediated GA deactivation promotes wall stiffening and restricts late growth, while BR signaling (BRI1/BAK1 → BES1/BZR1) can support seed coat activation *via* chromatin remodeling (Creff *et al.*, 2015; Creff *et al.*, 2023; Jiang *et al.*, 2013; Pankaj *et al.*, 2024b). Green arrows, activation; red bars, repression; dashed lines, indirect/putative links.

The coenocytic phase and the cavity volume of the coenocytic endosperm is often regarded as positive attributes behind the seed size. The internal turgor pressure of the endosperm can reach up

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to 1.5 MPa near the end of the coenocytic stage in Arabidopsis before dropping down to about 0.6 MPa and subsequent cellularization (Beauzamy *et al.*, 2016). The high turgor pressure confers positive effect on seed size while also contributing to the rigidity (wall stiffening) of the testa (Creff *et al.*, 2023).

5.4. Seed Size Regulation (Endosperm-Dependent)

Endosperm turgor pressure is a major mechanical determinant of seed growth, reflecting both promotion of expansion during the coenocytic phase and restriction as maternal tissues stiffen (Beauzamy *et al.*, 2016, Creff *et al.*, 2023). In Arabidopsis, paternally imprinted genes promote auxin biosynthesis and endosperm proliferation, increasing turgor, whereas maternally imprinted PRC2 members contribute to a later reduction (Figueiredo *et al.*, 2015, Beauzamy *et al.*, 2016). Elevated turgor reorients and bundles microtubules in outer integument L2 cells (oi L2) and is associated with stiffening of the adaxial inner wall (wall 3), a response linked to the wall-localized mechanosensitive CYP module *ELA1* and GA deactivation (**Figure 12** and **Figure 13**). Seed growth slows once cellularization begins (early heart stage), and *ELA1* expression peaks around torpedo stage, consistent with subsequent restriction of size expansion by wall-3 stiffening (Creff *et al.*, 2015, Creff *et al.*, 2023). Additionally, the wall *3* of *iku2* mutant exhibits relatively higher instances of demethylesterified homogalacturonans and higher stiffness as compared to col-0 suggesting the higher level of Ca^{2+}-mediated cell wall reinforcement by cross-linking demethylesterified homogalacturonans at the testa cell wall of the mutant ovule (Hamann, 2012, Creff *et al.*, 2023).

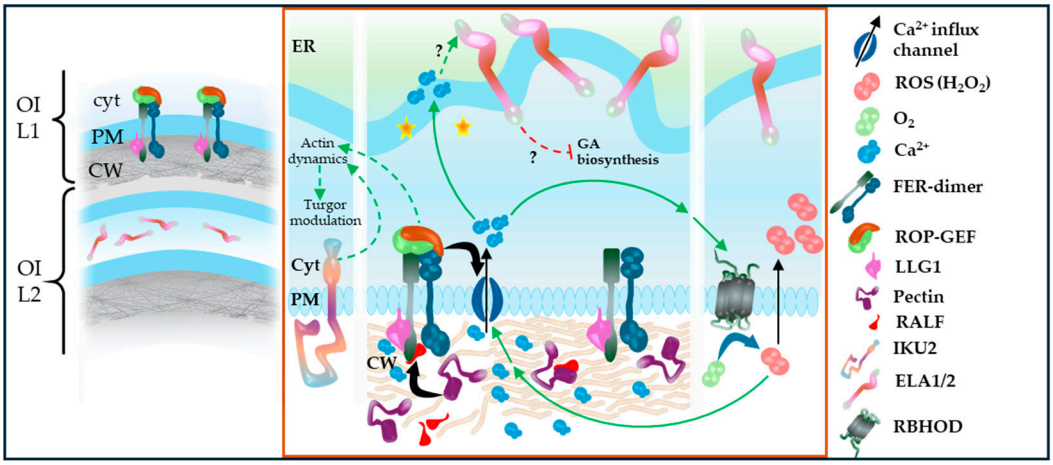

Figure 13. Schematic representation of putative mechanosensing in the fertilized ovule. The very initial seed enlargement is largely contributed by the outer integument, in which FER contributes to the process (Yu *et al.*, 2014). The expanding volume (cavity) of the coenocytic endosperm is perceived by the cell wall property change at the inner wall of the outer integument (OI) L1 layer. The FER-complex has been proposed to interact with the cell-wall freed RALF peptide and trigger actin dynamics, Ca2+ influx as well as RBOH-mediated ROS accumulation thereby contributing to the integument elongation and division. The actin dynamics is proposed to be regulated by IKU2 as well. The stiffening of the inner cell wall of the inner integument L2 layer and seed expansion limit is mediated by the ER-localized ELA1/2 via the deactivation of bioactive GA3 (Creff *et al.*, 2015, Creff *et al.*, 2023), which likely plays role during the final stage of the seed maturation.

FER is additionally known to mediate ROS production and TIR1/AFB1 oxidation thereby contributing to the auxin-signaling-dependent growth attributes (Duan *et al.*, 2010, Lu *et al.*, 2024). Arabidopsis seeds exhibit spike in FER accumulation in the abaxial layer (L1) of the outer integument (oi)/testa at globular stage (50 hours after pollination) and *FER* defect leads to an increase in seed size. Additionally, overexpression of *GEF1*, potential FER-interacting partner, leads to a significant decrease in the seed size suggesting its potential role in GEF-ROP/ARAC-facilitated mechanosensing (**Figure 13**) and seed size regulation. Furthermore, in both cases, visibly significant difference of the transgenic/mutant line to WT was observed at the globular stage, indicating that the seed size

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regulation up to the globular stage is largely controlled by FER-regulated oi L1 (Yu *et al.*, 2014). Later study by Creff *et al.* (2015) showed that this oi L1 layer cells exhibit less mechano-responsiveness to the endosperm turgor as compared to the oi L2 layer cells at later developmental stages.

FER is known to contribute to the elongation of hypocotyl by integrating brassinosteroid (BR) signaling (Deslauriers and Larsen, 2010). Furthermore, BR-signaling facilitates GA-mediated testa cell elongation in the developing seeds. BZR1, a BR signaling enzyme affecting the BR biosynthetic pathway in a negative feedback loop, interacts with a DELLA member GAI, which renders both of the interacting partners inactive (Figure 12) (Gallego-Bartolomé *et al.*, 2012) thereby facilitating the GA-responsive gene expression and cell elongation. Furthermore, BR acts as a non-cell autonomous seed coat-to-endosperm signal and contributes to the positive regulation of the endosperm proliferation associated genes such as *SHB1*, *IKU2*, and *MIN3* (Jiang *et al.*, 2013, Lima *et al.*, 2024, Pankaj *et al.*, 2024a, Pankaj *et al.*, 2024b). The active synthesis and signaling of BR in the maternal tissue (integuments/testa) has been suggested to potentiate the tissue to sustain endosperm proliferation (**Figure 12**) (Lima *et al.*, 2024).

The BR-effect in seed size regulation is likely due to its role in counteracting the PRC2-mediated repressive effect in the tissue. In the pre-fertilized ovules, seed coat development is actively repressed by the PRC2-mediated H3K27me3 marks deposition in the integuments. As observed in Arabidopsis, BR function couples with the demethylation activity of the seed coat localized JUMONJI-type (JMJ) histone demethylases (ELF6 and REF6) and BR effectors (BES1 and BZR1) recruit the JMJ proteins to the target loci for demethylation (Pankaj *et al.*, 2024b). The study postulated that BRI1, the BR-signaling associated protein involved in JMJs recruitment can facilitate the histone demethylation in a BR-independent manner unlike which, the process mediated by a BRI1 ortholog, BRL3 is BR-dependent (**Figure 12**). Studies indicate that the BR-mediated seed size regulation is likely mediated by microtubule reorganization (Abe *et al.*, 2010, Jiang *et al.*, 2013, Delesalle *et al.*, 2025)

Unlike auxin, GA, and BR, ABA is discussed here as a potentially growth-restrictive input that may limit endosperm cavity expansion. Non-seed-associated studies indicate that the GAMYB members contribute to ABA signaling by promoting the accumulation of *ABI5* transcript, a key ABA-signaling associated gene (Guo *et al.*, 2021, Wyrzykowska *et al.*, 2022). Earlier study additionally showed that ABA itself promotes miR159 accumulation in ABI3-dependent manner leading to GAMYB silencing (Figure 12) (Reyes and Chua, 2007). As observed in a maize study, its zma-miR159-target GAMYB members *ZmMYB74* and *ZmMYB139*, negatively affect seed size by enhancing the expression of key downstream genes *ABI5*, mir156, and CDK. Furthermore, knock-out of the GAMYB members or overexpression miR159 enhanced the grain size (Wang *et al.*, 2023).

5.5. Endosperm Cellularization

Endosperm cellularization typically commences after 10th consecutive nuclear division in Arabidopsis endosperm (Ali *et al.*, 2023). The PRC2-mediated drop in endosperm turgor pressure contributes to its proliferation halt followed by subsequent cellularization (Weinhofer *et al.*, 2010) *via* their putative downstream clustered ARFs (cARFs) (**Figure 12**) (Moreno-Romero *et al.*, 2016, Butel *et al.*, 2024).

The endosperm cellularization process is positively correlated with the activation of the genes associated with the ABA biosynthesis and signaling (Cheng *et al.*, 2014). However, its potential role in the endosperm turgor pressure reduction has remained unexplored. Nevertheless, a parallel can be made with the operation of stomata, which is typically regulated by the guard cell turgor pressure. ABA reportedly increases the cytosolic Ca^{2+} leading to activation of slow anion (S-type) channel and downregulation of K^+ influx channel in the guard cells thereby reducing its turgor and stomatal closure (Murata *et al.*, 2001).

Typically, cellularization initiates at the micropylar region and progresses towards the chalaza in a wave fashion (**Figure 10**) (Hehenberger *et al.*, 2012, Li *et al.*, 2022). The process also incorporates the cues from the chalazal region bridged by ABA signaling. In brief, *TFL1* - a PEBP member exclusively synthesized at the chalazal endosperm - is normally transported into the peripheral

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endosperm mediated by the small soluble GTP-binding RAN proteins, where it contributes to ABA signaling by stabilizing *ABI5* (Zhang *et al.*, 2020). *ABI5* suppresses the accumulation of a key gene involved in endosperm proliferation, *SHB1*, by binding to the ABRE element in its promoter in ABA-dependent manner (Cheng *et al.*, 2014). In alternate case, *SHB1* typically binds to the W-box within the promoters of a WRKY transcription factor *MIN3* and LRR protein family member *IKU2* facilitated by *MIN3* itself thereby promoting endosperm proliferation and the seed cavity volume increase (Kang *et al.*, 2013). Defect in ABA biosynthesis and signaling leads to de-repression of the SHB1-MIN3-IKU2 pathway leading to an increase in seed size (**Figure 12**) (Cheng *et al.*, 2014).

The SHB1-MIN3-IKU2 pathway is also negatively regulated by FIS-PRC2 in AGL9/15 dependent manner (**Figure 12**) in such a way that AGL9/15 binds to the CArG motifs located in the *IKU* and *MIN3* promoter and contribute to recruiting FIS-PRC2 by interacting with MEA for the H3K27me3 marks deposition. Defect in *AGL9* and *AGL15* leads to increased seed cavity and seed mass (Zhang *et al.*, 2024). Studies show that AGL15 is activated by auxin and it perturbs auxin signaling while contributing to somatic embryogenesis (Zheng *et al.*, 2016). Since defect in either of *AGL9*/*AGL15* and *TFL1* leads to increase in seed cavity and mass, whether both of them act on the same pathway during endosperm cellularization yet remains unclear.

5.6. Embryo Development

While the endosperm proliferation commences almost immediately after karyogamy, zygotic division initiates several hours after fertilization (~10 to 20+ hrs depending on species). Immediately after the egg cell-to-sperm cell fusion, the zygote contracts briefly before returning to its initial state as suggested by an *in vitro* observation made in maize (Antoine *et al.*, 2000). Some isolated-gamete or *in vitro* fertilization assays employ non-physiological medium conditions to enable handling and fusion (Faure *et al.*, 1994, Kranz and Lörz, 1994). In intact/semi-in vivo double fertilization, fertilization triggers egg-cell Ca^{2+} transients/spikes, supporting Ca^{2+} as an activation signal under near-physiological tissue context (**Figure 14**) (Antoine *et al.*, 2000, Denninger *et al.*, 2014, Hamamura *et al.*, 2014). The influx contributes to the cell wall formation around the freshly formed zygote (Denninger *et al.*, 2014). The fertilized egg cell secretes, the aspartic endopeptidases ECS1, and ECS2, upon its successful fertilization as observed in Arabidopsis. The peptides actively contribute to blocking polytubey primarily by destroying the pollen tube attraction-associated LURE1 peptides (**Figure 14**) (Yu *et al.*, 2021).

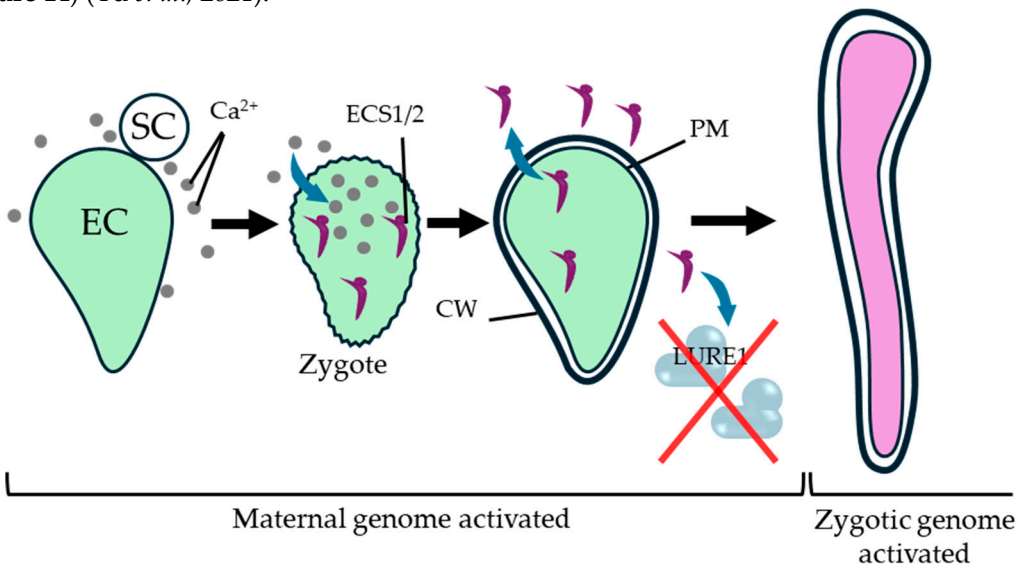

Figure 14. Early stages of sexual embryo development in angiosperm. Immediately after the egg cell (EC) and sperm cell (SC) fusion, the fresh zygotic cell contracts briefly before returning to the initial state (Antoine et al., 2000). Fertilization triggers Ca^{2+} influx (Denninger *et al.*, 2014, Hamamura *et al.*, 2014) and ECS1/2 proteins secretion. ECS1/2 actively contributing to the degradation of LURE1 peptide thereby hindering potential

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polytubey cases (Yu *et al.*, 2021). The zygote exhibits the expression of maternal genome up until it reaches to the elongated zygotic stage when it shows the expression of mixed genome of the zygote (Zhao *et al.*, 2019).

As mentioned earlier, RBR1 is inactive in mature egg cell. Nevertheless, RBR1-regulated cell cycle arrest in the tissue has been put forth by some studies based on the presence of G1/S phase-associated replication factors TMPK1 and DPB2, putative E2F-regulated outputs of the RBR1/E2F/DP gate, after fertilization (Ronceret and Devic, 2008). However, initiation of the first zygotic division requires APC/C-dependent turnover of mitotic cyclin activity, indicating that proteolysis-driven cell-cycle licensing is essential during early zygote activation (**Figure 9**) (Wang *et al.*, 2012, Guo *et al.*, 2016). Hence, while studies indicate that APC/C likely regulates the fertilization-dependent gating and RBR1/E2F regulates the embryo patterning, additional evidence is required to address the ongoing disagreement about whether the Arabidopsis egg cell is arrested in a pre-replicative (G0/G1-like) state or in G2 (Simonini, 2025).

Early embryogenesis involves a maternal-to-zygotic transition (MZT), during which maternally supplied transcripts are progressively removed and de novo zygotic transcription increases. In Arabidopsis, transcriptome dynamics support a transition from an egg/early-zygote program to a globular-stage program, followed by stronger zygotic genome activation (ZGA) coincident with elongation and patterning (**Figure 14**) (Kao and Nodine, 2019, Zhao *et al.*, 2019, Brantley and Di Talia, 2024). Although transcripts from both parental alleles are detected early, key specification events are largely governed by zygotic gene products (Nodine and Bartel, 2012). The zygotic transcription is initiated in both fertilized egg and central cells almost simultaneously, even though endosperm proliferation precedes embryonic divisions (Kao and Nodine, 2019).

The paternal and maternal regulatory inputs establish zygote polarity and instruct the first asymmetric division - the earliest visible mark of embryo patterning. One of the paternally derived triggers is an RLCK-II family member, *SHORT SUSPENSOR* (*SSP*), mRNA of which is synthesized in the pollen but translation occurs in the zygote after fertilization. SSP subsequently potentiates a zygote MAPK cascade comprising the MAPKKK YODA (YDA), the MAPKKs MKK4/MKK5, and the MAPKs MPK3/MPK6, which together promote zygote elongation and asymmetric division {Bayer, 2009 #2421;Ueda, 2017 #2423;Lukowitz, 2004 #2444;Wang, 2007 #2435;Zhang, 2017 #2434}. Normal embryo development requires functional maternal copy of at least *MPK6* during the process (Zhang *et al.*, 2017). An LRR-RLK member, ZAR1, after the activation of its kinase activity *via* its direct interaction with CaM1 and AGB1, is also required for *WOX8* expression and asymmetric division of the zygote (Yu *et al.*, 2016). The heterotrimeric G-protein β subunit AGB1 also interacts with YDA–MKK4/5–MPK3/6 components and functions as the scaffold for the complex {Yuan, 2017 #2470}. Additionally, the *WOX8* activation is also dependent on the endosperm-derived maternally imprinted factor, ESF1 (**Figure 15**) (Costa *et al.*, 2014). WOX8 activates the PLETHORA member *BBM* and other genes associated with embryogenesis (Zhao *et al.*, 2024). *BBM* and its close homolog, PLT2, are known to induce parthenogenesis independently upon their ectopic expression (**Figure 15**) (Chen *et al.*, 2022).

The activated YDA-MAPK phosphorylates WRKY2, which is required for repolarization of the zygote. It directly activates WOX8 transcription after fertilization, thereby linking fertilization to the earliest embryo axis programs (Ueda *et al.*, 2017). These paternal (SSP-to-YDA) and maternal (HDG11/12-to-WOX8) pathways are integrated into a framework in which independent parental cues converge to ensure robust zygote elongation and asymmetric division thereby initiating reliable apical-basal axis (**Figure 15**) (Ueda *et al.*, 2011, Ueda *et al.*, 2017). After the very first division, the PIN1-mediated auxin transport module facilitates to stabilizing the axis formation and embryo regionalization and patterning (**Figure 15**) (Möller and Weijers, 2009). For a normal hypophysis development in the embryo, *RKD2* needs to be repressed, which is normally active in the egg cell. The paternally derived one zinc finger domain and two repressive EAR motifs harboring proteins, DAZ3 and TREE1 redundantly facilitate the process by binding to a AGCTAAAG motif at RKD2 promoter (Cheng *et al.*, 2024). A separate study on TREE1-mediated shoot growth repression

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proposed the involvement of TREE1 EAR motif on HDA19 recruitment thereby leading to the deacetylation-mediated repression of the target gene (Wang *et al.*, 2020). While such case is also plausible on RKD2 regulation in the zygote (**Figure 15**), it requires direct evidence to confirm as such.

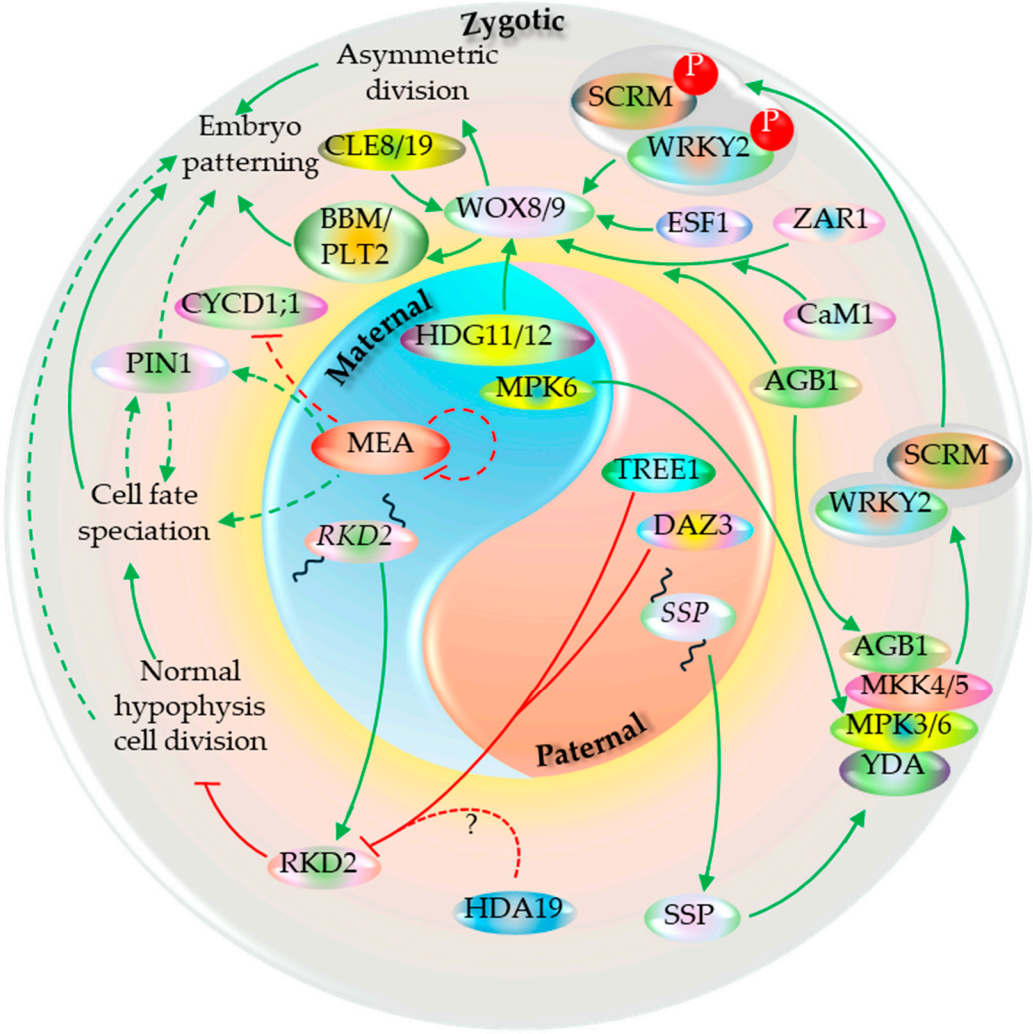

Figure 15. Factors associated with the maternal and paternal genome interaction in a zygote. WOX8/9 plays a central role in the very initial asymmetric division and subsequent embryo patterning. Its expression niche is controlled by the secretory CLAVATA peptides CLE8/19. Normal *WOX8* expression relies on the maternal (HDG11/12) and paternal (SSP-dependent YDA-MAPK) cascades. Sperm cell-derived *SSP* mRNA is translated in the zygote, which in turn activates YDA. AGB1 acts as a scaffold for the components of the MAPK complex by directly interacting with the associated components (Yuan et al., 2017), YDA forms complex with MPK3/6 and MKK4/5 thereby potentiating the complex for the phosphorylation of WRKY2 and SCRM, which directly contribute to WOX8 activation (Wang et al., 2007, Zhang et al., 2017). The activation is additionally facilitated by the Cam1 and AGB1-interaction-activated ZAR1 (Yu *et al.*, 2016) as well as the endosperm-derived maternally imprinted member ESF1 (Costa *et al.*, 2014). The egg cell constitutes normal *RKD2* expression, which hinders normal hypophysis cell division in the embryo. The process is alleviated by the sperm cell-derived TREE1 and DAZ3 (Cheng *et al.*, 2024), which are postulated to be facilitated *via* the HDA19-mediated deacetylation of RKD2 locus. MEA contributes to the patterning process by regulating CYCD1;1 and itself in a dose-dependent manner thereby affecting auxin distribution and cell fate speciation in the developing embryo (Baroux *et al.*, 2006, Simonini *et al.*, 2021, Simonini *et al.*, 2024). Promoting and suppressive effects are linked with green and red arrows respectively. The dotted arrows represent postulated/indirect links.

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Studies indicate that the WOX8/9-mediated patterning of early-stage embryo is facilitated by CLAVATA3/ESR-RELATED (CLE) proteins. *CLE19*, typically expressed in the abaxial epidermal layer of the embryo can confer embryo development defect non-cell autonomously when its antagonistic counterpart, CLE19$_{G6T}$ ectopically expressed in the endosperm, with no significant defect in the endosperm itself (Xu *et al.*, 2015). Defect in *CLE8*, normally expressed in both embryo and endosperm, confers development defect in embryo as well as endosperm (Fiume and Fletcher, 2012). The expression domain of *WUS* is regulated by accompanying CLE signals in *WUS* niches, including regulation of the *WOX8* expression domain by *CLE8* (**Figure 15**) (Laux *et al.*, 1996, Jun *et al.*, 2010, Fiume *et al.*, 2017). CLE is known to modulate *WUS* gradient at the post-transcriptional and post-translational level (Plong *et al.*, 2021). Whether the CLE8/19-mediated embryo development regulation is conferred by modulating *WOX8* gradient in similar way remains to be unraveled.

At the chromatin level, fertilization is accompanied by broad epigenetic reconfiguration of parental genomes, including resetting of histone variant landscapes and chromatin states that limit transgenerational carryover of gametic epigenetic information. In Arabidopsis, live cell imaging of histone H3 variants in fertilization products and subsequent studies support active remodeling/resetting in the zygote as part of early embryonic reprogramming. Furthermore, the zygote and endosperm exhibit distinctly different patterns of parental H3 marks. While the paternal H3 marks are gradually diluted in the endosperm over several rounds of nuclear divisions, zygote exhibits replication independent H3 mark removal (before its first division) as observed in Arabidopsis (Ingouff *et al.*, 2007). This indicates distinctly different PRC2-mediated regulation in the two tissues.

PRC2 component, MEA regulates *PIN1* to bring changes to the auxin distribution in the developing embryo and facilitates patterning via the regulation of cell cycle component, *CYCD1;1* (**Figure 15**) (Simonini *et al.*, 2021, Simonini *et al.*, 2024). Furthermore, it is autoregulated in a negative feedback loop (independent of the PRC2 complex); hence the heterozygous mutant (*MEA*/*mea*) ovules exhibit higher level of *MEA* expression than WT (Baroux *et al.*, 2006). Additionally, as transgenic study showed, translation of its transcript is dynamically regulated and pathogen infection leads to higher *MEA* protein synthesis (Rani *et al.*, 2025). However, the complete picture of MEA-PRC2 mode of action during MZT and ZGA phases of embryo development is not fully clear yet.

Unlike the fertilization-dependent sexual ovules, the apomictic ovules exhibit parthenocarpy (fertilization-independent seed development). A comparative assessment on the apomictic and sexual accessions of buffelgrass showed that the ovules of the former exhibits higher expression of the genes encoding calcium receptor protein and its effector, calcium/calmodulin-dependent protein kinases (Ke *et al.*, 2021). However, since apomixis is a convergent trait with its features appearing independently at different lineages (Goeckeritz *et al.*, 2024), it remains yet unclear how deterministic is the role of calcium receptor proteins in apomictic trait induction and persistence across species.

5.7. Embryo-Endosperm Growth Coordination

It has now been well accepted that molecular handshake between endosperm and embryo is essential for their timely progression in a developing seed. However, an Arabidopsis study with the developmental stage-specific marker genes observed that key developmental changes - endosperm cellularization, embryo growth, and embryo maturation - are not strictly correlated but primarily dependent on the developmental stage (O'Neill *et al.*, 2019). Nevertheless, normal embryo and endosperm development is essential for a typical seed development. While egg cell fertilization alone might be sufficient to trigger the division of the unfertilized central cell, its further development requires successful double fertilization as the seed development does not progress beyond post-globular stage (Nowack *et al.*, 2006). Similarly, the proliferation, cellularization, and developmental programmed cell death (dPCD) of the endosperm can progress in absence of successful zygote formation as well. The development of dicot seeds after the initiation of the endosperm degeneration, however, relies on the embryo growth as observed in Arabidopsis (Xiong *et al.*, 2021).

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Embryo growth is partly facilitated by LEC1, encoding a CCAAT-binding motif atypical subunit of NF-Y(B) transcription factor, which is normally expressed at both zygote and endosperm (**Figure 11**) (Lee *et al.*, 2003, Song *et al.*, 2025). It plays a positive role during gametic and somatic embryogenesis (SE) as well as in post-embryonic development (Zhao *et al.*, 2016, Brand *et al.*, 2019). It is expressed in the central cell (endosperm) immediately after fertilization, before exhibiting expression in the embryo. Furthermore, its endosperm expression is more pivotal than its expression within the embryo itself during embryogenesis. As observed in Arabidopsis, significant proportion (87%) of the LEC1-target-genes are uniquely active in embryos as compared to the endosperm at any developmental stage (Song *et al.*, 2025) while the LEC1-targets vary in the developing seeds depending on the endosperm growth phase- proliferation, cellularization, or degeneration (Pelletier *et al.*, 2017, Song *et al.*, 2025). The endosperm-derived LEC1 enters embryo suspensor symplastically *via* plasmodesmata (**Figure 16**) and is detectable by the two-celled zygotic stage (Song *et al.*, 2021). It promotes the expression of key maturity-associated genes including itself, LEC2, FUS3, and ABI3, which are essential for the seed storage protein accumulation during the later developmental stage (Song *et al.*, 2021). The across-cell-movement of the typical nuclear localizing LEC1 protein suggests that it either constitutes yet unidentified sorting signal within it - similar to that suggested for the KNOTTED1 protein (Lucas *et al.*, 1995) - or it is accompanied with yet unidentified signaling factor facilitating the transport. While LEC1-defect does not bring any pronounced defect during very early developmental stage, the mutant embryo exhibits desiccation intolerance and development halt at its linear cotyledonary stage (Lee *et al.*, 2003).

Additional endosperm-dependent embryo feature is its cuticle development, which typically commences at the globular stage in Arabidopsis (Szczuka and Szczuka, 2003, De Giorgi *et al.*, 2021). Embryo at globular stage and onwards expresses *GSO1/2* encoding putative leucine-rich repeat transmembrane-type receptor kinase (Tsuwamoto *et al.*, 2008), and *TWS1*, encoding an ER-localized peptide (Doll *et al.*, 2020b). The TWS1 needs to be activated before it is secreted to the apoplast and the process is facilitated by tyrosyl-protein sulfotransferase (TPST) by sulfating (Tyr-sulfating) TWS1 in the golgi complex. After being secreted, the sulfated TWS1 at the apoplast is further cleaved at the close downstream of its sulfation site by an endosperm-derived subtilase family protease, ALE1. Then the N-terminal TWS1 fragment harboring the sulfated site interacts with the extracellular region of the embryo plasma membrane-bound GSO1/2 (Tanaka *et al.*, 2001, Doll *et al.*, 2020b). The TWS1-to-GSO1/2 binding confirms the cuticle absence/damage and triggers the repair machinery (**Figure 16**). In later stage, the intact cuticle prevents TWS1 leakage to the apoplastic region thereby bringing the repair process to a halt (Doll *et al.*, 2020b).

GSO1/2 also contributes to later developmental stages by promoting formation of the 'sheath', a thin layer above the cuticle that supports non-sticky embryo growth during development and cotyledon emergence during germination (Moussu *et al.*, 2017). This process involves deposition of the ZHOUPI (*ZOU*)-regulated cysteine-rich protein KERBEROS (KRS) (**Figure 16**) (Doll *et al.*, 2020a). KRS expression initiates at the heart stage, peaks at the torpedo stage, and declines toward maturity in Arabidopsis. KRS shares sequence similarity with the tomato stigma-exudate protein STIG1, which functions as a ligand for the pollen-tube LRR receptor kinase LePRK2 and modulates pollen-tube redox potential during elongation (Tang *et al.*, 2004, Huang *et al.*, 2014). By analogy, KRS has been proposed to act as a ligand for an embryo-localized LRR receptor kinase to promote invasive embryo growth and ROS accumulation (**Figure 16**). However, direct biochemical evidence that KRS binds or activates GSO1/2 or other LRR receptor kinase is not yet available, which can be tested by genetic epistasis (krs × gso1/2), peptide rescue assays with synthetic KRS, and binding/activation measurements (SPR/ITC; receptor phosphorylation or reporter outputs) using GSO1/2 ± SERK coreceptors.

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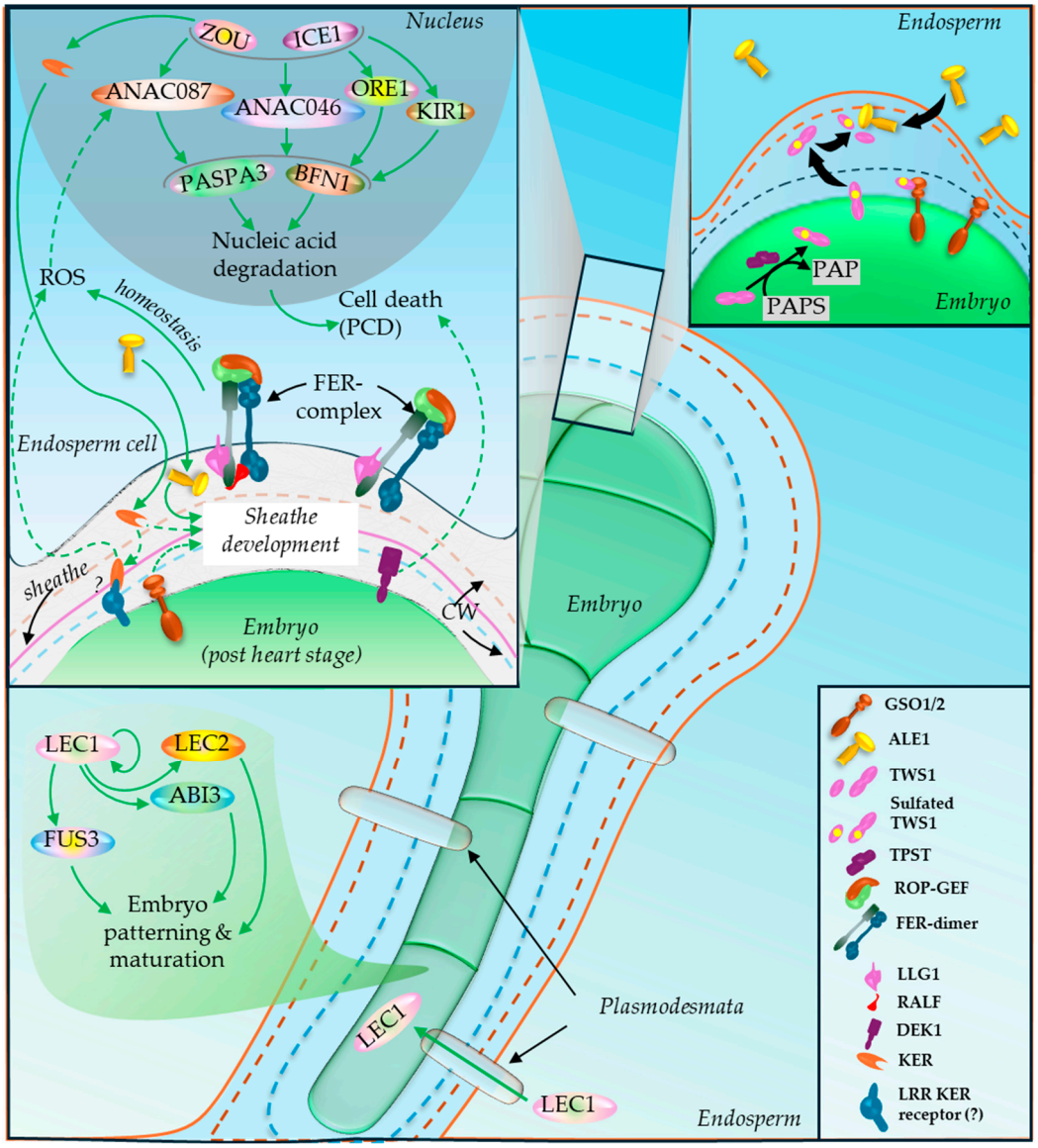

Figure 16. Factors associated with the embryo-endosperm interaction. The endosperm-derived LEC1 moves to the embryo by its binucleate stage and contributes to the expression of key genes including itself (*LEC2*, *ABI3*, *FUS3* regulating the embryo patterning and maturation processes during its later development stages (Song *et al.*, 2021). At the globular stage, embryo cuticle development commences, which is mediated by the embryo membrane-localized GSO1/2. The signal depends on the endosperm-derived ALE1-mediated cleavage of the embryo-derived sulfated peptide TWS1 (at the apoplast), which act as ligand for GSO1/2 (Tanaka *et al.*, 2001, Doll *et al.*, 2020b). In the later developmental stage, after endosperm cellularization, the invasive embryonic growth in the endosperm and developmental PCD (dPCD) of the latter are actively supported by the endosperm-expressed *ZOU*. *ZOU*, along with its ortholog *ICE1*, regulates the PCD-associated genes encoding NAC domain harboring proteins (*KIR1*, *ANAC087*, *ANAC046*, *ORE1*) involved in controlling the genes such as *PASPA3* and *BFN1* directly involved in the nucleic acid degradation (Yang *et al.*, 2008, Denay *et al.*, 2014, Fourquin *et al.*, 2016, Doll *et al.*, 2023). The process is further contributed by the embryo membrane bound DEK1 *via* mechanism yet unclear. ZOU also facilitates the deposition of KRS to the developing embryo sheath (Moussu *et al.*, 2017, Doll *et al.*, 2020a). Putative KER-interacting LRR receptor kinase (GSO1/2 or other) contributing to ROS accumulation and dPCD facilitation has been shown. Potential role of FER-complex in regulating controlled ROS homeostasis and downstream dPCD has been postulated. Factors with known mode of action has been shown in shapes with accompanying legend while others are presented in unique spheres. Promoting and suppressive effects are linked with green and red arrows respectively. The dotted arrows represent postulated/indirect links.

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Along with its interacting bHLH counterpart, INDUCER OF CBF EXPRESSION1 (*ICE1*), *ZOU* affects the embryo surrounding region (ESR) (Denay *et al.*, 2014). The perturbation of *ZOU* function affects the cell wall properties thereby increasing tissue-stiffness. Defect in either of *ZOU* and *ICE1* hinders the expression of the dPCD-associated NAC TFs (*KIR1*, *ANAC087*, *ANAC046*, *ORE1*) and their downstream nucleic acid degradation associated genes (*PASPA3* and *BFN1*) (**Figure 16**) leading to a halt to the embryo growth and shriveled seed development (Yang *et al.*, 2008, Denay *et al.*, 2014, Fourquin *et al.*, 2016, Doll *et al.*, 2023). The *ZOU* defect in Arabidopsis delays the expression the dPCD-associated genes by 4-5 days as compared to its wild type counterpart, which typically exhibits their expression by 8 DAP (Doll *et al.*, 2023). The cumulative defect in *KIR1* and additional ESR-expressed genes (*anac046-1 anac087-1 kir1-1 ore1-1*) leads to the persistence of uncleared corpse cells and halts the embryonic growth (Doll *et al.*, 2023). Analogous observation in maize showed that its KIR homologs KIL1/2 play role on PCD and corpse cell residues clearance at the endosperm region adjacent to the growing embryo, referred to as embryo scutellum. Defects in those genes affect the PCD and corpse cell clearance processes thereby strongly hindering the embryo growth. Furthermore, their expression is regulated by the paternally imprinted endosperm-specific DOSAGE-EFFECT DEFECTIVE1 (DED1), the closest maize homolog of Arabidopsis MYB64/119 (Dai *et al.*, 2022, Doll *et al.*, 2025).

The endosperm dPCD at the ESR, however, rely on the normal embryo development. The halt in post-globular stage embryonic growth led by the defect in *DEK1*, a gene encoding an integral membrane protein with a calpain protease domain, brings the normal endosperm development with no dPCD, even though the ZOU-dependent cell wall thinning and expression of pre-cell death markers remains normal in the *dek1* mutant (Johnson *et al.*, 2005, Fourquin *et al.*, 2016). Normal *DEK1* expression, however, is indispensable for its L1 layer development in Arabidopsis (Johnson *et al.*, 2005). The gene is attributed for its role in the endosperm aleurone cell (layer) development (Lid *et al.*, 2002). It strongly suggests that embryo L1 layer functions as the harbinger of the molecular factors more directly involved in embryo-endosperm communication at their interface. Such postulation also aligns with the globular stage growth arrest observed in the L1 layer development defective Arabidopsis dual mutant *pdf2; atml1* (Ogawa *et al.*, 2015). While the endosperm dPCD is essential for monocot seed development as well (Domínguez and Cejudo, 2014), a study showed that defect in two *FERONIA* homologs *FERONIA-LIKE RECEPTOR 3* (*FER3*) and *FER14* leads to a surge in ROS and upregulation of the PCD associated genes and caspases. Furthermore, the mutant endosperm exhibited disruption in starch biosynthesis, and decrease in seed storage protein accumulation (He *et al.*, 2023) suggesting potential role of FERONIA-like CrRLK1l members to perceive the pressure exerted by the growing embryo against endosperm and translating it to the controlled execution of PCD of the endosperm cells (**Figure 16**). Future monocots and dicots studies are expected to shed more light on the case

6. Bottlenecks and Future Perspectives

Despite rapid progress, seed development remains a patchwork of tissue-specific insights that are still difficult to unify into a predictive, cross-species framework. Below are key bottlenecks prevailing in the field.

6.1. Correlative vs Causative Evidence Behind MMC Speciation

Many proposed MMC speciation regulators (auxin threshold, SPL/NZZ-associated networks, small RNAs, chromatin states, and mechanics) are supported by strong correlations. Accompanying direct causal chains of evidence in a narrow spatial and temporal windows would benefit the case. Potential such avenues include (i) True spatiotemporal perturbation (cell-type specific, inducible, reversible): e.g., transient alteration of auxin transport or chromatin regulators specifically in L2 neighbors versus the enlarging candidate MMC, then measuring reversibility and fate stability. (ii) Integration of mechanics with gene regulation: primordium geometry, cell wall mechanics, and turgor-driven growth likely contribute to which L2 cell becomes MMC-competent. Study linking the

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mechanical changes to transcriptional/epigenetic switching would shed more light on the matter. Additionally, the coenocytic phase of endosperm development exhibit strong positive link with the microtubule and F-actin polymerization (Ali *et al.,* 2023). However, potential factors associated with the process in the endosperm is yet to be identified. Study on the cytoskeleton dynamics machineries with reference to the studies on other turgor-influenced tissues such as pollen tube (Adhikari *et al.,* 2020a), and stomata is expected to shed more light on the topic.

iku2 mutant exhibits increased testa stiffness in the developing seed (Hamann, 2012, Creff *et al.,* 2023). *IKU2* is a member leucine rich repeat (LRR) kinase *2* protein. A study on animal model (mouse fibroblast cell) showed that its LRR kinase *2* member, Lrrk2, modulates the intracellular cytoskeletal dynamics. The protein exhibits affinity to F-actin and its defect increases the cell's perimeter-to-area ratio (Meixner *et al.,* 2011). Furthermore, cellular auxin level is known to contributes to its actin dynamics in plant (**Figure 12**) (Adhikari *et al.,* 2020a). As observed in an Arabidopsis study, degradation of either microtubules or F-actin leads to a significant decrease in the coenocytic endosperm area and exhibits a visibly elongated size (Ali *et al.,* 2023). Whether *IKU2* defect confers cytoskeleton (F-actin in particular) dysregulation leading to the development of misshaped endosperm thereby affecting its turgor remains to be determined.

Regarding the ELA-controlled GA-regulation and testa stiffening, while GA biosynthesis is suppressed *via* the ELA1/2-mediated mechanosensing, their ER-localization (Zhang *et al.,* 2011) strongly suggests for upstream mechanosensory factors at play. In addition, ER is known to 'sense' the external mechanical stress based on Ca^{2+} homeostasis changes in its lumen potentially activated by the stress-triggered Ca^{2+} influx-led imbalance in cytoplasmic Ca^{2+} level (Howell, 2013, Yoshimura *et al.,* 2021). Mechanical strain that alters cell-wall/pectin status can be perceived by *FERONIA* (*FER*) and homologs at the cell surface, engaging downstream Ca^{2+}-channel activity to shape cytosolic Ca^{2+} signaling. This FER-dependent Ca^{2+} signaling can operate alongside tension-gated mechanosensitive channels to form the overall Ca^{2+} "mechano-signature" (**Figure 13**) (Feng *et al.,* 2018). Furthermore, Ca^{2+} is linked to GA biosynthesis and signaling (Du *et al.,* 2023, Gai *et al.,* 2024). It can rapidly elevate cytosolic Ca^{2+} in Arabidopsis via a pathway that is independent of DELLA degradation, indicating that GA signaling can interface directly with Ca^{2+} dynamics {Okada, 2017 #2467}. However, direct evidence linking FER-dependent Ca^{2+} signaling to ELA1/2-mediated GA deactivation in the seed coat is not yet available. In parallel, membrane tension–gated mechanosensitive Ca^{2+}-permeable channels (e.g., MCA-family, and other plant MS channel families such as MSL/OSCA/PIEZO) could contribute independently or alongside FER to shape the Ca^{2+} 'mechano-signature' that precedes downstream hormonal responses {Yoshimura, 2021 #2406;Guichard, 2022 #2469}.

6.2. Sporophyte-Gametophyte Cross-Talk Conduits

Multiple lines of evidence support sporophyte-gametophyte and embryo-endosperm cross-talk. However, the identity of mobile signals, their transport distances, and their routes (symplastic, apoplastic, or uptake-mediated) remain incompletely defined. Priority directions include: (i) identifying mobile molecules and directionality (small RNAs, peptides, hormones, metabolites) using lineage-restricted production and receiver-specific reporters; (ii) quantifying timing-dependent connectivity changes around fertilization as a measurable developmental variable; and (iii) testing conservation across taxa (eudicots vs monocots; persistent vs transient connectivity patterns).

6.3. Translational Perspective

Despite substantial progress on the molecular and mechanical basis of seed development, comparatively fewer studies translate these advances into crop improvement pipelines. Prioritizing translational frameworks could clarify cross-species conservation of regulatory modules while accelerating delivery of validated targets to breeding programs. Institutionally supported multi-team collaborations that integrate genetics, phenomics, and seed biology are expected to facilitate such efforts.

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7. Conclusion

The earliest phase of seed development is governed by coordinated hormonal, positional, and genetic/epigenetic regulators that enforce pre-fertilization developmental blocks and then enable rapid post-fertilization reprogramming. MMC restriction, ovule maturation, and fertilization-dependent activation of the coenocytic endosperm are coupled to maternal responses that initiate and pattern the seed coat, with auxin functioning as a central inter-tissue coordinator. Polycomb-associated chromatin states, small-RNA-linked silencing, and RBR1/APC-mediated cell-cycle gates provide robustness against ectopic proliferation, yet are selectively remodeled after fertilization to support parent-of-origin programs in endosperm and embryo. In parallel, mechanical forces generated by endosperm expansion and their perception by maternal tissues emerge as key determinants of seed growth and the timing of cellularization. Outstanding challenges include resolving causal signal flow across tissue interfaces (hormones, peptides, small RNAs, metabolites) and integrating mechanics with transcriptional and chromatin state changes at cell-type resolution.

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