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On the way to ovules: The hormonal regulation of ovule development

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ABSTRACT

This review focuses on the hormonal regulation of ovule development, especially on ovule initiation, patterning, and morphogenesis. Understanding of the genetic and molecular basis of ovule development is essential from both the scientific and economic perspective. The ovule represents an attractive system to study lateral organ development in plants, and, since ovules are the precursors of seeds, full comprehension of this process can be the key to the improvement of crops, especially those depending on high production of seeds and grains. Ovule initiation, patterning, and morphogenesis are governed by complex genetic and hormonal networks involving auxins, cytokinins, brassinosteroids, and gibberellins. These coordinate the determination of the ovule number, size, and shape through the regulation of the number of ovule primordia that arise from the placenta and/or ensuring their correct development into mature functional ovules. Here we summarize the current knowledge of how ovules are formed, paying special attention to the roles of these four plant hormones.

KEYWORDS

Arabidopsis, auxins, brassinosteroids, cytokinins, development, gibberellins, hormones, integument, ovule, primordia, regulation.

LIST OF ABBREVIATIONS

BRs	Brassinosteroids
CKs	Cytokinins
CMM	Carpel medial meristem
FM	Functional megaspore
GAs	Gibberellins
GWAS	Genome-Wide Association Study
IM	Inflorescence meristem
JA	Jasmonates
MMC	Megaspore mother cell
SAM	Shoot apical meristem
TF	Transcription factor

Introduction

In seeded plants, ovules play a central biological role during the plant life cycle. Ovules contain the female reproductive cells and, following fertilization, develop into seeds, which in turn hold, protect, nourish, and are the vehicles for dispersion of the embryos. In addition, seeds are of high economic importance because they are key for human and animal food. Therefore, understanding the molecular mechanisms that control ovule initiation and development is crucial not only from a scientific but also from an agricultural and economic point of view: most crop yield depends upon the high number and quality of seeds, and seed production per fruit heavily depends on the number and size of ovules and proper ovule development (Khan *et al.*, 2019).

In angiosperms, ovules develop inside the pistil, which forms the gynoecium or the female reproductive part of the flower, and are composed of three different morphological structures (Figures 1 and 2) (Schneitz *et al.*, 1995; Gasser and Skinner, 2019; Cucinotta *et al.*, 2020). The terminal region of the ovule is the nucellus, where megasporogenesis and megagametogenesis occur and the embryo sac is formed. Coming from chalazal tissue in the medial region, one or two integuments surround and encase the nucellus leaving an opening at the apex, the micropyle, through which the pollen tube can access the embryo sac to allow fertilization. The vascularized basal region corresponds to the funiculus, which connects the ovule to the septum and pistil.

Ovule initiation and development has been mainly studied in the reference plant *Arabidopsis thaliana* and follows a sequence of processes that typically characterize plant organ development: 1) primordium initiation from a meristem preceded, followed or accompanied by the specification of organ identity, 2) growth of the incipient primordia due to directional cell division and expansion, and 3) cellular differentiation and morphogenesis. Schneitz *et al*. (1995) provided a detailed ontogeny describing and classifying all the

processes of ovule development, in the context of flower development stages by Smyth *et al*. (1990), giving a basis for further ovule development studies (Figures 1 and 2).

Briefly, during stage 1 of ovule development, ovule primordia arise and elongate. From the distal to proximal regions, the nucellus, chalaza, and funiculus are defined (flower development stages 8 and 9). In stage 2, megasporogenesis and integument initiation take place (flower development stages 10 and 11). During stage 3, megagametogenesis occurs, the embryo sac is developed, and the two integuments grow and surround the nucellus (flower development stages 12 until anthesis). A fourth stage is also defined, corresponding with the postfertilization development of the ovule until the octant embryo is formed (embryo and seed development, recently reviewed by Armenta-Medina and Gillmor, 2019; Matilla, 2019; and Phillips and Evans, 2020). During this stage, double fertilization takes place, endosperm and embryo development initiate, and the transition of the integument into the seed coat begins.

Plant organ development involves a complex regulation scheme carried out by intricate genetic-hormonal networks. In the last years, several genes and hormones have been implicated in ovule development (Cucinotta *et al*., 2014; 2020; Shirley *et al*., 2019). The major objective of this review is to present a complete, updated description of hormonal regulation (and its interaction with genetic factors) of pre-fertilization ovule development. In order to present a clear overview, we have divided ovule development in three steps: i) the initiation of ovule primordia in the placenta; ii) patterning, the spatial arrangement of distinct regions with different cell fates within the primordia; and iii) morphogenesis that includes integument differentiation and growth. It must be kept in mind that ovule development is a continuous developmental process; therefore, many genes do not act in only one step but are active throughout the whole process. Detailed descriptions of the molecular control of the female gametophyte, the embryo sac, were recently provided by Pinto *et al.* (2019), Lora *et*

al. (2019), and Erbasol Serbes *et al*. (2019), which nicely complement the data reported in this review.

Coming into context: Pistil, carpel margin meristem, and placenta development

As was previously introduced, in flowering plants ovules develop inside the pistil. This structure is a key feature that allowed angiosperms to gain a great evolutionary advantage. The pistil protects, nourishes, and ensures the correct fertilization of the ovules. Upon fertilization, it becomes the fruit, which in addition to fulfilling the previous functions also facilitates the dispersion of seeds (Sauquet *et al*., 2017; Becker, 2020). The pistil (and consequently, the ovules) develops continuously during the reproductive state, after the shoot apical meristem (SAM) has transitioned into the inflorescence meristem (IM) (Pajoro *et al*., 2014). The IM produces the floral meristems, in which the sepals, petals, stamens, and pistils develop in concentric whorls (Denay *et al*., 2017; Thomson and Wellmer, 2018).

In *Arabidopsis* and most other flowering plants, the gynoecium is formed by a single pistil, composed of two congenitally fused carpels and divided into four different regions along its apical-basal axis: the stigma, the style, the ovary, and the gynophore (Figure 2). Among these structures, the ovary represents the largest section. During pistil development (recently reviewed by Zúñiga-Mayo *et al.*, 2019; Simonini and Østergaard, 2019 and Reyes-Olalde and de Folter, 2019), at the margin of the fused carpels, a group of meristematic cells called the carpel margin meristem (CMM) expands towards the center and gives rise to the placenta, among other tissues (Figure 1A) (Zúñiga-Mayo *et al.*, 2019; Reyes-Olalde and de Folter, 2019; Becker, 2020). Ovule primordia arise from this placental tissue.

Hence, ovule primordia initiation is directed in the context of carpel identity. *AGAMOUS* (*AG*) is a MADS-box gene that, together with *SEPALLATA* (*SEP*) genes, define carpel identity in the fourth whorl of the floral meristem (Thomson and Wellmer, 2018). Later

in carpel development, *AG* is expressed in the placenta as well as ovule primordia (Bowman *et al*., 1991a). In addition, Pinyopich *et al.* (2003) observed that AG could play a role in ovule identity and development. The combination of the *ag* mutant with *APETALA2* (*AP2*) mutant (*ap2*) (Bowman *et al*., 1991b) forms aberrant flowers with ectopic carpelloid structures instead of sepals. These carpelloid structures can develop ectopic ovules, some of them converted into carpelloid structures themselves.

However, this phenotype suggests that other AG-independent regulators had to be controlling carpel and ovule features. *SHATTERPROOF1* (*SHP1*), *SHP2*, and *SEEDSTICK* (*STK*) are closely related MADS-box genes that have overlapping expression patterns with *AG* in the placenta as well as ovule primordia (Pinyopich *et al*., 2003). Ectopic expression of these three genes promotes the formation of ectopic ovules on sepals (Favaro *et al*., 2003; Pinyopich *et al*., 2003), and the *stk shp1 shp2* triple mutant leads to the development of leaf- or carpel-like structures instead of ovules (Pinyopich *et al*., 2003, Brambilla *et al*., 2007), suggesting that *SHP1, SHP2*, and *STK* redundantly specify ovule identity. In addition, genetic and molecular analyses indicate that *SEP* activity is necessary in combination with *AG*, *SHP1, SHP2*, and *STK* for proper ovule development (Favaro *et al*., 2003; Brambilla *et al*., 2007). These MADS-box genes would assemble into ovule-specific complexes whose stoichiometry must be unaltered to define ovule identity (Favaro *et al*., 2003, Brambilla *et al*., 2007), similarly as the floral quartet hypothesis during floral organ specification (Smaczniak *et al*., 2012).

In recent years, some new layers of regulation for proper *SHP1, SHP2,* and *STK* activity have been reported, providing new insights into the molecular mechanism that regulates ovule identity. For instance, HUA-PEP proteins are RNA-binding proteins that compose a post-transcriptional regulatory module that regulates *SHP1, SHP2, STK*, and *AG* activity by affecting their pre-mRNA processing and production of functional proteins (Rodríguez-

Cazorla *et al*., 2018; 2020). Additionally, BASIC PENTACYSTEINE (BPC) C-box binding proteins cooperate with MADS-box factors and components of the Polycomb Repressive Complexes to ensure proper expression of *STK* during early flower development (Petrella *et al*., 2020).

First step: Ovule primordia initiation

Ovule primordia initiation (stage 1-I, according to Schneitz *et al*., 1995) can be visualized as small protrusions in the placenta of pistils at stage 8 of flower development (according to Smyth et al., 1990), when the pistil is still growing as an open-ended cylinder (Figures 1B and 3). This process is orchestrated after the determination of the primordium position by periclinal divisions of the placental subepidermal layers by anticlinal divisions, resulting in an expansion of a relatively homogeneous mass of cells (Schneitz *et al*., 1995). As a parallel example, for the initiation of lateral organs from the SAM, it is necessary to define the zone of primordia out-growth and the boundaries that separate the primordia from the meristem where they are initiated (Aida and Tasaka, 2006; Žádníková and Simon, 2014). These two zones are composed of groups of cells with very distinct gene expression programs and morphologies. In this way, the boundaries themselves express a set of genes that play a role to locally repress cell proliferation and physically separate organs. On the contrary, the zone of primordia out-growth is characterized by a high cell proliferation rate. Similarly, it should be necessary to define the boundaries between adjacent ovule primordia and the meristematic zone of primordia out-growth to determine the ovule primordia position. Failure in this regulation would imply an alteration of ovule number and/or development. In this context, several genes have been associated with ovule initiation as regulators of boundaries establishment or out-growth zones definition. Table 1 summarizes the genes that have been implicated in ovule primordia initiation.

On the one hand, CUP-SHAPED COTYLEDON1 (CUC1), CUC2, and CUC3 are NAC-domain transcription factors (TFs) that have major roles in defining the boundary regions in the SAM (Aida *et al*., 1997; Hibara *et al*., 2003; Vroemen *et al*., 2003) or between floral organs (Mallory *et al*., 2004; Baker *et al*., 2005), as well as during leaf margin serration (Nikovics *et al*., 2006) and are expressed in the placenta and the borders of the ovule primordia (Ishida *et al*., 2000; Vroemen *et al*., 2003; Galbiati *et al*., 2013; Gonçalves *et al*., 2015). The combination of mutations in *CUC1* and *CUC2* leads to a reduction in ovule number as well as aberrant spacing between ovules (Ishida *et al*., 2000; Galbiati *et al*., 2013), whereas the loss of *CUC3* in combination with the loss of *CUC2* induces ovule primordia fusions (Gonçalves *et al*., 2015). *CUC1* and *CUC2* are post-transcriptionally regulated by the *MIR164* microRNA (Rhoades *et al*., 2002; Laufs *et al*., 2004; Mallory *et al*., 2004; Baker *et al*., 2005), which has also been consistently described as influencing ovule number (Gonçalves *et al*., 2015). In addition, LATERAL ORGAN FUSION 1 (LOF1) is an MYB-domain TF with an overlapping function with CUC2 and CUC3 in lateral organ separation (Lee *et al*., 2009) that was also found to be expressed in ovule primordia boundaries (Gomez *et al*., 2011), suggesting a possible role of LOF1 in ovule boundary establishment.

On the other hand, AINTEGUMENTA (ANT) is an AP2 TF that positively regulates organ initiation and growth (Elliott *et al*., 1996; Krizek, 1999; Mizukami and Fischer, 2000), and was closely associated with ovule primordia formation and ovule development (Elliott *et al*., 1996; Klucher *et al*., 1996). During the early stages of ovule initiation, *ANT* is expressed in the placenta and ovule primordia (Elliott *et al*., 1996; Barro-Trastoy *et al*., 2020). Moreover, single *ant* mutations lead to a reduction in ovule number with no concomitant reduction in pistil length (Klucher *et al*., 1996; Liu *et al*., 2000; Barro-Trastoy *et al*., 2020), which results in decreased ovule density. Combinations of *ant* alleles with mutants of other transcriptional regulators (Table 1) aggravate the ovule number phenotype of the single *ant*

mutants. However, these double mutants also lead to disrupted pistil, disrupted CMM, and/or disrupted placenta development, hindering the discrimination between primary effects of these genes on ovule initiation rather than secondary effects due to pistil development malformations.

Although the initial understanding of the molecular control of ovule initiation mostly involved TFs, in recent years new studies have added layers of complexity in the regulation of this developmental process. As an example, Liao *et al*. (2020) have recently described the silencing of two cell wall sucrose invertases, *CWIN2* and *CWIN4*, which irreversibly catabolize the sucrose translocated to sink organs from phloem and are highly expressed in the placenta as well as ovule primordia, inhibits ovule initiation and, later, induces ovule abortion. Interestingly, the phenotype of *CWIN2/4*-silenced plants is not due to carbon starvation, as it cannot be rescued by supplying the ovules with more carbon nutrients, and the transcript levels of carbon starvation genes do not change in the *CWIN2/4*-silenced plants, suggesting that CWIN may play a role in this process through sugar signaling (Liao *et al*., 2020). Additionally, Yuan and Kessler (2019) identified *NEW ENHANCER OF ROOT DWARFISM* (*NERD1*) in a genome-wide association study (GWAS) as a gene associated with ovule number variation among different *Arabidopsis* accessions. This gene, expressed in both the placenta as well as the ovule primordia, encodes a membrane protein localized in the Golgi apparatus whose loss-of-function leads to a significant reduction of the number of ovules and disrupts megagametophyte development (Yuan and Kessler, 2019). However, the interplay of these genes with others in terms of the control of ovule initiation and development is unknown, and further analyses to uncover their molecular function are needed.

Role of plant hormones during ovule initiation

Hormones are signal molecules that participate in the control of plant growth and development. Among them, auxins, cytokinins (CKs), brassinosteroids (BRs) and gibberellins (GAs) have been described as being involved in ovule initiation (Table 2 and Figure 3).

Auxins are major hormones well known for participating in most growth and developmental processes regulating cell division, elongation, and differentiation (Weijers *et al*, 2018). One of their prominent functions is to promote organ primordia formation in both shoots (Wang and Jiao, 2018) and roots (Overvoorde *et al*., 2010). These processes occur due to auxin accumulation (also called auxin maximum) in the organ initiation sites, led by local auxin biosynthesis (Brumos *et al*., 2018) as well as polar auxin transport (Okada *et al*., 1991; van Berkel *et al*., 2013) facilitated mainly by the auxin efflux carriers named PIN-FORMED (PIN) (Zhou and Luo, 2018). For instance, the generation of an auxin maximum at the flank of the IM can promote the initiation of the floral meristem (Okada *et al*., 1991; Heisler *et al*., 2005; Heisler and Byrne, 2020). Likewise, during pistil development, auxin maxima define the sites of ovule primordia initiation along the placenta. Several arguments support this view. First of all, auxin-responsive DR5 reporter lines reveal that auxin-signaling maxima are detected only at the tip of the ovule primordia (Benkova *et al*., 2003; Ceccato *et al*., 2013). Second, the auxin biosynthesis gene *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1* (*TAA1*) is strongly expressed in the CMM and the epidermis of the incipient ovule primordia (Nole-Wilson *et al*., 2010a). Lastly, monitoring the GFP signal fused to different PIN proteins demonstrated that, among the eight PINs encoded in *Arabidopsis*, PIN1 and PIN3 are found in ovules (Benkova *et al*., 2003; Ceccato *et al*., 2013). PIN1 was localized at the membrane of the outer cell layer of ovule primordia, with its polarity pointing toward the primordium tip, most probably supplying the accumulation of auxins (Benkova *et al*.

2003, Ceccato *et al*., 2013). PIN3 has a similar but weaker pattern of expression in ovule primordia (Ceccato *et al*., 2013), and it is also found in clusters of a few cells in the placenta before the ovule primordia is observed (Larsson *et al*., 2014).

In addition, some components of the auxin signaling pathway are localized in the ovule primordia. Briefly, auxins are perceived within the cells by the F-box protein TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX (TIR1/AFB), which leads to the degradation of the Aux/IAA proteins that in turn repress the activators of the auxin-responsive genes, the AUXIN RESPONSE TRANSCRIPTION FACTOR (ARFs) (Leyser, 2018). Among all ARF proteins in *Arabidopsis*, MONOPTEROS (ARF5/MP) is broadly localized in the ovule primordia at stage 1-I and restricted to ovule primordia boundaries during stage 1-II (Galbiati *et al*., 2013).

The role of auxins in ovule initiation is also supported by genetic evidence, although it is sometimes difficult to discriminate between primary effects on ovule development and secondary effects due to pistil malformations in the corresponding mutants, since auxins are master regulators of pistil development (Marsch-Martinez and de Folter, 2016). For instance, the strong loss-of-function allele of PIN1, *pin1-1*, can occasionally induce the formation of flowers that have an empty pistil with no ovules and malformed style and stigma (Benkova *et al*., 2003). In contrast, the weak *pin1-5* mutant can develop flowers with pistils that have slightly reduced valves, normal styles, and stigmas (Sohlberg *et al*., 2006) but a high reduction in ovule number (Bencivenga *et al*., 2012). Moreover, young pistils treated with N-(1-naphthyl) phthalamic acid (NPA) to block auxin transport exhibit a reduction of ovule number, suggesting that auxin transport is required for ovule development (Okada *et al*., 1991; Nemhauser *et al*., 2000). Taken together, these data suggest that auxins play an important role during early ovule development, with PIN1 having a major role in both ovule initiation and pistil development.

Interestingly, Galbiati *et al*. (2013) found several pieces of evidence that point to both CUC1 and CUC2 as direct regulators of *PIN1* expression and correct PIN1 localization in ovule primordia. In addition, they also found that both *CUC1* and *CUC2* are directly and positively regulated by MP. Curiously, MP has also been shown to directly bind the promoter of *ANT* (Yamaguchi *et al*., 2013) and induce its expression (Galbiati *et al*., 2013). In turn, expression levels of the *Aux/IAA1, Aux/IAA17* and *TAA1* genes were significantly reduced in stage 8–10 pistils of the *ant-8* mutant, suggesting a role for ANT in auxin homeostasis, at least in young pistils (Nole-Wilson *et al*., 2010a). Among these, *TAA1* was recently described to be directly activated by ANT in floral buds (Krizek *et al*., 2020). The dynamic pattern of auxin synthesis and transport reflects a major role of auxins in ovule initiation. Also, auxins could be involved in a regulatory feedback loop between CUC1, CUC2, and ANT during ovule primordia outgrowth; auxins may be locally synthesized by the action of TAA1 and transported by PIN1 to the tip of the ovule primordia, where auxin maxima response is detected. PIN1 localization is controlled by CUC1 and CUC2, which in turn are regulated by MP. MP also regulates ANT, which could have a role in the control of auxin homeostasis in the primordia (Figure 3).

Cytokinins (**CKs**), which regulate cell division and differentiation, are also essential for ovule initiation. They are perceived by the ARABIDOPSIS HISTIDINE PROTEIN KINASES (AHKs), initiating a two-component signaling pathway characterized by a phosphorylation cascade (Hwang *et al*., 2012). Analysis of GUS expression driven by the promoters of the three AHKs encoded in *Arabidopsis* showed that these genes are active in the carpel and developing ovules (Nishimura *et al*., 2004; Bencivenga *et al*., 2012). In addition, the promoter of *CYTOKININ RESPONSE FACTOR 2* (*CRF2*) and *CRF6*, two AHK-downstream components of the CK signaling pathway (Hwang *et al*., 2012), are able to drive

expression in the placenta during ovule initiation and ovule primordia formation, respectively (Cucinotta *et al*., 2016).

Several studies demonstrate that CKs positively regulate ovule number. For instance, mutants with compromised CK perception present a reduction in ovule number. While the wild-type ecotype Col-0 develops around 63 ovules (Yuan and Kessler, 2019), the *cre1-12 ahk2-2 ahk3* triple receptor mutant develops an average of only 5 ovules per pistil (Bencivenga *et al*., 2012). For its part, the *crf2 crf3 crf6* triple mutant presents a significant reduction in ovule number with a mild shortening of placenta length, resulting in a decrease in ovule density (Cucinotta *et al*., 2016). The same occurs in *arr1 arr10 arr12*, a triple mutant of *ARABIDOPSIS RESPONSE REGULATOR 1* (*ARR1*), *ARR10,* and *ARR12* (Reyes-Olalde *et al*., 2017), other AHK-downstream components of the CK signaling pathway (Hwang *et al*., 2012).

On the contrary, when the CK catabolism is disrupted, an increase in ovule number is observed. The irreversible degradation of CKs is catalyzed by cytokinin oxidases/dehydrogenases (CKXs) enzymes. It was found that the double loss of *CKX3* and *CKX5* (*ckx3-1 ckx5-1*), which increases the CK level, produces significantly more flowers with larger pistils and almost twice as many ovules as the wild-type, indicating that CKs increase the meristem capacity of the IM and the placenta (Bartrina *et al*., 2011). A similar phenotype is observed in wild-type plants treated with 6-benzylaminopurine (BAP), a synthetic CK (Galbiati *et al*., 2013; Cucinotta *et al*., 2016).

Recent studies point to CUC1 and CUC2 as regulators of the CK homeostasis in ovule primordia. Plants of the *cuc2-1 pSTK:RNAi-CUC1* line (a null *CUC2* mutant with silenced *CUC1* in placenta and ovules) present a reduction in total active CKs and an increase in O-glucosylated CK ribosides (CKs reversible inactive forms) (Cucinotta *et al*., 2018). Similarly, Galbiati *et al*. (2013) had previously observed that BAP treatment alleviates the ovule number

phenotype of *cuc2-1 pSTK:RNAi-CUC1*. Moreover, both CUC1 and CUC2 can induce the expression of the *LUC* reporter, driven under the control of the two-component system signaling sensor (TCS), which reflects the CK response (Cucinotta *et al*., 2018). Furthermore, transcriptomic analysis by RNA-Seq and qPCR demonstrate that *UGT85A3* and *UGT73C1*, two genes that encode enzymes that catalyze the reversible inactivation of zeatin-type CKs by O-glucosylation, are upregulated in *cuc2-1 pSTK:CUC1-RNAi* (Cucinotta *et al*, 2018). Interestingly, the *ugt85a3* mutant had an increase in ovule number and an unaffected pistil, while the *35S:UGT73C1* line presented a reduction in ovule number and pistil length, suggesting that UGT85A3 may have a role in determining directly ovule density, and UGT73C1 may affect ovule development indirectly by controlling processes involved in pistil elongation (Cucinotta *et al*., 2018).

CKs also affect auxin polar transport during ovule initiation. For instance, BAP treatments are able to increase *PIN1* expression in pistils (Bencivenga *et al*., 2012, Cucinotta *et al*., 2016). In accordance, the *crf2 crf3 crf6* CK insensitive mutant presented a reduction in *PIN1* expression that cannot be restored by BAP treatments (Cucinotta *et al*., 2016). CRFs were found to be direct transcriptional regulators of *PIN1* by binding to *PIN CYTOKININ RESPONSE ELEMENT* (*PCRE*), a *cis*-regulatory sequence located in the *PIN1* promoter (Šimášková *et al*., 2015). Taken together all these data demonstrate that CKs also directly regulate *PIN1* expression during ovule initiation and highlight a convergence point between auxins and CKs in this developmental process (Figure 3).

Brassinosteroids (BRs) are a group of steroid plant hormones that control cell proliferation and elongation and are required for normal plant growth and development (Fridman and Savaldi-Goldstein, 2013). Their role in ovule initiation was mostly described by Huang *et al*. (2013). BRs are perceived in the plant membrane cells by the

BRASSINOSTEROID INSENSITIVE 1 (BRI1) homo-oligomer receptor. Upon BR binding, BRI1 forms a hetero-oligomer with BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), which activates a signal cascade that involves several phosphorylations and dephosphorylations (Planas-Riverola *et al*., 2019). Analysis of mutants with disrupted BR perception reveals that BRs are positive regulators of ovule and seed number.

For instance, the loss-of-function mutants *bri1-5* (Huang *et al*., 2013), *bri1-116* (Jia *et al*., 2020), and the gain-of-function mutant *bin2-1* of the BR signaling negative regulator *BRASSINOSTEROID-INSENSITIVE 2 (BIN2*) (Huang *et al*., 2013; Jia *et al*., 2020), have fewer ovules and seeds than wild-type plants. On the contrary, the gain-of-function mutant *bzr1-1D* of the positive BR signaling regulator *BRASSINAZOLE RESISTANT 1* (*BRZ1)* presents increased ovule and seed number (Huang *et al*., 2013; Barro-Trastoy *et al*., 2020). Supporting this, the BR biosynthesis defective mutant *det2-1* and brassinazole (a BR biosynthesis inhibitor) treatments decrease ovule number (Huang *et al*., 2013; Barro-Trastoy *et al*., 2020), while brassinolide (an endogenous natural BR) treatment increases ovule number (Barro-Trastoy *et al*., 2020). Nole-Wilson *et al*. (2010b) also found that the mutation of *CYP85A2*, a CYP450 involved in the last step of brassinolide biosynthesis (Nomura *et al*., 2005), causes a reduction in ovule number.

BRs appear to be involved in the regulation of ovule initiation through the up-regulation of *HLL* and *ANT* and down-regulation of *AP2* expression levels. Huang *et al*. (2013) found that both *HLL* and *ANT* have increased expression in brassinolide-treated plants and *bzr1-1D* (Figure 3). However, at least the up-regulation of *ANT* does not seem to be the cause of the increased ovule number in these plants, as the *ANT* over-expression (in *35S:ANT* plants) does not induce an increase in ovule number, but an increase in ovule size (Barro-Trastoy *et al*., 2020).

Gibberellins (GAs) are hormones that regulate a multitude of key developmental processes throughout the plant life cycle, such as seed germination, growth, flowering, and fruit development (Sun, 2011; Hedden and Sponsel, 2015; Rizza and Jones, 2019). GA signaling is mediated by the ubiquitin-dependent degradation of DELLA proteins, which belong to the GRAS family and act as negative regulators of GA signaling. The binding of bioactive GAs to the GIBBERELLIN INSENSITIVE DWARF 1 (GID1) receptors allows the formation of the GA-GID1-DELLA complex, which promotes the association of DELLA with F-box proteins, DELLA polyubiquitination and subsequent DELLA degradation (Sun, 2011; Daviere and Achard, 2016). Among these components, some GA INSENSITIVE DWARF (GID1) receptors (GID1A and GID1B) and DELLA proteins, GIBBERELLIC ACID INSENSITIVE (GAI), REPRESSOR OF GA (RGA), RGA-LIKE 1 (RGL1), and RGL2, are detected in placenta and/or ovule primordia using transcriptomic reporter lines and *in situ* mRNA hybridization (Gomez *et al*., 2016; 2018; 2019; 2020).

Genetic evidence points to DELLA proteins as positive regulators of the number of ovules, GA being detrimental for ovule initiation. The *global* mutant for the five DELLA of *Arabidopsis*, (*gaiT6 rgaT2 rgl1-1 rgl2-1 rgl3-1*), produces fewer ovules. A similar reduction is observed in the *quadruple* (*gaiT7 rgaT2 rgl1-1 rgl2-1*) and *triple* (*3xdella, gaiT6, rgaT2 rgl2-1*) mutants, which suggests that RGA, GAI, and RGL2 have a major role in ovule initiation. Reduced ovule number is also observed in GA-treated plants, which phenocopies the null *della* mutants (Gomez *et al*., 2018). By contrast, the gain-of-function DELLA mutants *gai-1* (Gomez *et al*., 2018) and *pRGL2:YPet-rgl2• 17* (Gomez *et al*., 2019) produce a significant increase in the number of ovules with minor or no effect on pistil length, suggesting that DELLAs specifically promote an increase in the number of ovule primordia in the developing pistil. A similar phenotype was also observed in the double *gid1a gid1b*

mutant, which lacks GA perception in the ovules (Gomez *et al*., 2018). This result is consistent with the fact that *GID1A* and *GID1B* are the only *GID1s* expressed in ovules (Gallego-Giraldo *et al*., 2014). Therefore, it seems that blockage of GA perception in the placenta (by knocking out both *GID1A* and *GID1B*) leads to a stabilization of DELLA proteins involved in ovule primordia initiation. Furthermore, ectopic expression of rga• 17, a version of RGA that cannot be degraded by GAs, driven by the *ANT* promoter is enough to increase ovule number (Gómez *et al*., 2018). Taken together, these results demonstrate that GAs are negative modulators of ovule number by promoting the degradation of DELLA proteins, whose activity is necessary to regulate ovule primordia formation.

Although GAs have been found to play an important role in this developmental process, there is still little evidence to elucidate the GA molecular mechanism in ovule number determination (Figure 3). Transcriptomic analysis of stage 8-9 pistils of *gai-1* and *global* identified two TFs, REPRODUCTIVE MERISTEM 22 (REM22) and UNFERTILIZED EMBRYO SAC 16 (UNE16), as possible DELLA targets that positively regulate ovule initiation, which can help to dissect the molecular mechanism of DELLA proteins in ovule number (Gomez *et al*., 2018). Moreover, it seems that GAs do not regulate ovule number by interfering with auxins, as neither DR5 nor PIN1 were affected by DELLA activity (Gomez *et al*., 2018). Nor do they seem to interfere with BRs in *Arabidopsis*, as DELLA proteins can still regulate ovule number independently of BR levels, and BR can still regulate ovule number in the absence of DELLA activity (Barro-Trastoy *et al*., 2020).

In summary, four major plant hormones appear to play key regulatory roles during ovule initiation, which strongly argues in favor of a complex interplay in the control of ovule primordia formation. Auxins and CKs are highly interconnected through *CUC1* and *CUC2*, and BRs directly regulate *ANT* expression, which in turn could be involved in a regulatory

feedback loop together with auxins (Figure 3). Little is known about how GAs could be involved in this molecular network. One mechanism could be that GAs are involved in the establishment of ovule primordia boundaries, as DELLA proteins are able to interact with CUC2 (Marin-de la Rosa *et al*., 2014). However, further work will be needed to uncover this hormone crosstalk to coordinate early ovule development.

Second step: Ovule patterning

Once ovule primordia have initiated and elongated (stage 1-II), they are spatially arranged in three different regions along their distal to proximal axis: the nucellus, the chalaza, and the funiculus (Schneitz *et al*., 1995). This process occurs when the pistil is constricted at the apex, at stage 9 of flower development (Figure 1C) (Smyth *et al*., 1990; Schneitz *et al*., 1995).

Located at the distal region, the nucellus is the site of formation of a single diploid germline cell, the megaspore mother cell (MMC), which can be visualized at stages 2-I to 2-III and is the precursor of the embryo sac. The chalaza is the medial region from which integuments develop. *Arabidopsis* ovules are bitegmic, since they form two integuments, inner and outer. These comprise protective layers of cells, three for the inner and two for the outer integument, that surround the nucellus and eventually the embryo sac. The proximal region gives rise to the funiculus, which attaches the ovules to the placenta and is characterized by the presence of a vascular strand that nurtures the ovule (Schneitz *et al*., 1995).

Ovule patterning heavily relies on a correct interpretation of the positional information of the three different regions of ovule primordia, as well as correct inter-region communication. Several genes have been identified to be involved in this process, most of them affecting both the development of the nucellus and the chalaza (Table 3 and Figure 4).

On the one hand, the homeobox gene *WUSCHEL* (*WUS*) is expressed in the nucellus in early ovule development and it was found to be required for both functional embryo sac and integument development (Gross-Hardt *et al*., 2002; Lieber *et al*., 2011; Yamada *et al*., 2016). In addition, *SPOROCYTELESS/NOZZLE* (*SPL/NZZ*) is a putative transcription factor also expressed in both the nucellus and integuments. The *spl-1* mutant leads to a reduction of both nucellar domain size and integument growth and loss of MMC formation (Yang *et al*., 1999; Schiefthaler *et al*., 1999, Balasubramanian and Schneitz, 2000).

On the other hand, *ANT* expression is restricted to the chalazal region during ovule patterning (Elliott *et al*., 1996). ANT is necessary for proper integument initiation and embryo sac maturation, since integuments are lacking and megagametogenesis does not occur in null *ant* mutants (Elliott *et al*., 1996; Klucher *et al*., 1996). Redundantly with *ant*, the *huellenlos* (*hll*) shows a blockage in early integument development and defective embryo sac formation (Schneitz *et al*., 1998). Another gene whose expression pattern specifically marks the chalazal region is *BELL1* (*BEL1*), a homeodomain gene (Reiser *et al*., 1995). However, in *bel1* mutants only the inner integument fails to initiate, while the outer integument develops into a carpelloid-like structure, forming a swollen collar structure that fails to cover the nucellus (Modrusan *et al*., 1994). This suggests that *BEL1* is required for inner integument development and outer integument identity (Robinson-Beers *et al*., 1992; Modrusan *et al*., 1994; Reiser *et al*., 1995).

A close relationship among several of these genes was described, revealing some pathways of communication between regions. For instance, BEL1 and SPL confine *WUS* expression to the nucellus (Brambilla *et al*., 2007; Sieber *et al*., 2004) and work together for proper chalaza formation (Balasubramanian and Schneitz, 2000). For its part, SPL regulates nucellus development, antagonizing BEL1 and ANT (Balasubramanian and Schneitz, 2000).

Finally, little is known about the establishment of the funiculus zone, as most patterning mutants appear seem to retain a normal funicular region with little or no alterations. For example, ovules homeotically transformed into sepaloid/carpeloid structures often occur connected to the placenta by umbilical structures with characteristic isodiametric funicular cells, although in other cases these are replaced by more elongated cell types (Pinyopich *et al.*, 2003; Rodriguez-Cazorla *et al.*, 2018; 2020). Moreover, in severe *hua-pep* mutant combinations in which ovules are converted into sepaloid organs, these occasionally arise directly from the placental tissue lacking any stalk-like structure (Rodriguez-Cazorla *et al.*, 2018; 2020). In any case, the role of *STK* as a negative modulator of funiculus development is well documented, and *stk* mutants display drastically enlarged funiculi (Pinyopich *et al.*, 2003).

Role of hormones during ovule patterning

Several studies point to the participation of auxins and CKs in ovule patterning (Figure 4). Table 4 summarizes all the components of auxin or CK biosynthesis, catabolism, and/or signaling that are found to be expressed in ovules at this stage.

The role of **auxins** in ovule patterning is highlighted by the fact that in the *pin1-5* mutant some ovules develop as finger-like structures that cannot reach the maturity stage (Bencivenga *et al.*, 2012). In accordance, *PIN1* is detected during stage 1-II in the outer cell layer of the elongated ovule primordia surrounding the nucellus, in the membranes of the inner integument cells and in the developing funiculus, most probably supplying auxins to the tip of the primordia, where a DR5 signal is still detectable (Benkova *et al.*, 2003; Ceccato *et al.*, 2013). In addition, it was described that both SPL (in the nucellus) and BEL1 (in the chalaza) could act upstream of auxins during patterning. Thus, it was shown that the *PIN1* and

516 *DR5* signals are reduced in the nucellus, inner integument, and funiculus of *spl*-1 ovules,
517 suggesting that SPL is a positive regulator of *PIN1* and auxin response in the early stage 2 of
518 ovule development (Bencivenga *et al*., 2012). In turn, SPL may regulate auxin homeostasis
519 during lateral organ morphogenesis, through the regulation of *YUCCA2 (YUC2)* and *YUC6*
520 auxin biosynthesis genes (Li *et al*., 2008). Moreover, loss of BEL1 induces the ectopic
521 expression of *PIN1* in the outer integument primordia and the epidermal layer of the
522 funiculus, as well as in the funiculus and the inner integument, where it is usually expressed,
523 indicating that BEL1 is important for the correct localization of PIN1 in the chalaza
524 (Bencivenga *et al*., 2012). Taken together, this evidence points to auxins acting downstream
525 of SPL and BEL1 during ovule patterning (Figure 4).

526 Regarding the implication of **CKs** in ovule patterning, as for the case of auxins, the fact
527 that the CK insensitive mutant *cre1-12 ahk2-2 ahk3-3* develops finger-like ovules with
528 disrupted development revealed that CKs are important for proper ovule pattern (Bencivenga
529 *et al*., 2012). Moreover, plants treated with BAP develop a single structure instead of two
530 integuments (Bencivenga *et al*., 2012). The similarity between *pin1-5* and *cre1-12 ahk2-2*
531 *ahk3-3* phenotypes and the role of CRF regulating the expression of *PIN1* (Šimaškova *et al*.,
532 2015), suggests crosstalk between auxins and CKs in ovule patterning. This was confirmed by
533 the analysis of PIN1 localization and expression levels in *cre1-12 ahk2-2 ahk3-3* mutant as
534 well as in plants treated with BAP (Bencivenga *et al*., 2012). In the first case, *PIN1* is
535 undetectable (Bencivenga *et al*., 2012), when it is usually detected in the central region of the
536 funiculus, the outer layer of the nucellus and the inner integument primordium in wild-type
537 plants (Benkova *et al*., 2003). In the second case, *PIN1* is detected in the nucellus, funiculus,
538 and inner integument and also the outer integument and the epidermal layer of the funiculus,
539 suggesting that CKs are important for proper *PIN1* expression and PIN1 localization
540 (Bencivenga *et al*., 2012). Interestingly, the *pin1-5* mutant is insensitive to BAP (Bencivenga

et al., 2012). All these results suggest that CKs mediate ovule patterning by the regulation of PIN1 distribution.

Additionally, both auxins and CKs were described to be implicated in a complex network that involves both *SPL* and *BEL1*, two major genes of ovule patterning described above. The ovule phenotype of *cre1-12 ahk2-2 ahk3-3* mutant was reminiscent of that of *spl-1*, and in both lines, *PIN1* is almost undetectable, as is the case for *cre1-12 ahk2-2 ahk3-3* (Bencivenga *et al*., 2012). It was found that *SPL* expression levels are drastically reduced in *cre1-12 ahk2-2 ahk3-3* plants, remaining only weakly detectable in the nucellus, while *SPL* expression increased in both nucellus and integument primordia of BAP-treated plants (Bencivenga *et al*., 2012). In addition, while *PIN1* is ectopically expressed in BAP-treated wild-type plants, it was undetectable in *spl-1* plants treated with BAP (Bencivenga *et al*., 2012), suggesting that the PIN1 regulation by SPL described above is mediated by CKs or that SPL is required for CK-induced *PIN1* expression during ovule patterning. It is highly appealing that BAP treatments can phenocopy the *bel1* mutant phenotype: the two integuments are replaced by a single structure. In accordance, *BEL1* expression is reduced in BAP-treated plants (Bencivenga *et al*., 2012). Moreover, *WUS* is ectopically expressed in the chalaza in both *bel1-1* ovules (Brambilla *et al*., 2007) and BAP-treated wild-type plants (Bencivenga *et al*., 2012). Finally, the *PIN1* expression profile is similar in both *bel1-1* and BAP-treated plants (Bencivenga *et al*., 2012), and *bel1-1* ovules treated with NPA develop as finger-like structures, similar to *pin1-5* (Bencivenga *et al*., 2012). Taken together, data suggest that *BEL1* is relevant for the CK regulation of PIN1 (Figure 4).

In summary, both auxins and CKs are required for proper ovule patterning (Figure 4). They are implicated in a complex crosstalk that involves both SPL and BEL1. CKs may be

565 regulating *SPL* and *BEL1* expression in the nucellus and chalaza, respectively. In turn, SPL
566 and BEL1 would regulate PIN1 in these two tissues.
567
568 **Third step: Integument morphogenesis**
569 As a pattern is correctly established, the inner and outer integuments grow in a coordinated
570 manner over the nucellus from stage 2-II to 3-IV, eventually enclosing the embryo sac and
571 leaving the micropyle at the apex of the mature ovule (Figures 1D-E, 2 and 5). The inner
572 integuments, which initiate before the outer integuments (Figure 1D), grow from both
573 gynoapical and gynobasal sides of the developing ovule as a radially symmetrical structure
574 that surrounds the nucellus. On the contrary, outer integuments grow asymmetrically, only
575 from the gynobasal side of the ovule and more extensively at its abaxial side (Figure 1E). This
576 asymmetric growth results in an anatropous ovule (Schneitz *et al*., 1995; Endress, 2011), in
577 which the resulting curvature causes the micropyle to be positioned close to the funiculus at
578 maturity stage (Figure 2). Furthermore, from stage 2-IV to 2-V (stage FG0 for
579 megagametophyte development, as described by Christensen *et al*., 1997), the MMC
580 undergoes megasporogenesis via meiosis and subsequent degeneration of three nuclei,
581 resulting in a single haploid functional megaspore (FM) at stage 3-I (FG1) (Figure 1E). Then,
582 from stages 3-II to 3-VI (FG2-FG6) the FM undergoes megagametogenesis via three rounds
583 of mitosis, forming the embryo sac (Figure 2). All these processes take place when the flower
584 is at stages 10 to 12 (Figures 1D, E, and 2) (Smyth *et al*., 1990; Schneitz *et al*., 1995,
585 Christensen *et al*., 1997).
586 Regarding integument development, some genes were identified as regulators of
587 integument polarity (Figure 5). *INNER NO OUTER* (*INO*) encodes a YABBY protein that is
588 expressed in the abaxial (or dorsal) side of the outer integument and is essential for its proper
589 development (Villanueva *et al*., 1999). In addition to *INO*, two KANADI genes, *KANADI1*

(*KAN1*) and *KAN2*, act redundantly to regulate outer integument development (Eshed *et al*., 2001; McAbee *et al*., 2006). *PHABULOSA* (*PHB*), *PHAVOLUTA* (*PHV*), and *CORONA* (*CNA*) are three HD-ZIP III genes expressed specifically in the adaxial (or ventral) side of the inner integument that redundantly induce its growth. *REVOLUTA* (*REV*) is a fourth HD-ZIP III that may be involved in the development of both integuments (Kelley *et al*., 2009).

Moreover, HD-ZIP III genes are post-transcriptionally regulated by the *MIR165/166* microRNAs (Rhoades *et al*., 2002; Jung *et al*., 2007), which were found to be strongly expressed in the incipient outer integument, suggesting another layer of regulation for correct integument development. Among these, *MIR166* microRNAs regulate and confine PHB expression to the inner integument (Hashimoto *et al*., 2018). *ABERRANT TESTA SHAPE* (*ATS*, another KANADI gene) is expressed at the boundary between integuments and plays a role in inner integument development and integument separation. In the *ats-1* mutant, there is a fusion of the inner and outer integuments that grow as a single structure (McAbee *et al*., 2006).

In addition, many genes are involved in the control of proper integument growth through the regulation of cell division, cell expansion, or cell organization. These are summarized in Table 5. Some of these genes were found to be closely related to each other and to ovule patterning genes as well. Thus, PHB, PHV, and CNB collaborate with BEL1 to regulate *WUS* (Yamada *et al*., 2016). INO contributes to the regulation of *SPL*, which in turn acts together with ATS to regulate *INO* expression (Balasubramanian and Schnetiz, 2002). UCN (Table 5) regulates growth patterns by interacting and repressing *ATS* (Enugutti *et al*., 2012). For its part, ATS acts in concert with PHB, PHV, and CNB to control the laminar growth of both the inner and outer integuments. In parallel, ATS in conjunction with REV restricts *INO* expression and outer integument growth, a mechanism that could relate to *SPL* action (Kelley *et al*., 2009). Moreover, INO is involved in a positive autoregulatory circuit

that is negatively regulated by SUPERMAN (SUP) (Table 5) (Meister *et al*., 2002) and can physically interact with the corepressors LUG and SEU and the coactivator ADA2b/PRZ1 to probably activate or repress different sets of target genes (Simon *et al*., 2017)

Role of hormones during morphogenesis

Among the four hormones mentioned in this review, auxins, GAs, and BRs have been described to be involved in integument morphogenesis (Table 6 and Figure 5).

Compared to the extensive knowledge gathered on the role of **auxins** in ovule initiation and patterning, much less is known regarding ovule morphogenesis. However, the fact that several auxin biosynthesis, transport, and signaling genes are expressed in ovule tissues during ovule morphogenesis suggests that auxins play a significant role in this process. These genes are summarized in Table 6. Among them, loss of *ARF3/ETTIN* (*ETT*), which is expressed in the abaxial region of the inner integument in young ovules (Kelley *et al*., 2012), induces malformations in the ovule integuments, as both the inner and the outer integument grow as a single fused structure (Kelley *et al*., 2012). This phenotype was also observed in the *ats-1* mutant (McAbee *et al*., 2006). In fact, bimolecular fluorescence complementation (BiFC) assays reveal that ATS and ARF3/ETT can physically interact in the plant cell nucleus (Kelley *et al*., 2012). Thus, it seems that auxins could be regulating the spacing between integuments via an ATS-ARF3/ETT complex (Figure 5). Interestingly, although UCN maintains planar growth of integuments by interacting and negatively regulating ATS, *UCN* and *ETT* act on different pathways, as is suggested by double mutant phenotypes: while *ats-1* is epistatic to *ucn-1* (Enugutti *et al*., 2012), *ucn-1 ett-1* exhibits an additive phenotype (Enugutti and Schneitz, 2013). Supporting this, suppression of integuments growth by *UCN* does not involve the regulation of auxin homeostasis (Enugutti and Schneitz, 2013). This

suggests that an intricate regulatory network involving interaction complexes may be needed for proper integument development.

The role of **GAs** during integument development is based on several pieces of evidence. Several DELLA proteins are localized in the integument primordia and funiculus (Table 6) (Gomez *et al*., 2016, 2019, 2020). However, the key evidence that points to GAs as regulators of the integument development comes from the analysis of the high-order multiple *della* null mutant. Both *global*, that lacks the five DELLA genes, and *quadruple*, which lacks *GAI, RGA, RGL1,* and *RGL2*, present an interesting phenotype: while the wild-type ovules normally form three layers of cells in the inner integument and two in the outer integument, in the two *della* mutants both the outer and inner integuments form two layers, which results in mature ovules with an irregular shape (Gomez *et al*., 2016).

Interestingly, these irregular ovules resemble those in the *ats-1* mutant, in which the outer and inner integuments are fused (McAbee *et al*., 2006). In fact, *ats-1* displayed other GA-signaling phenotypes, like higher germination rate and altered flowering time (Gomez *et al*., 2016), which could suggest that *ats-1* mutation may have altered GA levels. Indeed, the GA biosynthesis genes *GA3ox1*, *GA3ox2,* and *GA20ox2* are upregulated in *ats-1*. Among these, *GA3ox1* was found to be strongly expressed in *ats-1* ovules. According to this, RGA protein levels were decreased in the chalaza and integument of *ats-1* (Gomez *et al*., 2016), probably as a consequence of elevated GA levels. Moreover, yeast two-hybrid and BiFC analyses demonstrated that both GAI and RGA can physically interact with ATS. This result, and the fact that the GAI gain-of-function mutant, *gai-1*, do not rescue the ovule phenotype of *ats-1*, suggest that both ATS and DELLA would form a complex that is needed to regulate proper integument growth. In this regard, ATS would repress GA biosynthesis to promote the stabilization of DELLAs, strengthening the protein complex (Figure 5) (Gomez *et al*., 2016).

Over the past few years, some evidence has pointed to **BRs** as possible regulators of integument development. For instance, the *cyp85A2* mutant, with reduced BR levels, enhances *seu-1* mutant defects in the growth of the outer integuments (Nole-Wilson *et al*., 2010b). In addition, *in situ* hybridization analysis showed that *CYP85A1* (another BR biosynthesis gene), which is required for the initiation of female gametogenesis, is localized in both sporophytic and gametophytic tissues in mature ovules (Perez-España *et al*., 2011).

However, it has not been until recently that BRs have been clearly implicated in the development of the integuments. Jia *et al*. (2020) observed that in the *bri1-116* mutant, defective in BR perception, around 21% of the ovules had a severe outer integument growth arrest, in which the outer integument was not able to surround the inner integument. This phenotype, which was also found in BR mutants *det2-1* and *bri1-5*, was due to a reduction in both cell length and number. Moreover, the *bzr1-1D* mutation was able to partially restore *bri1-116* defects in outer integument growth (Jia *et al*., 2020), suggesting that BZR1 mediates the BRI1 outer integument growth regulation. According to this, in the sextuple null mutant of the six BZRs encoded in *Arabidopsis*, *bzr-h*, outer integument growth was completely arrested after initiation. Moreover, both *BRI1* and *BZR1* were broadly localized in all ovule tissues from stage 2-I to stage 3-V, including the initiated and out-growing outer integument cells (Jia *et al*., 2020).

Interestingly, using RNA-seq transcriptomic analysis, Jia *et al*. (2020) found that *INO,* which regulates outer integument development (Villanueva *et al*., 1999), is upregulated in the ovules of *bri1-116 bzr1-1D* when compared with *bri1-116*. Similarly, *INO* expression is repressed in *bri1-116* but its expression is restored to a wild type level in the *bri1-116 bzr1-1D* double mutant (Jia *et al*., 2020). Moreover, ChIP-qPCR analyses demonstrated that *INO* is a direct target of BZR1. All of these results suggest that downregulation of *INO* is the primary cause of the outer integument growth defects observed in *bri1-116* and *bzr-h* mutants (Figure

5). In line with this, the transformation of *bri1-116* with *pINO:INO-YFP*, which results in a slight increase of *INO* basal expression levels, leads to a reduction of defective outer integument growth phenotypes in the *bri1-116* mutant (Jia *et al*., 2020).

In summary, although more studies are needed to uncover the complex hormonal regulation of integument morphogenesis, evidence indicates that at least auxins, GAs, and BRs participate in this process (Figure 5). Auxins could be controlling the spacing between inner and outer integuments through the interaction with ATS, which in turn may be regulating GA levels. Likewise, the defects in integument development observed in multiple *della* null mutants may be partially due to an interaction of DELLA proteins with ATS. For its part, BRs could be involved in outer integument growth through the regulation of *INO* expression.

Beyond *Arabidopsis*: Ovule development in other plant species and future perspectives

The analysis of ovule development in *Arabidopsis* is a paradigmatic example of how a widely used model plant species, for which many experimental tools have been developed and implemented, has allowed the achievement of a deep knowledge about complex developmental processes. In this way, the understanding of the hormonal–genetic control of ovule development together with other crop-related traits could allow the discovery of promising targets and develop new strategies to improve crop seed yield. These issues have been recently reviewed by Shirley *et al*. (2019) and Cucinotta *et al*. (2020).

One of the most promising plant species to transfer knowledge gained from *Arabidopsis* would be *Brassica napus*, known as **rapeseed**, oilseed rape or canola. *Arabidopsis* and rapeseed are closely related plants that belong to the Brassicaceae family and have highly similar flower and pistil structures (Zuñiga-Mayo *et al*., 2018), including similar

anatropous and bitegmic ovules (Bouttier and Morgan, 1992). Additionally, rapeseed is an agronomically important crop widely cultivated in Europe, Asia, North America, and Australia for its oil-rich seed, used to produce vegetable oils for both nutritional and industrial purposes (Friedt *et al.*, 2018). As a consequence, rapeseed production has greatly increased in the last twenty years, mostly by increasing cultivated area, reaching a world production of 75 million tons and 37.6 million hectares of harvested area in 2018 (FAOSTAT 2020: Crops; http://www.fao.org/faostat/en/#data/QC/visualize). Thus, ovule and seed number are interesting and potential traits to increase canola/rapeseed crop yield (Mendham *et al.*, 1981; Bouttier and Morgan, 1991; Berry and Spink, 2009; Shi *et al.*, 2015; Cucinotta *et al.*, 2020). It was recently demonstrated that *B. napus* shares some well-conserved response mechanisms to CK treatments with *Arabidopsis* during flower development, including ovule number determination, as CK application increases rapeseed ovule number (Zuñiga-Mayo *et al.*, 2018). Moreover, GAs significantly reduce rapeseed ovule number in a dose-dependent manner (Gomez *et al.*, 2018). These are two examples indicating that common mechanisms may regulate ovule number in both *Arabidopsis* and *B. napus*, although further studies are needed to delineate similarities and differences.

Ovule development has also been well established in the fleshy fruit reference plant species **tomato** (*Solanum lycopersicum*), a member of the Solanaceae family. Tomato ovules are anatropous and unitegmic, with only one integument but follow the same sequence of processes as in *Arabidopsis thaliana*. Ovule primordia arise as protrusions from the placenta and, in the days following, the nucellus, chalaza, and funiculus are differentiated. Megasporogenesis and megagametogenesis then occur, eventually giving rise to the embryo sac and the integument growth surrounding it (Xiao et al., 2009; van der Knaap et al., 2014).

Several observations argue in favor of a similar role of auxins in the promotion of ovule initiation from the placenta in tomato as in *Arabidopsis*, despite anatomical and

738 developmental differences. For instance, a *DR5*-based signal is observed at the tip of ovule
739 primordia in tomato (Goldental-Cohen *et al*., 2017). Later, this signal is found in the area
740 corresponding to the micropylar pole of the embryo sac (Pattinson and Catala, 2012) and in
741 the vascular bundles connecting the ovules to the placenta (Goldental-Cohen *et al*., 2017).
742 Furthermore, NPA treatments during the early stages of flower development result in abortion
743 of ovule primordia, leading to an ovule-less phenotype (Goldental-Cohen *et al*., 2017). In
744 addition, tomato ovule number is negatively regulated by GAs (Gomez *et al*., 2018) and
745 positively regulated by BRs (Barro-Trastoy *et al*., 2020) as in *Arabidopsis*. GA-treatments, as
746 well as *procera*, the loss-of-function mutant of PROCERA, the only DELLA found in tomato,
747 induce a reduction in tomato ovule number (Gomez *et al*., 2018). The Micro-Tom (MT)
748 cultivar of tomato, which harbors a mutation in the *DWARF4* gene of BR biosynthesis,
749 causing reduced BR levels, has fewer ovules than the isogenic MT line that carries the wild-
750 type and functional *DWARF4* gene (Barro-Trastoy *et al*., 2020), which resembles the
751 phenotype of BR-deficient mutant *det2-1* of *Arabidopsis* (Huang *et al*., 2013).

752 However, Barro-Trastoy *et al*. (2020) have recently found that GA and BR crosstalk in
753 the determination of ovule primordia formation is quite different in tomato. As indicated
754 above, in *Arabidopsis* GAs and BRs down- and up-regulate, respectively, ovule number
755 regardless of the status of the other hormone. For example, GAs can still reduce ovule number
756 in plants with high or low BRs or BR responses, whereas BRs can promote the formation of
757 more ovule primordia in both *gai-1* or GA-treated plants, with high or low DELLA activity,
758 respectively. In contrast, in tomato, BRs control ovule number through the inhibition of GA
759 biosynthesis (Barro-Trastoy *et al*., 2020). BRs would reduce GA levels by repressing the
760 expression of GA biosynthesis genes, such as *SlGA20ox1*. This would lead to the stabilization
761 of PROCERA, which in turn would promote an increase in ovule number.

Additionally, it was recently described that jasmonates (JAs) regulate ovule development in tomato, since the lack of JA perception in the *jai1-1* mutant (the equivalent of the *Arabidopsis* CORONATIN-INSENSITIVE1 (COI1) mutant), results in abnormal ovule development (Schubert *et al*., 2019). Interestingly, JAs have not yet been implicated in ovule development in *Arabidopsis*. Therefore, it would be very interesting to know if JA also participates in the development of ovules in Arabidopsis and other species.

Final thoughts

Studies of ovule and seed development, as well as of the pistil and fruit, are key for creating new and innovative plant breeding techniques and genetic tools for tackling global challenges, like global warming and a growing world population. Since the beginning of agriculture, the improvement of seed and grain yield has been an essential and major goal, either through the manipulation of seed size, quality, or number. In recent times, the understanding of the genetic and hormonal control of these processes in *Arabidopsis* has been remarkably important to develop promising strategies for knowledge transfer, especially to closely related and agronomically important plants, through precision breeding. However, generalization of the knowledge gathered from *Arabidopsis* to other plant species must be done so with caution, because it will not always transfer. More efforts in understanding flower, seed, and fruit development in economically important species must thus be a goal for future research.

Acknowledgements and funding

We wish to thank Drs. C. Ferrandiz, A. Vera, and J. Carbonell for critical reading of the manuscript. Cambridge proofreading (https://proofreading.org/order/) provided proofreading

and editing of the text. D B-T received an FPU PhD fellowship (FPU18/00331) from the Spanish Ministry of Science and Innovation. This work was supported by the Spanish Ministry of Economy and Competitiveness-FEDER under grant BIO2017-83138R.

References

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. 1997. Genes involved in organ separation in Arabidopsis: an analysis of the cup-shaped cotyledon mutant. *Plant Cell*. **9**: 841-857.

Aida, M., and Tasaka, M. 2006. Genetic control of shoot organ boundaries. *Curr. Opin. Plant Biol*. **9**: 72-77.

Armenta-Medina, A., and Gillmor, C. S. 2019. Genetic, molecular and parent-of-origin regulation of early embryogenesis in flowering plants. *Curr. Top. Dev. Biol*. **131**: 497-543.

Azhakanandam, S., Nole-Wilson, S., Bao, F., and Franks, R. G. 2008. SEUSS and AINTEGUMENTA mediate patterning and ovule initiation during gynoecium medial domain development. *Plant Physiol*. **146**: 1165-1181.

Baker, C. C., Sieber, P., Wellmer, F., and Meyerowitz, E. M. 2005. The early extra petals1 mutant uncovers a role for microRNA miR164c in regulating petal number in Arabidopsis. *Curr. Biol*. **15**: 303-315.

Balasubramanian, S., and Schneitz, K. 2000. NOZZLE regulates proximal-distal pattern formation, cell proliferation and early sporogenesis during ovule development in *Arabidopsis thaliana*. *Development*. **127**: 4227-4238.

Balasubramanian, S., and Schneitz, K. 2002. NOZZLE links proximal-distal and adaxial-abaxial pattern formation during ovule development in *Arabidopsis thaliana*. *Development*. **129**: 4291-4300.

Barro•Trastoy, D., Carrera, E., Baños, J., Palau•Rodriguez, J., Ruiz•Rivero, O., Tornero, P., Alonso, J. M., Lopez-Díaz, I., Gomez, M. D., and Perez•Amador, M. A. 2020. Regulation of ovule initiation by gibberellins and brassinosteroids in tomato and Arabidopsis: two plant species, two molecular mechanisms. *Plant J*. **102**: 1026-1041

816 Bartrina, I., Otto, E., Strnad, M., Werner, T., and Schmülling, T. 2011. Cytokinin regulates
817 the activity of reproductive meristems, flower organ size, ovule formation, and thus
818 seed yield in *Arabidopsis thaliana*. *Plant Cell*. **23**: 69-80.
819 Bao, F., Azhakanandam, S., and Franks, R. G. 2010. SEUSS and SEUSS-LIKE
820 transcriptional adaptors regulate floral and embryonic development in Arabidopsis.
821 *Plant Physiol*. **152**: 821-836.
822 Becker, A. 2020. A molecular update on the origin of the carpel. *Curr. Opin. Plant Biol*. **53**:
823 15-22.
824 Bencivenga, S., Simonini, S., Benkova, E., and Colombo, L. 2012. The transcription factors
825 BEL1 and SPL are required for cytokinin and auxin signaling during ovule development
826 in Arabidopsis. *Plant Cell*. **24**: 2886-2897.
827 Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jürgens, G., and
828 Friml, J. 2003. Local, efflux-dependent auxin gradients as a common module for plant
829 organ formation. *Cell*. **115**: 591-602.
830 Berry, P. M., and Spink, J. H. 2009. Understanding the effect of a triazole with anti-
831 gibberellin activity on the growth and yield of oilseed rape (*Brassica napus*). *J. Agric.*
832 *Sci.* **147**: 273-285.
833 Bouttier, C., and Morgan, D. G. 1992. Ovule development and determination of seed number
834 per pod in oilseed rape (*Brassica napus* L.). *J. Exp. Bot.* **43**: 709-714.
835 Bowman, J. L., Drews, G. N., and Meyerowitz, E. M. 1991a. Expression of the Arabidopsis
836 floral homeotic gene AGAMOUS is restricted to specific cell types late in flower
837 development. *Plant Cell*. **3**: 749-758.
838 Bowman, J. L., Smyth, D. R., and Meyerowitz, E. M. 1991b. Genetic interactions among
839 floral homeotic genes of Arabidopsis. *Development*. **112:** 1-20.

840 Brambilla, V., Battaglia, R., Colombo, M., Masiero, S., Bencivenga, S., Kater, M. M., and
841 Colombo, L. 2007. Genetic and molecular interactions between BELL1 and MADS box
842 factors support ovule development in Arabidopsis. *Plant Cell*. **19**: 2544-2556.
843 Broadhvest, J., Baker, S. C., and Gasser, C. S. 2000. SHORT INTEGUMENTS 2 promotes
844 growth during Arabidopsis reproductive development. *Genetics*. **155**: 899-907.
845 Brumos, J., Robles, L. M., Yun, J., Vu, T. C., Jackson, S., Alonso, J. M., and Stepanova, A.
846 N. 2018. Local auxin biosynthesis is a key regulator of plant development. *Dev. Cell*.
847 **47**: 306-318.
848 Carter, B., Henderson, J. T., Svedin, E., Fiers, M., McCarthy, K., Smith, A., Guo, C., Bishop,
849 B., Zhang, H., Riksen, T., Shockley, A., Dilkes, B. P., Boutilier, K., and Shockley, A.
850 2016. Cross-talk between sporophyte and gametophyte generations is promoted by
851 CHD3 chromatin remodelers in *Arabidopsis thaliana*. *Genetics*. **203**: 817-829.
852 Ceccato, L., Masiero, S., Roy, D. S., Bencivenga, S., Roig-Villanova, I., Ditengou, F. A.,
853 Palme, K., Simon, R., and Colombo, L. 2013. Maternal control of PIN1 is required for
854 female gametophyte development in Arabidopsis. *PloS ONE*. **8**: e66148
855 Chevalier, D., Batoux, M., Fulton, L., Pfister, K., Yadav, R. K., Schellenberg, M., and
856 Schneitz, K. 2005. STRUBBELIG defines a receptor kinase-mediated signaling
857 pathway regulating organ development in Arabidopsis. *Proc. Natl. Acad. Sci. USA*. **102**:
858 9074-9079.
859 Christensen, C. A., King, E. J., Jordan, J. R., and Drews, G. N. 1997. Megagametogenesis in
860 Arabidopsis wild type and the Gf mutant. *Sex. Plant Reprod.* **10**: 49-64.
861 Conner, J., and Liu, Z. 2000. LEUNIG, a putative transcriptional corepressor that regulates
862 AGAMOUS expression during flower development. *Proc. Natl. Acad. Sci. USA*. **97**:
863 12902-12907.

Cucinotta, M., Colombo, L., and Roig-Villanova, I. 2014. Ovule development, a new model for lateral organ formation. *Front. Plant Sci.* **5**: 117.

Cucinotta, M., Di Marzo, M., Guazzotti, A., de Folter, S., Kater, M. M., and Colombo, L. 2020. Gynoecium size and ovule number are interconnected traits that impact seed yield. *J. Exp. Bot.* **71**: 2479-2489.

Cucinotta, M., Manrique, S., Cuesta, C., Benkova, E., Novak, O., and Colombo, L. 2018. CUP-SHAPED COTYLEDON1 (CUC1) and CUC2 regulate cytokinin homeostasis to determine ovule number in Arabidopsis. *J. Exp. Bot.* **69**: 5169-5176.

Cucinotta, M., Manrique, S., Guazzotti, A., Quadrelli, N. E., Mendes, M. A., Benkova, E., and Colombo, L. 2016. Cytokinin response factors integrate auxin and cytokinin pathways for female reproductive organ development. *Development*. **143**: 4419-4424.

Daviere, J. M., and Achard, P. 2016. A pivotal role of DELLAs in regulating multiple hormone signals. *Mol. Plant*. **9**: 10-20.

Denay, G., Chahtane, H., Tichtinsky, G., and Parcy, F. 2017. A flower is born: an update on Arabidopsis floral meristem formation. *Curr. Opin. Plant Biol*. **35**: 15-22.

Elliott, R. C., Betzner, A. S., Huttner, E., Oakes, M. P., Tucker, W. Q., Gerentes, D., Perez, P., and Smyth, D. R. 1996. AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. *Plant Cell*. **8**: 155-168.

Endress, P. K. 2011. Angiosperm ovules: diversity, development, evolution. *Ann. Bot.* **107**: 1465-1489.

Enugutti, B., Kirchhelle, C., Oelschner, M., Ruiz, R. A. T., Schliebner, I., Leister, D., and Schneitz, K. 2012. Regulation of planar growth by the Arabidopsis AGC protein kinase UNICORN. *Proc. Natl. Acad. Sci. USA.* **109**: 15060-15065.

888 Enugutti, B., and Schneitz, K. 2013. Genetic analysis of ectopic growth suppression during
889 planar growth of integuments mediated by the Arabidopsis AGC protein kinase
890 UNICORN. *BMC Plant Biol*. **13**: 2.
891 Eshed, Y., Baum, S. F., Perea, J. V., and Bowman, J. L. 2001. Establishment of polarity in
892 lateral organs of plants. *Curr. Biol*. **11**: 1251-1260.
893 Erbasol Serbes I, Palovaara J, Groß-Hardt R. Development and function of the flowering
894 plant female gametophyte 2019. *Curr. Top. Dev. Biol*. **131**: 401-434.
895 Favaro, R., Pinyopich, A., Battaglia, R., Kooiker, M., Borghi, L., Ditta, G., Yanofsky, M. F.,
896 Kater, M. M., and Colombo, L. 2003. MADS-box protein complexes control carpel and
897 ovule development in Arabidopsis. *Plant Cell*. **15**: 2603-2611.
898 Ferreira, L. G., de Alencar Dusi, D. M., Irsigler, A. S. T., Gomez, A. C. M. M., Mendes, M.
899 A., Colombo, L., and Campos Carneiro, V. T. 2018. GID1 expression is associated with
900 ovule development of sexual and apomitic plants. *Plant Cell Rep*. **37**: 293-306.
901 Franks, R. G., Wang, C., Levin, J. Z., and Liu, Z. 2002. SEUSS, a member of a novel family
902 of plant regulatory proteins, represses floral homeotic gene expression with LEUNIG.
903 *Development*. **129**: 253-263.
904 Frebort, I., Kowalska, M., Hluska, T., Frebortova, J., and Galuszka, P. 2011. Evolution of
905 cytokinin biosynthesis and degradation. *J. Exp. Bot.* **62**: 2431-2452.
906 Fridman, Y., and Savaldi-Goldstein, S. 2013. Brassinosteroids in growth control: how, when
907 and where. *Plant Sci.* **209**: 24-31.
908 Friedt, W., Tu, J., and Fu, T. 2018. Academic and economic importance of *Brassica napus*
909 (rapeseed). In *The Brassica napus Genome*; Liu, S., Snowdon, R., Chalhoub, B., Ed.
910 Springer: Cham, Switzerland, pp 1-20.

911 Gaiser, J. C., Robinson-Beers, K., and Gasser, C. S. 1995. The Arabidopsis SUPERMAN
912 gene mediates asymmetric growth of the outer integument of ovules. *Plant Cell*. **7**: 333-
913 345.
914 Galbiati, F., Sinha Roy, D., Simonini, S., Cucinotta, M., Ceccato, L., Cuesta, C., Simaskova,
915 M., Benkova, E., Kamiuchi, Y., Aida, M., Weijers, D., Simon, R., Masiero, S., and
916 Colombo, L. 2013. An integrative model of the control of ovule primordia formation.
917 *Plant J*. **76**: 446-455.
918 Gallego•Giraldo, C., Hu, J., Urbez, C., Gomez, M. D., Sun, T. P., and Perez•Amador, M. A.
919 2014. Role of the gibberellin receptors GID 1 during fruit•set in Arabidopsis. *Plant J*.
920 **79**: 1020-1032.
921 Gasser, C. S., and Skinner, D. J. 2019. Development and evolution of the unique ovules of
922 flowering plants. *Cur. Top. Develop. Biol.* **131:** 373-399.
923 Gifford, M. L., Dean, S., and Ingram, G. C. 2003. The Arabidopsis ACR4 gene plays a role in
924 cell layer organization during ovule integument and sepal margin development.
925 *Development*. **130**: 4249-4258.
926 Goldental-Cohen, S., Israeli, A., Ori, N., and Yasuor, H. 2017. Auxin response dynamics
927 during wild-type and entire flower development in tomato. *Plant Cell. Physiol*. **58**:
928 1661-1672.
929 Gonçalves, B., Hasson, A., Belcram, K., Cortizo, M., Morin, H., Nikovics, K., Vialette-
930 Guiraud, A., Takeda, S., Aida, M., Laufs, P., and Arnaud, N. 2015. A conserved role for
931 CUP•SHAPED COTYLEDON genes during ovule development. *Plant J*. **83**: 732-742.
932 Gomez, M. D., Barro-Trastoy, D., Escoms, E., Saura-Sanchez, M., Sanchez, I., Briones-
933 Moreno, A., Vera-Sirera, F., Carrera, E., Ripoll, J. J., Yanofsky, M. F., Lopez-Diaz, I.,
934 Alonso, J. M., Perez-Amador, M. A. 2018. Gibberellins negatively modulate ovule
935 number in plants. *Development*. **145**: dev163865.

936 Gomez, M. D., Barro-Trastoy, D., Fuster-Almunia, C., Tornero, P., Alonso, J. M., Perez-
937 Amador, M. A. 2020. Gibberellin-mediated RGL1 degradation regulates embryo sac
938 development in Arabidopsis. *J. Exp. Bot.*
939 Gomez, M. D., Fuster-Almunia, C., Ocaña-Cuesta, J., Alonso, J. M., and Perez-Amador, M.
940 A. 2019. RGL2 controls flower development, ovule number and fertility in Arabidopsis.
941 *Plant Sci.* **281**: 82-92.
942 Gomez, M. D., Urbez, C., Perez-Amador, M. A., and Carbonell, J. 2011. Characterization of
943 constricted fruit (ctf) mutant uncovers a role for AtMYB117/LOF1 in ovule and fruit
944 development in *Arabidopsis thaliana. PLoS ONE*. **6**: e18760.
945 Gomez, M. D., Ventimilla, D., Sacristan, R., and Perez-Amador, M. A. 2016. Gibberellins
946 regulate ovule integument development by interfering with the transcription factor ATS.
947 *Plant Physiol*. **172**: 2403-2415.
948 Gross-Hardt, R., Lenhard, M., and Laux, T. 2002. WUSCHEL signaling functions in
949 interregional communication during Arabidopsis ovule development. *Genes Develop.*
950 **16**: 1129-1138.
951 Hashimoto, K., Miyashima, S., Sato-Nara, K., Yamada, T., and Nakajima, K. 2018.
952 Functionally diversified members of the MIR165/6 gene family regulate ovule
953 morphogenesis in *Arabidopsis thaliana*. *Plant Cell. Physiol*. **59**: 1017-1026.
954 Hauser, B. A., He, J. Q., Park, S. O., and Gasser, C. S. 2000. TSO1 is a novel protein that
955 modulates cytokinesis and cell expansion in Arabidopsis. *Development*. **127:** 2219-
956 2226.
957 Hedden, P., and Sponsel, V. 2015. A century of gibberellin research. *J. Plant Growth Regul.*
958 **34**: 740-760.
959 Heisler, M. G., and Byrne, M. E. 2020. Progress in understanding the role of auxin in lateral
960 organ development in plants. *Curr. Opin. Plant Biol*. **53**: 73-79.

961 Heisler, M. G., Ohno, C., Das, P., Sieber, P., Reddy, G. V., Long, J. A., and Meyerowitz, E.
962 M. 2005. Patterns of auxin transport and gene expression during primordium
963 development revealed by live imaging of the Arabidopsis inflorescence meristem. *Curr.*
964 *Biol*. **15**: 1899-1911.
965 Hibara, K. I., Takada, S., and Tasaka, M. 2003. CUC1 gene activates the expression of
966 SAM•related genes to induce adventitious shoot formation. *Plant J*. **36**: 687-696.
967 Hill, T. A., Broadhvest, J., Kuzoff, R. K., and Gasser, C. S. 2006. Arabidopsis SHORT
968 INTEGUMENTS 2 is a mitochondrial DAR GTPase. *Genetics*. **174**: 707-718.
969 Huang, H. Y., Jiang, W. B., Hu, Y. W., Wu, P., Zhu, J. Y., Liang, W. Q., Wang, Z. Y., and
970 Lin, W. H. 2013. BR signal influences Arabidopsis ovule and seed number through
971 regulating related genes expression by BZR1. *Mol. Plant*. **6**: 456-469.
972 Hwang, I., Sheen, J., and Müller, B. 2012. Cytokinin signaling networks. *Annu. Rev. Plant*
973 *Biol*. **63**: 353-380.
974 Ishida, T., Aida, M., Takada, S., and Tasaka, M. 2000. Involvement of CUP-SHAPED
975 COTYLEDON genes in gynoecium and ovule development in *Arabidopsis thaliana*.
976 *Plant Cell. Physiol*. **41**: 60-67.
977 Jia, D., Chen, L. G., Yin, G., Yang, X., Gao, Z., Guo, Y., Sun, Y., and Tang, W. 2020.
978 Brassinosteroids regulate outer ovule integument growth in part via the control of
979 INNER NO OUTER by BRASSINOZOLE•RESISTANT family transcription factors. *J.*
980 *Integr. Plant Biol*. **0**: 1-19
981 Jung, J. H., and Park, C. M. 2007. MIR166/165 genes exhibit dynamic expression patterns in
982 regulating shoot apical meristem and floral development in Arabidopsis. *Planta*. **225**:
983 1327-1338.
984 Khan, S. U., Yangmiao, J., Liu, S., Zhang, K., Khan, M. H. U., Zhai, Y., Olalekan, A., Fan,
985 C., and Y Zhou, Y. 2019. Genome-wide association studies in the genetic dissection of

ovule number, seed number, and seed weight in *Brassica napus* L. *Ind. Crop. Prod*. **142:** 111877.

Kelley, D. R., Arreola, A., Gallagher, T. L., and Gasser, C. S. 2012. ETTIN (ARF3) physically interacts with KANADI proteins to form a functional complex essential for integument development and polarity determination in Arabidopsis. *Development*. **139**: 1105-1109.

Kelley, D. R., Skinner, D. J., and Gasser, C. S. 2009. Roles of polarity determinants in ovule development. *Plant J*. **57**: 1054-1064.

Klucher, K. M., Chow, H., Reiser, L., and Fischer, R. L. 1996. The AINTEGUMENTA gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene APETALA2. *Plant Cell*. **8**: 137-153.

Krizek, B. A. 1999. Ectopic expression of AINTEGUMENTA in Arabidopsis plants results in increased growth of floral organs. *Dev. Genet.* **25**: 224-236.

Krizek, B. A., Blakley, I. C., Ho, Y. Y., Freese, N., and Loraine, A. E. 2020. The Arabidopsis transcription factor AINTEGUMENTA orchestrates patterning genes and auxin signaling in the establishment of floral growth and form. *Plant J*. **103**: 752-768

Laufs, P., Peaucelle, A., Morin, H., and Traas, J. 2004. MicroRNA regulation of the CUC genes is required for boundary size control in Arabidopsis meristems. *Development*. **131**: 4311-4322.

Larsson, E., Roberts, C. J., Claes, A. R., Franks, R. G., and Sundberg, E. 2014. Polar auxin transport is essential for medial versus lateral tissue specification and vascular-mediated valve outgrowth in Arabidopsis gynoecia. *Plant Physiol*. **166**: 1998-2012.

Larsson, E., Vivian-Smith, A., Offringa, R., and Sundberg, E. 2017. Auxin homeostasis in Arabidopsis ovules is anther-dependent at maturation and changes dynamically upon fertilization. *Front. Plant Sci*. **8**: 1735.

1011 Lee, D. K., Geisler, M., and Springer, P. S. 2009. LATERAL ORGAN FUSION1 and
1012 LATERAL ORGAN FUSION2 function in lateral organ separation and axillary
1013 meristem formation in Arabidopsis. *Development*. **136**: 2423-2432.
1014 Leyser, O. (2018). Auxin signaling. *Plant Physiol*. **176**: 465-479.
1015 Liao, S., Wang, L., Li, J., and Ruan, Y. L. 2020. Cell wall invertase is essential for ovule
1016 development through sugar signaling rather than provision of carbon. *Plant Physiol*.
1017 **183**: 1126-1144.
1018 Lieber, D., Lora, J., Schrempp, S., Lenhard, M., and Laux, T. 2011. Arabidopsis WIH1 and
1019 WIH2 genes act in the transition from somatic to reproductive cell fate. *Curr. Biol*. **21**:
1020 1009-1017.
1021 Lituiev, D. S., Krohn, N. G., Müller, B., Jackson, D., Hellriegel, B., Dresselhaus, T., and
1022 Grossniklaus, U. 2013. Theoretical and experimental evidence indicates that there is no
1023 detectable auxin gradient in the angiosperm female gametophyte. *Development*. **140**:
1024 4544-4553.
1025 Li, L. C., Qin, G. J., Tsuge, T., Hou, X. H., Ding, M. Y., Aoyama, T., Oka, Z., Chen, Z., Gu,
1026 H., Zhao, Y., and Qu, L. J. 2008. SPOROCYTELESS modulates YUCCA expression to
1027 regulate the development of lateral organs in Arabidopsis. *New Phytol*. **179**: 751-764.
1028 Liu, H. H., Xiong, F., Duan, C. Y., Wu, Y. N., Zhang, Y., and Li, S. 2019. Importin ^{2}4
1029 mediates nuclear import of GRF-interacting factors to control ovule development in
1030 Arabidopsis. *Plant Physiol*. **179**: 1080-1092.
1031 Liu, Z., Franks, R. G., and Klink, V. P. 2000. Regulation of gynoecium marginal tissue
1032 formation by LEUNIG and AINTEGUMENTA. *Plant Cell*. **12**: 1879-1891.
1033 Lora, J., Yang, X., and Tucker, M. R. 2019. Establishing a framework for female germline
1034 initiation in the plant ovule. *J. Exp. Bot.* **70**: 2937-2949.

Mallory, A. C., Dugas, D. V., Bartel, D. P., and Bartel, B. 2004. MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. *Curr. Biol*. **14**: 1035-1046.

Marin-de la Rosa, N., Sotillo, B., Miskolczi, P., Gibbs, D. J., Vicente, J., Carbonero, P., Oñate-Sanchez, L., Holdsworth, M. J., Bhalerao, R., Alabadi, D., and Blázquez, M. A. 2014. Large-scale identification of gibberellin-related transcription factors defines group VII ETHYLENE RESPONSE FACTORS as functional DELLA partners. *Plant Physiol*. **166**: 1022-1032.

Marsch-Martínez, N., and de Folter, S. 2016. Hormonal control of the development of the gynoecium. *Curr. Opin. Plant Biol*. **29**: 104-114.

Matilla, A. J. 2019. Seed coat formation: its evolution and regulation. *Seed Sci. Res.* **29**: 215-226.

McAbee, J. M., Hill, T. A., Skinner, D. J., Izhaki, A., Hauser, B. A., Meister, R. J., Reddy, G. V., Meyerowitz, E. M., Bowman, J. L., and Gasser, C. S. 2006. ABERRANT TESTA SHAPE encodes a KANADI family member, linking polarity determination to separation and growth of Arabidopsis ovule integuments. *Plant J.* **46**: 522-531.

Mendham, N. J., Shipway, P. A., and Scott, R. K. 1981. The effects of delayed sowing and weather on growth, development and yield of winter oil-seed rape (*Brassica napus*). *J. Agric. Sci.* **96**: 389-416.

Mizukami, Y., and Fischer, R. L. 2000. Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. *Proc. Natl. Acad. Sci. USA*. **97**: 942-947.

Modrusan, Z., Reiser, L., Feldmann, K. A., Fischer, R. L., and Haughn, G. W. 1994. Homeotic transformation of ovules into carpel-like structures in Arabidopsis. *Plant Cell*. **6**: 333-349.

1060 Nemhauser, J. L., Feldman, L. J., and Zambryski, P. C. 2000. Auxin and ETTIN in
1061 Arabidopsis gynoecium morphogenesis. *Development*. **127**: 3877-3888.
1062 Nikovics, K., Blein, T., Peaucelle, A., Ishida, T., Morin, H., Aida, M., and Laufs, P. 2006.
1063 The balance between the MIR164A and CUC2 genes controls leaf margin serration in
1064 Arabidopsis. *Plant Cell*. **18**: 2929-2945.
1065 Nishimura, C., Ohashi, Y., Sato, S., Kato, T., Tabata, S., and Ueguchi, C. 2004. Histidine
1066 kinase homologs that act as cytokinin receptors possess overlapping functions in the
1067 regulation of shoot and root growth in Arabidopsis. *Plant Cell*. **16**: 1365-1377.
1068 Nole-Wilson, S., Azhakanandam, S., and Franks, R. G. 2010a. Polar auxin transport together
1069 with AINTEGUMENTA and REVOLUTA coordinate early Arabidopsis gynoecium
1070 development. *Dev. Biol*. **346**: 181-195.
1071 Nole-Wilson, S., and Krizek, B. A. 2006. AINTEGUMENTA contributes to organ polarity
1072 and regulates growth of lateral organs in combination with YABBY genes. *Plant*
1073 *Physiol*. **141**: 977-987.
1074 Nole-Wilson, S., Rueschhoff, E. E., Bhatti, H., and Franks, R. G. 2010b. Synergistic
1075 disruptions in *seuss cyp85A2* double mutants reveal a role for brassinolide synthesis
1076 during gynoecium and ovule development. *BMC Plant Biol*. **10**: 198.
1077 Nomura, T., Kushiro, T., Yokota, T., Kamiya, Y., Bishop, G. J., and Yamaguchi, S. 2005. The
1078 last reaction producing brassinolide is catalyzed by cytochrome P-450s, CYP85A3 in
1079 tomato and CYP85A2 in Arabidopsis. *J. Biol. Chem.* **280**: 17873-17879.
1080 Okada, K., Ueda, J., Komaki, M. K., Bell, C. J., and Shimura, Y. 1991. Requirement of the
1081 auxin polar transport system in early stages of Arabidopsis floral bud formation. *Plant*
1082 *Cell*. **3**: 677-684.
1083 Overvoorde, P., Fukaki, H., and Beeckman, T. 2010. Auxin control of root development. *Cold*
1084 *Spring Harb. Perspect. Biol.* **2**: a001537.

1085 Pajoro, A., Biewers, S., Dougali, E., Leal Valentim, F., Mendes, M. A., Porri, A., Coupland,
1086 G., de Peer, Y. V., van Dijk, A. D. J., Colombo, L., Davies, B., and Angenent, G. C.
1087 2014. The (r) evolution of gene regulatory networks controlling Arabidopsis plant
1088 reproduction: a two-decade history. *J. Exp. Bot.* **65**: 4731-4745.
1089 Park, S. O., Zheng, Z., Oppenheimer, D. G., and Hauser, B. A. 2005. The PRETTY FEW
1090 SEEDS2 gene encodes an Arabidopsis homeodomain protein that regulates ovule
1091 development. *Development*. **132**: 841-849.
1092 Pattison, R. J., and Catala, C. 2012. Evaluating auxin distribution in tomato (*Solanum*
1093 *lycopersicum*) through an analysis of the PIN and AUX/LAX gene families. *Plant J*. **70**:
1094 585-598.
1095 Perez-España, V. H., Sanchez-Leon, N., and Vielle-Calzada, J. P. 2011. CYP85A1 is required
1096 for the initiation of female gametogenesis in Arabidopsis thaliana. *Plant Signal. Behav*.
1097 **6**: 321-326.
1098 Petrella, R., Caselli, F., Roig•Villanova, I., Vignati, V., Chiara, M., Ezquer, I., Tadini, L.,
1099 Kater, M. M., and Gregis, V. 2020. BPC transcription factors and a Polycomb Group
1100 protein confine the expression of the ovule identity gene SEEDSTICK in Arabidopsis.
1101 *Plant J*. **102**: 582-599.
1102 Phillips, A. R., and Evans, M. M. 2020. Maternal regulation of seed growth and patterning in
1103 flowering plants. *Curr. Top. Dev. Biol*. **140**: 257-282.
1104 Pillitteri, L. J., Bemis, S. M., Shpak, E. D., and Torii, K. U. 2007. Haploinsufficiency after
1105 successive loss of signalling reveals a role for ERECTA-family genes in Arabidopsis
1106 ovule development. *Development*. **134**: 3099-3109.
1107 Pinyopich, A., Ditta, G. S., Savidge, B., Liljegren, S. J., Baumann, E., Wisman, E., and
1108 Yanofsky, M. F. 2003. Assessing the redundancy of MADS-box genes during carpel
1109 and ovule development. *Nature*. **424**: 85-88.

1110 Pinto, S. C., Mendes, M. A., Coimbra, S., and Tucker, M. R. 2019. Revisiting the female
1111 germline and its expanding toolbox. *Trends Plant Sci*. **24**: 455-457
1112 Planas-Riverola, A., Gupta, A., Betegon-Putze, I., Bosch, N., Ibañes, M., and Caño-Delgado,
1113 A. I. 2019. Brassinosteroid signaling in plant development and adaptation to stress.
1114 *Development*. **146**: dev151894.
1115 Reinhardt, D., Mandel, T., and Kuhlemeier, C. 2000. Auxin regulates the initiation and radial
1116 position of plant lateral organs. *Plant Cell*. **12**: 507-518.
1117 Reiser, L., Modrusan, Z., Margossian, L., Samach, A., Ohad, N., Haughn, G. W., and Fischer,
1118 R. L. 1995. The *BELL1* gene encodes a homeodomain protein involved in pattern
1119 formation in the Arabidopsis ovule primordium. *Cell*. **83**: 735-742.
1120 Reyes-Olalde, J. I., and De Folter, S. 2019. Control of stem cell activity in the carpel margin
1121 meristem (CMM) in Arabidopsis. *Plant Reprod.* **32**: 123-136.
1122 Reyes-Olalde, J. I., Zúñiga-Mayo, V. M., Serwatowska, J., Montes, R. A. C., Lozano-
1123 Sotomayor, P., Herrera-Ubaldo, H., Gonzalez-Aguilera, K. L., Ballester, P., Ripoll, J. J.,
1124 Ezquer, I., Paolo, D., Heyl, A., Colombo, L., Yanofsky, M. F., Ferrandiz, C., Marsch-
1125 Martinez, N., and de Folter, S. 2017. The bHLH transcription factor SPATULA enables
1126 cytokinin signaling, and both activate auxin biosynthesis and transport genes at the
1127 medial domain of the gynoecium. *PLoS Genet.* **13**: e1006726.
1128 Rhoades, M. W., Reinhart, B. J., Lim, L. P., Burge, C. B., Bartel, B., and Bartel, D. P. 2002.
1129 Prediction of plant microRNA targets. *Cell*. **110**: 513-520.
1130 Rizza, A., and Jones, A. M. 2019. The makings of a gradient: spatiotemporal distribution of
1131 gibberellins in plant development. *Curr. Opin. Plant Biol*. **47**: 9-15.
1132 Roe, J. L., Nemhauser, J. L., and Zambryski, P. C. 1997. TOUSLED participates in apical
1133 tissue formation during gynoecium development in Arabidopsis. *Plant Cell*. **9**: 335-353.

1134 Robinson-Beers, K., Pruitt, R. E., and Gasser, C. S. 1992. Ovule development in wild-type
1135 Arabidopsis and two female-sterile mutants. *Plant Cell*. **4**: 1237-1249.
1136 Rodriguez-Cazorla, E., Ortuño-Miquel, S., Candela, H., Bailey-Steinitz, L. J., Yanofsky, M.
1137 F., Martinez-Laborda, A., Ripoll, J. J., and Vera, A. 2018. Ovule identity mediated by
1138 pre-mRNA processing in Arabidopsis. *PLoS Genet.* **14**: e1007182.
1139 Rodriguez•Cazorla, E., Ripoll, J. J., Ortuño•Miquel, S., Martinez•Laborda, A., and Vera, A.
1140 2020. Dissection of the Arabidopsis HUA•PEP gene activity reveals that ovule fate
1141 specification requires restriction of the floral A•function. *New Phytol*. **227**: 1222-1234
1142 Sauquet, H., von Balthazar, M., Magallon, S., Doyle, J. A., Endress, P. K., Bailes, E. J.,
1143 Barroso de Morais, E., Bull-Hereñu, K., Carrive, L., Chartier, M., Chomicki, G., Coiro,
1144 M., Cornette, R., Ottra, J. H. L., Epicoco, C., Foster, C. S. P., Jabbour, F., Haevermans,
1145 A., Haevermans, T., Hernandez, R., Little, S. A., Löfstrand, S., Luna, J. A., Massoni, J.,
1146 Nadot, S., Pamperl, S., Prieu, C., Reyes, E., dos Santos, P., Schoonderwoerd, K. M.,
1147 Sontag, S., Soulebeau, A., Staedler, Y., Tschan, G. F., Leung, A. W. S., and
1148 Schönenberger, J.. 2017. The ancestral flower of angiosperms and its early
1149 diversification. *Nat. Commun.* **8**: 1-10.
1150 Schauer, S. E., Jacobsen, S. E., Meinke, D. W., and Ray, A. 2002. DICER-LIKE1: blind men
1151 and elephants in Arabidopsis development. *Trends Plant Sci.* **7**: 487-491.
1152 Schiefthaler, U., Balasubramanian, S., Sieber, P., Chevalier, D., Wisman, E., and Schneitz, K.
1153 1999. Molecular analysis of NOZZLE, a gene involved in pattern formation and early
1154 sporogenesis during sex organ development in *Arabidopsis thaliana*. *Proc. Natl. Acad.*
1155 *Sci. USA*. **96**: 11664-11669.
1156 Schneitz, K., Baker, S. C., Gasser, C. S., and Redweik, A. 1998. Pattern formation and growth
1157 during floral organogenesis: HUELLENLOS and AINTEGUMENTA are required for

the formation of the proximal region of the ovule primordium in *Arabidopsis thaliana*. *Development*. **125**: 2555-2563.

Schneitz, K., Hülskamp, M., Kopczak, S. D., and Pruitt, R. E. 1997. Dissection of sexual organ ontogenesis: a genetic analysis of ovule development in *Arabidopsis thaliana*. *Development*. **124**: 1367-1376.

Schneitz, K., Hülskamp, M., and Pruitt, R. E. 1995. Wild•type ovule development in *Arabidopsis thaliana*: a light microscope study of cleared whole•mount tissue. *Plant J*. **7**: 731-749.

Schubert, R., Dobritzsch, S., Gruber, C., Hause, G., Athmer, B., Schreiber, T., Marillonnet, S., Okabe, Y., Ezura, H., Acosta, I. F., Tarkowska, D., and Hause, B. 2019. Tomato MYB21 acts in ovules to mediate jasmonate-regulated fertility. *Plant Cell*. **31**: 1043-1062.

Shi, J., Zhan, J., Yang, Y., Ye, J., Huang, S., Li, R., Wang, X., Liu, G., and Wang, H. 2015. Linkage and regional association analysis reveal two new tightly-linked major-QTLs for pod number and seed number per pod in rapeseed (*Brassica napus* L.). *Sci. Rep.* **5**: 14481.

Shirley, N. J., Aubert, M. K., Wilkinson, L. G., Bird, D. C., Lora, J., Yang, X., and Tucker, M. R. 2019. Translating auxin responses into ovules, seeds and yield: Insight from Arabidopsis and the cereals. *J. Integr. Plant Biol.* **61**: 310-336.

Sieber, P., Gheyselinck, J., Gross-Hardt, R., Laux, T., Grossniklaus, U., and Schneitz, K. 2004. Pattern formation during early ovule development in *Arabidopsis thaliana*. *Dev. Biol.* **273**: 321-334.

Šimaškova, M., O'Brien, J. A., Khan, M., Van Noorden, G., Ötvös, K., Vieten, A., De Clercq, I., Van Haperen, J. M. A., Cuesta, C., Hoyerova, K., Vanneste, S., Marhavy, P., Wabnik, K., Van Breusegem, F., Nowack, M., Murphy, A., Friml, J., Weijers, D.,

1183 Beeckman, T., Benkova, E. 2015. Cytokinin response factors regulate PIN-FORMED
1184 auxin transporters. *Nature Commun.* **6**: 8717.
1185 Simon, M. K., Skinner, D. J., Gallagher, T. L., and Gasser, C. S. 2017. Integument
1186 development in Arabidopsis depends on interaction of YABBY protein INNER NO
1187 OUTER with coactivators and corepressors. *Genetics*. **207**: 1489-1500.
1188 Simonini, S., and Østergaard, L. 2019. Female reproductive organ formation: A multitasking
1189 endeavor. *Curr. Top. Dev. Biol*. **131**: 337-371.
1190 Smaczniak, C., Immink, R. G., Angenent, G. C., and Kaufmann, K. 2012. Developmental and
1191 evolutionary diversity of plant MADS-domain factors: insights from recent studies.
1192 *Development*. **139**: 3081-3098.
1193 Sohlberg, J. J., Myrenås, M., Kuusk, S., Lagercrantz, U., Kowalczyk, M., Sandberg, G., and
1194 Sundberg, E. 2006. STY1 regulates auxin homeostasis and affects apical–basal
1195 patterning of the Arabidopsis gynoecium. *Plant J*. **47**: 112-123.
1196 Smyth, D. R., Bowman, J. L., and Meyerowitz, E. M. 1990. Early flower development in
1197 Arabidopsis. *Plant Cell*. **2**: 755-767.
1198 Sun, T. P. 2011. The molecular mechanism and evolution of the GA–GID1–DELLA signaling
1199 module in plants. *Curr Biol*. **21**: 338-345.
1200 Tanaka, H., Watanabe, M., Sasabe, M., Hiroe, T., Tanaka, T., Tsukaya, H., Ikezaki, M.,
1201 Machida, C., and Machida, Y. 2007. Novel receptor-like kinase ALE2 controls shoot
1202 development by specifying epidermis in Arabidopsis. *Development*. **134**: 1643-1652.
1203 Thomson, B., and Wellmer, F. 2019. Molecular regulation of flower development. *Curr. Top.*
1204 *Dev. Biol*. **131**: 185-210.
1205 Truernit, E., and Haseloff, J. 2008. Arabidopsis thaliana outer ovule integument
1206 morphogenesis: ectopic expression of KNAT1 reveals a compensation mechanism.
1207 *BMC Plant Biol*. **8**: 35.

van Berkel, K., de Boer, R. J., Scheres, B., and ten Tusscher, K. 2013. Polar auxin transport: models and mechanisms. *Development*. **140**: 2253-2268.

van der Knaap, E., Chakrabarti, M., Chu, Y. H., Clevenger, J. P., Illa-Berenguer, E., Huang, Z., Keyhaninejad, N., Mu, Q., Sun, L., Wang, Y., and Wu, S. 2014. What lies beyond the eye: the molecular mechanisms regulating tomato fruit weight and shape. *Front. Plant Sci.* **5**: 227.

Villanueva, J. M., Broadhvest, J., Hauser, B. A., Meister, R. J., Schneitz, K., and Gasser, C. S. 1999. INNER NO OUTER regulates abaxial–adaxial patterning in Arabidopsis ovules. *Genes Develop*. **13**: 3160-3169.

Vroemen, C. W., Mordhorst, A. P., Albrecht, C., Kwaaitaal, M. A., and de Vries, S. C. 2003. The CUP-SHAPED COTYLEDON3 gene is required for boundary and shoot meristem formation in Arabidopsis. *Plant Cell*. **15**: 1563-1577.

Wang, H., Liu, Y., Bruffett, K., Lee, J., Hause, G., Walker, J. C., and Zhang, S. 2008. Haplo-insufficiency of MPK3 in MPK6 mutant background uncovers a novel function of these two MAPKs in Arabidopsis ovule development. *Plant Cell*. **20**: 602-613.

Wang, Y., and Jiao, Y. 2018. Auxin and above-ground meristems. *J. Exp. Bot.* **69:** 147-154.

Wei, S. J., Chai, S., Zhu, R. M., Duan, C. Y., Zhang, Y., and Li, S. 2020. HUA ENHANCER1 Mediates Ovule Development. *Front. Plant Sci.* **11**: 397.

Weijers, D., Nemhauser, J., and Yang, Z. 2018. Auxin: small molecule, big impact. *J. Exp. Bot.* **69**: 133-136.

Wynn, A. N., Seaman, A. A., Jones, A. L., and Franks, R. G. 2014. Novel functional roles for PERIANTHIA and SEUSS during floral organ identity specification, floral meristem termination, and gynoecial development. *Front. Plant Sci.* **5**: 130.

1231 Xiao, H., Radovich, C., Welty, N., Hsu, J., Li, D., Meulia, T., and van der Knaap, E. 2009.
1232 Integration of tomato reproductive developmental landmarks and expression profiles,
1233 and the effect of SUN on fruit shape. *BMC Plant Biol*. **9**: 49.
1234 Yamada, T., Sasaki, Y., Hashimoto, K., Nakajima, K., and Gasser, C. S. 2016. CORONA,
1235 PHABULOSA and PHAVOLUTA collaborate with BELL1 to confine WUSCHEL
1236 expression to the nucellus in Arabidopsis ovules. *Development*. **143**: 422-426.
1237 Yamaguchi, N., Wu, M. F., Winter, C. M., Berns, M. C., Nole-Wilson, S., Yamaguchi, A.,
1238 Coupland, G., Krizek, B. A., and Wagner, D. 2013. A molecular framework for auxin-
1239 mediated initiation of flower primordia. *Dev. Cell*. **24**: 271-282.
1240 Yang, W. C., Ye, D., Xu, J., and Sundaresan, V. 1999. The SPOROCYTELESS gene of
1241 Arabidopsis is required for initiation of sporogenesis and encodes a novel nuclear
1242 protein. *Genes Develop*. **13**: 2108-2117.
1243 Yuan, J., and Kessler, S. A. 2019. A genome-wide association study reveals a novel regulator
1244 of ovule number and fertility in *Arabidopsis thaliana*. *PLoS Genet.* **15**: e1007934.
1245 Žadnikova, P., and Simon, R. 2014. How boundaries control plant development. *Curr. Opin.*
1246 *Plant Biol*. **17**: 116-125.
1247 Zhou, J. J., and Luo, J. 2018. The PIN-FORMED auxin efflux carriers in plants. *Int. J. Mol.*
1248 *Sci.* **19**: 2759.
1249 Zuñiga-Mayo, V. M., Baños-Bayardo, C. R., Diaz-Ramirez, D., Marsch-Martinez, N., and de
1250 Folter, S. 2018. Conserved and novel responses to cytokinin treatments during flower
1251 and fruit development in Brassica napus and *Arabidopsis thaliana*. *Sci. Rep.* **8**: 1-10.
1252 Zuñiga-Mayo, V. M., Gomez-Felipe, A., Herrera-Ubaldo, H., and De Folter, S. 2019.
1253 Gynoecium development: networks in Arabidopsis and beyond. *J. Exp. Bot.* **70**: 1447-
1254 1460.

Tables

Table 1. Genes involved in ovule initiation.

Gene	Family or protein type	Involved in	Mutant phenotype aggravated by loss of	Reference
CUC1, *CUC2*	NAC transcription factors	Boundaries establishment		Ishida *et al*. (2000); Galbiati *et al*. (2013); Gonçalves *et al*. (2015)
CUC3	NAC transcription factors	Boundaries establishment		Vroemen *et al*. (2003) Gonçalves *et al*. (2015)
MIR164A	microRNA	Boundaries establishment		Gonçalves *et al*. (2015)
LOF1	MYB transcription factor	Boundaries establishment		Gomez *et al*. (2011)
ANT	AP2 transcription factor	Primordium growth		Elliott *et al*. (1996); Klucher *et al*. (1996)
			HULLENLOS (*HLL*)	Schneitz *et al*. (1998)
			LEUNIG (*LUG*)	Liu *et al*. (2000)
			FILAMENTOUS FLOWER (*FIL*)	Nole-Wilson and Krizek (2006)
			SEUSS (*SEU*)	Azhakanadam *et al*. (2008)
			SEUSS-LIKE 1 (*SLK1), SLK2*	Bao *et al*. (2010)
			REVOLUTA (*REV*)	Nole-Wilson *et al*. (2010a)
			PHERIANTHIA (*PAN*)	Wynn *et al*. (2014)

	NERD1	Golgi membrane protein	Ovule number	Yuan and Kessler (2019)
	CWIN2, *CWIN4*	Cell wall sucrose invertases	Ovule initiation	Liao *et al*. (2020)

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Table 2. Hormones involved in ovule initiation.

Hormone	**Role**			
Auxins	Promote ovule primordia initiation from placenta.			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	DR5rev:GFP	Monitors auxin response	Tip of primordia	Benkova *et al*. (2003); Ceccato *et al*. (2013)
	TAA1 (*in situ*)	Indicates auxin biosynthesis	CMM and epidermis of ovule primordia	Nole-Wilson *et al*. (2010a)
	pPIN1:PIN1-GFP, pPIN3:PIN3-GFP	Traces auxin efflux	Membrane of the ovule primordia outer cell layer.	Benkova *et al*. (2003); Ceccato *et al*. (2013)
	pMP:MP-GFP	Indicates auxin signaling	Ovule primordia (stage 1-I), primordia boundaries (stage 1-II)	Galbiati *et al*. (2013)
	Treatment/mutant	**Usage**	**Ovule number**	**Reference**
	NPA	Blocks auxin transport	"	Okada *et al*. (1991); Nemhauser *et al*. (2000)
	pin1-5	Compromises auxin transport	"	Bencivenga *et al*. (2012)
Related genetic factors	**Gene name**	**Family or protein type**	**Related with**	**Reference**
	CUC1, CUC2	NAC transcription factors	*PIN1*	Galbiati *et al*. (2013)
			MP	Galbiati *et al*. (2013)
	ANT	AP2 transcription factor	*MP*	Yamaguchi *et al*. (2013)

				Galbiati *et al*. (2013)
CKs	Positively regulate ovule number			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	pAHK2:GUS, *pAHK3:GUS*, *pAHK4:GUS*	Indicates CK signaling.	Carpel and ovule primordia	Nishimura *et al*. (2004), Bencivenga *et al*. (2012)
	pCRF2:3xGFP, *pCRF6:GUS*	Indicates CK signaling.	Placenta (*CRF2*) and ovule primordia (*CRG6*)	Nishimura *et al*. (2004), Bencivenga *et al*. (2012)
	CKX5 (*in situ*)	Indicates CK catabolism	Ovule primordia	Bartrina *et al*. (2011)
	Treatment/mutant	**Usage**	**Ovule number**	**Reference**
	BAP	Synthetic CK.	‘	Galbiati *et al*. (2013); Cucinotta *et al*. (2016)
	ckx3-1 ckx5-1	Compromises CK catabolism	‘	Bartrina *et al*. (2011)
	cre1-12 ahk2-2 ahk3	Compromises CK perception	“	Bencivenga *et al*. (2012)
	crf2 crf3 crf6	Compromises CK perception	“	Cucinotta *et al*. (2016)
	arr1 arr10 arr12	Compromises CK perception	“	Reyes-Olalde *et al*. (2017)
	ugt85a3	Compromises reversible CK inactivation	‘	Cucinotta *et al*. (2018)
Related genetic	**Gene name**	**Family or protein type**	**Related with**	**Reference**
	CUC1, CUC2	NAC transcription factors	*UGT85A3*	Cucinotta *et al*. (2018)

			UGT73C1	Cucinotta *et al*. (2018)
	PIN1	Auxin efflux carrier	*CRF*	Cucinotta *et al*. (2016)
BRs	Positively regulate ovule number			
Evidences	**Treatment/mutant**	**Usage**	**Ovule number**	**Reference**
	Brassinolide	Endogenous natural BR	‘	Barro-Trastoy *et al*. (2020)
	Brassinazole	Inhibits BR biosynthesis	“	Huang *et al*. (2013); Barro-Trastoy *et al*. (2020)
	bri1-5	Compromises BR perception	“	Huang *et al*. (2013)
	bri1-116	Compromises BR perception	“	Jia *et al*. (2020)
	bin2-1	Compromises BR perception	“	Huang *et al*. (2013); Jia *et al*. (2020)
	bzr1-1D	Enhances BR perception	‘	Huang *et al*. (2013); Barro-Trastoy *et al*. (2020)
	det2-1	Compromises BR biosynthesis	“	Huang *et al*. (2013); Barro-Trastoy *et al*. (2020)
	cyp85a2	Compromises BR biosynthesis	“	Nole-Wilson *et al*. (2010b)
Related genetic factors	**Gene name**	**Family or protein type**	**Related with**	**Reference**
	ANT	AP2 transcription factor	BZR1	Huang *et al*. (2013)
	HLL	Mitochondrial ribosome protein	BZR1	Huang *et al*. (2013)

	AP2	AP2 transcription factor	BZR1	Huang *et al*. (2013)
GAs	Negatively regulate ovule number			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	pGID1A:GID1A-GUS, pGID1B:GID1B-GUS	Indicates GA signaling	Placenta and ovule primordia	Gomez *et al*. (2018)
	GAI, RGA, RGL2 (*in situ*)	Indicates GA signaling	Placenta and ovule primordia	Gomez *et al*. (2018)
	Treatment/mutant	**Usage**	**Ovule number**	**Reference**
	$GA_4 + GA_7$	Bioactive GAs	“	Gomez *et al*. (2018)
	global	Induces constitutive GA response	“	Gomez *et al*. (2018)
	quadruple	Induces constitutive GA response	“	Gomez *et al*. (2018)
	triple	Induces constitutive GA response	“	Gomez *et al*. (2018)
	gai-1	Blocks GA perception	‘	Gomez *et al*. (2018)
	pRGL2:YPet-rgl2• 17	Blocks GA perception	‘	Gomez *et al*. (2019)
	gid1a gid1b	Blocks GA perception	‘	Gomez *et al*. (2018)

Arrows represent the ovule number phenotype of the different mutants or treatments compare to wild-type or mock, respectively.

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Table 3. Genes involved in ovule patterning.

Gene	Family or protein type	Expressed in	Required for	Mutant phenotype aggravated by loss of	Reference
WUS	Homeobox	Nucellus	Embryo sac and integuments development		Gross-Hard *et al*. (2002); Lieber *et al*. (2011); Yamada *et al*. (2016)
SPL/NZZ	Putative transcription factor	Nucellus, integuments	Nucellus MMC and integuments development		Yang *et al*. (1999); Schiefthaler *et al*. (1999); Balasubramanian and Schneitz, (2000, 2002)
ANT	AP2 transcription factor	Chalaza	Integuments and embryo sac development	*HLL* *SEU*	Elliott *et al*. (1996); Klucher *et al*. (1996) Schneitz *et al*. (1998) Azhakanandam *et al*. (2008)
BEL1	Homeodomain	Chalaza	Inner integument development, outer integument identity	*STK, SHP1, SHP2*	Robinson-Beers *et al*. (1992); Modrusan *et al*. (1994); Reiser *et al*. (1995) Brambilla *et al*. (2007)
STK		Funiculus	Funiculus development		Pinyopich *et al.* (2003)
HUA-PEP		Placenta and ovule	Funiculus development		Rodriguez-Cazorla *et al.* (2018); (2020)

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Table 4. Hormones involved in ovule patterning.

Hormone	**Role**			
Auxins	Proper pattern establishment			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	DR5rev:GFP	Monitors auxin response	Outer layer of the nucellus	Benkova *et al*. (2003); Ceccato *et al*. (2013)
	pTAA1:GFP	Indicates auxin biosynthesis	Boundary between nucellus and chalaza.	Ceccato *et al*. (2013)
	pPIN1:PIN1-GFP	Traces auxin efflux	Outer layer of the nucellus.	Benkova *et al*. (2003); Ceccato *et al*. (2013)
	Mutant	**Usage**	**Ovule phenotype**	**Reference**
	pin1-5	Compromises auxin transport	Finger-like structure	Bencivenga *et al*. (2012)
Related genetic factors	**Gene name**	**Family or protein type**	**Related with**	**Reference**
	SPL/NZZ	Putative transcription factor	*PIN1*	Bencivenga *et al*. (2012)
	BEL1	Homeodomain protein	*PIN1*	Bencivenga *et al*. (2012)
CKs	Proper pattern establishment			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	pIPT1:GUS	Indicates CK biosynthesis	Stage 2-III ovules	Nishimura *et al*. (2004); Bencivenga *et al*. (2012)

	CKX5	Indicates CK catabolism	Chalaza	Bartrina *et al*. (2011)
	pAHK4:GUS, pCRE1:GUS	Indicates CK signaling	Chalaza and developing inner integument	Bencivenga *et al*. (2012)
	pAHK2:GUS	Indicates CK signaling	All tissues, in all stages	Bencivenga *et al*. (2012)
	Treatment/mutant	**Usage**	**Ovule phenotype**	**Reference**
	BAP	Synthetic CK. Increases CK levels	Ovule primordia develops a single structure instead of two integuments	Bencivenga *et al*. (2012)
	cre1-12 ahk2-2 ahk3	Compromises CK perception	Finger-like structure	Bencivenga *et al*. (2012)
Related genetic factors	**Gene name**	**Family or protein type**	**Related with**	**Reference**
	PIN1	Auxin efflux carrier	*CRE1, AHK2, AHK3*, BAP treatments	Bencivenga *et al*. (2012)
	SPL/NZZ	Putative transcription factor	*CRE1, AHK2, AHK3*, BAP treatments	Bencivenga *et al*. (2012)
	BEL1	Homeodomain protein	BAP treatments	Bencivenga *et al*. (2012)

Table 5. Genes involved in integuments (int) morphogenesis.

Gene	**Family or protein type**	**Expressed/Localized in**	**Required for**	**Reference**
*INO**	YABBY gene	Abaxial side of the outer integument	Outer integument development	Gaiser *et al*. (1995) Villanueva *et al*. (1999)
KAN1, KAN2**	KANADI genes		Outer integument development	Eshed *et al*. (2001)
PHB, PHV, CNA	HD-ZIP III genes	Adaxial side of the inner integument	Outer and inner integuments development	Kelley *et al*. (2009)
REV	HD-ZIP III gene	Chalaza	Outer and inner integuments development	Kelley *et al*. (2009)
MIR166	microRNA	Outer integument primordia	Inner integument development	Hashimoto *et al*. (2018)
*ATS**	KANADI gene	Boundary between integuments	Inner integument development and integument separation	McAbee *et al*. (2006)
UNICORN (*UCN*)*	AGC VIII kinase		Maintenance of planar growth of integuments. Correct integument growth orientation	Enugutti *et al*. (2012); Enugutti and Schneitz (2013)
SUPERMAN (*SUP*)	Similar to zinc finger transcription factor		Asymmetric growth of outer integument	Gaiser *et al*. (1995)

STRUBBELIG (*SUB*)*	Receptor-like kinase	Mature ovules, at stage 13	Outer integument development	Chevalier *et al*. (2005)
TOUSLED (*TSL*)*	Nuclear serine/threonine protein kinase		Inner and outer integument development	Roe *et al*. (1997)
PRETTY FEW SEEDS2 (*PFS2*)*	Homeodomain protein	Ovule primordia. Chalaza and nucellus	Directional integuments cell expansion	Park *et al*. (2005)
*LUG**	Glutamine-rich protein with seven WD repeats, transcriptional co-regulator	Developing ovules, at stage 12	Outer integument development	Roe *et al*. (1997); Conner and Liu (2000); Simon *et al*. (2017)
SEU, SLK1, SLK2*	Transcriptional co-regulator		Outer integument development	Franks *et al*. (2002); Bao *et al*. (2010); Simon *et al*. (2017)
KNAT1	Homeodomain protein		Outer integument development	Truernit and Haseloff (2008)
ADA2b/PROPORZI (*PRZ1*)	Transcriptional co-activator	Mature ovules, at stage 13	Outer integument development	Simon *et al*. (2017)
ERECTA (*ER*)*, *ERECTA-LIKE 1*	ERECTA-family genes	Ovule primordia and developing	Outer and inner integument development	Pillitteri *et al*. (2007)

(*ERL1*)*, *ERL2**		integuments		
PICKLE (*PKL*)*	CHD3 chromatin remodeler		Asymmetric integuments growth	Carter *et al*. (2016)
DICER-LIKE1/SHORT INTEGUMENTS 1 (*DCL1/SIN1*)*	RNA helicase/nuclease		Directional integument cell expansion. Asymmetric integuments growth	Robinson-Beers *et al*. (1992); Schauer *et al*. (2002)
HUA ENHANCER1 (*HEN1*)*	miRNAs and siRNAs methyltransferase		Asymmetric integuments growth	Wei *et al*. (2020)
HYPONASTIC LEAVES 1 (*HYL1*)*	dsRNA-binding protein		Asymmetric integuments growth	Wei *et al*. (2020)
*TSO1**	CHC protein	Ovule primordia and funiculus, chalaza and nucellus	Directional integument cell expansion	Hauser *et al*. (2000)
SHORT INTEGUMENTS 2 (*SIN2*)*	Mitochondrial DAR GTPase		Integuments cell division	Broadhvest *et al*. (2002); Hill *et al*. (2006)
ARABIDOPSIS CRINKLY4 (*ACR4*)*	Receptor kinase	Apoplastic compartments between inner and outer cell layer of the outer integument	Integuments cell organization	Gifford *et al*. (2003)

ABNORMAL LEAF SHAPE 2 (*ALE2*)	Receptor kinase, ACR4 homolog		Integuments cell morphology and division	Tanaka *et al*. (2007)
*MPK3, MPK6**	Mitogen-activated protein kinases	Ovule primordia and ovule integuments	Integuments cell division	Wang *et al*. (2008)
IMPORTIN ² 4 (*IMB4*)	Karyopherin, importin	Ovule primordia, chalaza and integuments	Asymmetric integuments growth	Liu *et al*. (2019)
BLASIG (*BAG*)*	Unknown		Inner and outer integuments growth	Schneitz *et al*. (1997)
MOLLIG (*MOL*)*	Unknown		Integuments cell enlargement	Schneitz *et al*. (1997)
LAELLI (*LAL*)*	Unknown		Inner integument development	Schneitz *et al*. (1997)

*Genes known to also affect female gametophyte development

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Table 6. Hormones involved in integument morphogenesis.

Hormone	**Role**			
Auxins	Regulation of the spacing between integuments			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	pTAA1:GFP	Indicates auxin biosynthesis	Inner integument primordia (stage 2-III), funiculus (stages 2-III to 3-II)	Ceccato *et al*. (2013)
	pYUC4:GUS	Indicates auxin biosynthesis	Distal nucellus (stages 3-II to 3-VI)	Ceccato *et al*. (2013)
	pYUC1:n3xGFP, pYUC6:eGFP	Indicates auxin biosynthesis	Funiculus (stage 3-V)	Larsson *et al*. (2017)
	pYUC4:3xGFP	Indicates auxin biosynthesis	Inner integuments (stage 3-V)	Larsson *et al*. (2017)
	pYUC5:eGFP, pYUC8:eGFP	Indicates auxin biosynthesis	Micropylar end of the inner integument (stage 3-V)	Larsson *et al*. (2017)
	DR5rev:GFP	Monitors auxin response	Nucellus, near the micropylar end (stage 3-III)	Ceccato *et al*. (2013)
	pPIN1:PIN1-GFP	Traces auxin efflux	Chalaza and funiculus vascular strand (after stage 3-II)	Ceccato *et al*. (2013) Larsson *et al*. (2017)
	pPIN3:PIN3-GFP	Traces auxin efflux	Funiculus vascular strand (after stage 3-II)	Ceccato *et al*. (2013)
	pPGP1:PGP1-GFP	Traces auxin efflux	Integuments, chalaza, funiculus (stage 3-III to 3-VI)	Lituiev *et al*. (2013)
	pPGP19:PGP19-GFP	Traces auxin efflux	Integuments, chalaza (stage 3-VI)	Lituiev *et al*. (2013)

	pAUX1:AUX1-YFP	Traces auxin influx	Integuments (stage 3-II to stage 3-IV)	Lituiev *et al*. (2013)
	ARF3/ETT1 (*in situ*)	Indicates auxin signaling	Abaxial region of the inner integument (stage 2-IV)	Kelley *et al*. (2012)
Related genetic factors	**Gene name**	**Family or protein type**	**Related with**	**Reference**
	ATS	KANADI gene	*ARF3/ETT*	Kelley *et al*. (2012)
GAs	Regulation of proper integuments development			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	pGAI:GUS, pRGA:GUS, pRGL1:GUS	Indicates GA signaling	Integuments primordia and funiculus (stage 2-IV)	Gomez *et al*. (2016)
	rgl2-5 allele (with *Ds-GUS* insertion)	Indicates GA signaling	Integuments primordia, nucellus, funiculus (stage 2-IV)	Gomez *et al*. (2016)
	pRGL1:YPet-rgl1• 17	Indicates GA signaling	Integuments, nucellus and funiculus (stage 3)	Gomez *et al*. (2020)
	pRGL2:YPet-rgl2• 17	Indicates GA signaling	Funiculus and chalaza (stage 3-IV)	Gomez *et al*. (2019)
	pGID1a:GID1a-GUS	Indicates GA signaling	Integuments (stage 2-III). Embryo sac and integuments (mature ovules).	Gallego-Giraldo *et al*. (2014) Ferreira *et al*. (2018)
	pGID1b:GID1b-GUS	Indicates GA signaling	Integuments (stage 2-III). Chalaza (mature ovules).	Gallego-Giraldo *et al*. (2014) Ferreira *et al*. (2018)
	Treatment/mutant	**Usage**	**Ovule phenotype**	**Reference**

	global	Induces constitutive GA response	Both integuments form two cell layers	Gomez *et al*. (2016)
	quadruple	Induces constitutive GA response	Both integuments form two cell layers	Gomez *et al*. (2016)
Related genetic factors	**Gene name**	**Family or protein type**	**Related with**	**Reference**
	ATS	KANADI gene	*GAI, RGA, GA3ox1*	Gomez *et al*. (2016)
BRs	Regulation of outer integument growth			
Evidences	**Gene reporter line/ *In situ* hybridization**	**Usage**	**Expression**	**Reference**
	pBRI1:BRI1-YFP, pBZR1:BZR1-YFP	Indicates BR signaling	All ovule tissues (stages 2-I to 3-V)	Jia *et al*. (2020)
	Treatment/mutant	**Usage**	**Ovule phenotype**	**Reference**
	bri1-116	Compromises BR perception	Outer integument growth arrest	Jia *et al*. (2020)
	bri1-5	Compromises BR perception	Outer integument growth arrest	Jia *et al*. (2020)
	det2-1	Compromises BR biosynthesis	Outer integument growth arrest	Jia *et al*. (2020)
	bzr-h	Compromises BR perception	Outer integument growth arrest	Jia *et al*. (2020)

	Gene name	Family or protein type	Related with	Reference
Related genetic factors	*INO*	AP2 transcription factor	BZR1	Jia *et al*. (2020)

Figures and Figure captions

Figure 1

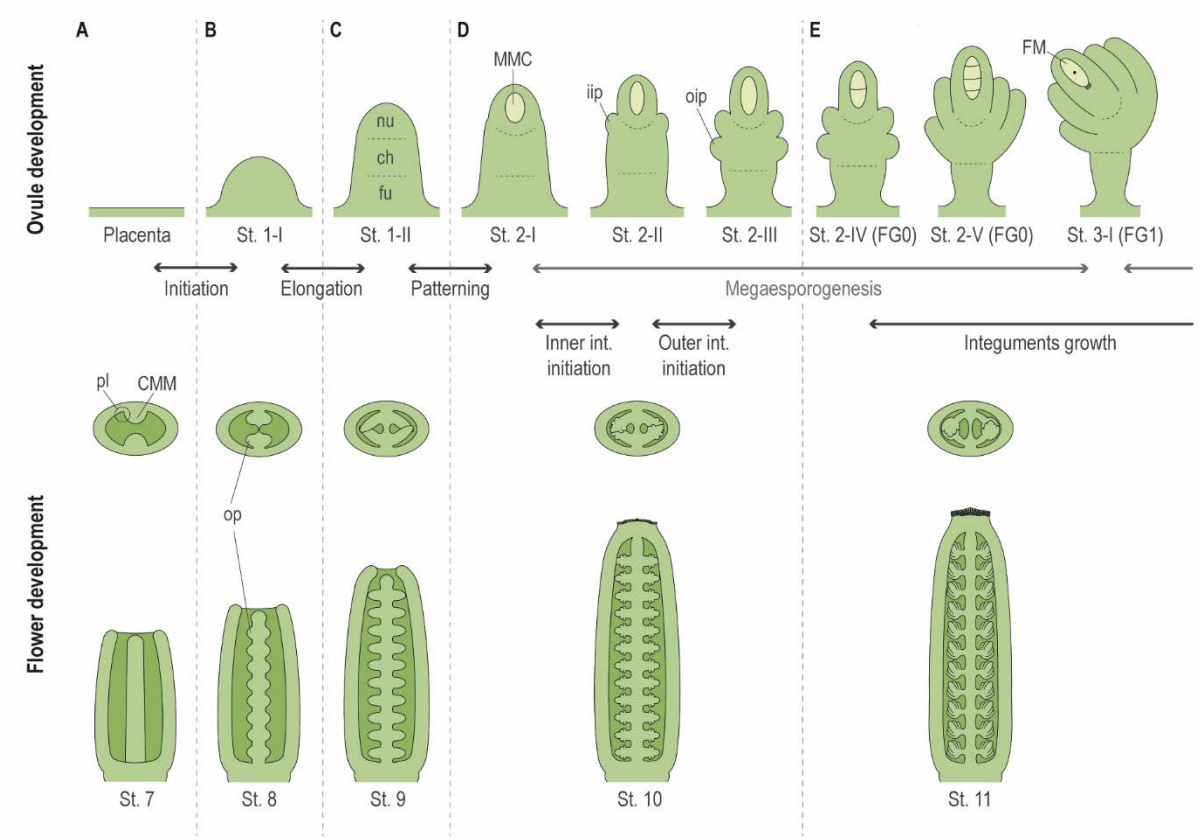

Figure 1. Schematic illustrations of early- and mid- ovule development related to pistil development in *Arabidopsis thaliana*. The illustrations represent ovule development stages (top), transversal sections (middle), and longitudinal (bottom) sections of stages 7 to 11 of pistil development. Stages and processes of ovule development are indicated below each illustration. Abbreviations: ch, chalaza; CMM, carpel margin meristem; fu, funiculus; FM, functional megaspore; iip, inner integument primordium; MMC, megaspore mother cell; nu, nucellus; op, ovule primordium; oip, outer integument primordium; pl, placenta; St., stage. Ovule development stages are according to Schneitz *et al*. (1995), pistil development stages according to Smyth *et al*. (1990), and FG0–FG1 are stages of megagametophyte development, according to Christensen *et al*. (1997).

Figure 2

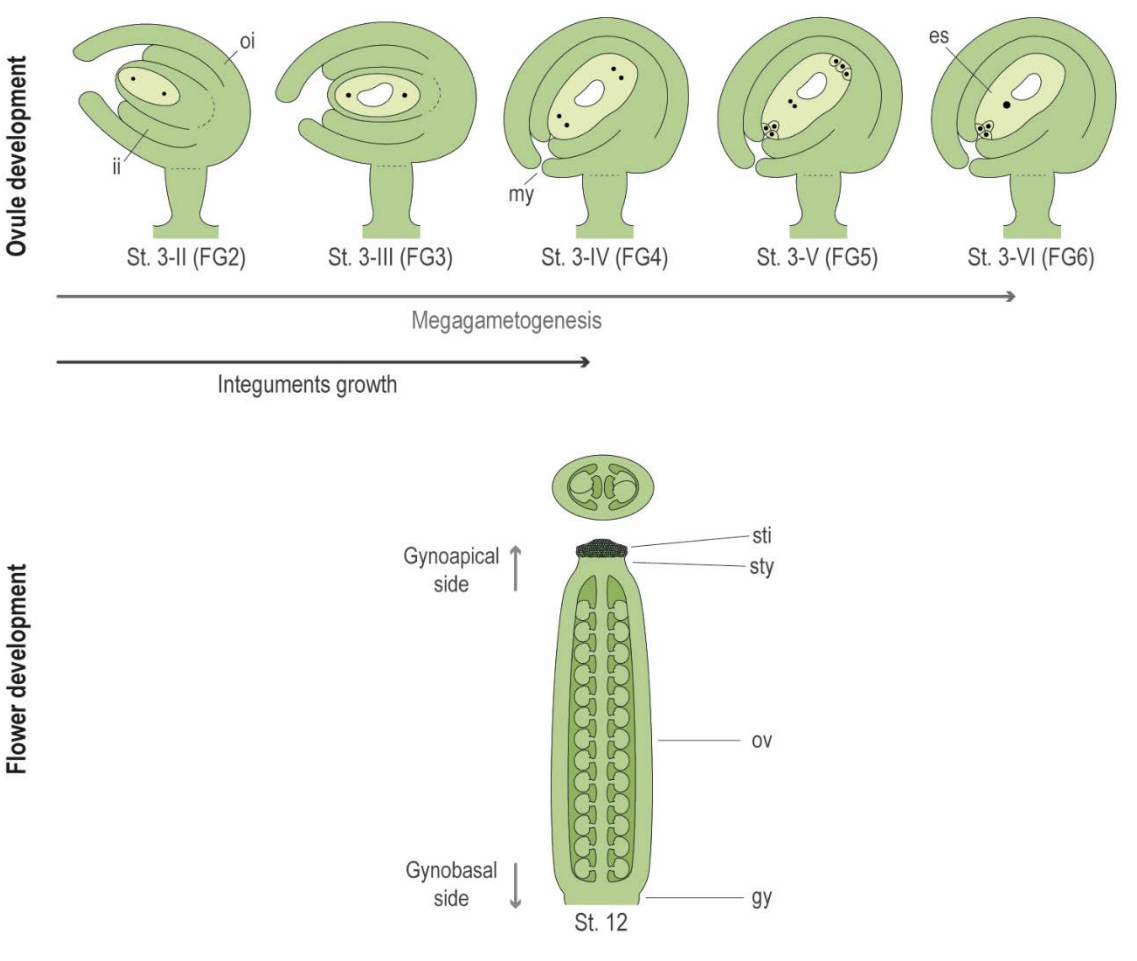

Figure 2. Schematic illustrations of late ovule development related to pistil development in *Arabidopsis thaliana*. The illustrations represent ovule development stages (top), transversal sections (middle), and longitudinal (bottom) sections of stage 12 of pistil development. Stages and processes of ovule development are indicated below each illustration. Abbreviations: es, embryo sac; gy, gynophore; ii, inner integument; mi, micropyle; oi, outer integument; ov, ovary; St., stage; sti, stigma; sty, style. Ovule development stages are according to Schneitz *et al*. (1995); pistil development stages according to Smyth *et al*. (1990); and FG2–FG6 are stages of megagametophyte development, according to Christensen *et al*. (1997).

Figure 3

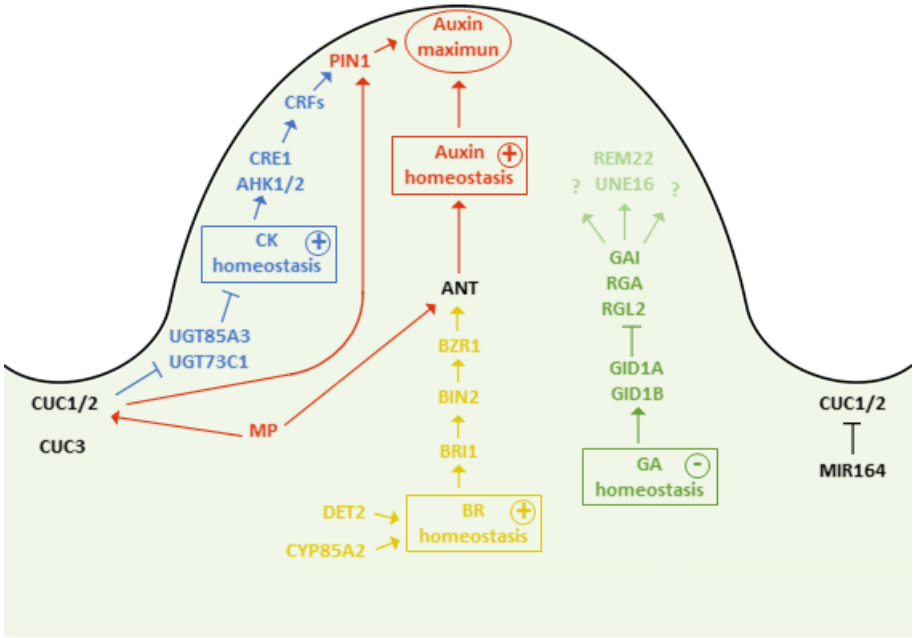

Figure 3. Proposed model for the regulation of ovule primordia initiation. The illustrations represent current knowledge of the hormonal control of the initiation of ovule primordia. See the text for further details.

Figure 4

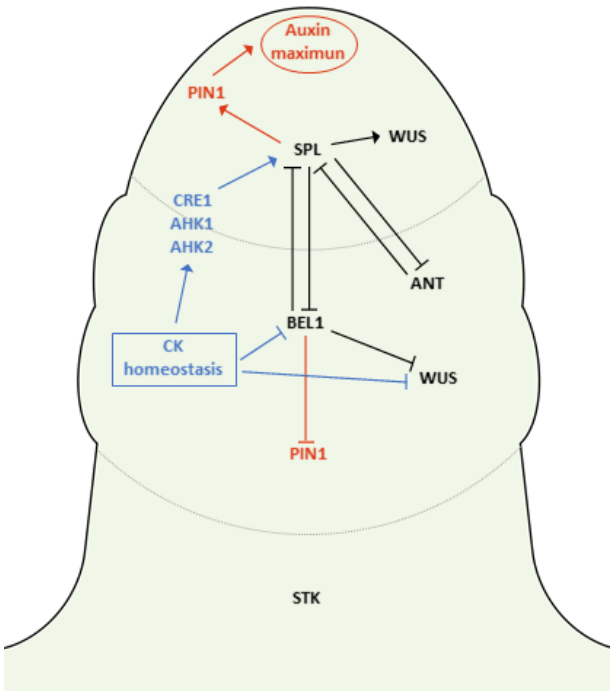

Figure 4. Proposed model for the regulation of ovule patterning. The illustrations represent current knowledge of the hormonal control of the initiation of ovule primordia. See the text for further details.

Figure 5

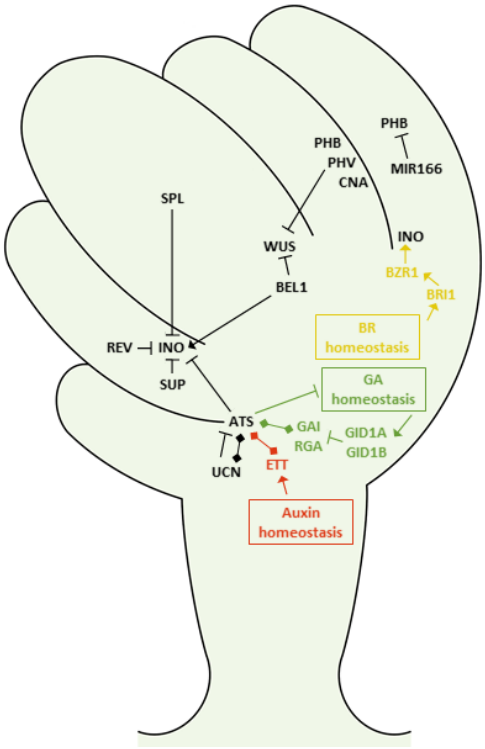

Figure 5. Proposed model for the regulation of ovule morphogenesis. The illustrations represent current knowledge of the hormonal control of the initiation of ovule primordia. See the text for further details.