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.

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

KEYWORDS
Arabidopsis; auxins; brassinosteroids; cytokinins; development; gibberellins; hormones; integument; ovule; primordia; regulation

I. Introduction
In seed 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 gynecium 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 placenta in the ovary.

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 ontology 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 prefertilization ovule development. In order to present a clear overview, we have divided ovule development into the following three steps: (1) the initiation of ovule primordia in the placenta; (2) patterning, the spatial arrangement of distinct regions with different cell fates within the primordia; and (3) 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.

II. 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, 2019).

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 Zuñiga-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 toward the center and gives rise to...
the placenta, among other tissues (Figure 1A) (Zuñiga-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, 2019). Later in carpel development, AG is expressed in the placenta as well as ovule primordia (Bowman, Drews, et al., 1991). 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, Smyth, et al., 1991) 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 premRNA 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).

III. First step: ovule primordia initiation

Ovule primordia initiation (stage 1-I, according to Schnitz 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 (Schnitz 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; Žadnikova 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).

### Table 1. Genes involved in ovule initiation.

| Gene     | Family or protein type | Involved in                        | Mutant phenotype aggravated by loss of | Reference                      |
|----------|------------------------|------------------------------------|----------------------------------------|--------------------------------|
| CUC1, CUC2 | NAC                   | Boundaries establishment             |                                       | Ishida et al. (2000); Galbiati et al. (2013); Gonçalves et al. (2015) |
| CUC3     | NAC                   | Boundaries establishment             |                                       | Vroemen et al. (2003); Gonçalves et al. (2015) |
| MIR164A  | microRNA              | Boundaries establishment             |                                       | Gonçalves et al. (2015) |
| LOF1     | MYB                   | Boundaries establishment             |                                       | Gomez et al. (2011) |
| AN1      | AP2                   | Primordium growth                   |                                       | Elliott et al. (1996); Klucher et al. (1996) |
| AN2      | AP2                   | Primordium growth                   |                                       | Schneitz et al. (1998); Liu et al. (2000) |
| HULLENLOS (HLL) |                     |                                    |                                       | Nole-Wilson and Krizek (2006) |
| LEUNIG (LUG) |                        |                                    |                                       | Azhakanandam et al. (2008) |
| FILAMENTOUS FLOWER (FIL) |                |                                    |                                       | Bao et al. (2010) |
| SEUSS (SEU) |                        |                                    |                                       | Wynn et al. (2014) |
| SEUSS-LIKE 1 (SLK1), SLK2 |              |                                    |                                       | Nole-Wilson, Azhakanandam, et al. (2010) |
| PHERINANTHIA (PAN) |                  |                                    |                                       | Yuan and Kessler (2019) |
| REVOLUTA (REV) |                       |                                    |                                       | Liao et al. (2020) |

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.
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.

**A. 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 (Brunos 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, Azhakanandam, et al., 2010). 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 TRANSFER 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...
Table 2. Hormones involved in ovule initiation.

| Hormone | Role | Evidences | Treatment/mutant | Related genetic factors |
|---------|------|-----------|------------------|-------------------------|
| **Auxins** | Promote ovule primordia initiation from placenta. | Gene reporter line/In situ hybridization | Usage | Expression | Reference | Gene name | Family or protein type | Related with | Reference |
| **Evidences** | | | | | | | | | |
| DR5rev:GFP, TAA1 (in situ) | Monitors auxin response | Tip of primordia | Okada et al. (1991); Nemhauser et al. (2000) | Benkova et al. (2003); Ceccato et al. (2013) | | | | | |
| pPIN1:PIN1-GFP, pPIN3:PIN3-GFP, pMP:MP-GFP | Indicates auxin biosynthesis | CMM and epidermis of ovule primordia | Nole-Wilson, Azhakanandam, et al. (2010) | Benkova et al. (2003); Ceccato et al. (2013) | | | | | |
| | Traces auxin efflux | Membrane of the ovule primordia outer cell layer. | | | | | | | |
| | Indicates auxin signaling | Ovule primordia (stage 1-I), primordia boundaries (stage 1-II) | | | | | | | |
| **Treatment/mutant** | Usage | Ovule number | Reference | |
| NPA | Blocks auxin transport | | Okada et al. (1991); Nemhauser et al. (2000) | Bencivenga et al. (2012) |
| pin-1-S | Compromises auxin transport | | | |
| **Related genetic factors** | Gene name | Family or protein type | Related with | Reference | |
| CUC1, CUC2 | NAC | PIN1 | | Galbiati et al. (2013) |
| ANT | AP2 | MP | | Galbiati et al. (2013) |
| **CKs** | Positively regulate ovule number | Gene reporter line/In situ hybridization | Usage | Expression | Reference | |
| **Evidences** | | | | | | | | | |
| pAHK2:GUS, pAHK3:GUS, pAHK4:GUS | Indicates CK signaling | Carpelt 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) |
| cks-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 factors**

| Gene name | Family or protein type | Related with | Reference |
|-----------|------------------------|--------------|-----------|
| CUC1, CUC2 | NAC | UGT85A3 | Cucinotta et al. (2018) |
| PIN1 | Auxin efflux carrier | CRF | Cucinotta et al. (2016) |

(Continued)
| Hormone | Role | Treatment/mutant | Usage | Ovule number | Reference |
|---------|------|------------------|-------|--------------|-----------|
| BRs     | Positively regulate ovule number | **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) |
|         |      | **bri2-1**       | 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, Rueschhoff, et al. (2010) |

| Related genetic factors | Gene name | Family or protein type | Related with | Reference |
|-------------------------|-----------|------------------------|--------------|-----------|
|                         | **ANT**   | AP2                    | BZR1         | Huang et al. (2013) |
|                         | **HLL**   | Mitochondrial ribosome protein | BZR1         | Huang et al. (2013) |
|                         | **AP2**   | AP2                    | BZR1         | Huang et al. (2013) |

| GAs | Negatively regulate ovule number | Gene reporter line/in situ hybridization | Usage | Expression | Reference |
|-----|---------------------------------|----------------------------------------|-------|-----------|-----------|
|     | **pGID1A:GID1A-GUS**           | Indications GA signaling               | Placenta and ovule primordia | Gomez et al. (2018) |
|     | **pGID1B:GID1B-GUS**           | Indications GA signaling               | Placenta and ovule primordia | Gomez et al. (2018) |
|     | **GA1, RGA, RGL2 in situ**     |                                        |       |           |           |

| Treatment/mutant | Usage | Ovule number | Reference |
|------------------|-------|--------------|-----------|
| **GA4 + GA7**    | Bioactive GAs | ↑ | Gomez et al. (2018) |
| **global della** | Induces constitutive GA response | ↓ | Gomez et al. (2018) |
| **quadruple della** | Induces constitutive GA response | ↓ | Gomez et al. (2018) |
| **triple della** | Induces constitutive GA response | ↓ | Gomez et al. (2018) |
| **gai-1**        | Blocks GA perception | ↑ | Gomez et al. (2018) |
| **pRGL2:YPet-rgl2Δ17** | Blocks GA perception | ↑ | Gomez et al. (2018) |
| **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.
Floral buds (Krizek recently described to be directly activated by ANT in Azhakanandam, ant-8 mutant, suggesting a role for ANT in auxin homeostasis in the primordia (Figure 3).

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, because 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, Azhakanandam, et al., 2010). 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, 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 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, whereas 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-oligomeric 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 bzip1-1D 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), whereas brassinolide (an endogenous natural BR) treatment increases ovule number (Barro-Trastoy et al., 2020). Noles-Wilson, Rueschhoff, et al. (2010) 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 bzip1-1D (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; 2020; Gómez et al., 2019).

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-rgl2Δ17 (Gómez 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 rgaA17, a version of RGA that cannot be degraded by GAs, driven by the ANT promoter is enough to increase ovule number (Gomez 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 (Marín-de la Rosa et al., 2014). However, further work will be needed to uncover this hormone cross-talk to coordinate early ovule development.

IV. 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 \textit{WUSCHEL} (\textit{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 \textit{et al.}, 2002; Lieber \textit{et al.}, 2011; Yamada \textit{et al.}, 2016). In addition, \textit{SPOROCYTELESS/NOZZLE} (\textit{SPL/NZZ}) is a putative transcription factor also expressed in both the nucellus and integuments. The \textit{spl-1} mutant leads to a reduction of both nucellar domain size and integument growth and loss of MMC formation (Yang \textit{et al.}, 1999; Schiefthaler \textit{et al.}, 1999, Balasubramanian and Schneitz, 2000).

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

A close relationship among several of these genes was described, revealing some pathways of communication between regions. For instance, \textit{BEL1} and \textit{SPL} confine \textit{WUS} expression to the nucellus (Sieber \textit{et al.}, 2004; Brambilla \textit{et al.}, 2007) and work together for proper chalaza formation (Balasubramanian and Schneitz, 2000). For its part, \textit{SPL} regulates nucellus development, antagonizing \textit{BEL1} and \textit{ANT} (Balasubramanian and Schneitz, 2000).
Finally, little is known about the establishment of the funiculus zone, as most patterning mutants appear 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).

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

Regarding the implication of CKs in ovule patterning, as for the case of auxins, the fact that the CK insensitive mutant cre1-12 ahk2-2 ahk3-3 develops fingerlike ovules with disrupted development revealed that CKs are important for proper ovule pattern (Bencivenga et al., 2012). Moreover, plants treated with BAP develop a single structure instead of two integuments (Bencivenga et al., 2012). The similarity between pin1-5 and cre1-12 ahk2-2 ahk3-3 phenotypes and the role of CRF regulating the expression of PIN1 (Šimasková et al., 2015), suggests cross-talk between auxins and CKs in ovule patterning. This was confirmed by the analysis of PIN1 localization and expression levels in cre1-12 ahk2-2 ahk3-3 mutant as well as in plants treated with BAP (Bencivenga et al., 2012). In the first case, PIN1 is undetectable (Bencivenga et al., 2012), when it is usually detected in the central region of the funiculus, the outer layer of the nucellus and the inner integument primordium in wild-type plants (Benkova et al., 2003). In the second case, PIN1 is detected in the nucellus, funiculus, and inner integment and also the outer integument and the epidermal layer of the funiculus, suggesting that CKs are important for proper PIN1 expression and PIN1 localization (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, whereas 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
Table 4. Hormones involved in ovule patterning.

| Hormone | Role | Evidences | Usage | Expression | Reference |
|---------|------|-----------|-------|------------|-----------|
| **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 | Oxal phenotype | Reference |
| pin1-5 | Compromises auxin transport | Fingerlike 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-II ovules | | Nishimura et al. (2004); Bencivenga et al. (2012) |
| CKX5 | Indicates CK catabolism | Chalaza and developing inner integument | | Bartrina et al. (2011) |
| pAHK4:GUS, pCRE1:GUS | Indicates CK signaling | All tissues, in all stages | | Bencivenga et al. (2012) |
| pAHK2:GUS | Indicates CK signaling | | | Bencivenga et al. (2012) |
| **Treatment/mutant** | Usage | Oxal 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 | Fingerlike 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) |
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 fingerlike 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 cross-talk that involves both SPL and BEL1. Cks may be regulating SPL and BEL1 expression in the nucellus and chalaza, respectively. In turn, SPL and BEL1 would regulate PIN1 in these two tissues.

V. Third step: integument morphogenesis

As a pattern is correctly established, the inner and outer integuments grow in a coordinated manner over the nucellus from stage 2-II to 3-IV, eventually enclosing the embryo sac and leaving the micropyle at the apex of the mature ovule (Figures 1D, E, 2, and 5). The inner integuments, which initiate before the outer integuments (Figure 1D), grow from both gynoapical and gynobasal sides of the developing ovule as a radially symmetrical structure that surrounds the nucellus. On the contrary, outer integuments grow asymmetrically, only from the gynobasal side of the ovule and more extensively at its abaxial side (Figure 1E). This asymmetric growth results in an anatropous ovule (Schneitz et al., 1995; Endress, 2011), in which the resulting curvature causes the micropyle to be positioned close to the funiculus at maturity stage (Figure 2). Furthermore, from stage 2-IV to 2-V (stage FG0 for megagametophyte development, as described by Christensen et al., 1997), the MMC undergoes meiosporogenesis via meiosis and subsequent degeneration of three nuclei, resulting in a single haploid functional megaspore (FM) at stage 3-I (FG1) (Figure 1E). Then, from stages 3-II to 3-VI (FG2-FG6) the FM undergoes megagametogenesis via three rounds of mitosis, forming the embryo sac (Figure 2). All these processes take place when the flower is at stages 10 to 12 (Figures 1D, E, and 2) (Smyth et al., 1990; Schneitz et al., 1995, Christensen et al., 1997).

Regarding integument development, some genes were identified as regulators of integument polarity (Figure 5). INNER NO OUTER (INO) encodes a YABBY protein that is expressed in the abaxial (or dorsal) side of the outer integument and is essential for its proper 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 and Park, 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 CNA 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 Schneitz, 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 CNA 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).

A. 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, 2020; Gomez et al. 2019). 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 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
| Gene | Family or protein type | Expressed/Localized in | Required for | Reference |
|------|------------------------|------------------------|--------------|-----------|
| INO* | YABBY                  | Abaxial side of the outer integument | Outer integument development | Gaiser et al. (1995); Villanueva et al. (1999) |
| PHB, PHV, can | KANADI | Adaxial side of the inner integument | Outer and inner integumets development | Eshed et al. (2001); Kelley et al. (2009) |
| REV | HD-ZIP III | Chalaza | Outer and inner integumets development | Kelley et al. (2009) |
| MR166 | microRNA | Outer integument primordia | Inner integument development | Hashimoto et al. (2018) |
| ATS* | KANADI | 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 | Mature ovules, at stage 13 | Asymmetric growth of outer integument | Gaiser et al. (1995) |
| STRUBE9L (SUB)* | Receptor-like kinase | Mature ovules, at stage 13 | Outer integument development | Chevalier et al. (2005) |
| TOUSLED (TSL)* | Nuclear serine/threonine protein kinase | Mature ovules, at stage 13 | Inner and outer integument development | Roe et al. (1997) |
| PRETTY FEW SEED52 (PFS2)* | Homeodomain protein | Ovule primordia, Chalaza and nucellus | Directional integuments | Park et al. (2005) |
| LUG* | 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 | Developing ovules, at stage 12 | Outer integument development | Franks et al. (2002); Ba et al. (2010); Simon et al. (2017) |
| KNAT1 | Homeodomain protein | Mature ovules, at stage 13 | Outer integument development | Truenit and Hasekoff (2008) |
| ADA2B/PROPORZ1 (PRZ1) | Transcriptional co-activator | Mature ovules, at stage 13 | Outer integument development | Simon et al. (2017) |
| ERECTA (ER), ERECTA-LIKE 1 (ERL1)*, ERL2* | ERECTA-family | Ovule primordia and developing integuments | Outer and inner integument development | Pillitteri et al. (2007) |
| PICKLE (PKL)* | CHD3 chromatin remodeler | Ovule primordia and developing integuments | Asymmetric integments growth | Carter et al. (2016) |
| DICER-LIKE1 SHORT INTEGMENTS 1 (DCL1/SIN1)* | RNA helicase/nuclease | | Directional integument cell expansion, Asymmetric integments growth | Robinson-Beers et al. (1992); Schauer et al. (2002) |
| HUA ENHANCER1 (HEN1)* | microRNAs and siRNAs methyltransferase | | Asymmetric integments growth | Wei et al. (2020) |
| HYPONASTIC LEAVES 1 (HYL1)* | dsRNA-binding protein | Ovule primordia and funiculus, chalaza and nucellus | Asymmetric integments growth | Wei et al. (2020) |
| TSOY* | CHC protein | Ovule primordia and funiculus, chalaza and nucellus | Directional integument cell expansion | Hauser et al. (2000) |
| SHORT INTEGMENTS 2 (SIN2)* | Mitochondrial DAR GTPase | Apoplastic compartments between inner and outer cell layer of the outer integument | Integuments cell division | Broadhvest et al. (2000); Hill et al. (2006) |
| ARABIDOPSIS CRINKLY4 (ACR4)* | Receptor kinase | Apoplastic compartments between inner and outer cell layer of the outer integument | Integuments cell division | 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 6 (IMB6) | Karyopherin, importin | Ovule primordia, chalaza and integuments | Asymmetric integments growth | Liu et al. (2019) |
| BLASIG (BAG)* | Unknown | | Inner and outer integuments growth | Schnitz et al. (1997) |
| MOLLIG (MOL)* | Unknown | | Integuments cell enlargement | Schnitz et al. (1997) |
| LAELLI (LAL)* | Unknown | | Inner integument development | Schnitz et al. (1997) |

*Genes known to also affect female gametophyte development.
### Table 6. Hormones involved in integument morphogenesis.

| Hormone | Role | Evidences | Usage | Expression | Reference |
|---------|------|-----------|-------|------------|-----------|
| **Auxins** | Regulation of the spacing between integuments | | | | |
| | | 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) |
| | | pYUC1p3:eGFP, 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:PIN1-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) |
| | | ATS KANADI | Induces constitutive auxin response | Both integuments form two cell layers | Kelley et al. (2012) |
| **GAs** | Regulation of proper integument development | | | | |
| | | pGAI:GUS, pRGA:GUS, pRGL1:GUS | Indicates GA signaling | Integuments primordia and funiculus (stage 2-II) | Gomez et al. (2016) |
| | | rgl2-5 allele (with Ds-GUS insertion) | Indicates GA signaling | Integuments primordia, nucellus, funiculus (stage 2-II) | Gomez et al. (2016) |
| | | pRGL1:YPet-rgl1D17 | Indicates GA signaling | Funiculus and chalaza (stage 3-V) | 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) |
| **BRs** | Regulation of outer integument growth | | | | |
| | | pBRI1:BRI1-YFP, pBZR1:BZR1-YFP | Indicates BR signaling | All ovule tissues (stages 2-1 to 3-V) | Jia et al. (2020) |
| | | 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) |
| | | barh-1 | Compromises BR perception | Outer integument growth arrest | Jia et al. (2020) |
| **Related genetic factors** | Gene name | Family or protein type | Related with | Reference |
| **Auxins** | pTAA1 | KANADI | | Kelley et al. (2012) |
| | pYUC4 | KANADI | | Kelley et al. (2012) |
| | pYUC1p3 | KANADI | | Kelley et al. (2012) |
| | pYUC5 | KANADI | | Kelley et al. (2012) |
| | DR5rev | KANADI | | Kelley et al. (2012) |
| | pPIN1 | KANADI | | Kelley et al. (2012) |
| | pPIN3 | KANADI | | Kelley et al. (2012) |
| | pPGP1 | KANADI | | Kelley et al. (2012) |
| | pPGP19 | KANADI | | Kelley et al. (2012) |
| | pAUX1 | KANADI | | Kelley et al. (2012) |
| **GAs** | pGAI | KANADI | | Gomez et al. (2016) |
| | pRGA | KANADI | | Gomez et al. (2016) |
| | pRGL1 | KANADI | | Gomez et al. (2016) |
| | | GA3ox1 | | Gomez et al. (2016) |
| **BRs** | pBRI1 | KANADI | | Gomez et al. (2016) |
| | pBZR1 | KANADI | | Gomez et al. (2016) |
| | | GA1, RA1, GA3ox1 | | Gomez et al. (2016) |
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, Azhakanandam, et al., 2010). 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 (Pérez-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 bzip1-1D 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, bzip1-h, outer integument growth was completely arrested after initiation. Moreover, both BRI1 and BZR1 were widely 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 bzip1-1D 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 bzip1-1D 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 bzip1-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 del1 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.

VI. 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 bitetramic 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, 1992; Berry and Spink, 2009; Shi et al., 2015; Cucinotta et al., 2020). 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 (Zúñiga-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. 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 developmental differences. For instance, a DR5-based signal is observed at the tip of ovule primordia in tomato (Goldental-Cohen et al., 2017). Later, this signal is found in the area corresponding to the micropylar pole of the embryo sac (Pattison and Catala, 2012) and in the vascular bundles connecting the ovules to the placenta (Goldental-Cohen et al., 2017). Furthermore, NPA treatments during the early stages of flower development result in abortion of ovule primordia, leading to an ovule-less phenotype (Goldental-Cohen et al., 2017). In addition, tomato ovule number is negatively regulated by GAs (Gomez et al., 2018) and positively regulated by BRs (Barro-Trastoy et al., 2020) as in Arabidopsis. GA-treatments, as well as procera, the loss-of-function mutant of PROCERA, the only DELLA found in tomato, induce a reduction in tomato ovule number (Gomez et al., 2018). The Micro-Tom (MT) cultivar of tomato, which harbors a mutation in the DWARF4 gene of BR biosynthesis, causing reduced BR levels, has fewer ovules than the isogenic MT line that carries the wild-type and functional DWARF4 gene (Barro-Trastoy et al., 2020), which resembles the phenotype of BR-deficient mutant det2-1 of Arabidopsis (Huang et al., 2013).

However, Barro-Trastoy et al. (2020) have recently found that GA and BR cross-talk in the determination of ovule primordia formation is quite different in tomato. As indicated above, in Arabidopsis GAs and BRs down- and up-regulate, respectively, ovule number regardless of the status of the other hormone. For example, GAs can still reduce ovule number in plants with high or low BRs or BR responses, whereas BRs can promote the formation of more ovule primordia in both gai-1 or GA-treated plants, with high or low DELLA activity, respectively. In contrast, in tomato, BRs control ovule number through the inhibition of GA biosynthesis (Barro-Trastoy et al., 2020). BRs would reduce GA levels by repressing the expression of GA biosynthesis genes, such as SLGA20ox1. This would lead to the stabilization 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.

VII. 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.

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