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REVIEW

Gynoecium and fruit development in *Arabidopsis*

Humberto Herrera-Ubaldo and Stefan de Folter*

ABSTRACT

Flowering plants produce flowers and one of the most complex floral structures is the pistil or the gynoecium. All the floral organs differentiate from the floral meristem. Various reviews exist on molecular mechanisms controlling reproductive development, but most focus on a short time window and there has been no recent review on the complete developmental time frame of gynoecium and fruit formation. Here, we highlight recent discoveries, including the players, interactions and mechanisms that govern gynoecium and fruit development in *Arabidopsis*. We also present the currently known gene regulatory networks from gynoecium initiation until fruit maturation.

KEY WORDS: Gynoecium, Fruit, Gene regulatory networks, Transcription factors, Hormones

Introduction

Multicellular life on Earth is inconceivable without plants. Our planet harbors close to 400,000 vascular plant species, of which approximately 94% are seed plants (Govaerts, 2001; Willis, 2017), which produce and reproduce themselves via seed. The vast majority of seed plants are angiosperms, which produce flowers, such as *Arabidopsis thaliana* of the Brassicaceae family (Fig. 1A). Flower and seed formation are adaptive advantages of plant sexual reproduction, which requires male and female gametes. In general, flowers have four different types of floral organs placed in four whorls, from outside to inside: sepals, petals, stamens and carpels (see Glossary, Box 1). The stamen, also called the androecium, is the 'male' part of the flower that produces the male gametophyte from which gametes differentiate. The 'female' part of the flower is formed by one or more carpels. In *Arabidopsis*, two congenitally fused carpels form the pistil, which is also called gynoecium (see Glossary, Box 1). Ovules are formed inside the gynoecium and the female gametophyte, which produces the female gametes, develops within each ovule (see Glossary, Box 1). Upon fertilization, seed development begins and, in most plant species, the gynoecium turns into a fruit (reviewed by Alvarez-Buylla et al., 2010; Dresselhaus et al., 2016).

The identification of AGAMOUS (AG) as a transcription factor controlling male and female reproductive organ development in plants (androecium and gynoecium, respectively), marked the beginning of a journey towards the understanding of the molecular aspects of flower formation (Bowman et al., 1989; Irish, 2017; Yanofsky et al., 1990). During three decades of research, many master regulators, such as SEPALLATAs (SEPs), CRABS CLAW (CRC), SPATULA (SPT), SHATTERPROOFs (SHPs), FRUITFULL (FUL) and SEEDSTICK (STK), among many others, have emerged as crucial regulators of reproductive development, especially of gynoecium development (reviewed by Alvarez-Buylla et al., 2010; Bowman et al., 1999; Ferrándiz et al., 2010; Reyes-Olalde et al., 2013; Roeder and Yanofsky, 2006; Simonini and Østergaard, 2019; Zúñiga-Mayo et al., 2019). Besides the identification of more transcription factors involved in gynoecium development, information on genes acting downstream of them have also been discovered (reviewed by Pajoro et al., 2014). Together, these discoveries have opened the road for charting gene regulatory networks (GRNs). In addition, several datasets from chromatin immunoprecipitation followed by sequencing (ChIP-seq) experiments, have allowed genome-wide analysis of binding events for many master regulators that participate in the transition to reproduction and flower development (Chen et al., 2018). Now, the fine-tuning aspects of flower development are starting to be revealed.

Over the years, many review articles on flower and fruit development have been published (Alvarez-Buylla et al., 2010; Ballester and Ferrándiz, 2017; Bowman et al., 1999; Ferrándiz et al., 2010; Marsch-Martínez and de Folter, 2016; Reyes-Olalde and de Folter, 2019; Reyes-Olalde et al., 2013; Roeder and Yanofsky, 2006; Sehra and Franks, 2015; Simonini and Østergaard, 2019; Smyth et al., 1990; Zúñiga-Mayo et al., 2019). However, there is currently no recent review that includes all the current GRNs known to date for gynoecium and fruit development. In 2015, we proposed approaches and tools to construct a comprehensive GRN for gynoecium development (Chávez Montes et al., 2015). Therefore, this Review focuses on recent discoveries and the integration of the GRNs that guide the development of *Arabidopsis*, starting from gynoecium initiation until fruit maturation.

From a floral meristem to gynoecium initiation (stages 1-6)

Floral meristem

Plants possess the ability to form new organs continuously (reviewed by Gaillochet and Lohmann, 2015; Sablowski, 2007). When environmental and endogenous genetic cues are met, the shoot apical meristem (SAM; see Glossary, Box 1) transitions to an inflorescence meristem (IM; see Glossary, Box 1) (Fig. 1B,E), marking the beginning of the reproductive phase of the plant (reviewed by Andrés and Coupland, 2012). At the flanks of the IM, auxin induces differentiation to give rise to flower primordia, each with a floral meristem (FM; see Glossary, Box 1). In *Arabidopsis*, the most recently formed flower primordium is referred to as 'stage 1' (Smyth et al., 1990). When the next flower primordium is formed, the previous one is called stage 2, and so on. At floral stage 3, the flower primordium increases in size and sepal primordia become visible (Fig. 1B,C,F). The FM gives rise to the different floral organs present in a mature flower (Fig. 1D,F) (reviewed by Denay et al., 2017). During the next stages, sepals continue to grow; during stage 5, petal and stamen primordia appear, whereas stage 6 is characterized by the complete coverage by the sepals of the FM and internal flower primordia, including the gynoecium

Unidad de Genómica Avanzada (UGA-Langebio), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Km. 9.6 Libramiento Norte, Carretera Irapuato-León, Irapuato 36824, Guanajuato, México.

*Author for correspondence (stefan.defolter@cinvestav.mx)

H.H., 0000-0002-5408-4022; S.d., 0000-0003-4363-7274

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Box 1. Glossary

Carpel. The female reproductive structure of a flower, consisting of an ovary, a stigma and a style.
Carpel margin meristem (CMM). A meristematic region in the medial domain, a zone located where the carpel margins fused. The CMM is the origin of the medial tissues: placenta, ovules, septum, style and stigma. Clearly visible at stages 7 and 8.
Chalaza. The basal part of an ovule opposite to the micropyle; where the integuments, the nucellus and the funiculus are joined.
Floral meristem (FM). The progenitor of all the flower organs.
Fruit dehiscence. The opening of a mature fruit to release the seeds.
Funiculus. The stalk that attaches an ovule or seed to the placenta.
Gynoecium. The female part of a flower. In *Arabidopsis*, the gynoecium consists of two congenitally fused carpels.
Gynoecium primordium. A dome-shaped group of cells, first visible at stage 6.
Inflorescence meristem (IM). The region at the tip of the growing shoot containing meristematic cells that generates the flowers.
Lateral domain. The two regions that are on either side of the medial domain, corresponding to the carpel walls or valves. Visible from stage 7 onwards.
Medial domain. In *Arabidopsis*, the two carpels are fused vertically at their margins, and these fused margins correspond to the medial domain of the gynoecium. Visible from stage 7 onwards (oval-shaped hollow tube).
Nucellus. The central part of the ovule that contains the embryo sac.
Ovule. A structure that contains the female reproductive cells, after fertilization ovules become seeds. Ovule primordia arise at stage 9.
Placenta. The region within the ovary to which the ovules and seeds are attached.
Replum. Located at the outer parts of the septum in the medial domain. The valves are attached to them.
Septum. In *Arabidopsis*, there is a false septum that divides the two carpels. Contains the transmitting tract.
Shoot apical meristem (SAM). The region at the tip of the growing shoot containing meristematic cells that generates the aerial organs (leaves and branches).
Silique. A type of dry fruit that has two fused carpels in *Arabidopsis*, characteristic of the Brassicaceae family.
Stigma. Specialized epidermal cells located at the top of the gynoecium, which capture the pollen grains, allowing germination and the first steps of pollen tube growth.
Style. The tissue that connects the stigma with the ovary.
Transmitting tract. Specialized tissue derived from PCD in the style and some septum cells in the ovary. Possesses ECM that provides nutrients, guidance and support to the growing pollen tubes.
Valves. The outer tissue of the ovary; the carpel walls.

primordium (see Glossary, Box 1), which becomes visible at stage 6 (Fig. 1F,G) (Alvarez-Buylla et al., 2010; Denay et al., 2017; Smyth et al., 1990).

Floral meristem maintenance and termination

In general, the genes that regulate meristem maintenance in the SAM and the FM are shared (reviewed by Chang et al., 2020; Gaillochet and Lohmann, 2015). The role of the WUSCHEL-CLAVATA3 (WUS-CLV3) circuit in plant stem cell niche maintenance is key (Brand et al., 2000; Schoof et al., 2000). The activity of WUS, together with LEAFY (LFY) and others, converges on the regulation of AG, which starts to be expressed at stage 3 in the FM (Fig. 2A). In turn, AG starts to repress *WUS* and activates several genes that negatively regulate *WUS* expression (Fig. 2).

The meristematic activity of the FM ends when the gynoecium primordium is formed at stage 6; however, the programs controlling FM termination begin at stage 3. The molecular mechanisms underlying FM termination have recently been reviewed (Chang et al., 2020; Lee et al., 2019; Shang et al., 2019; Xu et al., 2019; Zúñiga-Mayo et al., 2019). The GRNs that control FM termination (Fig. 2) and gynoecium initiation (Fig. 2B) contain at least 15 transcription factors and various other proteins. AG plays a leading role in both these processes, as illustrated in the ABC and quartet model for floral organ formation (reviewed by Coen and Meyerowitz, 1991; Theißen and Saedler, 2001). AG is therefore observed in the GRNs as the main hub (Fig. 2A). AG activates pathways related to auxin and cytokinin signaling (Ó'Maoiléidigh et al., 2018; Yamaguchi et al., 2017). Cytokinin signaling, in turn, also controls AG (Gómez-Felipe et al., 2021; Rong et al., 2018). The effects of AG in the control of FM termination and gynoecium development must be tightly controlled to perform those functions properly. Indeed, the mechanisms that control *AG* expression are diverse, involving transcription factors, microRNAs, histone deacetylase complexes and RNA-binding complexes (reviewed by Pelayo et al., 2021). Interactions with other proteins and the formation of protein complexes provide additional layers of control of AG activity (Box 2). For example, the interaction with SEP3, via the formation of dimers or tetramers, controls different biological processes (Hugouvieux et al., 2018; Lai et al., 2020).

New nodes in the GRNs

Over the past few years, some new nodes have been included in the networks that underlie gynoecium initiation. AINTEGUMENTA (ANT), which has reported roles in the establishment of flower primordia and the development of floral organs, has now been identified to function at very early stages of flower formation (stages 3-6), and the roles of ANT are shared with AINTEGUMENTA-LIKE 6 (AIL6) (Krizek et al., 2020, 2021). At stage 3, both proteins directly regulate *AG*, as well as other genes, such as *MONOPTEROS* (*MP*) and *REVOLUTA* (*REV*), which are related to meristem activity regulation and new primordia formation (Krizek et al., 2021) (Fig. 2A). At stage 6, ANT positively regulates *SPT*, an important gene for tissue development in the medial domain, as described below (Fig. 2B) (Krizek et al., 2020).

Epigenetic silencing of *AG* is illustrated by the participation of POLYCOMB-GROUP (PcG) complexes with CURLY LEAF (CLF), EMBRYONIC FLOWERING 1 (EMF1) and EMF2. In a similar way, APETALA 2 (AP2) recruits TOPLESS (TPL) and HISTONE DEACETYLASE 19 (HDA19) to repress *AG* (Fig. 2A) (Pelayo et al., 2021). Now, some evidence points to the involvement of PcG in silencing of *WUS* (Sun et al., 2019) and the modification of chromatin in the control of auxin biosynthesis by AG and CRC (Fig. 2) (Yamaguchi et al., 2018).

KNUCKLES (KNU), a C2H2 zinc-finger transcription factor, also functions during FM termination in addition to its role in repressing *WUS* in the FM (Fig. 2B). A recent analysis of its expression pattern has revealed the presence of *KNU* in the CLV3 expression domain. The extended functions of KNU include suppression of the expression of several floral meristem regulators, such as CLV1 and CLV3, at stage 6 (Kwaśniewska et al., 2021; Shang et al., 2021).

The transcription factor ETTIN (ETT), also known as AUXIN RESPONSE FACTOR 3 (ARF3), is involved in gynoecium patterning (Heisler et al., 2001). An additional function of ETT is to inhibit cytokinin biosynthesis by repressing *ISOPENTENYLTRANSFERASE* (*IPT*) and *LONELY GUY* (*LOG*) genes, and the gene encoding the cytokinin receptor ARABIDOPSIS HISTIDINE KINASE 4 (AHK4) during stages 5

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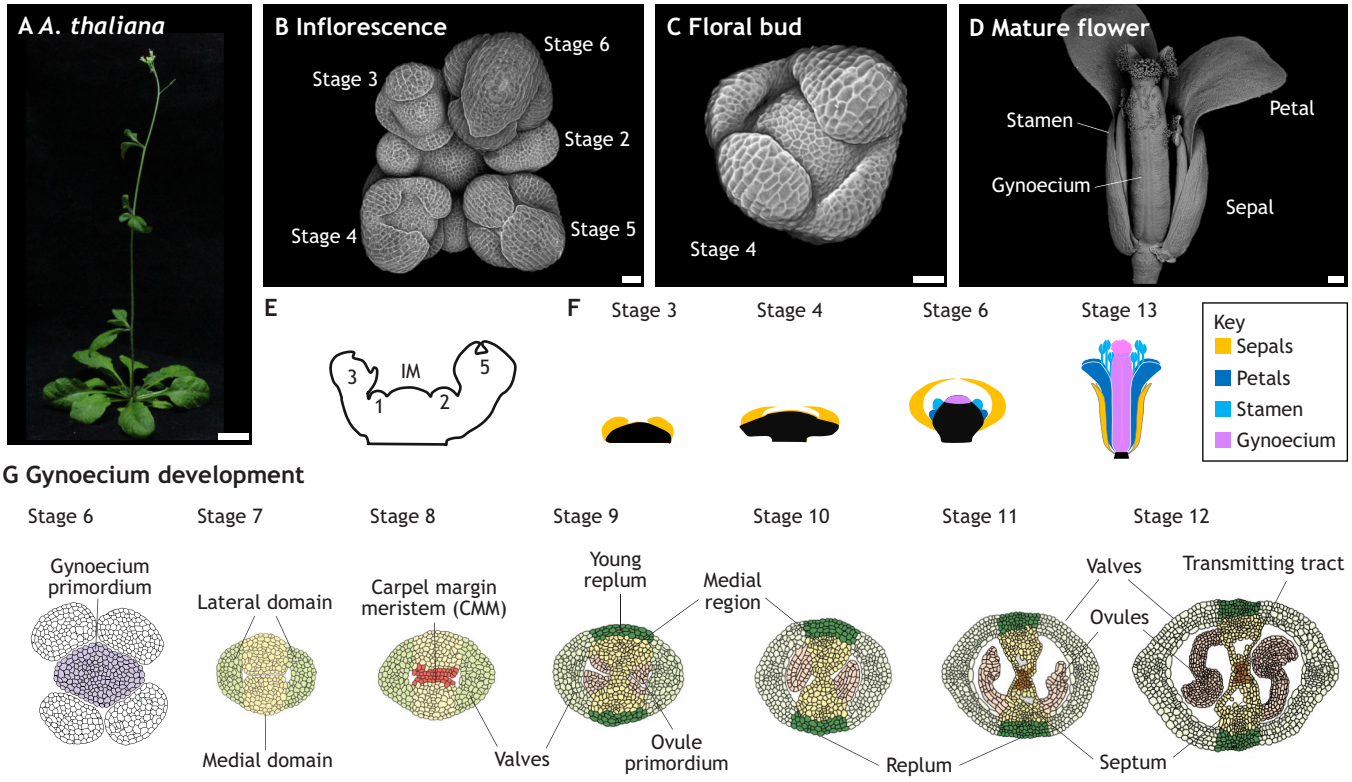

Fig. 1. Overview of gynoecium development in *Arabidopsis*. (A-F) Once the reproductive phase has initiated, the plant *Arabidopsis thaliana* (Col-0) (A) produces an inflorescence with an inflorescence meristem (IM) at the apical tip (top view; B), and the IM produces on its flanks floral buds with floral meristems (FMs) (B,C,E). The FM gives rise to all the floral organs present in a mature flower: sepals, petals and stamens, with the gynoecium at the center (D,F). (E) Schematic of an IM and the initial stages of flower development (longitudinal view). Numbers indicate different stages of FMs. At floral stage 3, sepal primordia become visible and at stage 6 the primordia of petal, stamens and gynoecium are visible. (G) Transverse gynoecia sections showing key tissues and regions during gynoecium development at stages 6-12. Scale bars: 1 cm (A); 25 µm (B); 40 µm (C); 200 µm (D). Images in B and C are taken from Zúñiga-Mayo et al. (2019). Image in D is from Zúñiga-Mayo et al. (2012).

and 6. Through these mechanisms, ETT contributes to FM termination (Fig. 2B) (Zhang et al., 2018).

Conversely, cytokinin also activates transcription factors important for gynoecium initiation and patterning, which starts around stage 6 (Fig. 2B). For example, the master regulator *AG* is regulated by cytokinin via the *Arabidopsis* response regulators type-B (ARRs) (Gómez-Felipe et al., 2021; Rong et al., 2018). Furthermore, other transcription factors that act in parallel downstream of AG are *CRC*, *SHP2* and *SPT*, which are important for gynoecium initiation and patterning, as well as being positively regulated by ARR type-B transcription factors (Fig. 2B) (Gómez-Felipe et al., 2021).

Gene expression: additional nodes in the GRNs?

Many transcription factors involved in gynoecium development (Reyes-Olalde et al., 2013) are also expressed during gynoecium initiation. In total, over 60 genes are expressed at stage 4 and over 65 genes at stage 6 (Table S1) (Herrera-Ubaldo et al., 2018 preprint; Jiao and Meyerowitz, 2010). Current studies are revealing novel roles for well-known regulators; additionally, previously unreported regulators and interactions are continuously being discovered. Therefore, we expect that these GRNs will expand in the future. This expansion of GRNs also applies to the GRNs described for the subsequent stages of gynoecium and fruit development.

Gynoecium domains, medial and lateral (stages 7 and 8)

The transition from a gynoecium primordium into two, well-defined domains (lateral and medial; see Glossary, Box 1) marks the beginning of patterning and the formation of internal tissues (Fig. 1G; Fig. 3). During stages 7 and 8, the activation and maintenance of the carpel margin meristem (CMM; see Glossary, Box 1) is crucial for the subsequent development of specialized structures: in the lateral domains, valves (see Glossary, Box 1) develop to protect the ovules, and seeds are subsequently formed in the medial domain. Additional tissues, such as the septum and the transmitting tract (see Glossary, Box 1), also form in the medial domain to facilitate fertilization (Fig. 1G).

The process of gynoecium differentiation into medial and lateral domains requires several transcription factors and hormonal signals (Reyes-Olalde and de Folter, 2019; Reyes-Olalde et al., 2013). One important regulatory module involves the integration of auxin and cytokinin signaling with the activity of the transcription factors HECs and SPT in the medial domain around floral stages 7 and 8 (Müller et al., 2017; Reyes-Olalde et al., 2017; Schuster et al., 2015) (Fig. 3C). To summarize, auxin signaling is present in the lateral domains and cytokinin signaling in the medial domain in the CMM. In the medial domain, cytokinin signaling induces auxin biosynthesis and auxin transporters to create an auxin flux to transport auxin to the lateral domain. Auxin-induced proteins in the lateral domain repress cytokinin signaling genes to limit them to the medial domain, such as the cytokinin signaling inhibitor ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6) and the auxin response factor ETT (Heisler et al., 2001; Reyes-Olalde et al., 2017). The created auxin flux is important for the apical-basal growth of the gynoecium. Cytokinin biosynthesis in

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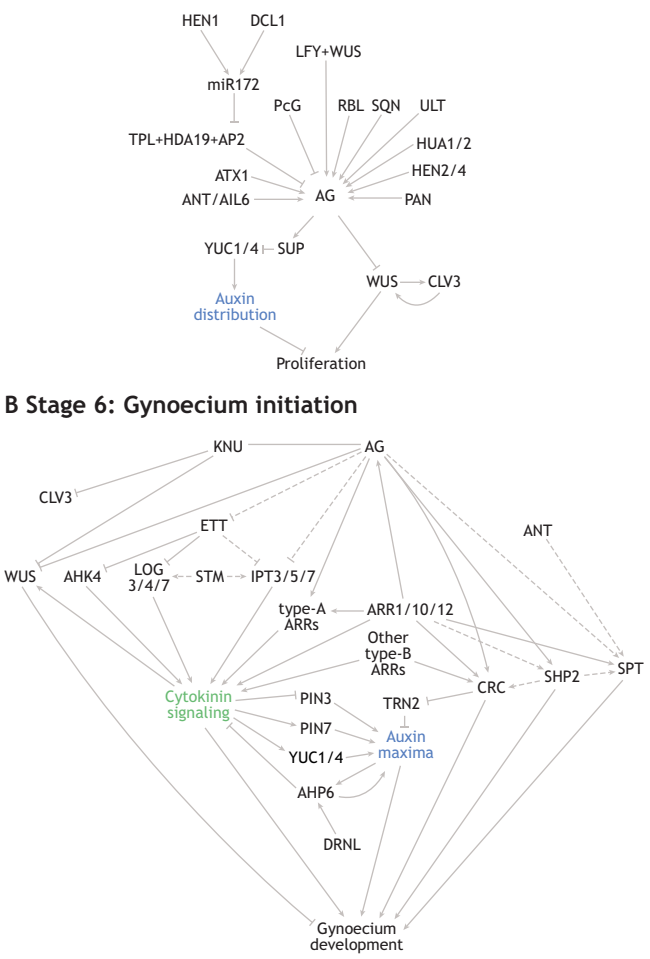

Fig. 2. Floral meristem termination and gynoecium establishment (stages 3-6). (A) A gene regulatory network for floral meristem (FM) maintenance and termination at stage 3. (B) AGAMOUS (AG) orchestrates gynoecium identity establishment by integrating hormonal signals with the activation/repression of transcription factors (Xu et al., 2019; Zúñiga-Mayo et al., 2019). Dashed lines indicate indirect regulation. Colored words indicate different hormone-related processes: auxin signaling (blue); cytokinin signaling (green). AHK4, ARABIDOPSIS HISTIDINE PROTEIN KINASE 4; AHP6, HISTIDINE PHOSPHOTRANSFER PROTEIN 6; AIL6, AINTEGUMENTA-LIKE6; ANT, AINTEGUMENTA; AP2, APETALA2; ARRs, ARABIDOPSIS RESPONSE REGULATORs; ARR1/10/12, RESPONSE REGULATOR 1, 10, 12; ATX1, ARABIDOPSIS HOMOLOG OF TRITHORAX 1; CLV3, CLAVATA3; CRC, CRABS CLAW; DCL1, DICER LIKE1; DRNL, DORNROSCHEN-LIKE; ETT, ETTIN; HEN1/2/4, HUA ENHANCER 1, 2, 4; HDA19, HISTONE DEACETYLASE 19; HUA1/2, HUA1, 2 (At5g23150); IPT3/5/7, ISOPENTENYLTRANSFERASE 3, 5, 7; KNU, KNUCKLES; LFY, LEAFY; LOG3/4/7, LONELY GUY 3, 4, 7; miR172, microRNA172; PAN, PERIANTHIA; PcG, Polycomb-group; PIN3/7, PIN-FORMED 3, 7; RBL, REBELOTE; SHP2, SHATTERPROOF 2; SPT, SPATULA; SQN, SQUINT; STM, SHOOT MERISTEMLESS; SUP, SUPERMAN; TPL, TOPLESS; TRN2, TORNADO 2; ULT, ULTRAPETALA; WUS, WUSCHEL; YUC1/4, YUCCA 1, 4.

the CMM is assumed to be regulated by SHOOT MERISTEMLESS (STM), as has been shown for other meristems (Jasinski et al., 2005; Yanai et al., 2005). STM is a *KNOTTED* (*KNAT*) *1-LIKE* homeodomain transcription factor important for CMM formation and activity (Scofield et al., 2007). The *STM* gene is activated by the CUP-SHAPED COTYLEDON proteins (CUCs) (Kamiuchi et al., 2014). A positive regulatory loop between *STM* and *CUC* genes has been reported in the SAM and could be present in the CMM as well (Spinelli et al., 2011). The CUC transcription factors seem to also be important for *SPT* expression in the basal part of the gynoecium (Nahar et al., 2012).

The current GRN that controls activities in the CMM has been derived from functional studies of genes and reporter lines (reviewed by Reyes-Olalde and de Folter, 2019; Reyes-Olalde et al., 2013). The study and integration of additional regulators is currently in progress; there are more than 50 transcription factors related to gynoecium development with reported expression in this region (Table S1) (Herrera-Ubaldo et al., 2018 preprint). The coordination of biochemical and genetic processes underlying meristematic activity in the CMM, and its further differentiation, probably involves the participation of many proteins and pathways that are yet to be characterized (Kivivirta et al., 2021; Villarino et al., 2016; Wynn et al., 2011).

Ovule initiation and patterning (stages 9 and 10)

One of the most important functions of the gynoecium is to provide protection to the developing ovules. The determination of ovule identity occurs at the flanks of the CMM. Ovule primordia are formed at stage 9 by periclinal cell divisions within the epidermal tissue of the placenta (see Glossary, Box 1) (Fig. 1G; Fig. 4). Like previous examples, the GRNs involve the coordinated action of several transcription factors and hormonal signaling pathways that control the main aspects of ovule development: the establishment of boundaries and primordia (Fig. 4A), the control of ovule initiation and number, and ovule patterning (Fig. 4B,C) (Barro-Trastoy et al., 2020b; Cucinotta et al., 2014, 2020).

Regulation of ovule initiation

The intricate mechanisms that guide ovule initiation are far from fully understood, but advances have been made (Barro-Trastoy et al., 2020b; Cucinotta et al., 2020). Indeed, the identification of enzymes and proteins that act at different levels outside the GRN has shed light on fine aspects of the molecular mechanisms, and opens new questions and directions regarding our understanding of ovule development. For example, factors have been recently identified that control the spacing of ovules: two secreted peptides and their ERECTA (ER) family receptor kinases coordinate regular ovule primordia initiation coupled to fruit growth from the placenta and carpel walls (Kawamoto et al., 2020).

Processes that occur at the cell-wall level have also been discovered. A recent study has reported on the involvement of placenta-expressed genes encoding CELL WALL INVERTASE (CWIN) 2 and 4 as positive regulators of ovule initiation. CWINs hydrolyze sucrose into glucose and fructose, and have additional roles in sugar signaling. Specific suppression of the activity of these enzymes leads to a significant reduction in ovule number, as well as ovule and seed abortion. Based on transcriptome analysis in *CWIN2*- and *CWIN4*-silenced lines, it has been shown that the ovule-identity gene *STK*, as well as auxin signaling-related genes and genes encoding hexose transporters are downregulated (Liao et al., 2020).

Also at the membrane level, the localization of PIN auxin transporters in the placenta epidermis is crucial for ovule initiation (Fig. 2A). The distribution of PIN1 and auxin fluxes is very dynamic in the placenta and affects not only one ovule primordium, but also the neighboring ovule primordium. Analysis of marker lines and auxin response has revealed that ovules initiate asynchronously and follow this trend through later stages (Yu et al., 2020).

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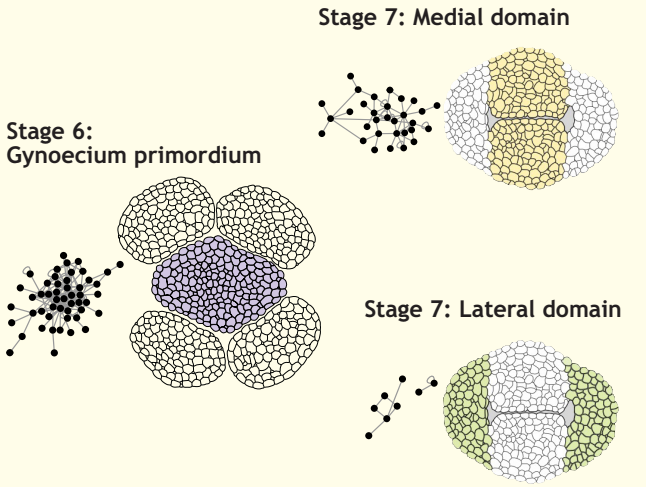
Box 2. Protein interactions: a combinatorial model for gynoecium patterning?

The concerted action of transcription factors (MADS-box and APETALA 2), to guide the establishment of floral organ identity is elegantly represented by the 'ABCDE' model (Theißen and Saedler, 2001). For gynoecium patterning, the involvement of numerous transcription factor families surpasses those for floral organ specification. The crucial action and effect of transcription factors may suggest a combinatorial model for gynoecium patterning; some crucial transcription factors [i.e. SEUSS (SEU) and LEUNIG (LUG)] may dimerize to act as a chassis for higher-order complexes in the early stages (Azhakanandam et al., 2008). In terms of protein interactions, the best-studied tissues are the stigma and style, where transcription factors, such as INDEHISCENT (IND), SPATULA (SPT) and NGATHA (NGA) participate (Ballester et al., 2021; Simonini et al., 2018).

We have made efforts to find such protein complexes. The identification of physical interactions between several transcription factors in combination with gene expression and functional information is useful to represent the networks and interaction dynamics during the development of the gynoecium (Herrera-Ubaldo et al., 2018 preprint). Figure shows protein-protein interaction networks at two early stages of gynoecium development.

The involvement of these transcription factors is just the beginning of the story; previous works have extended regulatory networks beyond the range of transcription factors alone. For example, AGAMOUS (AG) interacts with histone deacetylases and other proteins (Smaczniak et al., 2012).



Ovule number

On average around 60 ovules are formed inside the gynoecium (reviewed by Barro-Trastoy et al., 2020b; Cucinotta et al., 2014, 2020; Yuan and Kessler, 2019). However, ovule numbers can range between 40 and 80, depending on the accession of *Arabidopsis* (Yuan and Kessler, 2019). Some modules of the GRN that control ovule number can also control gynoecium size (reviewed by Cucinotta et al., 2020). A recent study has uncovered a previously unidentified positive regulator of ovule number: NEW ENHANCER OF ROOT DWARFISM1 (NERD1) (Yuan and Kessler, 2019).

Transcription factors, such as MP, ANT and CUCs, are interconnected with hormone homeostasis mechanisms, mainly with auxins, cytokinins, brassinosteroids and gibberellins, to regulate ovule number (Fig. 4A) (reviewed by Barro-Trastoy et al., 2020b; Cucinotta et al., 2020; Qadir et al., 2021). As mentioned above, auxin positively regulates ovule primordia initiation and cytokinin signaling positively regulates ovule number (Bartrina et al., 2011; Cerbantez-Bueno et al., 2020; Cucinotta et al., 2018; Galbiati et al., 2013; Reyes-Olalde et al., 2017; Zúñiga-Mayo et al., 2018). It has now been shown that cytokinin metabolism in the epidermis of the placenta is also important for ovule number (Werner et al., 2021). Furthermore, brassinosteroids and gibberellins positively and negatively regulate ovule number, respectively (Barro-Trastoy et al., 2020a; Huang et al., 2013; Nole-Wilson et al., 2010; Gomez et al., 2018, 2019).

Ovule patterning

After the establishment of ovule identity and ovule number definition (Fig. 4A), ovule patterning takes place, which involves differentiation of the funiculus, chalaza and nucellus (see Glossary, Box 1) at stage 10 (Fig. 4B), and continues with the formation of the inner and outer integuments at stage 11 (Fig. 4C). Various recent reviews on these stages are available (Barro-Trastoy et al., 2020b; Pinto et al., 2019), and an in-depth discussion of this topic goes beyond the scope of this article. However, one important transcription factor, STK, is the master regulator of ovule identity (Favaro et al., 2003; Pinyopich et al., 2003), although this gene has many additional functions, as described below. Various other genes involved in ovule patterning have additional functions during gynoecium development.

Gynoecium patterning (stages 11 and 12)

During stages 11 and 12, gynoecium patterning continues to form all the tissues, and the gynoecium attains the final shape suitable for pollination (Fig. 1D). During this period, there is active patterning in the main axes: the abaxial-adaxial with funiculus, chalaza and nucellus (summarized in Fig. 5); the medial-lateral with the valves, valve margins, replum (see Glossary, Box 1), septum and transmitting tract (Fig. 5A); and the apical-basal with stigma (see Glossary, Box 1), style (see Glossary, Box 1), ovary and gynophore (Fig. 5B) (Chávez Montes et al., 2015; Deb et al., 2018; Marsch-Martínez and de Folter, 2016; Simonini and Østergaard, 2019; Zúñiga-Mayo et al., 2019). Most of the best-characterized regulators in these GRNs are transcription factors that affect tissue differentiation.

Medial-lateral patterning

In a mature gynoecium, the medial-lateral axis is composed of the valves (the carpel walls), which protect the developing ovules. After pollination, the valves are attached to the replum (Fig. 1F,G; Fig. 5A). The formation of this axis (valves-replum-valves) requires the concerted action of medial factors, such as BP, RPL, WUSCHEL-RELATED HOMEOBOX 13 (WOX13) and/or NO TRASMITTING TRACT (NTT), which possess meristematic-related functions and repress the action of lateral factors, such as FILAMENTOUS FLOWER/YABBY3 (FIL/YAB3) and the ASYMMETRIC LEAVES 1/2 (AS1/2) (Alonso-Cantabrana et al., 2007; Dinneny et al., 2005; Marsch-Martínez et al., 2014; Romera-Branchat et al., 2013). Additionally, the so-called 'boundary factors' (CUCs and KNAT2/6) participate in between, marking the division of the meristematic and lateral organ functions (Fig. 5A) (reviewed by Ballester and Ferrándiz, 2017; Simonini and Østergaard, 2019).

Inside the ovary, transmitting tract formation is controlled by NTT (Crawford et al., 2007), the basic helix-loop-helix (bHLH) proteins CESTA/HALF FILLED (CES/HAF), BRASSINOSTEROID ENHANCED EXPRESSION 1 and 3 (BEE1, BEE3) (Crawford and Yanofsky, 2011), HECs (Gremski et al., 2007), SPT

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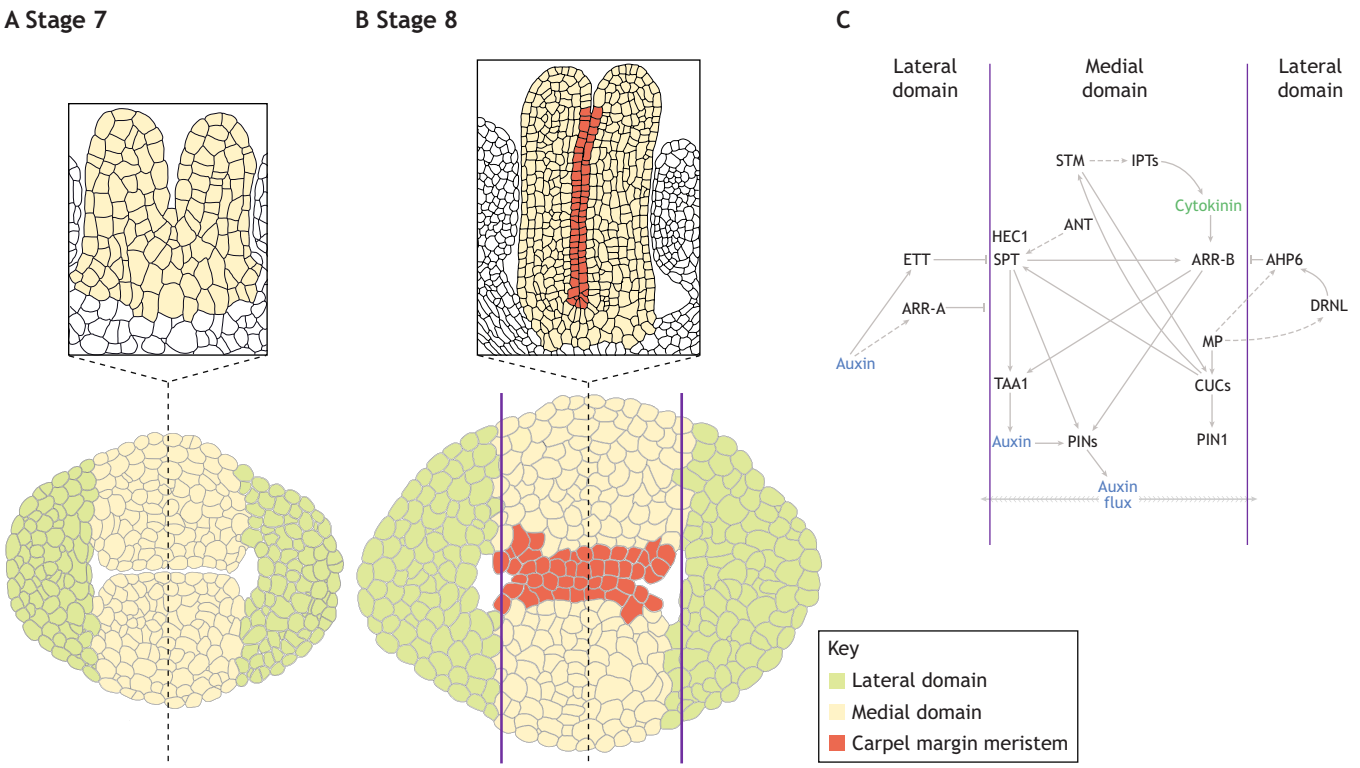

Fig. 3. Carpel margin meristem activation and maintenance (stages 7 and 8). (A) Schematic of a young gynoecium at stage 7, when two different domains – lateral (green) and medial (yellow) – are visible. (B) At stage 8, the carpel margin meristem (red) becomes specified. (C) The activity of the CMM is maintained by the activity of the bHLH transcription factors SPATULA (SPT) and HECATE 1 (HEC1), via the activation of auxin synthesis and transport. Genes related to cytokinin signaling are also involved (Reyes-Olalde and de Folter, 2019). Upper panels represent longitudinal sections of the dashed lines in the bottom panels of the gynoecium images. Dashed lines in the regulatory network indicate indirect regulation, or interactions found in other tissues that are likely occurring in the gynoecium. Colored words indicate different hormone-related processes: auxin signaling (blue); cytokinin signaling (green). Purple lines in B and C indicate the regions in which the processes occur. AHP6, HISTIDINE PHOSPHOTRANSFER PROTEIN 6; ANT, AINTEGUMENTA; ARRs, ARABIDOPSIS RESPONSE REGULATORs; CUCs, CUP-SHAPED COTYLEDONs; DRNL, DORNROSCHEN-LIKE; ETT, ETTIN; HEC1, HECATE 1; IPTs, ISOPENTENYLTRANSFERASEs; MP, MONOPTEROS; PIN1, PIN-FORMED 1; SPT, SPATULA; STM, SHOOT MERISTEMLESS; TAA1, TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1.

(Heisler et al., 2001) and STK (Di Marzo et al., 2020b; Herrera-Ubaldo et al., 2019) (Fig. 5A). In addition, several of these factors (NTT, SPT and STK) control the development of other tissues in the gynoecium that are related to transmitting tract formation; they control programmed cell death (PCD) and the deposition of extracellular matrix (ECM) (reviewed by Crawford and Yanofsky, 2008; Pereira et al., 2021).

Some processes occurring between stages 11 and 12 highlight the importance of cell wall modifications in medial-lateral gynoecium patterning. The leading role of ETT in gynoecium morphology is clear; ETT is known to participate, in coordination with auxin input, in the guidance of early patterning events (Fig. 2B; Fig. 3) (Sessions et al., 1997; Simonini et al., 2016). Now, an additional pathway of ETT participation has been identified, the regulation of pectin methylesterase (PME) activity in the valves (Andres-Robin et al., 2018). PME activity in valve cell walls increases the levels of demethylesterified pectins, allowing the reduction of cell wall stiffness and the elongation of the valves.

Inside the gynoecium, other modifications need to occur in the medial domain to allow the formation of the transmitting tract. Recently, NTT and STK have been shown to participate in the modulation of the cell wall composition by regulating a gene encoding a putative mannanase enzyme, as well as other genes encoding proteins involved in lipid biosynthesis and transport (Herrera-Ubaldo et al., 2019).

Apical-basal patterning

Major changes must be coordinated to achieve the distinct apical-basal features (stigma, style and ovary) observed in a mature gynoecium. The ovary is a bilateral structure – in contrast to the style, which exhibits radial symmetry (Fig. 1F,G). During floral stage 12, the style differentiates and the bilateral-to-radial transition is controlled by the modulation of auxin flux, as well as cytokinin sensitivity (Fig. 5B) (Carabelli et al., 2021; Moubayidin and Ostergaard, 2014). First, the transcription factors SPT and INDEHISCENT (IND) promote apolar localization of PIN proteins (auxin efflux transporters normally localized to cell poles to create polar auxin transport; Moubayidin and Ostergaard, 2014). SPT and HECs then control the expression of the adaxial-identity genes *HOMEOBOX ARABIDOPSIS THALIANA 3* (*HAT3*) and *ARABIDOPSIS THALIANA HOMEOBOX 4* (*ATHB4*), which orchestrate the final steps of the radicalization process (Fig. 5B) (Carabelli et al., 2021). In another study focusing on style development using genetics and protein-protein interaction experiments, it has been suggested that ETT, IND, BREVIPEDICELLUS (BP), REPLUMLESS (RPL) and SEUSS (SEU) work synergistically to regulate style morphology (Fig. 5B) (Simonini et al., 2018).

Sequential activation and function of transcription factors is required for stigma development: the NGATHA (NGA) and HECATE (HEC) protein families cooperatively regulate stigma

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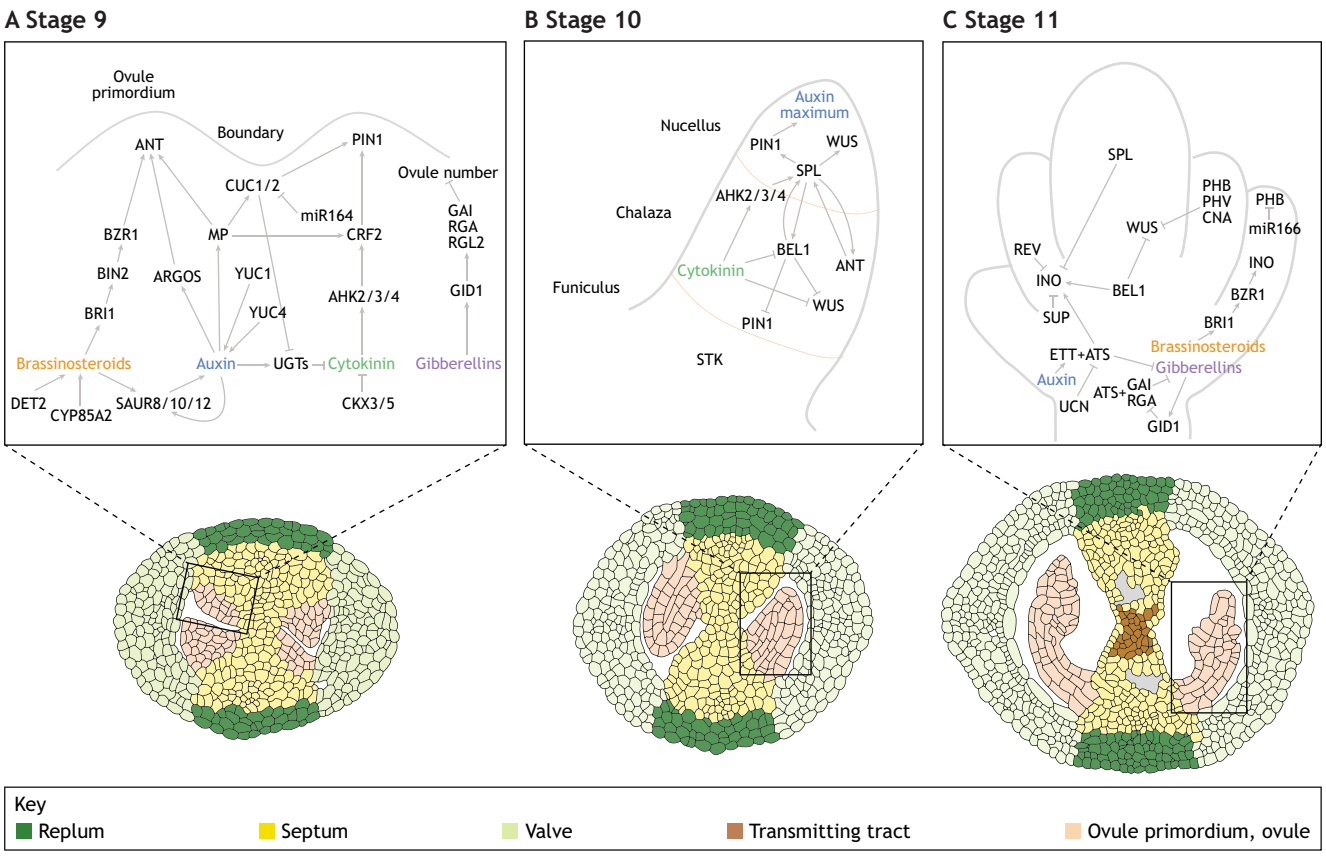

Fig. 4. Ovule identity specification and patterning (stages 9-11). (A-C) The beginning of ovule development (determination of number and position) is controlled by a complex network that involves the action of at least four hormones and several transcription factors. The regulatory networks controlling ovule primordia initiation (A), patterning (B) and morphogenesis (C) (Barro-Trastoy et al., 2020b; Cucinotta et al., 2020) are summarized. Colored words indicate different hormone-related processes: auxin signaling (blue); cytokinin signaling (green); gibberellins (purple); brassinosteroids (orange). AHK2/3/4, ARABIDOPSIS HISTIDINE PROTEIN KINASE 2, 3, 4; ANT, AINTEGUMENTA; ARGOS, AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE; ATS, ABERRANT TESTA SHAPE; BEL1, BELL 1; BIN2, BRASSINOSTEROID-INSENSITIVE 2; BRI1, BRASSINOSTEROID INSENSITIVE 1; BZR1, BRASSINAZOLE RESISTANT 1; CKX3/5, CYTOKININ OXIDASE/DEHYDROGENASE 3, 5; CNA, CORONA; CRF2, CYTOKININ RESPONSE FACTOR 2; CUC1/2, CUP-SHAPED COTYLEDON 1, 2; CYP85A2, cytochrome p450 enzyme; DET2, ATDET2; ETT, ETTIN; GAI, GIBBERELLIC ACID INSENSITIVE; GID1, GA INSENSITIVE DWARF; INO, INNER NO OUTER; miR164, microRNA164; miR166, microRNA166; MP, MONOPTEROS; PHB, PHABULOSA; PHV, PHAVOLUTA; PIN1, PIN-FORMED 1; REV, REVOLUTA; RGA, REPRESSOR OF GA; RGL2, RGA-LIKE 2; SAUR8/10/12, SAUR-like auxin-responsive protein family; SPL, SPOROCYTELESS; STK, SEEDSTICK; SUP, SUPERMAN; UCN, UNICORN; UGTs, UDP-glucosyl transferases; WUS, WUSCHEL; YUC1/4, YUCCA 1, 4.

development through the activation of IND, which later interacts with NGA (and probably HECs) to activate SPT. SPT is then probably integrated into the NGA-IND-HEC complex to activate target genes (Ballester et al., 2021). Recently, three angiosperm-specific regulators of the style and stigma have been identified called STIGMA AND STYLE STYLIST 1-3 (SSS1-3), which belong to a previously unreported family. They are expressed in the apical tissues of the gynoecium and act downstream of the NGA transcription factors to fine-tune the development of the stigma and style (Fig. 5B) (Li et al., 2020).

Pollination and fertilization (stage 13)

Pollination and fertilization involve a continuous and active communication between female and male tissues over several steps: pollen hydration, pollen germination, pollen tube growth, pollen tube attraction to the ovule, pollen tube reception, sperm cell delivery and gamete activation (reviewed by Cascallares et al., 2020).

As discussed earlier, the formation of the transmitting tract includes regulation of PCD and ECM deposition (reviewed by Pereira et al., 2021), as well as other changes in cell wall composition in the medial region (Herrera-Ubaldo and de Folter, 2018). The ECM provides nutrition, adhesion and guidance during pollen tube growth. During fertilization, the transmitting tract secrets chemical signals, such as arabinogalactan-proteins (AGPs), whereas other signals, such as LURE proteins, derive from the ovules to attract pollen tubes (reviewed by Johnson et al., 2019; Pereira et al., 2021).

Checkpoints during the pollination process control the acceptance or rejection of the pollen prior to fertilization (reviewed by Dresselhaus et al., 2016) and various small peptides and receptors are involved in these processes (reviewed by Zhang et al., 2021). Recently, some new discoveries have been made towards the understanding of communication and signaling cascades during pollination. One such discovery is the existence of an autocrine signaling pathway acting at the surface of stigmatic papillae, inducing reactive oxygen species (ROS) production; ROS levels are reduced upon pollination owing to an antagonistic peptide from the pollen coat, allowing pollen hydration and germination (Liu et al., 2021; Zhou et al., 2021). Another discovery also involves

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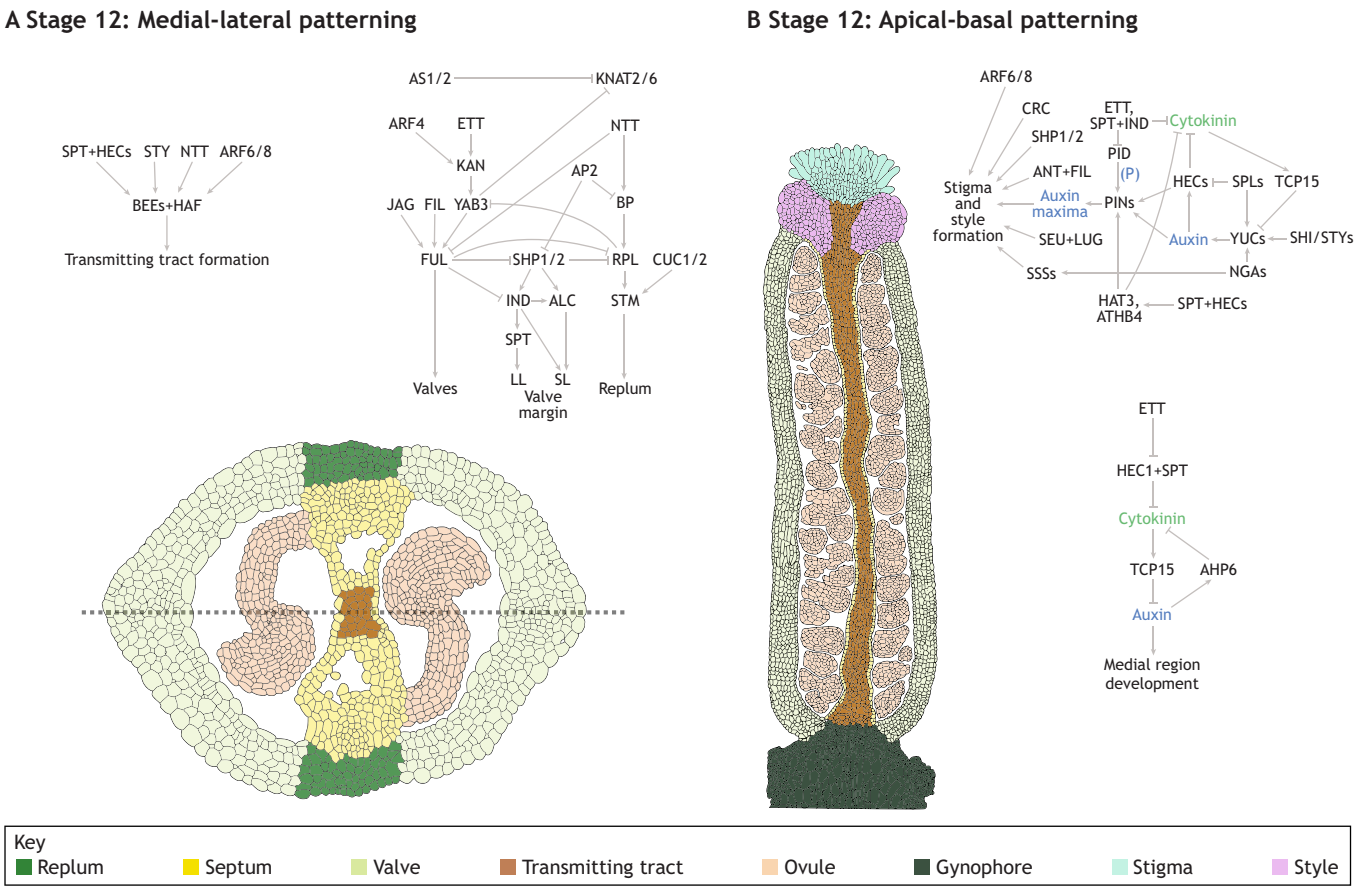

Fig. 5. Medial-lateral and apical-basal gynoecium patterning fertilization (stage 12). (A) In the outer part of the gynoecium, lateral and medial factors cooperate or compete to define the valves, valve margins and replum. The valve margin is divided in the lignification (LL) and separation layer (SL). The formation of the transmitting tract is coordinated by several transcription factors. Dashed line represents the transverse section depicted in B. (B) In the apical-basal axis, stigma and style formation is guided by regulation that converges in the production of auxins. Cooperation between transcription factors and hormones participates in maintaining the ovary (Alonso-Cantabrana et al., 2007; Deb et al., 2018; Marsch-Martínez and de Folter, 2016; Simonini and Østergaard, 2019). Colored words indicate different hormone-related-processes: auxin signaling (blue); cytokinin signaling (green). '(P)' indicates that PID phosphorylates PIN proteins. AHP6, HISTIDINE PHOSPHOTRANSFER PROTEIN 6; ALC, ALCATRAZ; ANT, AINTEGUMENTA; AP2, APETALA 2; ARF4/6/8, AUXIN RESPONSE FACTOR 4, 6, 8; AS1/2, ASYMMETRIC LEAVES 1, 2; ATHB4, ARABIDOPSIS THALIANA HOMEOBOX 4; BEEs, BR-ENHANCED EXPRESSIONS; BP, BREVIPEDICELLUS; CRC, CRABS CLAW; CUC1/2, CUP-SHAPED COTYLEDON 1, 2; ETT, ETTIN; FIL, FILAMENTOUS FLOWER; FUL, FRUITFULL; HAF, HALF FILLED; HAT3, HOMEOBOX ARABIDOPSIS THALIANA 3; HECs, HECATEs; IND, INDEHISCENT; JAG, JAGGED; KAN, KANADI; KNAT2/6, KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 2, 6; LUG, LEUNIG; NGAs, NGATHAs; NTT, NO TRANSMITTING TRACT; PID, PINOID; PINs, PIN-FORMED; RPL, REPLUMLESS; SEU, SEUSS; SHI, SHORT INTERNODES; SHP1/2, SHATTERPROOF 1, 2; SPLs, SQUAMOSA PROMOTER BINDING PROTEIN-LIKEs; SPT, SPATULA; SSSs, STIGMA AND STYLE STYLISTs; STM, SHOOT MERISTEMLESS; STYs, STYLISHs; TCP15, TEOSINTE BRANCHED, CYCLOIDEA/PCF 15; YAB3, YABBY 3; YUCs, YUCCAs.

the receptor kinase FERONIA (FER) in the control of male-female interactions. FER, located at the entrance to the female gametophyte, mediates de-esterified pectins and the production of nitric oxide (NO), which is necessary for when the first pollen tube arrives at the ovule; NO accumulation stops the attraction of further pollen tubes (Duan et al., 2020). The process of pollination, and the subsequent events, can only occur in a specific time window, delimited by stigmatic receptivity. Two transcription factors, *KIRA1* (*KIR1*) and *ORESARA1* (*ORE1*), belonging to the NAC family promote PCD in the stigma cells, thereby controlling stigma longevity (Gao et al., 2018).

Seed and fruit development (stages 14-20)

After fertilization, the gynoecium becomes a fruit, and its development in *Arabidopsis* continues over 12 days to finally release fully formed, fertile seeds. During the final stages of fruit development, the fruit grows, the seeds mature and developmental programs prepare for fruit dehiscence (see Glossary, Box 1) and seed dispersal (Alvarez-Buylla et al., 2010; Ballester and Ferrándiz, 2017; Roeder and Yanofsky, 2006). After fertilization (stage 13), the fruit grows mainly longitudinally to reach its maximum size by stage 17, followed by the start of senescence of the fruit (stage 18), and finally fruit dehiscence and seed release (stage 20) (Fig. 6A-C).

Longitudinal growth

The involvement of cytokinin in fruit elongation has recently been reported: phenotypic alterations observed in fruit length of the *stk* mutant, which produces short fruits, resemble those of fruits of the *ckx7* mutant (encoding for the enzyme CYTOKININ OXIDASE/DEHYDROGENASE 7), in which cytokinin degradation is affected (Di Marzo et al., 2020a). Therefore, cytokinin has a negative effect on fruit elongation. This additional function of STK also integrates pathways that control fruit size because STK positively regulates *CKX7* expression in the fruit and indirectly controls FUL, which is

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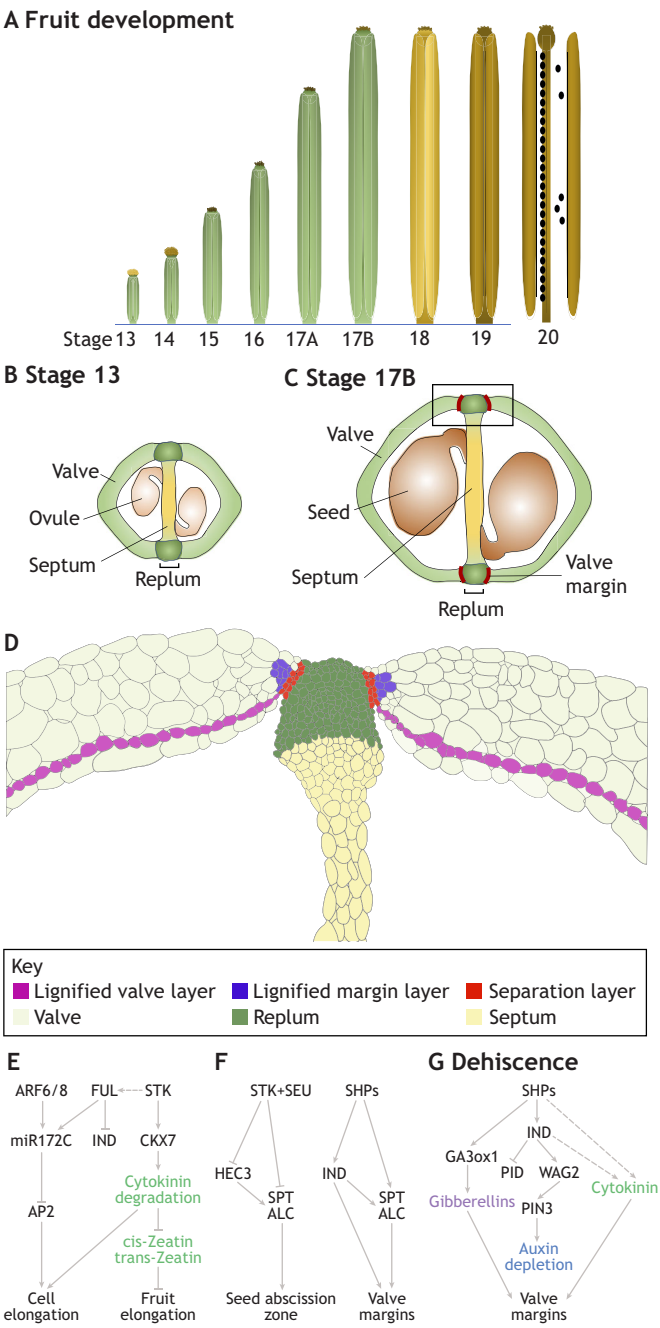

Fig. 6. Fruit growth and dehiscence (stages 14-20). (A) After fertilization, the gynoecium becomes a fruit. During stages 14-19, the fruit grows and prepares to release the seeds. (B-D) Internally, the gynoecium-to-fruit transition involves changes in tissue conformation. One of the most relevant is the formation of the seeds, as well as the differentiation of the lignification (LL) and separation (SL) layers in the valve margins prior to dehiscence. D shows a detailed view of the boxed area in C. (E-G) At the molecular level, fruit elongation relies on cell elongation (E) and dehiscence (F), which are controlled by transcription factors with hormonal input. (G) The specification of the seed abscission zone and the valve margins share some regulatory elements, such as SEEDSTICK (STK) and SHATTERPROOFs (SHPs). Dashed lines indicate indirect regulation. Colored words indicate different hormone-related processes: auxin signaling (blue); cytokinin signaling (green); gibberellins (purple) (Balanzà et al., 2016; Di Marzo et al., 2020a; Marsch-Martínez and de Folter, 2016; van Gelderen et al., 2016). ALC, ALCATRAZ; AP2, APETALA2; ARF6/8, AUXIN RESPONSE FACTOR 6, 8; CKX7, CYTOKININ OXIDASE/DEHYDROGENASE 7; FUL, FRUITFULL; GA3ox1, GIBBERELLIN 3-beta-dioxygenase 1; HEC3, HECATE 3; IND, INDEHISCENT; miR172C, microRNA172C; PID, PINOID; PIN3, PIN-FORMED3; SPT, SPATULA; SEU, SEUSS; WAG2, WAG2.

involved in a pathway with miR172 and AP2 (Fig. 6E) (Di Marzo et al., 2020a; Ripoll et al., 2015). Additionally, STK regulates fruit growth by controlling the expression of α-*XYLOSIDASE 1* (*XYL1*), which is involved in the modification of xyloglucans (XyGs), which form a component of cell walls. Indeed, XyG modifications contribute to the mechanical properties of the cell wall, affecting its extensibility and thus cell growth. The activity of XYL1 is also required in the growing seeds in order for them to achieve their final size (Di Marzo et al., 2021).

The involvement of hormones in fruit development and maturation goes beyond just auxin and cytokinin. Gibberellins induce fruit growth in *Arabidopsis* (Dorcey et al., 2009) and regulate the formation of the separation layer in the valve margins (Fig. 6D,F) (Arnaud et al., 2010). The role of hormones, such as abscisic acid, ethylene and brassinosteroids, are better described for fleshy fruit development (reviewed by Li et al., 2021; McAtee et al., 2013; Sotelo-Silveira et al., 2014).

Related to longitudinal growth of the silique (see Glossary, Box 1) or fruit, a recent study has reported a spatiotemporal map of fruit growth at the cellular level that captures quantitative data to measure cell division and cell expansion. Analysis of the *ntt* mutant affected in seed-set revealed that final fruit length correlates with the number of seeds (Ripoll et al., 2019).

Preparation for dehiscence

Early studies on fruit development have described the initial models for fruit patterning related to fruit opening (dehiscence or pod shattering), highlighting the key roles of the transcription factors FUL, SHPs, RPL, ALC and IND (Ballester and Ferrándiz, 2017; Liljegren et al., 2004). The dehiscence zone, located on the valve margins, are specialized cell layers that allow valve detachment. SHP proteins control the expression of IND, which guides the formation of the lignification layer (Fig. 6D,F). Additionally, SHPs control ALCATRAZ (ALC), which guides the formation of the separation layer (reviewed by Ballester and Ferrándiz, 2017). Moreover, FUL controls the formation of a lignified layer in the valves (Liljegren et al., 2004).

Another role of STK is the control of lignification in the funiculus, which is necessary for seed abscission (Balanzà et al., 2016). In the funiculus, STK, together with the co-repressor SEU, represses the expression of *HEC3*, *SPT* and *ALC* (Fig. 6F). A rather similar process occurs in valve margin lignification, whereby SHPs regulate *IND*, *SPT* and *ALC*, but the regulation is inverted (i.e. SHPs activate gene expression) (Fig. 5A; Fig. 6F).

Important roles of hormones have been uncovered as well. IND regulates the expression of PINOID (PID) and WAG2 to control auxin transport outside the separation layer in the valve margins, creating a so-called ‘auxin minimum’ that is important for dehiscence zone formation (Sorefan et al., 2009). However, further studies regarding the participation of auxin in the control of dehiscence, using reporter lines to track auxin distribution have revealed that, at early fruit-development stages (stage 14-16), auxin is present at the valve margin for specifying the dehiscence zone (van Gelderen et al., 2016). Perhaps the ‘auxin minimum’ at later fruit stages (e.g. stage 17B) is still functionally relevant for cell separation (reviewed by Ballester and Ferrándiz, 2017; de Folter, 2016). The involvement of cytokinin signaling during fruit development has also been studied. The valve margins display cytokinin signaling, which is lost in mutants that are indehiscent (*ind* and *shp1 shp2*). When cytokinin is applied to these mutants, fruit dehiscence is restored (Marsch-Martínez et al., 2012). Furthermore, replum development is also dependent on the presence of cytokinin; with less cytokinin or

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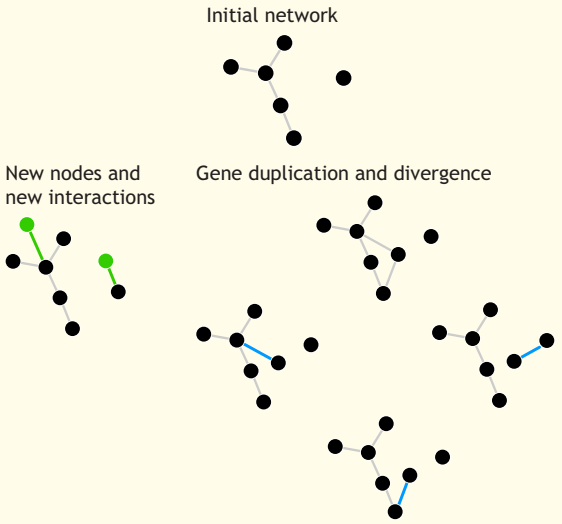
Box 3. Evolution of gene regulatory networks (GRNs) directing gynoecium development

The study of the origin of the carpel at the morphological level is an active topic of research (Endress, 2019; Endress and Doyle, 2015; Endress and Igersheim, 2000; Scutt et al., 2006). Some evidence points to the tube-like (ascidiate) or leaf-like (plicate) origin of the carpel. The information from *Arabidopsis* is the reference for comparative studies. The identification of key regulators (as individuals or families) and their conservation across the tree of life and its evolution (Pfannebecker et al., 2017a,b) has allowed the tracing of the history of the main regulators (Becker, 2020; Ferrándiz and Fourquin, 2014).

Current work is focused on deciphering the origin of regulators and their connections in GRNs that guide the variation in the shape and function of fruits. Two basic aspects of GRN evolution are: (1) the rise of nodes and connections (see figure, green dots and lines); and (2) rewiring of the edges (blue lines). Furthermore, variation in cell division rates or cell expansion may explain shape diversity in related species (Dong et al., 2020; Łangowski et al., 2016).

The construction of genetic and protein interaction networks that retrieves proteins of different clades gives an idea of how regulatory networks gain complexity. Furthermore, information from other fruit species, such as *Solanaceae* (Ortiz-Ramírez et al., 2018) and others (Gomariz-Fernández et al., 2017; Pabón-Mora et al., 2014; Simonini et al., 2018), could give insights into gynoecium evolution.



cytokinin signaling, the width of the replum is reduced and vice versa (Marsch-Martínez et al., 2012; Reyes-Olalde et al., 2017). In a double *Arabidopsis ntt rpl* mutant that lacks the replum, replum formation is restored upon cytokinin application, suggesting that cytokinin signaling acts downstream of NTT and RPL (Zúñiga-Mayo et al., 2018). In addition, increased replum width has also been observed when *NTT* is overexpressed (Marsch-Martínez et al., 2014).

In addition to the hormonal and genetic control of dehiscence, a recent study adds the importance of environmental input. Temperature affects the expression of IND; higher temperature causes chromatin modifications at the *IND* locus, which results in increased *IND* expression that accelerates valve margin development (Fig. 6G) (Li et al., 2018).

Gene expression: additional nodes in the GRNs?

The concerted action of GRNs during these stages will ultimately guide fruit growth and shape. Although a lot of information is available, further players are likely still to be discovered. A recent study has conducted transcriptome analysis to reveal gene expression dynamics during fruit growth and maturation, including silique samples, from 3, 6, 9 and 12 days after pollination (Mizzotti et al., 2018). This dataset is supported by another study, which has analyzed the pre-fertilization stages: carpel initiation (stage 5), elongation of carpel walls (stage 9), gynoecia during female meiosis (stage 11), and gynoecia before anthesis (stage 12) (Kivivirta et al., 2021). These two studies (and others, e.g. Klepikova et al., 2016; Villarino et al., 2016; Wynn et al., 2011) now provide a broad gene expression atlas for the complete developmental window from gynoecium-fruit development. In both works, samples included complete gynoecia and fruits, so additional work is required to resolve gene expression further or dissect transcriptomic data at the tissue- and single-cell level.

Conclusions

The current knowledge on gynoecium and fruit development is expanding. We have given an overview of the GRNs known to date. In the future, it is likely that these GRNs will expand because we know that many more transcription factors are expressed at the specific stages discussed (Table S1). Furthermore, many recent works have provided new datasets and information related to gynoecium formation, such as transcriptomics during development (Kivivirta et al., 2021; Mizzotti et al., 2018) or at specific times or scenarios (Krizek et al., 2021; Liao et al., 2020; Martínez-Fernández et al., 2020; Yu et al., 2020); variation by genome-wide association studies (Yuan and Kessler, 2019) or quantitative trait locus analysis (Kawamoto et al., 2020); and protein-protein interactions (Herrera-Ubaldo et al., 2018 preprint). The presented GRNs are based on data of *Arabidopsis* it is likely that these GRNs have variations and/or rewiring that may explain existing diversity in gynoecia and fruits of other species (Box 3).

Current technologies allow the study of morphogenesis in *Arabidopsis* in unprecedented detail. Live imaging, lineage tracking and geometric analysis, in combination with single-cell transcriptome profiling, are changing how we study plants and paving the way to study development in four dimensions. Some important advances have been made during early flower development, such as the construction of a 4D atlas that integrates cell growth quantification and gene expression data (Refahi et al., 2021); a 3D gene expression atlas of the FM based on the spatial reconstruction of single-nucleus RNA-sequencing data (Neumann et al., 2021 preprint); and a study of sepal (Zhu et al., 2020) and stamen growth (Silveira et al., 2021). In the case of ovule formation, the generation of a 3D reference atlas (Vijayan et al., 2021) or the analysis of the relationship between organ geometry and cell fate (Hernandez-Lagana et al., 2021) provide comprehensive views of ovule development.

These works are related to reproductive structures, but the field can also take advantage of broader studies performed at the whole-plant level. For example, the protein interactome for hormone-related proteins (Altmann et al., 2020) or the *Arabidopsis* proteome with samples from whole flowers and fruits (as well as carpels, seeds, valves and septum samples; Mergner et al., 2020), provide useful resources and valuable information to be integrated. Additionally, a recent dataset has facilitated the study of protein complexes at the pan-plant level (McWhite et al., 2020), and another allows the comparison of transcriptomic programs across land plants (Julca et al., 2021). Finally, the Plant Cell Atlas initiative will provide insight into the content and processes taking place in each cell type in the plant (Plant Cell Atlas Consortium et al., 2021; Rhee et al., 2019). The integration of all these data in the

coming years will reveal many hidden aspects of morphogenesis during gynoecium and fruit formation and will achieve a detailed picture of the mechanisms involved, at a similar level to other systems, such as plant embryo patterning (Harnvanichvech et al., 2021).

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Competing interests
The authors declare no competing or financial interests.

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