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Involvement of *CUP-SHAPED COTYLEDON* Genes in Gynoecium and Ovule Development in *Arabidopsis thaliana*

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When mutations in *CUP-SHAPED COTYLEDON1* (*CUC1*) and *CUC2* are combined, severe defects involving fusion of sepals and of stamens occur in Arabidopsis flowers. In addition, septa of gynoecia do not fuse along the length of the ovaries and many ovules have their growth arrested. *CUC2* is expressed at the tips of septal primordia during gynoecium development and at the boundary between nucellus and chalaza during ovule development. These expression patterns are partially consistent with the phenotype of the mutant gynoecium. *CUC2* mRNA is also shown to be expressed at the boundaries between meristems and organ primordia during both the vegetative and reproductive phases. This expression pattern indicates that *CUC2* is generally involved in organ separation in shoot and floral meristems.

Key words: *Arabidopsis thaliana* — *CUC* — Gynoecium — Ovule — Organ separation — Postgenital fusion.

In higher plants, the flower usually consists of four types of organ (sepals, petals, stamens and carpels) which are sequentially produced from the floral meristem in a whorled manner. Analyses of homeotic flower mutants of Arabidopsis and snapdragon have led to a genetic model which suggests how identities of floral organs are established (Coen and Meyerowitz 1991, Weigel and Meyerowitz 1994). In Arabidopsis, the flower has four separate sepals, four separate petals, six separate stamens and two fused carpels. Organs in the outer two whorls have a comparatively simple structure, while organs in the inner two whorls, especially carpels, have a complex structure. Carpels form a gynoecium which consists of three different regions along its longitudinal axis, (from top to base) stigma, style and ovary. The ovary is partitioned into two locules by a septum. Within the ovary, ovules develop from placentae. The ovule consists of three distinct elements, a funiculus, a chalaza, and a nucellus arranged along the proximal-distal axis. These elements differentiate sequentially from the distal to the proximal end (Schneitz et al. 1995). Inner and outer integuments differentiate from the chalazal region and megasporogenesis and megagametogenesis occur in the nucellus.

There are a number of mutations which disrupt development of the gynoecium in Arabidopsis. Mutations in the *ETT* gene cause defects in apical-basal and internal-external patterning within the gynoecium (Sessions and Zambryski 1995). In *tsl* mutants, the gynoecium is unfused at its top, and in the ovary the septum is unfused (Roe et al. 1997). Unfused gynoecia and unfused septa are also observed in *spatula* (*spt*) and *crabs claw* (*crc*) mutants (Alvarez and Smyth 1999). With respect to ovule development, recent molecular genetic analyses have identified genes that regulate differentiation of the chalazal region (Gasser et al. 1998, Schneitz et al. 1998b). For example, *BELL1* (*BEL1*) plays an important role in determining integument identity (Modrusan et al. 1994). *AINTEGUMENTA* (*ANT*) functions to promote initiation and growth of both integument primordia and the ovule primordium itself (Klucher et al. 1996, Elliott et al. 1996, Baker et al. 1997, Schneitz et al. 1998a).

We previously reported that flowers with mutations in both *CUP-SHAPED COTYLEDON1* (*CUC1*) and *CUC2* have severely fused sepals and stamens (Aida et al. 1997). In the double mutant seedling, cotyledons are fused along the edges of their sides, resulting in a cup-shaped cotyledon. These phenotypes suggest that *CUC1* and *CUC2* are involved in the separation of cotyledons during embryogenesis and in the separation of sepals and stamens during flower development. Each single mutant has almost the same phenotype as wild type, suggesting *CUC1* and *CUC2* are functionally redundant. *CUC2* encodes a member of the NAC domain proteins.

In this study, we examined the phenotype of the gynoecium and the ovule in *cuc1 cuc2* double mutants, because flowers of the double mutant are female sterile (Aida et al. 1997). The gynoecium of *cuc1 cuc2* was defective in the formation of the septum and in the development of ovules. We also examined the expression pattern of the *CUC2* gene after germination, from the vegetative stage to ovule formation. *CUC2* was expressed at boundaries between organ primordia and meristems from the vegetative stage to early flower development, supporting presumptive roles in organ separation for *CUC1* and *CUC2*. Addition-

Abbreviations: IM, inflorescence meristem; SAM, shoot apical meristem.

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ally, *CUC2* was expressed at the tips of septal primordia and at the boundary between the nucellus and chalaza. These expression patterns were partially consistent with the morphological changes in double mutant combinations of *cuc1* and *cuc2*.

Materials and Methods

Plants and growth conditions—Arabidopsis thaliana ecotype Landsberg *erecta* was used as the wild type. The origin of the *cuc* mutants was described previously (Aida et al. 1997). Seeds were surface sterilized, sown on Murashige and Skoog plates, and germinated as previously described (Aida et al. 1997). Seedlings about 2 weeks after germination were transplanted into soil and usually grown at 23°C under constant white light as previously described (Fukaki et al. 1996). When plants were grown for examination of the septum phenotype, photoperiod conditions were changed after transplantation from constant light to long days (16-h light/8-h dark).

Shoot regeneration—Shoot regeneration was performed as described by Aida et al. (1997).

Microscopy and in situ hybridization—Gynoecia and ovules at anthesis or 1 d after anthesis were observed. For scanning electron microscopy, intact or dissected gynoecia were fixed in FAA overnight at 4°C. Samples were then subjected to dehydration and critical point drying. Samples were mounted on stubs and coated with gold in an ion sputter coater before observation. For tissue sections, gynoecia were embedded in 5% agar gel. After hardening the gel in a refrigerator for 1 h, agar blocks containing samples were sectioned. 40-μm sections were cut with a microslicer DTK-1500 (Dosaka EM, Kyoto, Japan).

In situ hybridization was performed as described by Aida et al. (1999) with the following modifications. Tissues were fixed in FAA for 4 h at room temperature, and, for flower tissues, hybridization was performed at 42°C. The template for *CUC2* antisense probes was the 558-bp fragment corresponding to the third exon or the 1,140-bp fragment containing the whole coding region of the *CUC2* cDNA (Aida et al. 1999). The two probes gave identical results. Control experiments were performed with sense probes of *CUC2* made from the above templates, and no significant signal was detected.

Results

Septum defects in cuc1 cuc2—Wild-type Arabidopsis flowers have four sepals, four petals, six stamens and two carpels. Organs in the outer three whorls (sepals, petals and stamens) are separate. On the other hand, two carpels are initiated as a fused cylinder-like structure and make a gynoecium. The mature gynoecium is composed of a stigma, a style and an ovary which is partitioned into two locules by a septum (Fig. 1A, B). The septum is formed by postgenital fusion of two septal primordia, which arise at the boundaries between the two carpel primordia. The septal primordia elongate inward and fuse together to make the septum. At maturity, outer epidermal cells of the ovary in the region of the septum are regular in shape and smaller than those of the valves themselves, and the site of the septum can be recognized as furrows between the two carpel valves.

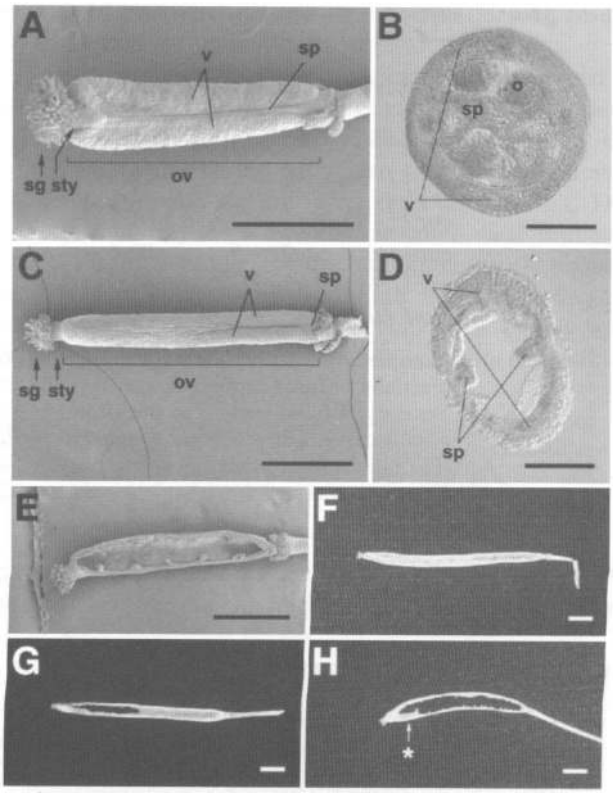

Fig. 1 Gynoecia of wild type and *cuc1 cuc2* mutant flowers. (A): Scanning electron micrograph (SEM) image of wild-type gynoecium 1 d after anthesis. (B): Transverse section of wild-type gynoecium at anthesis. (C): SEM image of gynoecium of *cuc1 cuc2* double mutant lacking septum-specific outer epidermis in the upper part of the ovary. (D): Transverse section of gynoecium of *cuc1 cuc2* double mutant. (E): SEM image of gynoecium of *cuc1 cuc2* double mutant. A carpel valve has been removed to show the inside of the ovary. (F) to (H): Septa of a *cuc1/+ cuc2/cuc2* plant after dehiscence. (F) shows a normal septum, (G) shows a partially unfused septum and (H) shows a severely unfused septum. Asterisk indicates an elongated septal primordium. sg, stigma; sty, style; ov, ovary; v, carpel valve; sp, septum; o, ovule. Bars = 1 mm in (A), (C), and (E) to (H); Bars = 100 μm in (B) and (D).

Double mutations in *CUC1* and *CUC2* cause defects in the formation of a shoot apical meristem and the separation of cotyledons during embryogenesis. However, adventitious shoots can be induced from calli derived from hypocotyls of *cuc1 cuc2* double mutants, and flowers derived from these shoots are defective in the separation of sepals and stamens (Aida et al. 1997). *cuc1 cuc2* flowers are sterile, although their stamens produce fertile pollen (data not shown), suggesting that there may be defects in the gynoecia. In *cuc1 cuc2*, the stigma, the style and the ovary differentiate. However, all ovaries of *cuc1 cuc2* lacked fused septa, and 143 of 292 gynoecia scored lacked septum-specific outer epidermis in the upper part of their

Table 1 Frequencies of septal phenotypes

Genotype	Normal (%)[a]	Unfused		Number of gynoecia observed
		Partial (%)[b]	Severe (%)[c]	
Ler[d]	99.5	0.5	0	379
cuc1[d]	97.8	1.8	0.4	499
cuc2[d]	93.1	4.8	2	596
cuc1/cuc1 cuc2/+[d]	90.6	8.1	1.3	554
cuc1/+cuc2/cuc2[e]	51.6	25.8	22.6	1820
cuc1 cuc2[f]	0	0	100	239

[a] Completely fused septum as shown in Fig. 1F.
[b] Partially unfused septum as shown in Fig. 1G.
[c] Severely unfused septum as shown in Fig. 1E or H.
[d] Five independent plants were analyzed.
[e] Ten independent plants were analyzed.
[f] Plants regenerated from ten independent calli were analyzed.

ovaries (Fig. 1C). Inward bulges were formed at the boundaries of carpel valves but they did not elongate or fuse (Fig. 1D, E and Table 1). These defects suggest that *CUC1* and *CUC2* are involved in the formation of septum.

We compared septa of wild type, *cuc1, cuc2, cuc1/cuc1 cuc2/+* and *cuc1/+ cuc2/cuc2* with those of the *cuc1 cuc2* double mutant. Most septa were normal in wild type, *cuc1, cuc2* and *cuc1/cuc1 cuc2/+* (Fig. 1F, G, H and Table 1). The proportion of normal septa in *cuc1* was slightly lower than that in the wild type and the proportions in *cuc2* or *cuc1/cuc1 cuc2/+* were lower than that in *cuc1*. The proportion of normal septa was further reduced to nearly 50% in *cuc1/+ cuc2/cuc2*. These data suggest that the effect of each single mutation on septum formation is subtle and that the *cuc2* mutation affects septum formation more severely than the *cuc1* mutation. The length of the unfused region in the septum varied among different gynoecia; some septa failed to fuse in part (Fig. 1G) and some

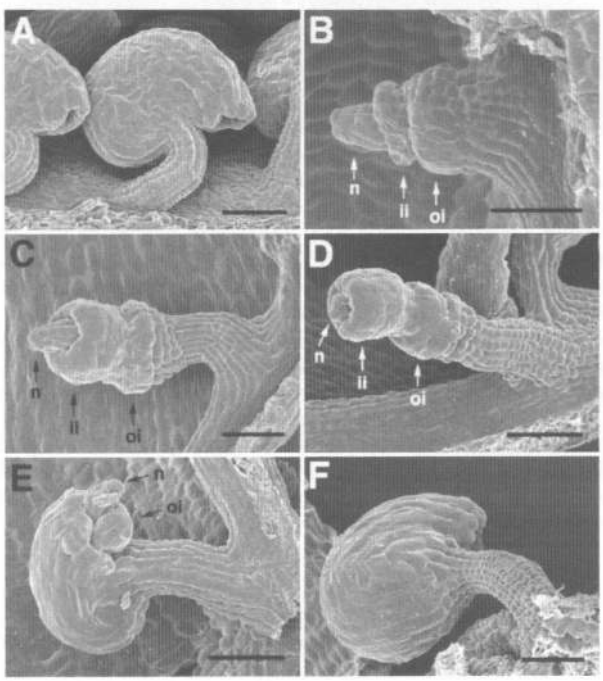

Fig. 2 Ovules of the *cuc1 cuc2* double mutant. (A): Wild-type ovule at anthesis. (B) to (F): Ovules of the *cuc1 cuc2* double mutant. The ovule shown in (B) has a short funiculus and has failed to develop inner and outer integuments soon after the differentiation. Ovules shown in (C) and (D) have failed to develop an outer integument soon after differentiation but growth of the inner integument has occurred. The extent of inner integument growth varies between different ovules. The ovule shown in (E) has developed an outer integument which does not completely cover the nucellus. The ovule shown in (F) is similar to the wild-type ovule. n, nucellus; ii, inner integument; oi, outer integument. Bars = 50 μm.

Table 2 Classification of ovules in *cuc1 cuc2* double mutants

Phenotype of ovule	Number of ovules
I[a]	76
II[b]	32
III[c]	24
II or III[d]	5
IV[e]	1

[a] Outer integuments stopped their growth soon after differentiation as shown in Fig. 2B, C, D.
[b] Outer integuments grew but did not cover nucelli as shown in Fig. 2E.
[c] Integuments covered nucelli normally as shown in Fig. 2F.
[d] Outer integuments grew but it was not confirmed that they covered nucelli completely.
[e] Integuments did not differentiate.

were almost fully unfused (Fig. 1H). The septal primordia in these more severely affected gynoecia elongated but failed to fuse, whereas those in *cuc1 cuc2* double mutants arrested before elongation.

Aberrant ovule development in cuc1 cuc2—The ovule consists of three different regions, nucellus, chalaza and funiculus. Development of the ovule has been reported in detail (Schneitz et al. 1995) and it has been divided into four steps. Ovule primordia arise from the placenta located between two carpels (step 1). The primordia elongate (step 2) and three sub-regions are established. Inner and outer integuments differentiate in the chalazal region (step 3) and grow asymmetrically to cover the nucellus (step 4), resulting in the mature anatropous ovule.

Approximately 30 ovules per gynoecium developed in wild type or *cuc1* or *cuc2* single mutants when plants were regenerated from calli. In the *cuc1 cuc2* double mutant, the number of ovules varied from zero to a normal number with most gynoecia having fewer than 10 (Fig. 1E). This suggests that step 1 is inhibited in *cuc1 cuc2* mutants. Almost all the *cuc1 cuc2* ovules had integuments, suggesting that *cuc1 cuc2* double mutations had little effect on integument differentiation. Some *cuc1 cuc2* ovules were shorter (Fig. 2B) and the position of integuments in these

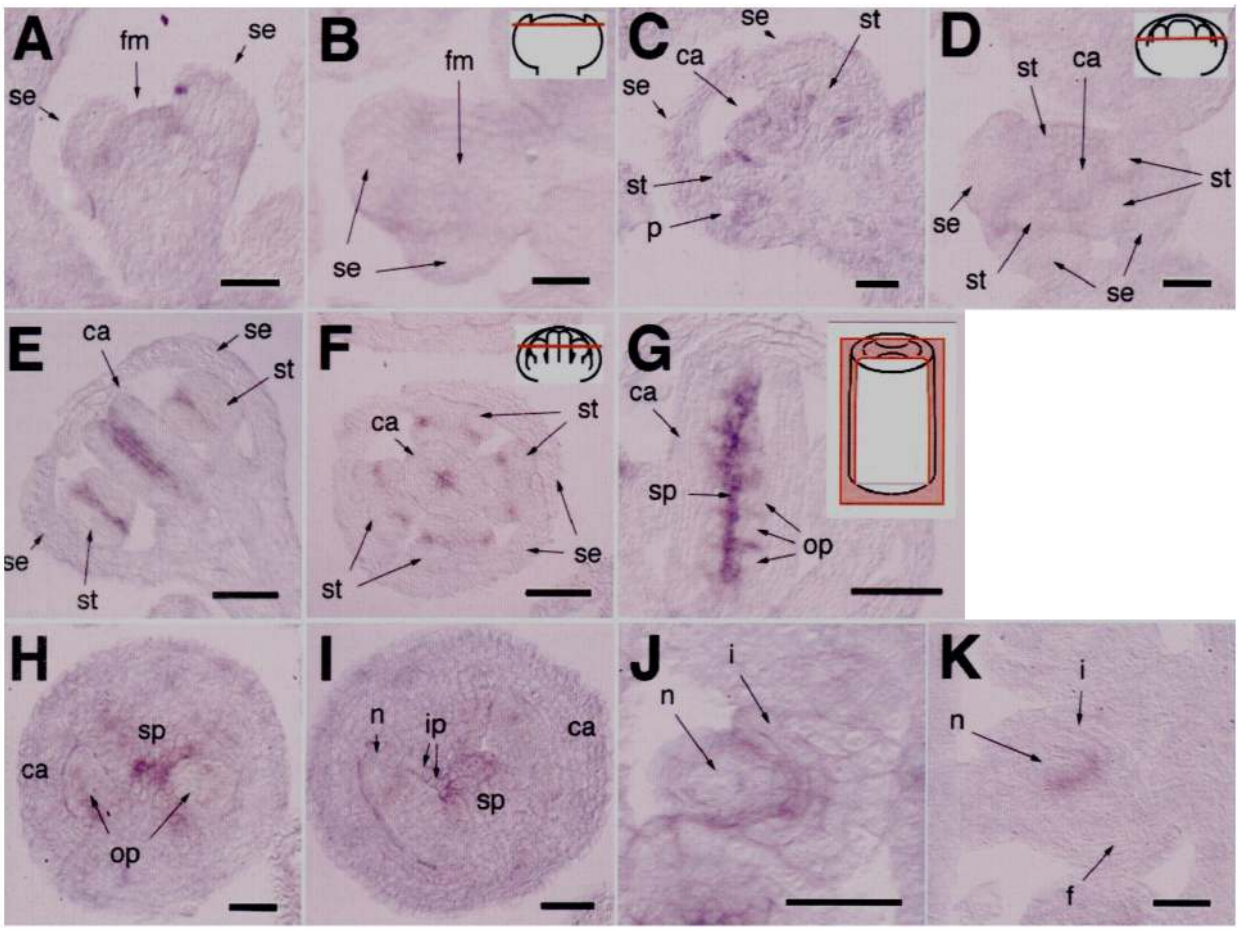

Fig. 3 Patterns of *CUC2* mRNA expression during flower and ovule development in wild type. (A): Longitudinal section of flower bud at floral stage 4. (B): Transverse section of flower bud at floral stage 4. (C): Longitudinal section of flower bud at floral stage 6. (D): Transverse section of flower bud at floral stage 6. (E): Longitudinal section of flower bud at early floral stage 8. (F): Transverse section of flower bud at early floral stage 8. (G): Longitudinal section of gynoecium at late floral stage 8. *CUC2* is not expressed in ovule primordia but at the boundaries of ovule primordia. (H): Transverse section of gynoecium at floral stage 9. Fusion of septal primordia occurs and expression of *CUC2* continues in the fused region. (I): Transverse section of gynoecium at floral stage 10. Integument primordia are differentiating at the chalazal region and *CUC2* is expressed at the boundary between nucellus and chalaza. (J): Oblique section of a developing ovule. (K): Longitudinal section of a mature ovule. Diagrams in (B), (D) and (F) represent longitudinal views of each flower bud with a red line indicating the plane of section. Diagram in (G) represents a stereoscopic view of the gynoecium, with a red plane indicating the plane of section. fm, flower meristem; se, sepal; st, stamen; p, petal; ca, carpel; sp, septum; op, ovule primordium; n, nucellus; ip, integument primordia; i, integuments; f, funiculus. Bars = 20 μm in (A) to (D) and (H) to (K); Bars = 50 μm in (E) to (G).

ovules was apparently more basal. When such ovules were cleared and observed using light microscopy, nucellar and chalazal regions were connected to the placenta by short funiculi (data not shown). This phenotype suggests that the formation of the funicular region is affected by *cuc1 cuc2* double mutations. After the integuments differentiated, growth of ovules was arrested at a range of different steps. According to their final phenotype, ovules were classified into three types. Type I ovules had outer integuments that stopped growth soon after differentiation (Fig. 2B, C, D). The extent of growth of inner integuments varied. In type II ovules, integuments did not completely cover the nucellus although curvature of ovule was observed (Fig. 2E). Type III ovules were similar to the wild-type (Fig. 2F). More than half of ovules scored were type I (Table 2), suggesting that the *cuc1 cuc2* double mutations strongly affect step 4. About 17% of ovules were close to wild type (type III), but *cuc1 cuc2* double mutants did not generate any seed.

CUC2 expression during flower development—CUC2 has been cloned and encodes a member of the NAC protein family (Aida et al. 1997). The expression of *CUC2* during wild-type flower development was analyzed by in situ hybridization.

CUC2 was first detected at stage 3 or 4 within floral primordia (Fig. 3A, B). Serial transverse sections showed that *CUC2* appeared between sepal primordia and the floral meristem. *CUC2* is also expressed at the boundaries of individual sepal primordia in whorl 1. At stage 6, *CUC2* was detected in the region surrounding each petal and stamen primordium (Fig. 3C, D), indicating that, for whorls 1, 2 and 3, it is expressed between whorls as well as between organ primordia. The expression at the boundaries of individual organ primordia could be traced until stage 8. It was not clear whether *CUC2* was expressed before each primordium arose.

At stage 8, four anther locules appear at the top of the stamens, and primordia of septa and ovules arise in the gynoecium (Hill and Lord 1989, Smyth et al. 1990, Modrusan et al. 1994, Schneitz et al. 1995). *CUC2* was detected at the boundaries between locules of each theca in anthers (Fig. 3E, F). This expression disappeared by stage 10. *CUC2* was also expressed at the inner part of presumptive septal regions before swelling of septal primordia occurred (Fig. 3E, F). This expression broadened to include presumptive placenta and was localised to the tips of septal

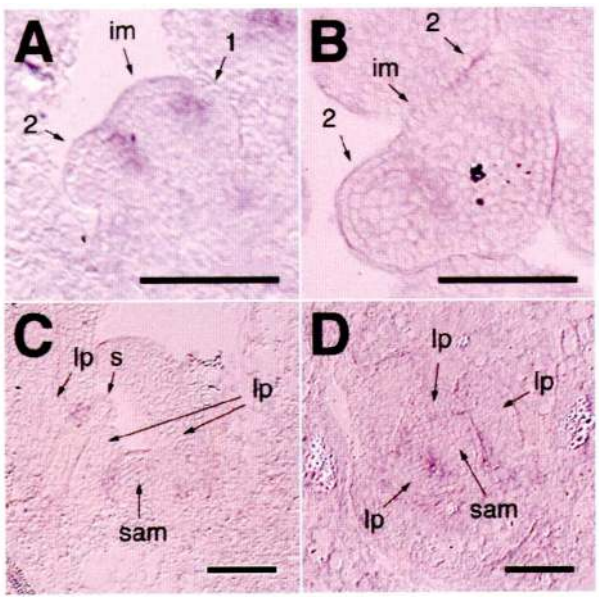

Fig. 4 *CUC2* expression in inflorescence and vegetative meristems. (A): Longitudinal section of inflorescence meristem. (B): Transverse section of inflorescence meristem. (C): Longitudinal section of vegetative shoot apical meristem. (D): Transverse section of vegetative shoot apical meristem. im, inflorescence meristem; sam, shoot apical meristem; lp, leaf primordia; s, stipule. Numbers indicate stages of flower development as described by Smyth et al. (1990). Bars = 50 μm.

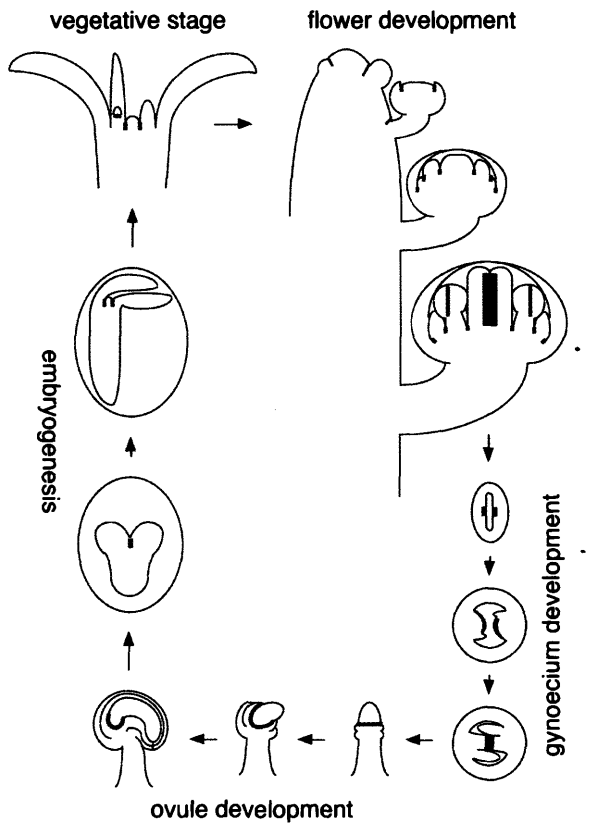

Fig. 5 Summary of *CUC2* expression patterns throughout the life of *Arabidopsis thaliana*. The pattern is shown in blue in each diagram. Diagrams representing gynoecium development are transverse views and the other diagrams are longitudinal views. The expression pattern during embryogenesis is adopted from Aida et al. (1999).

primordia as the septum grew. Fusion of the septum occurred at stage 9 (Modrusan et al. 1994) and *CUC2* was expressed at the fused region (Fig. 3H, I). Expression in the septum continued until stage 11. *CUC2* signal at the boundaries of anther locules and septal primordia was much stronger than that at the boundaries of floral organ primordia.

CUC2 expression during ovule development—When ovule primordia arose from the placenta, *CUC2* was expressed at the boundaries of ovule primordia (Fig. 3G, H). *CUC2* was not detected in ovules themselves until integument primordia differentiated. At this stage, *CUC2* was detected at the boundary between the nucellus and the chalaza (Fig. 3I). This expression pattern continued until ovules matured (Fig. 3J, K).

CUC2 expression at the boundaries between meristem and organ primordia—Flower primordia arise on the flank of the inflorescence meristem (IM) and are arranged in a spiral manner. *CUC2* was expressed at the boundaries between the IM and young flower primordia from stage 1 to 3 (Fig. 4A, B). *CUC2* signal in the axils of flower primordia became weak after stage 4 but it could be detected until stage 8. It was anticipated that *CUC2* may also be expressed earlier at boundaries between the vegetative shoot apical meristem (SAM) and leaf primordia because IMs are derived from vegetative SAMs. As expected, *CUC2* mRNA was detected at these boundaries (Fig. 4C, D). Additionally, *CUC2* mRNA was detected at boundaries between leaf primordia and stipules (Fig. 4C).

Discussion

We have previously reported that the *cuc1 cuc2* double mutation affects embryogenesis and flower development (Aida et al. 1997). In flowers, we mainly focused on the phenotype of sepals and stamens. In this study we describe the phenotype of the gynoecium. We also demonstrate that *CUC2* is expressed throughout the life of Arabidopsis plants (Fig. 5).

Role of CUC1 and CUC2 in shoot meristems—In *cuc1 cuc2* double mutants, ectopic bulging occurs at the boundaries of sepals in whorl 1 and of stamens in whorl 3 (Aida et al. 1997). These phenotypes suggest that *CUC1* and *CUC2* function to repress bulging at the boundaries of individual floral organ primordia in order to separate them. This idea is supported by *CUC2* expression pattern in flowers. It is indeed expressed at all the boundaries of organ primordia as shown in Fig. 3A to 3D. Additionally, this expression pattern suggests that *CUC2* separates organ primordia from the floral meristem. Indeed, occasional fusion of stamens to the gynoecium in *cuc1 cuc2* double mutants was reported previously (Aida et al. 1997). Ectopic bulging also occurs at the boundaries of cotyledons in *cuc1 cuc2* seedlings and *CUC2* is expressed at the boundaries of cotyledon primordia (Aida et al. 1997, 1999). These results suggest that a common mechanism functions to separate organ primordia during both flower development and embryogenesis.

CUC2 is detected at the boundaries between shoot meristems and organ primordia in both the vegetative and reproductive phases, suggesting that *CUC2* also functions to separate leaves and flowers from vegetative and inflorescence meristems in the same manner. However, no defect is observed in these regions in *cuc1 cuc2* double mutants, raising the possibility that redundant factors other than *CUC1* or *CUC2* function in these regions. There are many NAC box containing genes in Arabidopsis genome and at least one of them shows an expression pattern similar to *CUC2* (S. Takada, T. Ishida and M. Tasaka. unpublished data). One possibility is that such *CUC2* homologs are the factors working redundantly with *CUC2* in the vegetative and inflorescence meristems.

SHOOT MERISTEMLESS (*STM*) encodes a member of the KNOTTED1 class of homeodomain proteins which is essential both for the formation of the shoot apical meristem (SAM) during embryogenesis and for SAM maintenance after germination (Endrizzi et al. 1996, Long et al. 1996). It has been suggested that interaction of *CUC1, CUC2* and *STM* regulates cotyledon separation and SAM formation during embryogenesis (Aida et al. 1999). In addition, *STM* is detected not only in the central zone of the SAM but also in the peripheral zone between organ primordia at the vegetative stage (Evans et al. 1997). It is highly likely that interaction between *CUC2* and *STM* may also regulate organ separation after germination.

Role of CUC1 and CUC2 in septum formation—The fused parts of two carpel primordia differentiate to form the septal primordia. These primordia grow inward and fuse to each other postgenitally. All gynoecia of the *cuc1 cuc2* double mutant have unfused septa along the length of their ovaries. Consistent with this, *CUC2* mRNA is detected in the inner part of presumptive septal regions and at the tips of septal primordia. These results indicate that *CUC1* and *CUC2* are essential for septum development. *cuc1 cuc2* mutant gynoecia often lack the outer epidermis of the ovary that lies external to the septum. However, *CUC2* mRNA is not detected in the outer parts of the septal primordia, suggesting two possibilities. One is that *CUC1* and *CUC2* function to differentiate the outer part of the septum. Interestingly, *STM* is expressed in the whole presumptive septal regions before septum primordia are apparent (Long et al. 1996). We have confirmed that *STM* and *CUC2* signals overlap in the inner region (data not shown). This observation suggests that the presumptive septal regions are distinguished at the molecular level before septal primordia appear, and that *CUC1, CUC2* and *STM* may interact during septum initiation and subsequent differentiation.

The other possibility is that the differentiation of the outer part of the septum is a secondary consequence of the development of the inner part. In *cuc1 cuc2*, inward bulges of septal primordia are not observed in the regions where septum-specific outer epidermis is absent, and the formation of septal primordia occurs earlier than the differentiation of the epidermis. It is possible that the outer epidermal characteristics simply depend on the formation of septal primordia.

CUC1 and CUC2 are required for ovule development—In many cases, the gynoecium of *cuc1 cuc2* double mutants forms fewer ovules than wild-type, *cuc1* or *cuc2*. Abnormal development of the septum may partially reduce ovule formation in *cuc1 cuc2* double mutants because ovules arise from placentae that arise at the base of the septum. However, it is possible that *CUC1* and *CUC2* directly regulate ovule initiation as *CUC2* is expressed at the boundaries of ovule primordia as they arise from the placenta. Reduction of ovule number is also observed in *aintegumenta* (*ant*) mutants (Elliott et al. 1996). Before ovule initiation, both *CUC2* and *ANT* mRNA are detected in the placenta. After ovule initiation, *CUC2* mRNA is not detected in the initiated ovule primordia themselves whereas *ANT* mRNA is still present (Elliott et al. 1996).

Almost all the observed *cuc1 cuc2* ovules have integuments, suggesting that *CUC1* and *CUC2* are not involved in integument differentiation. Some mutant ovules have short funiculi with normal sized nucelli. This defect may have been caused by insufficient growth of the funiculus. However, *CUC2* mRNA is not detected in the funiculus itself. Rather, it is detected in the placenta before integument differentiation, and at the boundary between the nucellus and the chalaza after integument differentiation. Reduction in the length of the funiculus is also observed in *ant* and *huellenlos* (*hll*) mutants, and *ANT* and *HLL* are functionally redundant in ovule development (Schneitz et al. 1998a). *ANT* mRNA is expressed in the funicular region throughout ovule development (Elliott et al. 1996). It is possible that *CUC1* and *CUC2* interact with *ANT* and *HLL* to regulate growth of the funiculus.

More than half of *cuc1 cuc2* ovules demonstrated arrested integument development. However, *CUC2* mRNA was detected at the boundary between the nucellus and the chalaza, not in the integuments themselves. Recent studies have revealed several genes that regulate integument development (Gasser et al. 1998, Schneitz et al. 1998b). Interaction of *CUC1, CUC2* and these genes may be required for integument growth.

Although there are some normal-appearing ovules in *cuc1 cuc2* gynoecia, *cuc1 cuc2* plants do not generate any seed. There are at least two possibilities why no seed is formed. One is that fertilization does not occur in *cuc1 cuc2* gynoecia. After pollination, pollen tubes normally grow towards the ovules, passing through the fused region of the septum corresponding to the transmitting tract. Because *cuc1 cuc2* gynoecia do not form transmitting tract tissues, an immediate outcome would be that fertilization does not occur. Another possibility is that even though some ovules appear outwardly normal they do not have a functional female gametophyte.

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