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The *WIGGUM* gene is required for proper regulation of floral meristem size in *Arabidopsis*

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SUMMARY

The study of cell division control within developing tissues is central to understanding the processes of pattern formation. The floral meristem of angiosperms gives rise to floral organs in a particular number and pattern. Despite its critical role, little is known about how cell division is controlled in the floral meristem, and few genes involved have been identified. We describe the phenotypic effects of mutations in *WIGGUM*, a gene required for control of cell proliferation in the floral and apical meristem of *Arabidopsis thaliana*. *wiggum* flowers contain more organs, especially sepals and petals, than found in wild-type flowers. This organ number phenotype correlates with specific size changes in the early floral meristem, preceding organ initiation. Genetic studies suggest that *WIGGUM* acts on a similar process but in a separate pathway than the *CLAVATA1* and *CLAVATA3* genes in meristem size regulation, and reveal interactions with other genes affecting meristem structure and identity. Analysis of double mutant phenotypes also reveals a role for *WIGGUM* in apical meristem function. We propose that *WIGGUM* plays a role in restricting cell division relative to cellular differentiation in specific regions of the apical and floral meristems.

Key words: *Arabidopsis*, *WIGGUM* (*WIG*), Floral meristem, Flower development, Pattern formation

INTRODUCTION

The flower meristem in angiosperms gives rise to floral organs in numbers and patterns characteristic of an individual species. In *Arabidopsis thaliana*, floral meristems initiate four concentric rings, or whorls, of organs, with 4 sepals in the first (outer) whorl, 4 petals in the second whorl, 6 stamens in the third whorl, and 2 fused carpels in the central gynoecium. The organs arise in defined positions as well, presumably due to stereotypic changes in cell division patterns at organ initiation sites that give rise to organ primordia, which then undergo further cell divisions and cell differentiation to form the mature organ.

The mechanisms by which the sites of organ initiation in the floral meristem are determined is unknown. One recent approach to studying this problem has been to isolate mutations that change the number and position of organs. Mutations in the *CLAVATA1* (*CLV1*) and *CLAVATA3* (*CLV3*) genes increase organ number in all 4 floral whorls, especially the inner whorls, as well as increasing the total number of whorls (Leyser and Furner, 1992; Clark et al., 1993, 1995; Crone and Lord, 1993; Alvarez and Smyth, 1994). Mutations in the *PERIANTHIA* (*PAN*) gene cause an increase in sepal and petal number and a slight decrease in stamen number (Running and Meyerowitz, 1996). Mutations in the *FASCIATA1* (*FAS1*) and *FASCIATA2* (*FAS2*) genes show a variable organ number change in the first three whorls of the flower (Leyser and Furner, 1992). Mutations in *TOUSLED* (*TSL*; Roe et al., 1993, 1997), *REVOLUTA* (*REV*; Talbert et al., 1995), *SHOOT MERISTEMLESS* (*STM*; Barton and Poethig, 1993; Clark et al., 1996; Endrizzi et al., 1996), *AINTEGUMENTA* (*ANT*; Elliot et al., 1996; Klucher et al., 1996) and *WUSCHEL* (*WUS*; Laux et al., 1996) lead to decreased organ numbers in all 4 whorls.

In *clv1* and *clv3* mutants, the increase in organ and whorl number is correlated with specific changes in floral meristem size at the time of organ initiation (Clark et al., 1993, 1995; Alvarez and Smyth, 1994). Defects in the apical and/or floral meristem size or structure have also been reported in *fas1*, *fas2*, *stm* and *wus* mutants (Leyser and Furner, 1992; Barton and Poethig, 1993; Clark et al., 1996; Laux et al., 1996; Endrizzi et al., 1996).

In this paper we describe the effects of mutations in *WIGGUM (WIG*), a gene required for maintenance of apical and floral meristem structure, floral organ number, and organ patterning. *wig* mutant plants show an increase in organ number similar to those seen in *pan* mutants, with extra sepals and petals, and some effects on stamen and carpel number as well. However, detailed studies of the *wig* mutant phenotype, as well as analysis of double mutant combinations of *wig* with other mutants, suggest that *WIG* acts in manners distinct from those of previously described genes, and uncovers a novel

mechanism for cell division control in apical and floral meristems.

MATERIALS AND METHODS

Seeds were sown at least 2 cm apart on an approximately 1:1:1 mix of soil:vermiculite:perlite, imbibed at 4°C for 4-5 days, then placed under 600 foot-candles of continuous cool-white fluorescent light at 23°C. Plants were given Miracle-Gro fertilizer (1:1500 dilution) at approximately 2 week intervals. For tests of the germination rate and time, seeds that were freshly harvested and dried for 2 weeks were sown on water-saturated Whatman no. 3 filter paper in closed Petri dishes, imbibed at 4°C for 5 days, and placed under the same light conditions as above.

All mutant alleles of *wig* were isolated using ethyl methanesulphonate (EMS) as a mutagen. *wig-1* was isolated in the Landsberg *erecta* (L-*er*) ecotype and was backcrossed to wild-type L-*er* two times before analysis. *wig-2* and *wig-3* were isolated in the Nossen (No-0) ecotype and were backcrossed to L-*er* two times before analysis. All *wig* plants were homozygous for the *erecta* mutation. The *hanaba taranu* and *snowball* mutations arose independently in a population of *Agrobacterium tumefaciens*-mediated transformation mutants of ecotype Wassilewskija (Ws; Feldmann, 1992). All other mutant lines have been previously described.

F_2 plants from a cross of *wig-1* to the wild-type Columbia (Col-2) ecotype were used in determining the chromosomal map position of *WIG* relative to simple sequence length polymorphisms described by Bell and Ecker (1994). Two loci on chromosome 5 showed linkage: nga76 was found to be 12.2 cM from *wig-1*, and nga139 was found to be 25 cM from *wig-1*. Linkage was also noted with the visible markers *fas2* (Leyser and Furner, 1992) and *pinhead* (*pnh*; McConnell and Barton, 1995).

Scanning electron microscopy was performed as described by Bowman et al. (1989). Confocal laser scanning microscopy was performed as described by Running et al. (1995).

RESULTS

wiggum affects floral organ number

Mutants in the *WIGGUM* gene were isolated on the basis of their increased floral organ number compared to wild type (Table 1; Fig. 1). All four whorls are affected, though petal and sepal number is increased the most. Organ number is more variable than in wild type: most flowers in *wig-1* plants have 4-6 sepals, 4-6 petals, and 5-7 stamens (Table 1), and occasionally flowers with up to 8 of one type of organ are seen. Variation in sepal, petal and carpel number is rarely seen in wild-type L-*er* (Smyth et al., 1990). The organ number increase is greatest in the basal flowers, and decreases in apical flowers to numbers closer to wild type, except that carpel number remains stable. *wig-3* floral organ number is similar to that of *wig-1*, while *wig-2* has more sepals and petals (Table 1). All 3 alleles are recessive.

The number of floral organs found in *wig-1* plants seems to be dependent on environmental conditions and genetic background. *wig-1* plants grown under suboptimal conditions, such as overcrowding or higher temperatures, develop fewer organs in all four whorls, sometimes resembling wild-type in organ number. *wig-1* was isolated in the Landsberg *erecta* (L-*er*) ecotype; when crossed to the Columbia (Col-2) ecotype, *wig-1* mutant flowers showed greater numbers of organs, especially in basal flowers, than were found in a pure L-*er* background (Fig. 1E). The increase in organ number in *wig-2* compared to *wig-1* and *wig-3* may also be due to ecotypic differences. Wild type No-0 plants, unlike L-*er* plants, occasionally produce flowers with 5 sepals or petals, suggesting the possibility that genes enhancing perianth organ number present in No-0 may have been carried through two crosses of *wig-2* into L-*er*.

Fig. 1. Flowers of wild type and *wiggum* mutants. (A) Wild-type *Arabidopsis* flower, Landsberg *erecta* ecotype, with 4 sepals, 4 petals, 6 stamens and 2 carpels in stereotypical positions. (B) A *wig-1* mutant flower, in L-*er*. *wig-1* flowers have greater numbers of floral organs, especially sepals and petals. (C) A *wig-2* mutant flower, in L-*er*. (D) A *wig-3* mutant flower, in L-*er*. (E) *wig-1* plant in a mixed Columbia (Col-2)-L-*er* background, with more floral organs than found in a pure L-*er* background. (F) *wig-1* in L-*er*, showing petals in abnormal orientations, possibly due to abnormal growth of sepals that restrict petal growth.

Flower organ initiation and development in *wiggum* mutants

Scanning electron microscopy (SEM) reveals that the sepals in *wig* flowers initiate in an aberrant manner. In wild-type flowers, 4 sepals initiate evenly spaced from each other, with 2 in lateral positions and 2 in medial positions. The medial sepals are larger and arise higher on the floral meristem than the lateral

Table 1. Mean number of *wig* floral organs

Genotype	Sepals	Petals	Stamens	Carpels
Flowers no. 1-5*				
L*er*	4.0±0	4.0±0	5.8±0.6	2.0±0
wig-1	5.3±0.8	5.4±0.8	6.3±0.8	2.4±0.5
wig-2	6.0±1.0	6.5±0.9	6.4±0.8	2.4±0.5
wig-3	4.9±0.8	5.4±1.0	6.3±0.7	2.7±0.5
Flowers no. 6-10†				
L*er*	4.0±0	4.0±0	5.8±0.6	2.0±0
wig-1	4.7±0.6	5.1±0.7	5.6±0.6	2.6±0.6
wig-2	5.9±0.9	6.2±0.9	6.0±0.8	2.3±0.5
wig-3	4.8±0.8	5.3±0.9	6.0±0.8	2.5±0.5

The average number of organs per flower is given. The standard error is indicated.

*n=60 L*er* plants, 68 *wig-1* plants, 15 *wig-2* plants and 15 *wig-3* plants.

†n=40 L*er* plants, 41 *wig-1* plants, 15 *wig-2* plants and 15 *wig-3* plants.

L*er*, Landsberg *erecta*.

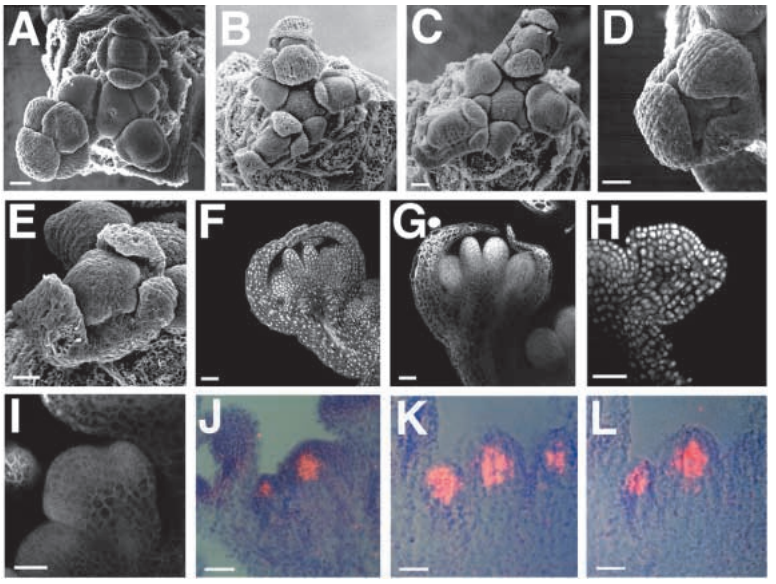

Fig. 2. Microscopic analysis of developing wild-type and *wig* flowers. (A) SEM of a wild-type inflorescence with very young flowers (up to stage 6). Flowers arise in a spiral pattern approximately 140° from each other. On each flower, 2 large medial sepals and 2 small, lower lateral sepals are visible, and the sepals grow to enclose the bud. (B) SEM of a *wig-1* inflorescence. The phyllotaxy of *wig-1* plants sometimes deviates from that seen in wild type. The sepals do not arise in a particular pattern and can vary greatly in size. They are generally shorter and thicker than wild type and fail to enclose the developing flower. (C) SEM of a *wig-1* inflorescence, showing deviations in phyllotaxy and aberrant sepal initiation. The floral meristem is noticeably wider than wild type at these stages of flower development. (D) SEM of a wild-type stage 5 flower. Two of the large medial stamen primordia are visible. Two small petal primordia are visible between the sepals. Two large medial sepal primordia are more prominent and higher on the flower meristem. (E) SEM of a *wig-1* flower at stage 5, with irregular stamen and petal initiation patterns. (F) CLSM of a wild-type flower at late stage 6, after sepals have enclosed the bud. (G) CLSM of a *wig-1* flower at late stage 6. The sepals often do not elongate sufficiently to enclose the bud, and the flower is wider than a late stage 6 wild-type flower. (H) CLSM of wild-type flower at mid stage 3, when sepals have initiated. (I) CLSM of *wig-1* flower at mid stage 3, when the sepals are beginning to initiate. The flower is wider than a mid stage 3 wild-type flower. (J) Expression of *CLV1* RNA in a wild-type inflorescence apex. (K-L) Expression of *CLV1* RNA in *wig-1* inflorescence apeces. Scale bars, A-I, 10 μm; J-L, 40 μm.

sepals, indicating that sepals have positional identity around the whorl (Fig. 2A, Smyth et al., 1990). In *wig* mutants, sepals do not arise in a predictable pattern (Fig. 2B-C), and they are often not evenly spaced from each other. They also tend to lose positional identity; that is, the size of the sepal does not necessarily depend on its position. Sepal size is variable, ranging at maturity from much smaller to somewhat larger than wild-type sepals.

In a wild-type flower, all petals are the same size and are evenly spaced from each other, arising alternately with and interior to the sepals (Smyth et al., 1990). Petals in *wig* plants are usually close to normal in size, but arise in positions unrelated to the positions of the sepals. There are more petals than sepals on average, and the number of petals in a particular flower shows no correlation to the number of sepals in that flower. Wild type flowers generally have 2 short lateral stamens and 4 taller medial stamens, though one or both lateral stamens are missing in about 25% of flowers (Smyth et al., 1990). In *wig* flowers, stamens, like the other floral organs, are not positioned in a regular manner, and their size is not necessarily related to their position, as it is in wild-type flowers (Fig. 2D,E).

Some effects on later floral organ development are observed in *wig* flowers. The flowers tend to open precociously, and the sepals are shorter and rougher than in wild type, and sometimes wrinkled, with surface cells enlarged (Fig. 2E). SEM and confocal laser scanning microscopy (CLSM) analysis showed that sepals in *wig-1* are shorter and thicker than wild-type sepals at a comparable developmental stage (Fig. 2A-G). Petal and stamen morphology appear fairly normal; however, petals on some flowers emerge from the flower in abnormal orientations and sometimes appear wrinkled (Fig. 1F), though these effects might have resulted from physical constraints imposed by the abnormal sepals during petal elongation. In addition, larger, compound stamens are sometimes observed, with a thicker filament and a large single anther or 2 separate anthers. The gynoecium is typically shorter than wild type at maturity, and contains fewer seeds. Thus the overall fecundity of *wig* mutants is reduced, though *wig* is both male and female fertile.

In *wig* mutants, the floral meristem is larger than wild type, including at stage 6, just after petal primordia have emerged (Fig. 2F,G; all stages according to Smyth et al., 1990), and at stage 3, when sepals begin to emerge (Fig. 2H,I). At this stage, wild-type flowers are 30±3 μm wide while *wig-1* flowers are 37±3 μm wide (10 flowers counted per genotype). In the case of *wig*, the organ number alteration is most dramatic in the outer whorls, which is the area most enlarged in the meristem before organ initiation occurs.

Expression of *CLAVATA1* RNA in *wig-1* mutants

Like *wig* mutants, *clavata1 (clv1)* mutant plants also have enlarged floral meristems (Clark et al., 1995), and *CLV1* is thought to play a role in regulating cell proliferation in meristematic regions (Clark et al., 1997). Since the *clv1* and *wig* mutant phenotypes display some similarities, we investigated *CLV1* expression in *wig* mutants to see if the enlarged floral meristem phenotype was due to misexpression of the *CLV1* gene. The *CLV1* gene is expressed primarily in L3 of the presumptive central zone of apical and floral meristems, and downregulated of the peripheral zone from which flowers and floral organs are initiated (Clark et al., 1997; Fig. 2J). The general pattern of *CLV1* expression is not substantially different in *wig-1* compared to wild type (Fig. 2K,L). Expression is seen in the L3 of the presumptive central zone, with no expression in the periphery of apical and floral meristems. The domain of both the expressing and non-expressing cells, however, appears to be increased in size, suggesting that both central and peripheral zones may be larger in *wig-1* than in wild type.

Effects of *wig* on general plant growth and development

Overall, *wig* mutant plants have a growth pattern and body plan similar to wild-type plants. There are, however, notable differences in certain aspects of growth and development. *wig-1* plants germinate less well than wild type: under controlled germination conditions (see Materials and Methods) where nearly all wild-type seeds germinate, only 64% of *wig* seeds germinate (Table 2). In addition, germination is delayed under ideal conditions: the median time of germination of wild-type L-*er* seedlings is 2 days, while in *wig-1* it is 4 days, with some *wig* seeds delaying germination 2 weeks or more. *wig-2* and *wig-3* also affect germination rate and timing, with *wig-2* showing a more dramatic effect (Table 2). Decreased and delayed germination of *wig-1* is also seen in the absence of cold treatment (stratification), which speeds germination and increases the germination rate of wild-type seeds. This decrease and delay of germination is due to zygotic effects, since plants heterozygous for *wig-1* show a lower than expected number of *wig-1* homozygous progeny. However, *wig-1* mature embryos appear morphologically normal.

Of *wig-1* plants that germinate, flowering (bolting) is also delayed, and rosette leaf number is slightly increased (Table 2). Maturation and senescence are also delayed, with *wig-1* life spans longer than wild type. *wig-2* and *wig-3* also show delayed flowering time (Table 2) and longer life span.

While the apical meristem of *wig-1* plants usually appears fairly normal in size and structure, occasional fasciation of the apical meristem is observed. In addition, internode length is decreased in *wig*, resulting in shorter plants, and *wig* roots are shorter than wild-type roots at comparable ages. Phyllotaxy defects are sometimes observed as well, with the angle between flowers occasionally deviating from the approximately 140 degree angle found in wild type (Fig. 2A-C). Nevertheless, the plants are generally healthy and male and female fertile.

Double mutants of *wiggum* and other genes controlling meristem and flower development

wig shows synergistic interactions with other mutations affecting meristem size

Strong alleles of *clv1* lead to an increase in apical and floral meristem size as well as floral organ number (Fig. 3A; Leyser and Furner, 1992; Clark et al., 1993), and mutations in the *clv3* gene have similar effects (Fig. 3E; Alvarez and Smyth, 1994; Clark et al., 1995). The double mutant of *wig-1* and *clv1-4*, a strong *clv1* allele, shows an additive phenotype in early flowers, with more enlarged apical meristems and more of each type of floral organ (Fig. 3B). Later in development the double mutant phenotype becomes much more severe: the apical meristem is greatly disrupted, often increasing to a diameter of 1 cm or more, and then either differentiates into tissue that resembles stigmatic tissue (Fig. 3C) or remains undifferentiated overproliferating meristem tissue (Fig. 3D). In addition, the plants are shorter and have greatly reduced internode length and severe phyllotaxy disruptions, and fewer flowers are formed compared to wild type or either single mutant. Later flowers sometimes have reduced numbers of organs compared to wild type, indicating a severe disruption in floral meristem function. This is also seen by the lack of determinacy in *wig-1 clv1-4* flowers, with undifferentiated floral meristem tissue sometimes emerging from the carpels. Because of this, *wig-1 clv1-4* plants are much less fertile than wild type or either single mutant.

The double mutant of *clv3-2* (a strong *clv3* allele) with *wig-1* shows similar effects: the phenotype is additive in the earliest flowers (Fig. 3F), but soon the apical meristem greatly enlarges (Fig. 3G,H), and later flowers have reduced organ number and loss of determinacy resulting in undifferentiated floral meristem tissue emerging from the carpels (Fig. 3H). The double mutant combination of *wig-1* with the intermediate *clv1-1* allele does not have as severe a phenotype as with the strong *clv1* and *clv3* alleles, with no enhancement of apical meristem defects compared to either single mutant. The *clv2-1* mutant resembles weak *clv1* alleles in its severity in organ number increase, affecting only the carpel number (Koornneef et al., 1983); double mutants of *wig-1* with *clv2-1* appear additive.

Mutations in *fas2* cause changes in floral organ number, producing flowers with slightly more sepals and slightly fewer petals than wild type (Fig. 3I; Leyser and Furner, 1992). In addition, *fas2* plants show some similarities to *wig* in their vegetative growth: *fas2* mutants flower about 1 week later than wild type, and produce an extra leaf before flowering, and the leaves are slightly smaller and darker green. The *fas2 wig-1* double mutant shows a synergistic interaction (Fig. 3J): sepal, petal and stamen number are all reduced compared to wild type or either single mutant (Table 3). In addition, the plants take longer to flower than either single mutant alone, and are much less fertile. *fas1* shows a similar but less severe change in organ number compared to *fas2* (Leyser and Furner, 1992). The *fas1 wig-1* double mutant appears additive with respect to organ number and plant growth.

wig is additive with *perianthia* and *superman*

Mutations in the *PERIANTHIA* gene lead to a similar change in organ number as do mutations in *WIG*. Sepal, petal, and stamen number all average 5 in number, and 2 carpels are usually present (Fig. 3K). The mechanism of *PAN* gene activity appears different, however, since no floral meristem size change is detected in *pan* mutants, and organs arise in a regular, predictable pattern (Running and Meyerowitz, 1996). The *wig -1 pan-1* double mutant phenotype is additive (Fig. 3L). Flowers average 6 sepals and petals, along with 6-7 stamens and 2-3 carpels (Table 3). This suggests that *wig* and *pan* act in separate processes in the determination of organ number.

The *SUPERMAN* (*SUP*; also called *FLO10*) gene affects the position of the boundary between the third and fourth whorl, and *sup* mutants show increased stamen number at the expense of carpel tissue (Fig. 3M; Schultz et al., 1991; Bowman et al., 1992; Sakai et al., 1995). The *wig-1 sup-1* double mutant phenotype (Fig. 3N) is additive with respect to sepal, petal and

Table 2. *wig* germination frequency and flowering time

Genotype	% Germination*	Days to bolt†	Rosette leaf no.†
L*er*	99	22.4±1.5	8.6±0.8
wig-1	64	30.2±1.5	9.1±1.1
wig-2	59	38.1±2.3	14.9±3.0
wig-3	64	34.2±2.4	9.4±1.7

*Percentage germination of 200 seeds scored per allele.
†Mean and standard error of 30 plants scored per allele.
L*er*, Landsberg *erecta*.

Table 3. Mean number of floral organs in *wig* double mutants

Genotype	*n*	Sepals	Petals	Stamens	Carpels
Flowers no. 1-5					
fas2	50	4.6±0.8	4.0±0.6	5.7±0.7	2.3±0.3
wig-1 fas2	35	4.0±0.7	3.1±0.9	5.1±0.9	2.4±0.6
pan-1	49	5.1±0.7	4.8±0.8	5.6±0.6	2.0±0.0
wig-1 pan-1	50	5.9±0.6	5.8±0.6	6.7±0.8	2.7±0.5
sup-1	25	4.0±0	4.0±0	12.4±2.3	1.2±0.7
wig-1 sup-1	20	4.8±0.8	5.1±0.9	28.6±5.0	0.9±0.7
Flowers no. 6-10					
fas2	50	4.3±0.6	4.0±0.6	5.4±0.7	2.2±0.4
wig-1 fas-2	34	3.8±0.7	3.0±1.1	4.4±1.4	2.0±0.7
pan-1	49	4.9±0.6	5.0±0.8	5.4±0.8	2.0±0.0
wig-1 pan-1	50	5.5±0.6	5.6±0.6	6.2±0.9	2.3±0.5
sup-1	25	4.0±0.0	4.0±0.0	10.0±1.3	1.3±0.7
wig-1 sup-1	20	4.7±0.9	4.9±0.9	26.5±4.9	0.7±0.5

The average number of organs per flower is given. The standard error is indicated.
n, number of flowers analyzed.

carpel number. However, stamen number in the double mutant is significantly increased over that in either single mutant (Table 3), revealing that *wiggum* may play a role in controlling cell proliferation between as well as within floral whorls.

wig is additive with the floral homeotic genes

Several flower mutants are known to show a homeotic conversion from one organ type to another type found elsewhere in the flower (reviewed in Weigel and Meyerowitz, 1994; Yanofsky, 1995). These fall into 3 classes, with each class affecting the identity of organs in 2 adjacent whorls. The class A genes include *APETALA1* (*AP1*), which also affects floral meristem identity, and *APETALA2* (*AP2*). Mutations in both genes cause the conversion of sepals to leaf-like organs or carpels, and the partial conversion of petals to stamens in some alleles. Double mutant combinations of *wig-1* with *ap2-2* (a strong *ap2* allele) are additive, with organ number intermediate between the two single mutants, and organ identity the same as found in *ap2-2*. Similar additive effects are seen in the double mutant *wig-1 ap1-1* (a strong *ap1* allele). The class B genes are *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), mutations in either of which cause a conversion of petals to sepals and of stamens to carpels. *wig-1* in combination with either *ap3-3* (a strong *ap3* allele) or *pi-1* (a strong *pi* allele) is additive, with organ number per whorl characteristic of *wig-1* single mutants, and organ identity characteristic of *ap3-3* or *pi-1*. The class C gene is *AGAMOUS* (*AG*), which when mutated causes the conversion of stamens to petals and carpels to sepals, as well as the generation of additional whorls in the center of the flower (Fig. 3O). The double mutant *wig-1 ag-3* (a strong *ag* allele) also has an additive phenotype. The organ number in the first and second whorl is increased as in *wig-1*, with the organ type the same as seen in the *ag-3* mutant (Fig. 3P). Thus *wig* acts independently of the floral homeotic genes in determining flower pattern.

wig shows synergistic interactions with mutations affecting floral meristem identity

The *AP1* and *LEAFY* (*LFY*) genes have been shown to affect floral meristem identity (reviewed by Weigel, 1995). Mutation in these genes leads to a partial conversion of flowers into shoot structures. Combinations of mutations in genes affecting floral meristem structure with those affecting floral meristem identity, particulary *lfy*, have been shown to have a severe synergistic effect, increasing the severity of both the disruption of floral meristem structure and loss of flower meristem identity (Clark et al., 1993, 1994; Clark and Meyerowitz, 1994; Levin and Meyerowitz, 1995). Similar interactions are seen with meristem structure mutants in combination with three other mutants having weaker effects on floral meristem identity: *unusual floral organs* (*ufo*; Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995), *hanaba taranu* (*han*; H. Sakai, M. P. R. and E. M. M., unpublished) and *filamentous flower* (*fil*, also called *fl54*, Komaki et al., 1988; Okada and Shimura, 1994; Clark et al., 1994; Clark and Meyerowitz, 1994).

wig mutants also show synergistic interactions in double mutant combinations with *lfy*, *ufo*, *han*, and *fil*, with some differences from those seen in previous studies with other mutants. The most severe phenotype is observed in the *wig-1 lfy-6* mutant (Fig. 4A,B). The primary apical meristem terminates prematurely in a carpel-like structure after producing a few reduced flowers and several filamentous structures. Secondary inflorescences give rise only to filaments before terminating. Double mutants of *wig-1* with *ufo-2* also produce reduced flowers with fewer organs or flowers completely replaced by filaments (Fig. 4C,D). The shoots terminate in carpelloid structures, much earlier than in *ufo* alone. Flower development is not as disrupted as in *wig lfy* plants, since many flowers with flower parts, predominantly sepals and carpels, are formed. Similar results are seen in double mutants of *wig fil*. *fil* single mutant flowers have smaller and fewer organs, and occasional filaments are initiated from the inflorescence (Fig. 4E; Komaki et al., 1988; Okada and Shimura, 1994). The double mutant of *fil* with *wig* shows more filaments in place of flowers, fewer organs in flowers that do form, and earlier termination of the main stem, compared to either single mutant (Fig. 4F). Mutations in *han* lead to flowers with fewer organs, especially petals and stamens (Fig. 4G,H. Sakai, M. P. R. and E. M. M., unpublished); in combination with *wig-1*, filaments are occasionally formed in place of flowers (Fig. 4H). In general, double homozygotes of *wig* with these mutants are less severe in phenotype than strong *clv* allele double mutants with these genes, since *wig ufo*, *wig han* and *wig fil* still produce flowers.

wig shows synergistic interactions with several other mutations affecting flower development

leunig (*lug*) mutant plants show a variety of defects, including slightly later flowering, narrow leaves and floral organs, and ectopic homeotic gene expression (Fig. 4I; Liu and Meyerowitz, 1995). The double mutants *lug lfy* and *lug ufo* also have reduced flowers and filamentous structures (Levin and Meyerowitz, 1995). Double mutants of *wig-1* and *lug-3*, a strong *lug* allele, do not produce filaments, but flowers often lack petals and have fewer organs than in *lug-3* alone (Fig. 4J).

Mutations in the *SNOWBALL* gene lead to flowers with narrow organs, with 4-5 sepals and 3-5 petals in most flowers (Fig. 4K; R. W. Williams, M. P. R. and E. M. M., unpublished). The *sno wig-1* double mutant shows a severe disruption in floral and apical meristem structure. The main stem initiates

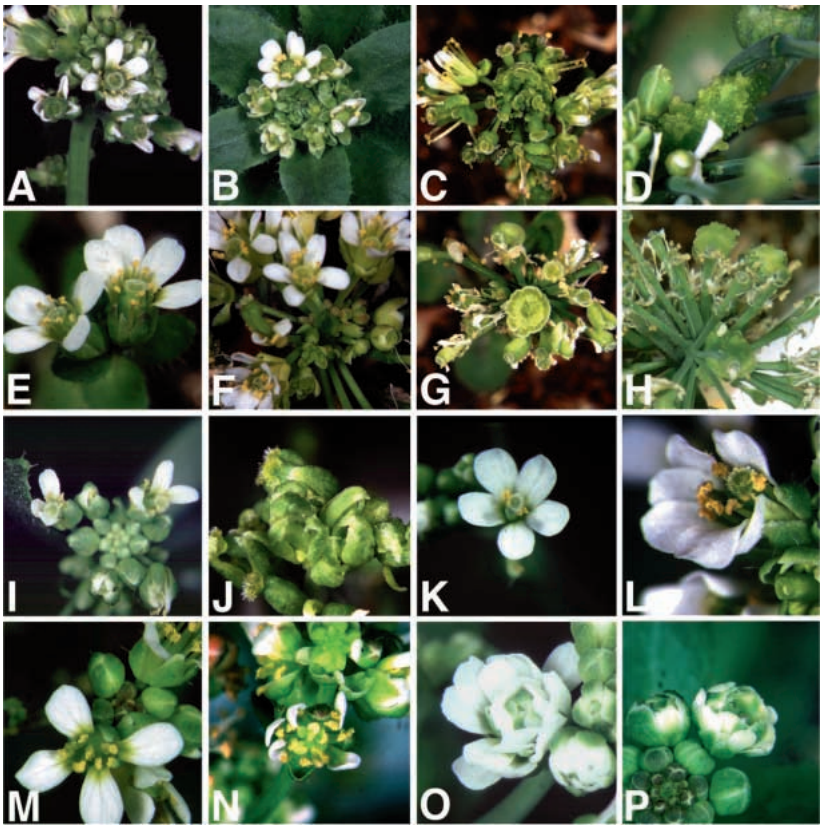

Fig. 3. Double mutants of *wig* with other genes affecting floral organ number. (A) *clv1-4* mutant flowers, which have extra organs in all 4 whorls, especially carpels. (B) The first flowers of a *wig-1 clv1-4* double mutant, showing an additive phenotype with respect to floral organ number (more organs in each whorl compared to either single mutant). (C) *wig-1 clv1-4* apex at a later stage, with the apical meristem overproliferating and differentiating into stigmatic tissue. Flowers have fewer organs than in either single mutant, seen here in the reduced number of carpels in the gynoecium. (D) Another *wig-1 clv1-4* apex, with massive overproliferation of apparently undifferentiated meristem tissue. (E) *clv3-2* flowers, which display a phenotype very similar to *clv1-4*. (F) Early flowers of a *wig-1 clv3-2* double mutant plant, showing an additive phenotype with respect to organ number. (G) An older *wig-1 clv3-2* plant, with overproliferation of the apical meristem. (H) Another older *wig-1 clv3-2* plant with overproliferation of both the apical meristem and undifferentiated floral meristem cells that have grown out of carpels. (I) A *fas2* mutant, which has flowers with 4-5 sepals and 3-5 petals. (J) *wig-1 fas2* double mutant, which has flowers with fewer organs, especially petals and stamens, compared to *wig-1* and *fas2* single mutants. (K) A *pan-1* flower, which has floral organs similar in number to *wig-1* mutants, including extra sepals and petals and occasionally extra carpels. (L) A *wig-1 pan-1* flower, which shows an additive organ number phenotype. (M) A *sup-1* flower, with more stamens and a reduced gynoecium compared to wild type. (N) A *wig-1 sup-1* flower, with an additive phenotype with respect to organ number. (O) An *ag-3* mutant flower, in which stamens are converted to petals and carpels converted to sepals, with additional whorls of petals and sepals forming interior to the fourth whorl. (P) A *wig-1 ag-3* flower, which shows an additive phenotype: homeotic conversions of floral organ type and extra whorls as seen in *ag-3*, with extra organs in each whorl as in *wig-1*.

numerous filaments or sepal-like organs and occasionally structures recognizable as incomplete flowers, missing most floral organs (Fig. 4L).

DISCUSSION

We have studied the effects of mutations in the *WIGGUM* gene on *Arabidopsis* development. *wig* mutant flowers form more floral organs than wild type, particularly sepals and petals, a phenotype correlated with an increase in floral meristem width. *wig* mutants also have some vegetative growth defects, indicating that *WIG* is likely to be active in all stages of plant development. Many of the visible pleiotropic phenotypes, including reduced germination, slower growth, and faulty initiation of floral organs leading to abnormal sepal shape and position, may be due primarily to underlying defects in the apical and floral meristems of *wig* mutant plants.

Similarities and differences between *wiggum* and previously described mutants affecting flower development

Certain aspects of the *wiggum* mutant phenotype resemble those reported for other *Arabidopsis* mutants. In terms of floral organ number, the mutant most closely resembling *wig* is *pan* (Running and Meyerowitz, 1996), which also shows an increase in sepal and petal number without dramatic increases in stamen or carpel number. In most other ways, though, *wig* resembles mutants that have been shown to affect apical and floral meristem structure.

The organ number increases seen in *pan* and *wig* flowers seem to be due to different mechanisms. *pan* mutations do not cause disruptions in overall apical and floral meristem structure, and changes in organ number in *pan* mutants are presumably caused by changes in the spacing and position of floral organs (Running and Meyerowitz, 1996). *wig* mutations, on the other hand, cause the formation of floral meristems wider in girth than wild type, which allows for the formation of more organs in the outer whorls. This is analogous to the mechanism proposed for the increase in organ number seen in *clv1* and *clv3* mutants (Clark et al., 1993, 1995; Alvarez and Smyth, 1994). In *clv* plants the floral meristem is taller than wild type, leading to extra organs in the inner whorls as well as extra whorls, and alleles with more severe organ number defects have a greater size increase in the floral meristem. The fact that organ number changes in *wig*, *clv1*, and *clv3* can be correlated with specific changes in floral meristem size at the time of organ initiation indicates that one mechanism flowering plants use to regulate floral organ number is the regulation of floral meristem size.

wig mutants do not initiate organs in a consistent or predictable pattern, which is also the case for *clv1* and *clv3* (Clark et al., 1993, 1995). In wild-type flowers, organs arise in stereotypical positions, and in *pan* flowers certain aspects of

wild-type organ position are maintained, like the presence of an adaxial sepal and the dependence of petal position on sepal position. Sepals of *wig* mutant flowers can arise in any position around the floral meristem, with petal position not necessarily showing any relationship to sepal position. Also, positional differences in floral organs seem to be lost in *wig* and *clv* mutants; for instance, the size of an organ is not strictly dependent on where it arises, as they are in wild type and *pan*. These results suggest that disruptions of floral meristem structure, as seen in *wig* and *clv*, lead to defects in organ positioning and positional identity.

Unlike *pan* and *clv* plants, mutant *wig* plants show a number of abnormalities outside of the apical and floral meristem, including lower germination rate, slower germination, longer time to bolting, decreased internode length, and decreased root length. This suggests that *WIG* is required in areas outside the apical and floral meristem, in wider domains than *PAN* and *CLV*. Other mutations affecting meristem structure have pleiotropic effects as well: the *fas2*, *tsl*, *lug*, and *rev* mutations cause defects in vegetative leaf and floral organ development, and later flowering (Leyser and Furner, 1992; Roe et al., 1993; Liu and Meyerowitz, 1995; Talbert et al., 1995).

Genetic interactions between *wig* and other *Arabidopsis* mutants are consistent with a role for *wig* in control of meristematic cell division

Studies of genetic interactions between *wig* and previously described mutants give results consistent with a role for *WIG* in the control of floral and apical meristem structure deduced by phenotypic studies of *wig* single mutants. Synergistic interactions are seen with mutants that have been shown to play a role in the same or similar processes, while additive interactions are seen with mutants that do not disrupt meristem structure.

The most striking example of genetic synergy is seen in interactions between *wig* and strong *clv1* and *clv3* alleles. Both *CLV1* and *CLV3* have been implicated in the control of apical and floral meristem size, and mutations in either gene lead to an overproliferation of cells in these regions (Leyser and Furner, 1992; Clark et al., 1993, 1995; Alvarez and Smyth, 1994), although the changes induced by *clv* mutations are distinct from those seen in *wig*. *CLV1* and *CLV3* have similar phenotypes and appear to act in the same pathway, since double mutants of strong *clv1* and *clv3* alleles resemble both single mutants (Clark et al., 1995). Double mutants of strong *clv* alleles with *wig* show exaggerated proliferation of cells in older apical and floral meristems, seemingly due to a much greater loss of control of cell proliferation than that seen in either single mutant. However, the apical meristem on younger double mutant plants does not show gross morphological defects, and the early flowers show an additive phenotype. This phenotype, along with the absence of any epistatic interactions, indicates that *WIG* acts in a separate pathway from *CLV1* and *CLV3* in control of cell division in apical and floral meristems. Expression of *CLV1* in *wig-1* mutants is not substantially different from that seen in wild type, supporting the notion that *CLV1* function is important in restricting cell proliferation in *wig-1*.

The *FAS2* gene has also been shown to play a role in the control of apical meristem structure, and shows a slight change in floral organ number (Leyser and Furner, 1992). *wig fas2* double mutant plants have flowers with fewer organs than either single mutant, suggesting that *WIG* is involved in a similar process but in a different pathway than *FAS2*. In contrast, *wig* shows an additive interaction with *pan*, a mutation that affects floral organ number without affecting meristem size or structure. This supports the idea that *PAN* and *WIG* control floral patterning via two separable mechanisms, *WIG* by controlling, in the floral meristem, the amount of cell proliferation relative to withdrawal of cells into developing

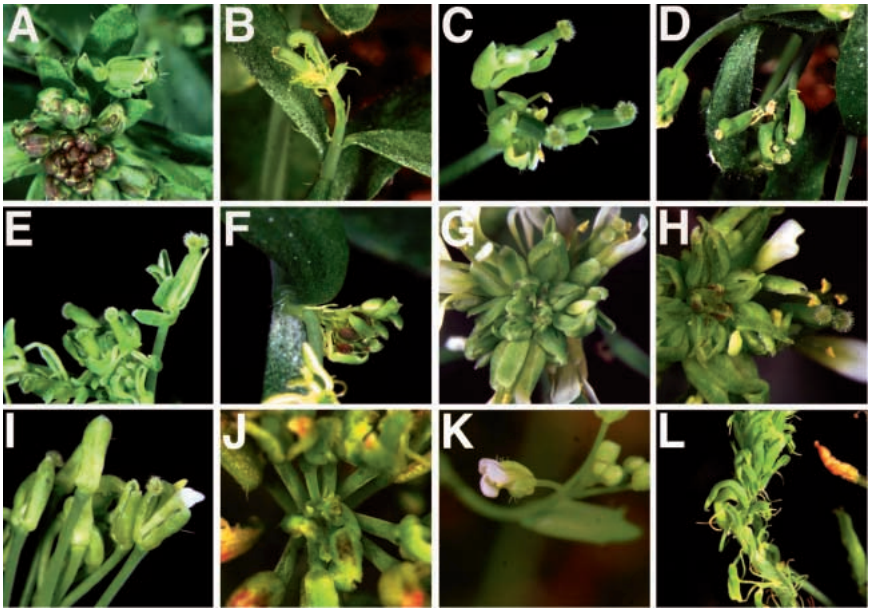

Fig. 4. Enhancement of meristem defects of *wig* in combination with other mutations affecting flower development. (A) A *lfy-6* mutant apex. *lfy* mutants lead to a partial conversion of flowers into shoot structures, accompanied by loss of some floral organ identity. (B) A *wig-1 lfy-6* double mutant, which shows filamentous structures in place of flowers, and early termination of the apical meristem in carpel structures. (C) *ufo-2* mutant inflorescence, which is also defective in floral meristem identity, though not as severely as in *lfy* mutants. (D) *wig-1 ufo-2* double mutant plants show early termination of the main stem into carpels, and some flowers are replaced by filaments. (E) *fil* mutant flowers, which have defects in organ number, size and position. (F) A *wig-1 fil* double mutant apex, with many flowers reduced in organ number or replaced by filaments. The inflorescence displays both phyllotaxy defects and early termination of the apical meristem. (G) *han-1* mutant flowers, showing reduced organ number, especially in petal and stamen whorls. (H) *wig-1 han-1* double mutant apex, with some flowers replaced by filaments. (I) *lug-3* mutant flowers, in which floral organs are narrower. (J) *wig-1 lug-3* double mutant flowers, which have reduced organ numbers, though no filament structures are seen. (K) *sno* mutant flowers, which have extra sepals and 3-5 petals. (L) A *wig-1 sno* double mutant inflorescence, which produces many filaments and occasionally flowers with dramatically reduced organ number.

organs, and *PAN* by controlling the distance between organ initiation sites. In addition, *ag*, *ap1*, *ap2*, *ap3* and *pi*, mutations affecting floral organ identity, are additive in combination with *wig*, indicating that *WIG* acts independently of the floral homeotic genes in establishing flower pattern. *CLV1* also acts independently of *PAN* and the homeotic genes (Clark et al., 1993), which is consistent with *CLV* genes acting in the same process as *WIG*, distinct from genes that do not affect meristem structure. *wig-1* also shows largely additive phenotypes with *sup-1*, though the number of stamens in the double mutant is dramatically increased, indicating a role for *WIG* in restricting proliferation in *sup-1*.

Enhancement of meristem defects in *wig* by *lfy* and other mutations causing defects in floral meristem identity

Previous studies have shown that certain combinations of meristem mutations show a dramatically enhanced phenotype, leading to severe disruptions in apical meristem function (Clark et al., 1993, 1994; Clark and Meyerowitz, 1994; Levin and Meyerowitz, 1995). Defects include extreme phyllotaxy abnormalities and failure to initiate complete flowers, with filamentous structures formed in their place. Most commonly, these filamentous structures are seen in combinations of mutations defective in apical meristem structure (such as *clv1*, *clv3*, and *han*) and mutations affecting floral meristem identity (such as *lfy*, *ufo*, and *fil*), though not all double mutants show this phenotype. The *wig* mutation also causes filamentous structure formation and other apical meristem defects in combination with most of these mutations. The most severe defects are seen with *wig* and strong *lfy* mutants, which rarely form flowers, while *wig* with *ufo*, *fil*, or *han* mutants show occasional filament structures. The *sno* mutant also seems to fall into the filament producing class, with a particularly severe effect in combination with *wig*.

The fact that certain *wig* double mutant combinations lead to filament structures is surprising, given that *lfy*, *ufo* and *fil* show single mutant phenotypes seemingly unrelated to those seen in *wig*. It is possible that these genes also play a role in maintenance of meristem structure, and their role becomes more important in the background of mutations that also disrupt meristem structure, such as *wig*, *clv1* and *clv3*. This implies a partial redundancy among these genes, and suggests a threshold of meristem maintenance activity, below which meristems become increasingly abnormal. This idea is supported by the fact that double mutant combinations of *wig* and weaker mutant alleles of other genes do not show a severe disruption of meristem activity, as does *wig* in combinations with stronger alleles. For instance, while *wig-1 clv1-4* plants have greatly disrupted meristems, *wig-1 clv1-1* plants do not, nor do *wig-1 clv2-1* plants. Also, while *wig* interacts with many of the same mutations as *clv1*, it does so in a less severe fashion, perhaps reflecting the more severe phenotypes found in *clv1* single mutants compared to *wig*. Similar threshold effects are seen in *clv1 ap1-1* double mutants, with *clv1-4* showing a synergistic effect, and *clv1-1* showing an essentially additive phenotype with *ap1-1* (Clark et al., 1993). There are indications this threshold increases as the shoot gets older, since meristem disruption in the double mutants becomes progressively worse in all cases, especially notable in the *wig clv* doubles.

Model of *wig* function in apical and floral meristems

The phenotype of *wig* plants suggests at least two models predicting the mechanism of action and expression pattern of *WIG*. Both are based on the hypothesis that *WIG* is responsible for controlling cell proliferation relative to cell differentiation in certain regions of the floral meristem. Traditional histological studies have divided apical and floral meristems into three zones: the central zone (CZ), a region of less mitotically active cells in the center of the meristem, the peripheral zone (PZ), a more densely staining region of more actively dividing cells surrounding the central zone and giving rise to leaf and flower primordia, and the rib meristem, below the CZ (reviewed by Meyerowitz, 1997). *WIG* may be responsible for negatively regulating cell proliferation or increasing the rate of cell differentiation in the PZ, and, in the absence of *WIG* function, the number of cells in this region increases relative to wild type, leading to meristem expansion and occasional fasciation.

Apical and floral meristems have another level of organization, being composed of three cell layers termed L1, L2, and L3. Both L1 and L2 are single layers of cells, with L1 cells forming the epidermal layer, and L2 cells immediately below. Cells in both layers divide almost exclusively anticlinally, and are clonally distinct. The remaining cells are found in the L3, or corpus, where cells divide in many different planes (reviewed by Meyerowitz, 1997). It is possible that *WIG* is responsible for limiting cell proliferation in the L1 and L2 layers, and when this control is lost, the resulting increases in cell division lead to a widening of the floral meristem, because of the increased number of anticlinal cell divisions relative to cell divisions in different planes in the meristem. Cell communication between different cell layers, presumably carried out by a mechanism unrelated to *WIG*, would keep the overall meristem structure intact.

These models are complementary to models proposed for *CLV1* action (Clark et al., 1993, 1997). *CLV1* is also required for limiting cell division relative to cellular differentiation in the apical and floral meristems, but seemingly by a different mechanism than *WIG*, since its mutant phenotype is distinct: a much larger increase in height than in width. It has been proposed that *CLV1* is responsible for controlling cell proliferation in the CZ, such that when *CLV1* is mutant the CZ is increased in size (Clark et al., 1993). An alternate model is that *CLV1* is required for restricting cell division in the L3 layer, such that in *clv1* mutants, the ratio of periclinal to anticlinal cell divisions increases, leading to a greater meristem height. The latter model is consistent with the expression pattern of *CLV1* RNA, which is found in the L3 layer, but not in the L1 and likely not in the L2 (Clark et al., 1997). Both models help explain the dramatic phenotype seen in double mutant combinations of *clv1* and *wig*. Both single mutant phenotypes are less severe, since cell division control in one area of the meristem is still intact, and cell-cell communication between different layers of the meristem may help limit the total number of cells in the meristem. However, if cell division control in most or all of the meristem is disrupted, overproliferation of the entire meristem may be more likely.

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