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# The *indeterminate floral apex1* gene regulates meristem determinacy and identity in the maize inflorescence

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## SUMMARY

**Meristems may be determinate or indeterminate. In maize, the indeterminate inflorescence meristem produces three types of determinate meristems: spikelet pair, spikelet and floral meristems. These meristems are defined by their position and their products. We have discovered a gene in maize, *indeterminate floral apex1* (*ifa1*) that regulates meristem determinacy. The defect found in *ifa1* mutants is specific to meristems and does not affect lateral organs. In *ifa1* mutants, the determinate meristems become less determinate. The spikelet pair meristem initiates more than a pair of spikelets and the spikelet meristem initiates more than the normal two flowers. The floral meristem initiates all organs correctly, but the ovule primordium, the terminal product of the floral meristem, enlarges and proliferates, expressing both meristem and ovule marker genes. A role for *ifa1* in meristem identity in addition to meristem determinacy was revealed by double mutant analysis. In *zea agamous1* (*zag1*) *ifa1* double mutants, the female floral meristem converts to a branch meristem whereas the male floral meristem converts to a spikelet meristem. In *indeterminate spikelet1 (ids1) ifa1* double mutants, female spikelet meristems convert to branch meristems and male spikelet meristems convert to spikelet pair meristems. The double mutant phenotypes suggest that the specification of meristems in the maize inflorescence involves distinct steps in an integrated process.**

Key words: Determinacy, Meristem, Maize, Ovule, Floral reversion, Spikelet

## INTRODUCTION

Plant development depends largely on the activities of meristems, pools of undifferentiated cells that divide to maintain the meristem and to provide cells for lateral organs, branches, and stems (Steeves and Sussex, 1989). Indeterminate meristems continuously produce organs until senescence, whereas determinate meristems cease producing new cells after a fixed number of primordia initiate. Inflorescence meristems often exemplify indeterminate meristems by producing an indefinite number of floral meristems. Flower meristems are usually determinate; after initiating four whorls of floral organs, they are consumed in the production of carpels. Maize and other related grasses provide an excellent system to study the transition from indeterminate to determinate meristem owing to the presence of intermediate branching steps prior to the formation of flowers.

The arrangement of floral meristems on the inflorescence varies broadly. In some species, floral meristems are born directly on the inflorescence and in others they arise from lateral branches. The spacing and phyllotactic pattern of the floral meristems can also provide great diversity in form. Many species change from one phyllotactic pattern in the vegetative stage to a different pattern in the production of flowers. The inflorescence meristem of maize produces primordia in a polystichous (or multi-rowed) pattern. The early arising primordia develop into branch meristems and the later formed primordia become spikelet pair meristems (Fig. 1). The branch meristem (BM) is similar to the inflorescence meristem in the continuous production of spikelet pair meristems (SPM) but differs in the pattern of initiation, which is distichous, or two-rowed. SPMs form two spikelet meristems (SM), each of which forms two floral meristems (Fig. 1A). As a monoecious plant, maize has two types of inflorescence meristems, a terminal male inflorescence (tassel, Fig. 1D) and a lateral female inflorescence (ear, Fig. 1E) (Cheng et al., 1983; McSteen et al., 2000). The tassel is formed by conversion of the vegetative meristem into an inflorescence, whereas, the ear arises from an axillary meristem of a vegetative leaf. Both the tassel and the ear produce SPM but the production of BM is suppressed in the ear.

Like the floral meristem, both SPM and SM are determinate, producing a fixed number of derivatives. A number of mutations in maize affect the determinacy of these meristems. In *ramosa 1 (ra1)* mutants, SPM on the main axis of the tassel become indeterminate, producing multiple spikelets (Postlethwait and Nelson, 1964; Veit et al., 1993). SM themselves are affected in a number of mutations including *indeterminate spikelet1* (*ids1*), *Tasselseed6* (*Ts6*), and *reverse germ orientation1* (*rgo1*) (Irish, 1997; Chuck et al., 1998;

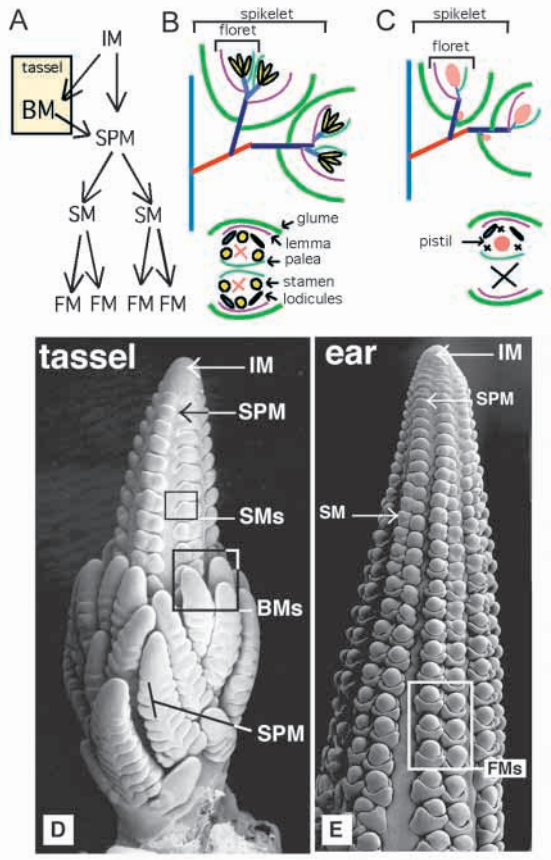


**Fig. 1.** Maize inflorescence development. (A) Different meristem types in maize. The inflorescence meristem (IM) forms spikelet pair meristems (SPM) which form two spikelet meristems (SM), each of which forms two floral meristems (FM). Tassels also form branch meristems (BM) at the base. (B,C) Schematic of a spikelet pair. Each spikelet contains two glumes and two florets. The mature floret comprises the lemma, a palea, three lodicules (one of which is rudimentary and not shown), three stamens and a pistil. The pistil aborts in male spikelets (B). All stamens and the lower floret abort in female spikelets (C). (D) Male inflorescence. The basal branches on the tassel form indeterminate BMs, which give rise to SPMs in a distichous pattern. SPMs give rise to two SMs that are side by side. Each SM forms two floral meristems (FM). (E) Female inflorescence development proceeds similarly to that of male inflorescence development, only BMs are suppressed in the ear. The boxed area contains six spikelets. In each spikelet, the upper FM and outer glume are visible. The lower FM and inner glume are obscured.

McSteen et al., 2000). In *ids1*, *Ts6* and *rgo1*, additional flowers are produced, suggesting that the SM is indeterminate. Determinacy of the floral meristem itself is affected in the maize *AGAMOUS* homolog, *zea agamous1* (*zag1*), *knotted1* (*kn1*), and *thick tassel dwarf1* (*td1*) (Mena et al., 1996; Kerstetter et al., 1997; McSteen et al., 2000). In each of these three mutants, more carpels are produced.

Several mutations in maize also affect the identity of meristem products. In *tasselseed4 (ts4)* mutants, SPM initiate SPMs and thus have the identity of BMs (Irish, 1997). In *branched silkless1* (*bd1)* mutant ears, SM identity is altered and floral meristems are not produced (Kempton, 1934; Colombo et al., 1998). In *indeterminate1 (id1)*, the SM is converted into a vegetative meristem after producing two flowers (Galinat and Naylor, 1951) (D. L. C. and J. Colasanti, unpublished).

The mutations previously described affect only one particular type of meristem. Here we describe a new mutation *indeterminate floral apex1* (*ifa1*) that affects all determinate meristems of the maize inflorescence; the number of spikelets and flowers is increased, and ovule primordia proliferate. Double mutants with *zag1* and *ids1* reveal an additional role for *ifa1* in meristem identity. The specification of meristem identity is distinct in male and female flowers.

## MATERIALS AND METHODS

### Genetics

Pollen from plants homozygous for *ifa1-r* (r for reference) was crossed onto the standard set of *waxy*-marked reciprocal translocation lines (Laughnan and Gabay-Laughnan, 1993). Segregation distortion using T1-9c showed evidence of linkage, suggesting that *ifa1-r* mapped to chromosome 1. Several RFLP markers were used to further define the location of *ifa1* on chromosome 1S. *ifa1-r* mapped 3 cM from *umc76* and less than 1 cM from *zmm14* (zero recombinants in 110 chromosomes). *ifa1-r* has been introgressed four to six generations into several inbred lines including B73, Mo17, W23, A188, A619 and A632.

*zag1-mum1* was a gift from R. Schmidt (University of California, San Diego). *zag1* and *ifa1* double mutants were generated by crossing pollen from plants homozygous for *ifa1-r* onto *zag1-mum1*/+ ears. $F_1$ plants were self-pollinated and $F_2$ progenies were scored for the segregation of both *ifa1-r* and *zag1* phenotypes in the family. The genotype of the double mutants was verified by crossing putative double mutant plants as male to plants heterozygous for *zag1* and *ifa1-r* or by scoring for diagnostic RFLP markers associated with each mutation. Double mutants of *ifa1-r* with *ids1-mum1* (Chuck et al., 1998) were constructed in a similar fashion. The alleles used in constructing double mutants are likely to be null based on the fact that *ifa1-r/–* plants are similar to *ifa1-r/ifa1-r* plants when compared in the same background, and that gene-specific transcripts are absent in *ids1-mum1* and *zag1-mum1* (Mena et al., 1996; Chuck et al., 1998).

### Microscopy

Young ear primordia used for RNA in situ experiments and for scanning electron microscopy were harvested from plants grown in the greenhouse. In situ hybridization was performed as described (Jackson et al., 1994) using *kn1* or *zag2* cDNA as probes. A 650 bp fragment downstream of the MADS box domain was amplified from a *zag2* cDNA subclone using the following primer pairs: 5′-TAA TAC GAC TCA CTA TAG GCC TTA ACC TGA TCA CAG CCT-3′ and 5′-ATT TAA CCC TCA CTA AAG GCA TCT CTA AGA TCA GGG-3′, where the underlined sequences are the T7 and T3 promoter sequences, respectively. The probes were generated by in vitro transcription using T7 polymerase to generate the antisense strand and the T3 polymerase to generate the sense strand. Young ear primordia were fixed in FAA (50% ethanol, 5% acetic acid, 3.7% paraformaldehyde) at 4°C overnight and dehydrated in a graded ethanol series to 100%. The samples were critical point dried and sputter-coated with palladium. Samples were viewed on an ISI 30 scanning electron microscope at 10 kV accelerating voltage.

## RESULTS

### *ifa1* maps to chromosome 1

*ifa1-r* (r for reference) was identified in a maize line carrying the *Ac* transposon but is unlinked to *Ac* (data not shown). We

mapped it to chromosome 1 using waxy translocation lines (Laughnan and Gabay-Laughnan, 1993) and showed that it behaves as a single recessive mutation. Crosses of *ifa1-r* to B-A translocation lines that result in chromosomal deficiencies (Beckett, 1993) support its position on the short arm of chromosome 1. Further mapping using RFLP markers placed *ifa1* within 3 cM of *umc76* and less than 1 cm from *zmm14*.

The phenotype of plants carrying the *ifa1-r* allele uncovered by a deficiency is similar to *ifa1-r* homozygotes suggesting that *ifa1-r* is a null mutation. *ifa1-r* has been backcrossed to several inbred lines and is fully penetrant. Aspects of the phenotype are more expressive in the original lines in which it was identified than after introgressing into a given inbred. Additional alleles have been recovered by screening $F_1$ populations for transposon and EMS induced alleles. The double heterozygotes are not as severe as the *ifa1-r* homozygotes. This paper reports the phenotype of *ifa1-r*.

### *ifa1* regulates determinacy of spikelet pair, spikelet and floral meristems

The inflorescence meristems of the tassel and ear produce spikelet pair meristems (SPM) in a polystichous branching pattern (Fig. 1D,E). SPMs produce a pair of spikelet meristems (SM). In the tassel, the upper spikelet, which has a longer pedicel, is referred to as the pedicellate spikelet, and the lower spikelet is sessile (Fig. 2A). Each SM produces four leaf-like organs in a distichous pattern. The first two, also known as glumes, are sterile. The next two (called lemma) are fertile and contain floral meristems in their axil (Fig. 1B,C). The lemma and products of the floral meristem constitute the floret. Thus, a spikelet is composed of a pair of glumes that enclose two florets (Fig. 1B, Fig. 2B).

In *ifa1-r* plants, the SPM and SM become less determinate, producing more spikelets and florets (Fig. 2). The extra spikelets, as with normal spikelets, contain florets enclosed by glumes. The additional spikelet(s) in the tassel are pedicellate and initiated consecutively. Homozygous *ifa1-r* mutants can make more than two florets per spikelet, which like normal florets, are separated by short internodes and are contained within two sterile glumes (Fig. 2C). Extra spikelets and florets are not as frequent after repeated back-crossing into a single inbred and are more likely to be found towards the tip of the main tassel and the tips of the lateral branches. The formation of additional spikelets and florets in *ifa1* mutants suggests that *ifa1* regulates determinacy of the SPM and SM, respectively.

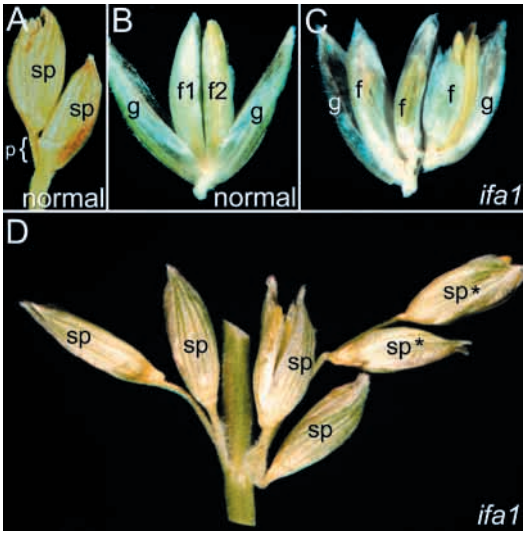


**Fig. 2.** *ifa1* mutants produce additional spikelets and extra florets. (A) A pedicellate and sessile spikelet from a normal tassel. (B) A male spikelet showing two florets within a pair of glumes. (C) In *ifa1* mutants, more than two florets are observed in a spikelet. (D) Three or more spikelets form instead of a pair of spikelets. g, glume; f, floret; sp, spikelet; sp*, extra spikelet, p, pedicel.

The initial steps of male and female floral development are similar, differentiating into unisexual flowers at later stages. The first organ formed from the floral meristem is the palea, a membranous leaf-like organ, followed by three lodicules (one suppressed), three stamens and a pistil (Fig. 1B,C). Lodicules are considered rudimentary petals based on expression of marker genes (Ambrose et al., 2000). The pistil is formed from three carpels (one rudimentary and two fused) that elongate to form the silk (Fig. 3A) (Nickerson, 1954). Within the fused carpels is a single ovule that consists of integuments and nucellar tissue surrounding the female gametophyte (Fig. 3B). The pistil aborts early in floret development in the tassel (Fig. 1B). In the ear, all stamens as well as the lower floret of each

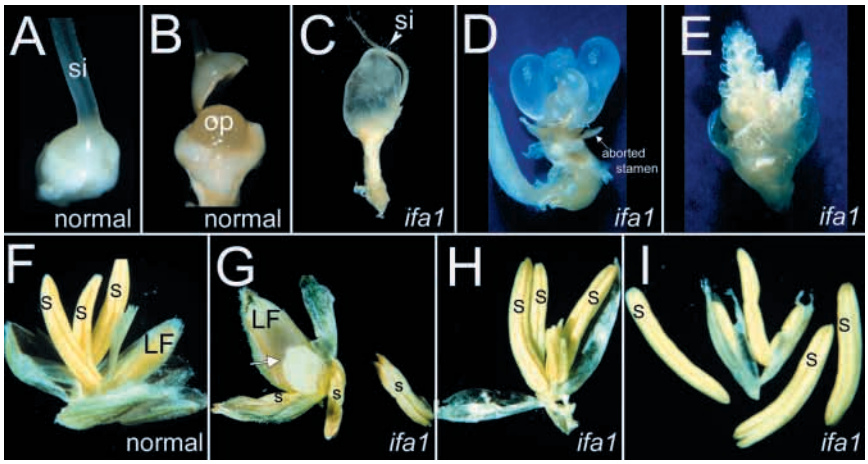


**Fig. 3.** Floral phenotype of *ifa1* mutants. (A) Normal pistil. Two of the carpels fuse and elongate to form the silk. (B) The ovule primordium is exposed inside the fused carpels. (C) *ifa1-r* pistil. A nucellar-like proliferative tissue arises from the floral apex, vestiges of the carpel wall and rudimentary silk can be seen. (D) Occasionally, multiple ovule-like protrusions form at the center of the *ifa1-r* pistil. (E) *ifa1-r* spikelet showing ectopic inflorescence from both florets. This phenotype occurs at a low frequency. (F) Normal male spikelet. The stamens of the upper floret are visible, the stamens of the lower floret remain enclosed by the lemma and palea. (G) *ifa1-r* male floret. Nucellar-like proliferative tissue (arrow) is present inside the stamen whorl. (H) *ifa1-r* male floret with extra stamens at the center of the stamen whorl. (I) The dissected floret from H. Inside the stamen whorl are leaf-like organs and more stamens. si, silk; op, ovule primordium; s, stamen; lf, lower floret.

spikelet abort, thus producing a single female flower per spikelet (Fig. 1C).

In *ifa1* mutants, the floral meristem becomes less determinate. Female flowers are characterized by short silks and undeveloped carpels that surround a mass of protruding nucellus-like tissue (Fig. 3C). The identity of the tissue as a product of the floral meristem was determined by its placement within the whorl of the aborted stamens (Fig. 3D). The severity of the protruding nucellus phenotype is greatest at the tip of the ear. Occasionally, more than one protruding nucellar mass is seen (Fig. 3D) or the ovule primordium appears to be transformed into an inflorescence structure (Fig. 3E). These transformations were seen in the original *Ac* transposon lines and occur at less than 0.1% of the time in any introgressed material. The protruding nucellus phenotype is 100% penetrant when *ifa1-r* is introgressed into all inbreds tested.

A similar phenotype was observed in florets of male spikelets. In normal flowers, three stamens are found at maturity inside the enclosing palea and lemma (Fig. 3F). In *ifa1* mutants, an enlarged nucellar-like mass sometimes forms within the whorl of the three stamens (Fig. 3G). In florets closer to the tip of the tassel or tassel branches, extra stamens form in the center of the flower instead of the enlarged nucellar mass (Fig. 3H). Each of the extra stamens appear to develop in the axil of a lemma-like structure (Fig. 3I) suggesting that the extra stamens are not due to the formation of an additional whorl of stamens but rather arise from a sustained floral meristem. The nucellar mass or extra stamens is found in both the upper and lower male florets. Thus, both male and female floral meristems continue to proliferate. This result suggests that *ifa1* regulates determinacy of the floral meristem.

### Scanning electron microscopy reveals early events in *ifa1* mutants

In order to determine when *ifa1-r* flowers first deviate from normal, we examined developing female inflorescences from mutants (Fig. 4F-I) and their normal siblings (Fig. 4A-D) using scanning electron microscopy (SEM). Because formation of the stamen whorl is not affected in *ifa1-r*, we used the stage of stamen development as a standard to compare developing florets. In Fig. 4A,F, both upper and lower floral meristems have initiated. Although the lower floral meristem is initiated first (Cheng et al., 1983), it lags in development. Normal and *ifa1-r* floral meristems appear similar, both in size and morphology, as stamen primordia initiate. Normal florets form a ridge of carpel tissue that arises on the adaxial side (relative to the inflorescence meristem) of the floral meristem. The carpels surround the floral meristem, which at this stage terminally differentiates into an ovule primordium (Fig. 4B,C) and elongates to make the silk (Fig. 4D).

In *ifa1* mutants, the carpel ridge does not enclose the ovule primordium (Fig. 4G). The enlarged and misshapen ovule primordium produces additional organs in a distichous pattern (Fig. 4H,I). These organs are presumed to be carpels because of their thickened ridge. The lower floral meristem is also affected in *ifa1* mutants. In Fig. 4I, the lower floral meristem has produced one carpel and is producing a second one in an opposite position.

We also examined the origin of the extra spikelets in *ifa1* mutants using SEM. As in normal plants, the SPM divides unequally to form two primordia. In *ifa1-r*, the larger primordium divides again into two unequal primordia one of which develops into an extra spikelet (data not shown). Fig. 4E shows a spikelet pair from a normal individual. The upper floral meristems have already initiated stamens and pistils. In the *ifa1* mutant, an additional spikelet appears between a spikelet pair (Fig. 4J). Extra spikelets were always found between two normally placed spikelets (data not shown). This extra spikelet is in an opposite orientation to the other spikelets as revealed

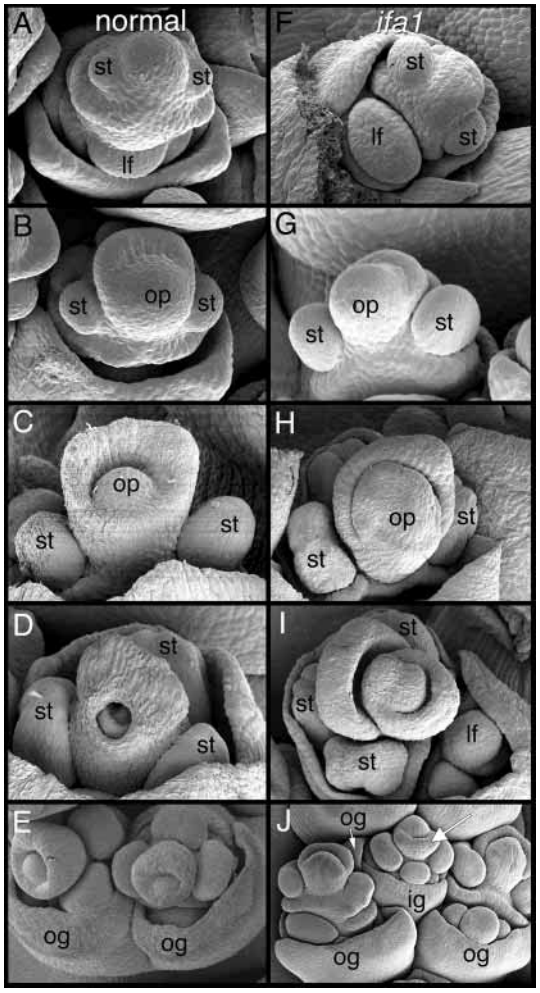


**Fig. 4.** SEM of normal and *ifa1-r* spikelets and florets. (A-D) Normal floret. (F-I) *ifa1-r* floret. (A,F) The upper FMs have initiated three stamens, one of which is hidden. Part of the glume is removed from the spikelet in F to reveal more of the lower FM. (B,G) The carpel ridge forms on the adaxial side of the FM. (C,H) The carpel ridge begins to envelope the ovule primordium in normal (C) but not in *ifa1* mutants (H). (D) The carpels have almost completely enveloped the ovule. (I) The ovule primordium in *ifa1* mutants is forming organs in a distichous pattern. The lower FM is also abnormal. (E) Spikelets develop in pairs in normal plants with the outer glumes forming at the abaxial side. The upper floret, which develops ahead of the lower floret, forms at the adaxial side of the spikelet. Both the inner glume and lower floret are obscured. (F) In *ifa1* mutants, an extra spikelet forms between the spikelet pair. This extra spikelet develops in a reverse orientation such that the outer glume (see small arrow) is adaxial and the inner glume and the carpel ridge of the upper floret (see large arrow) are abaxial. lf, lower floret; st, stamen; op, ovule primordium; og, outer glume; ig, inner glume.

by the orientation of the carpel ridge on the upper floret (arrow in Fig. 4J). The carpel ridge, which is normally adaxial in position, is now abaxial. The extra spikelet appears to be slightly delayed in development as compared to the other spikelets. This result suggests that branching, rather than subdivision, produces the extra spikelet.

Thus, SEM reveals that many aspects of normal development persist in the *ifa1* mutant. The SPM makes SMs, which make FMs, which initiate the correct complement of organs. In each case, however, the determinate meristems produce more products than normal. The distichous patterns of initiation suggest that the defect is one of determinacy rather than meristem size.

## Expression of carpel and meristem marker genes in *ifa1* mutants

In order to evaluate the determinacy of *ifa1* meristems at the molecular level, we carried out in situ hybridization with the homeobox gene, *knotted1* (*kn1*) (Smith et al., 1992; Jackson et al., 1994). *kn1* is expressed in all shoot meristems and shoot axes and is down regulated as lateral organs form. It is expressed in spikelet pair, spikelet and floral meristems but not in glumes or other floral primordia. As shown in Fig. 5A, *kn1* expression in these spikelets recedes from the upper floral meristem as the ovule primordium develops. At the same time, *kn1* expression is still strong in the lower floral meristem, which is in an abaxial position. Later in development, neither the silk nor the ovule expresses *kn1*, although the base of the floral apex and undifferentiated lower floral meristem continue to do so (Fig. 5D).

The expression pattern of *kn1* in a longitudinal section of an *ifa1-r* inflorescence reveals the presence of an extra spikelet (* in Fig. 5B). Spikelet pairs develop side by side in a normal inflorescence, thus, only one column of spikelets can be observed per longitudinal section (Fig. 5A). The presence of an extra spikelet can be observed in a longitudinal section of an *ifa1-r* inflorescence because a portion of the extra spikelet overlaps with the normal spikelet pair during development (Fig. 5B). A young floral meristem is branching from one of these extra spikelets. Based on its developmental stage, this adaxially located meristem is the lower floral meristem. This observation is consistent with our SEM of an *ifa1-r* inflorescence (Fig. 4J), indicating that the orientation of the extra spikelet is opposite from that of normal spikelets.

We examined *kn1* expression in ovule primordia of *ifa1-r* flowers. In wild-type floral meristems, *kn1* is expressed until the ovule primordia initiate (Fig. 5D). At the stage in which the carpel ridge begins to elongate, *kn1* expression forms a sharp line between the ovule primordium and the axis of the floral meristem. In *ifa1* mutants, *kn1* expression is also restricted to shoot meristems and not found in floral organs. Expression disappears from the ovule primordium as in normal siblings, however, expression returns in a few cells in the center of the enlarging ovule-like structure. A serial section through an *ifa1-r* ovule primordium reveals a small group of centrally located cells that express *kn1* (Fig. 5E-H). This pattern of expression was observed in several sectioned ovules (data not shown). Thus, *ifa1* functions directly or indirectly, to negatively regulate *kn1* in a subset of cells at the center of the ovule primordium.

As *kn1* expression disappears in incipient ovule primordia, carpel and ovule specific genes, such as *zea agamous2* (*zag2*), are expressed (Schmidt et al., 1993). We utilized *zag2*, as an in situ hybridization probe to determine whether the *ifa1-r* primordium gained ovule identity. As in wild-type female flowers, *zag2* mRNA was detected in the ovule primordium and inner carpel wall of the *ifa1-r* pistil (Fig. 6A,B). Although the *ifa1-r* primordium is greatly enlarged compared to normal ovules, *zag2* expression is still visible. We also observed megaspore mother cells in some *ifa1-r* ovule primordia (data not shown). These results indicate that the floral meristem in *ifa1* mutants has acquired ovule-like identity. Thus, the expression of *kn1* in a few cells of the *ifa1-r* ovule primordium

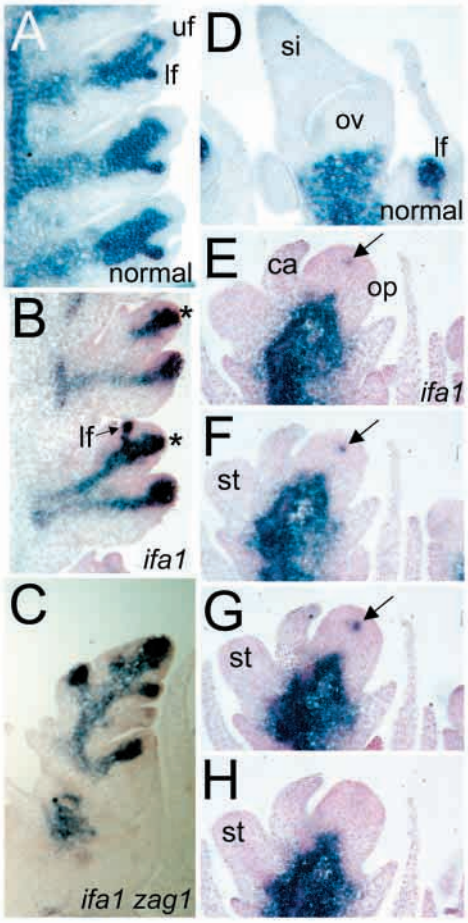


**Fig. 5.** *kn1* gene expression in ear spikelets and florets. (A) *kn1* expression in developing spikelets. A longitudinal section of a normal inflorescence shows only one spikelet of the spikelet pair. The lower floret forms at the abaxial side of each spikelet. (B) *kn1* expression in developing *ifa1-r* spikelets. A longitudinal section reveals a portion of an extra spikelet (marked with *) that overlaps with the developing spikelet pair. The lower floret develops on the adaxial side in extra spikelets. (C) *kn1* expression in an ectopic BM arising from within an *ifa1-r zag1* ear floret. (D) *kn1* is not expressed in the silk or ovule of normal plants. Expression is detected in the lower floral meristem and floral axis below the ovule. (E-H) Serial section of *ifa1* mutant ovule primordium. *kn1* is also down-regulated as the ovule primordium initiates, however, as shown in a series of 10 μm sections from the same floral apex, *kn1* is expressed in a small group of cells at the center of the primordium. uf, upper floret; lf, lower floret; *, extra spikelet; ov, ovule; op, ovule primordium; ca, carpel; ov, ovule.

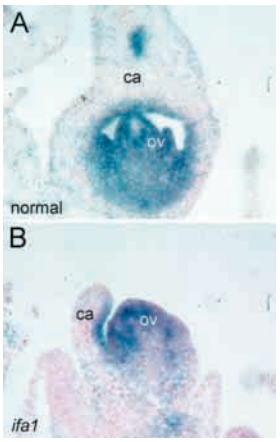


**Fig. 6.** *zag2* gene expression in normal and *ifa1-r* flower. (A) *zag2* is expressed in the ovule and carpel wall. (B) In *ifa1* mutants, *zag2* is also expressed in the carpel wall and initiating ovule primordium. ca, carpel; op, ovule primordium.

is not simply due to a failure to differentiate into ovule tissue. It appears that a subset of *ifa1-r* ovule primordium cells regain, or retain meristematic identity.

### Genetic interaction of *ifa1-r* with mutants affecting floral and spikelet determinacy

To gain further insights into the function of *ifa1* in meristem determinacy, we constructed double mutants between *ifa1-r* and two other mutations that affect floral and spikelet meristem determinacy, *zag1-mum1* and *ids1-mum1*. Mutations in *zag1*, a maize homologue of *AGAMOUS* (Yanofsky et al., 1990) in *Arabidopsis* and *PLENA* in *Antirrhinum* (Bradley et al., 1993), result in a loss of determinacy of the female floral meristem (Mena et al., 1996). *zag1* mutant flowers form extra carpels and extra silks (Fig. 7B), and occasionally form a mass of tissue within the carpel whorl. Mutations in *zag1* do not affect male flower morphology.

Female flowers of *ifa1 zag1* double mutants reveal a phenotype not found in either single mutant (Fig. 7A,B). All female flowers in plants defective for *ifa1* and *zag1* develop a branching structure at the center of the flower (Fig. 7C,D). The branching structures form spikelet pairs in a distichous pattern and therefore are considered BM. These secondary BM arise within the whorl of stamens (data not shown). Flowers produced by the secondary BM reiterate the defective phenotype, giving rise to tertiary branches. Consistent with this indeterminate phenotype, *kn1* is expressed in the SPM that arise from this ectopic BM (Fig. 5C). The phenotype is completely penetrant and very expressive in both inbred and mixed backgrounds (data not shown).

The tassels of *ifa1 zag1* double mutants (Fig. 7F) are clearly distinct from *zag1-mum1* tassels, which display normal morphology, and *ifa1-r* tassels, which are slightly bushier (Fig. 7E,F). Dissection of a floret from a double mutant tassel, shown in Fig. 7G, reveals an ectopic spikelet within the stamen whorl. This spikelet is in the position of the pistil, which normally aborts in male flowers. The ectopic spikelet has leaf-like glumes not observed in normal spikelets. When the leaf-like glumes of the ectopic spikelet are opened, several stamens enclosed by additional leaf-like organs are revealed (Fig. 7H). This phenotype suggests that the ectopic spikelet made multiple florets. Thus *ifa1* and *zag1* independently regulate floral meristem determinacy, but act redundantly to regulate the identity of the floral meristem. When both *ifa1* and *zag1* genes

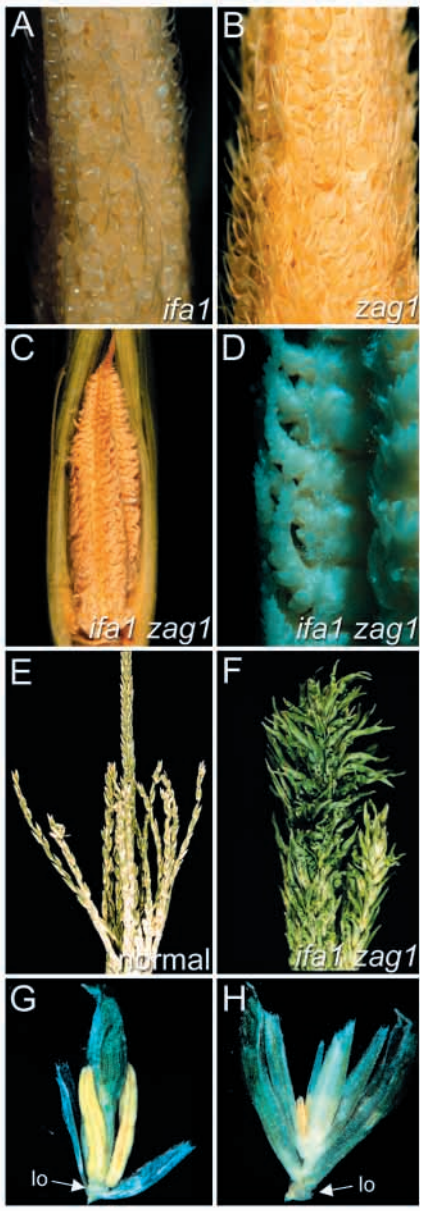


**Fig. 7.** *ifa1 zag1* double mutant phenotype. (A) *ifa1-r* ear. (B) *zag1-mum1* ear. (C) *ifa1-r zag1-mum1* ear shows inflorescences arising from the center of every floret. (D) Close-up of (C). (E) Normal tassel. (F-H) *ifa1-r zag1-mum1* tassel. (G) *ifa1-r zag1-mum1* floret reveals an ectopic spikelet arising from the center of the stamen whorl where the aborted pistil should have been. (H) The ectopic spikelet from G was opened to reveal staminoid florets arising from the axils of leaf-like organs. Arrows in G and H point to one of the lodicules (lo).

are mutated, the floral meristem converts to a branch meristem in the ear and to a spikelet meristem in the tassel.

*indeterminate spikelet1 (ids1)*, which encodes an AP2-like protein, controls spikelet meristem determinacy in maize (Chuck et al., 1998). The *ids1-mum1* allele results in multiple florets forming from the spikelet meristem. This phenotype is similar to one aspect of the *ifa1-r* phenotype (Fig. 2C). Both female and male florets are affected in *ids1* mutants. Florets in the ear make silks (Fig. 8A) but are usually infertile (Chuck et al., 1998).

Ears of the *ifa1 ids1* double mutant show a phenotype that is distinct from either single mutant (Fig. 8A-D). A highly branched structure arises from within the spikelets. Dissecting a spikelet pair (Fig. 8E) and spikelet (Fig. 8F) reveals the basis of the highly branched phenotype; multiple florets and a branching structure form within the enclosing glumes of the spikelet (Fig. 8F). This branched structure forms spikelet pairs in a distichous pattern and thus is considered to be a branch

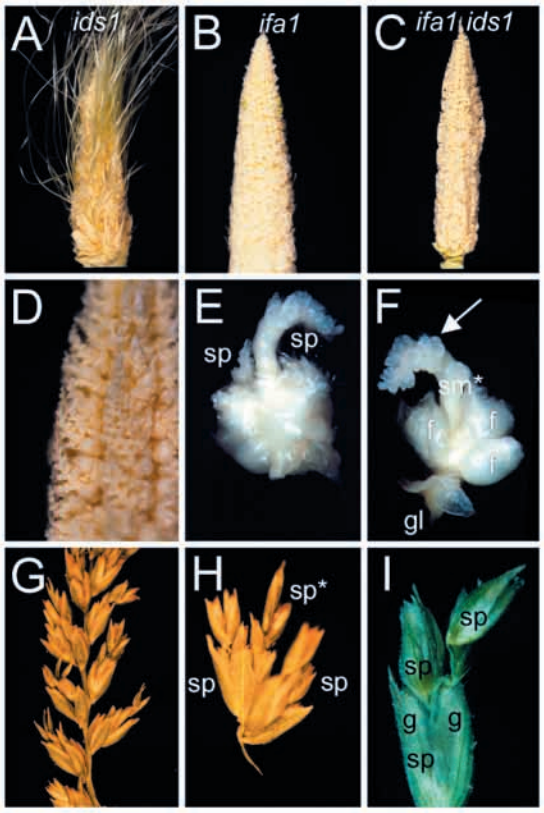


**Fig. 8.** *ifa1 ids1* double mutant phenotype. Individuals are from a family segregating for the *ifa1 ids1* double mutant phenotype. (A) *ids1-mum1* ear. (B) *ifa1-r* ear. (C-F) *ifa1-r ids1-mum1* ear. Each spikelet forms an ectopic inflorescence. (E) *ifa1-r ids1-mum1* spikelet pair. Both spikelets in a pair are affected. (F) *ifa1-r ids1-mum1* spikelet with three florets and an ectopic inflorescence. The ectopic inflorescence forms spikelet pairs along its axis (arrow points to a spikelet pair). (G-I) *ifa1-r ids1-mum1* tassel. (H) Spikelet pair of an *ifa1-r ids1-mum1* plant. Many more spikelets form than in normal plants. (I) A series of spikelets form inside the glumes of the spikelet. sp, spikelet; sp*, extra spikelet; f, floret; g, glume; sm*, ectopic inflorescence that arises from an SM.

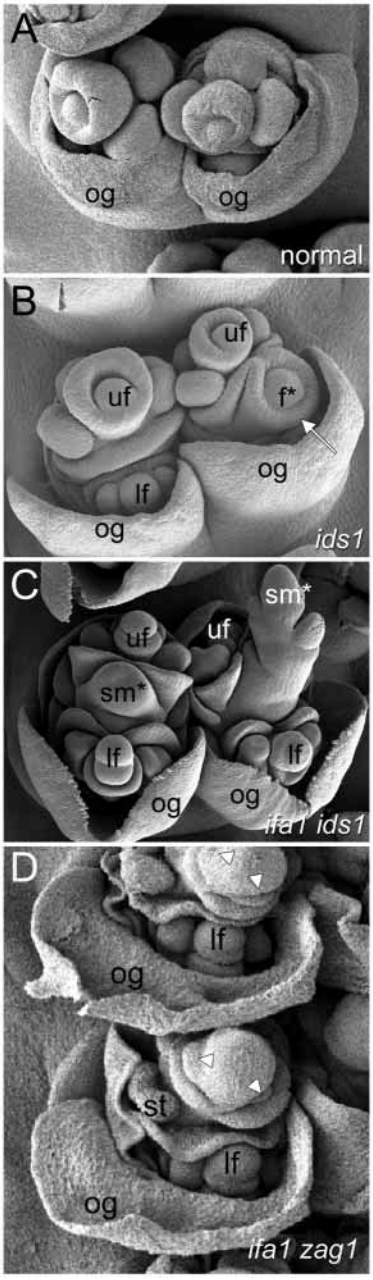


**Fig. 9.** SEM of developing spikelets. (A) Normal spikelet pair. The more advanced upper floret develops on the adaxial side and the lower floret, which is partially covered by the outer glume, forms on the abaxial side. (B) *ids1-mum1* spikelet pair. One of the spikelets is developing a third floret between the upper and the lower floret. The carpel ridge (arrow) of the extra floret (f*) has an orientation opposite to that of the upper floret and similar to that of the lower floret. (C) Spikelet pair from an *ifa1-r ids1-mum1* ear. An indeterminate meristem arises between two florets (sm*). The floral meristems produce additional carpel ridges as seen in the single *ifa1* mutant. (D) Longitudinal row of *ifa1-r zag1-mum1* spikelets. Lateral organs form from the ovule primordium (arrowheads). uf, upper floret; lf, lower floret; og, outer glume; ig, inner glume; st, stamen; sm*, indeterminate meristem.

meristem. Florets on the secondary branch reiterate the *ifa1-r* floral phenotype. Both spikelets are affected (Fig. 8E).

Tassels of *ifa1 ids1* double mutants show a highly branched phenotype in which spikelets form within the spikelets (Fig. 8G). Fig. 8I shows a single spikelet that contains spikelets emerging from within the encircling glumes. The presence of spikelets arising within spikelets is found in both the pedicellate and sessile spikelet as well as in extra spikelets that form (Fig. 8H). The ectopic spikelets develop singly and consecutively, suggesting that they arise from a spikelet pair meristem. Thus, *ifa1* and *ids1* act independently to maintain the determinacy of the SM and redundantly to maintain its identity. The fate of the SM depends on whether it is born on a male or female inflorescence. In the double mutant, the ear SM becomes a BM, whereas, in the tassel, the SM becomes a SPM.

SEM was used to follow the meristems as they initiate in the double mutants (Fig. 9). As shown in Fig. 9B, *ids1* mutants make extra florets in a spikelet, which arise in a distichous branching pattern. The extra floret is between the upper and lower floret. In *ifa1 ids1* double mutants, a bulging meristem forms between the upper and lower floral meristem (Fig. 9C). The spikelet on the right (Fig. 9C) is slightly more advanced and reveals an elongating meristem that appears to be initiating spikelets. The younger spikelet on the left clearly shows that this meristem arises between the two florets. SEM of the *ifa1 zag1* double mutant shows an abnormal upper and lower floral meristem that has initiated a few ridges (arrows in Fig. 9D). No meristems arise between the upper and lower flower. These results emphasize the fact that the secondary inflorescences in *ifa1 zag1* or *ifa1 ids1* mutants arise from different locations.

We also examined expression of both *zag1* and *ids1* in *ifa1* mutants and found that their expression patterns were indistinguishable from wild type (data not shown). These results support the genetic analysis that failed to show an epistatic interaction.

## DISCUSSION

We have discovered a gene in maize that regulates meristem determinacy in the inflorescence. Maize has three types of determinate meristems, the spikelet pair meristem (SPM), the spikelet meristem (SM) and the floral meristem (FM). The *ifa1* mutation affects the floral meristem most severely, but also affects the other two determinate meristems. In an *ifa1* single mutant, each determinate meristem produces additional subsequent meristems or organs that are normally specified. However, in a background in which either *zag1* or *ids1* is mutated, each affected meristem produces its normal organs and then converts to earlier meristem types. Which meristem converts, and to what, is genetically controlled. The conversions are distinct without gradients of transformation.

### *ifa1* is required for determinacy of the floral meristem

Flower meristems produce four whorls of organs in most Angiosperm flowers, starting with sepals as the outer whorl, followed by petals, stamens and lastly, carpels. Carpels produce ovules, which contain the female gametophyte. Ovules are regarded by some as a fifth whorl (Angenent et al., 1994; Ray et al., 1994). In maize, the fused carpels constitute the pistil, which contains a single ovule that is the terminal product of the floral meristem (Sharman, 1945; Barnard, 1964). In male flowers, the pistil aborts and in female flowers, the stamens are arrested in growth (Cheng et al., 1983; DeLong et al., 1993).

*ifa1* mutants appear normal at early stages of floral development with properly specified floral organs. They begin to differ from wild type as the carpels form and the ovule primordium is initiated. The carpels fail to enclose the ovule primordium, which continues to expand. The expanding ovule primordium has many features of a meristem although it still expresses the carpel/ovule marker, *zag2* (Schmidt et al., 1993). Male flowers of *ifa1* mutants are also defective at the floral apex. In *ifa1* mutants, a protruding nucellar-like mass or additional male florets are occasionally found in the position normally occupied by the aborted pistil. The frequency of indeterminacy is not as high in male flowers as it is in female flowers, but dramatically increases when the pistil does not undergo abortion. Such a condition occurs in mutants such as *tasselseed2* (ts2) and *Tasselseed5* (*Ts5*) that have feminized flowers on the tassel (Irish and Nelson, 1989; DeLong et al., 1993; Veit et al., 1993). In *ifa1 ts2* or *ifa1 Ts5* double mutants, all the tassel florets have a protruding nucellus-like mass within the stamen whorl (data not shown). Thus the indeterminate nature of the floral meristem may override abortion of the pistil in the male flower and is enhanced when pistil abortion is suppressed.

### *ifa1* is required to specify the identity of spikelet and floral meristems

*zag1* also regulates floral meristem determinacy (Mena et al., 1996). It is first expressed in floral meristems and then in stamens and carpels, similar to the expression of *AG* in *Arabidopsis* (Yanofsky et al., 1990). Female florets of *zag1* mutants have an indeterminate floral meristem in which extra carpels are produced (Mena et al., 1996). This defect is similar to one aspect of *ifa1* mutants. In *ifa1 zag1* double mutants, the floral meristem is converted to a branch meristem in female flowers and converted to a spikelet in male flowers after production of normal whorls of floral organs (Fig. 10). This phenotype reveals additional roles for both *zag1* and *ifa1* in female and male flowers. Each gene functions independently to maintain determinacy of the female floral meristem but functions redundantly to maintain its identity. Although *zag1* plays no apparent role in male flowers, both *zag1* and *ifa1* function redundantly to maintain identity of the floral meristem in male flowers as well.

The penetrance of the large nucellus phenotype in *ifa1* single mutants is 100%, as is the penetrance of the branch meristem phenotype in *ifa1 zag1* double mutants. Occasionally, a single

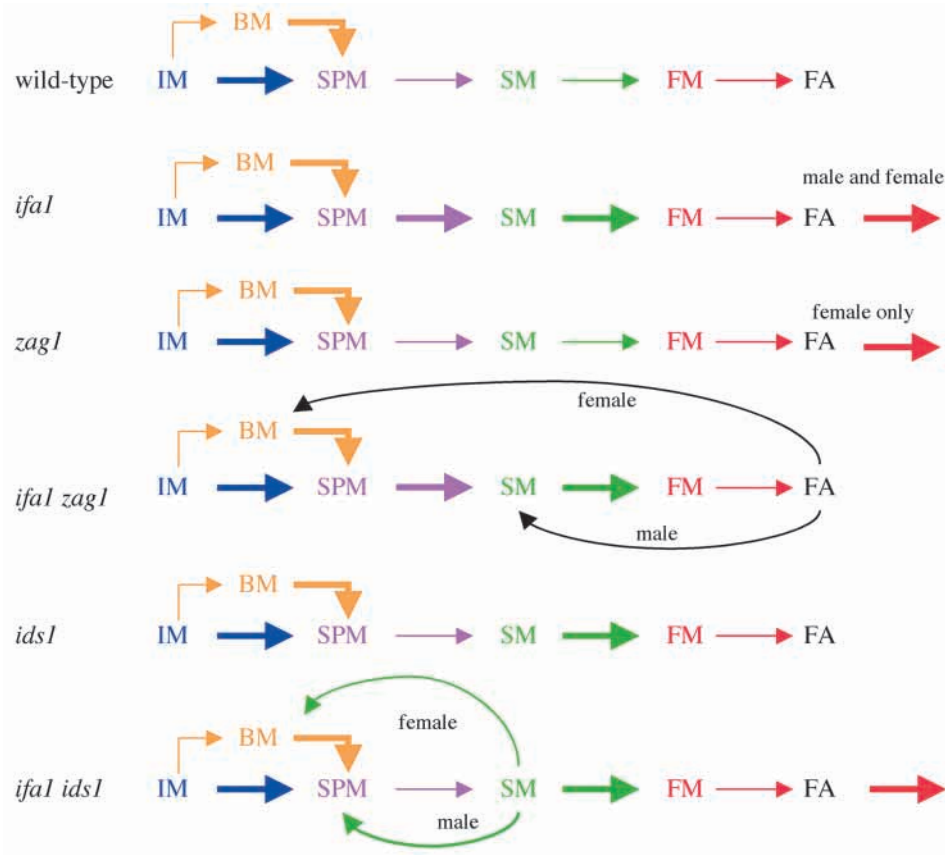


**Fig. 10.** Meristem identity in different mutants. IM, inflorescence meristem; BM, branch meristem; SPM, spikelet pair meristem; SM, spikelet meristem; FM, floral meristem; FA, floral apex; thin arrow, determinate meristem; thick arrow, indeterminate meristem producing extra organs or meristems.

kernel on an *ifa1* mutant ear may show the branching phenotype. While this finding could suggest that *ifa1* alone can regulate identity, it is also possible that *zag1*, or genes downstream of *zag1*, fail to function at some stochastic frequency.

A synergistic interaction also occurs in *ifa1 ids1* double mutants. *ids1* single mutants have extra florets in both male and female spikelets (Chuck et al., 1998). *ids1* is expressed in immature floral organs, spikelet pair and spikelet meristems. In the *ifa1 ids1* double mutant, the SM in the ear produces floral meristems as usual but then is converted to a branch meristem. The SM in the tassel is also converted after producing floral meristems. This male meristem produces a series of spikelets that are initiated singly and thus may be considered a SPM.

Despite the effect of *ifa1-r* on all determinate meristems, *ids1-mum1* and *zag1-mum1* limit the conversion seen in double mutants to a single meristem type. *ids1* plays a role in SM determinacy and thus the double mutant affects conversion of the SM. *zag1* plays a role in floral meristem determination and thus the double mutant affects floral meristem conversion (Fig. 10). Whether the meristems of the double mutants revert to branch, spikelet pair or spikelet, the reversions are complete and not a mixture of meristem types. This result suggests that the identity of each meristem type is distinctly defined. This finding is intriguing given that *ifa1* affects all meristems and *ids1* is expressed at all stages, yet their dual absence affects one meristem type most significantly. It suggests that some other unidentified genes must function only at the SM stage.

Male and female conversions are distinct in both double mutants (Fig. 10). In each case, the converting female meristem becomes a branch meristem and the converting male meristem becomes a spikelet or spikelet pair meristem. We also found that the severity of the *ifa1-r* phenotype differed between male florets that were feminized or not. Other maize mutants are also more severe in the ear than the tassel. For example, *bd1* mutant ears have SM that produce BM and never reach the stage of floral meristem. *bd1* mutant tassels, on the other hand, are capable of producing floral meristems (G. Chuck, S. H. and R. Schmidt, unpublished) (Colombo et al., 1998). The difference between male and female inflorescence may result from different hormone levels or different sensitivities to hormones due to the different positions on the plant (Dellaporta and Calderon-Urrea, 1994; Irish, 1996).

## Meristem conversion

A classic example of meristem conversion is found in *Impatiens balsamina* (Battey and Lyndon, 1990). *Impatiens* requires constant short days for flowering. If plants are returned from inductive short days to long days, the floral meristem begins producing vegetative leaves from the floral meristem. Chimaeric organs that are part petal and part leaf sometimes form. A switch back to short days results in the immediate production of petals again. Reversion to vegetative development is also seen in the *indeterminate1* (*id1*) mutation of maize (Colasanti et al., 1998). *id1* mutants fail to flower under most normal growing seasons and produce two to three times more than the usual number of leaves. Eventually, the shoot apical meristem is converted to a tassel that is highly abnormal. Ears do not form on these plants. Dissection of an *id1* tassel shows that vegetative seedlings are initiated from the spikelet meristem after initiation of two florets (Galinat and Naylor, 1951) (D. L. C. and J. Colasanti, unpublished). Thus, the *id1* spikelet meristem has converted to a vegetative meristem.

A number of mutations affect determinacy and identity of the flower meristem in *Arabidopsis*. *AGAMOUS* (*AG*) plays a crucial role in floral meristem determinacy in *Arabidopsis* (Bowman et al., 1989). Under certain conditions, the *ag* floral meristem produces an inflorescence meristem at the center of the carpel whorl similar to *ifa1 zag1* double mutants. Okamura and colleagues showed that *agamous* homozygotes or *leafy* (*lfy*) heterozygotes in short day conditions produce shoots from within the carpel whorl (Okamuro et al., 1993). Similar results were obtained using *ag constans* double mutants (Mizukami and Ma, 1997). CONSTANS regulates LFY (Simon et al., 1996), which itself is an activator of AG (Busch et al., 1999), thus explaining why limiting amounts of LFY might result in the same phenotype. Short day conditions delay the activation of LFY (Busch et al., 1999), and so perhaps also reduce the amount of AG.

Recently, two groups have shown that LFY together with WUSCHEL, a homeodomain protein that is required for meristem function (Lenhard et al., 2001; Lohmann et al., 2001), positively regulate AG and that AG negatively regulates WUS. WUS expression persists in the indeterminate meristems of *ag* flowers (Lohmann et al., 2001). We have shown that *kn1* is expressed in ovule primordia of *ifa1* mutants, suggesting that IFA1 negatively regulates KN1. Given the parallels between *ifa1 zag1* and weak *ag* alleles of *Arabidopsis*, it is likely that IFA1 also negatively regulates a WUS ortholog.

## Models for maize inflorescence architecture

Different models have addressed the relationships of the branch meristems in maize. The conversion model proposes that the SPM converts to an SM after initiation of the sessile spikelet, and the SM converts to the upper floral meristem after initiating the lower floret (Irish, 1997). With this model, the *ifa1-r* phenotype could be explained as a delay in the conversion of one meristem type to another. This delay would result in the initiation of more meristems before conversion. The SPM would initiate more than one SM and the SM would initiate more than one floral meristem before converting. Perhaps, if an SM can convert to a floral meristem, then it can also convert back to a SPM, depending on amounts of gene product. The other model proposes that a residual SM is present after production of both floral meristems (Chuck et al., 1998; Irish, 1998). In mutants such as *ids1-mum1* or *ifa1-r*, the residual meristem continues to produce lateral organs. Our SEM analysis of *ifa1 ids1* lends support to the residual meristem model, in which a meristem is clearly present between the two floral meristems.

## Conclusion

The spikelet is a unifying feature of all grasses, yet the branches upon which the spikelets are born vary considerably, depending on the species (Clifford, 1987). Spikelets may arise directly on the primary inflorescence, or on secondary or tertiary branches, and they may produce one, two or many florets. The Andropogoneae, a subgroup of the grasses that include maize, have two florets in the spikelet and two spikelets in a modified branch called the spikelet pair. The unique structure of the Andropogoneae is considered to be evidence

of monophyly of the subfamily (Kellogg and Campbell, 1987). *ifa1* and a handful of other genes are responsible for determinacy in the SPM and SM. It is interesting to speculate whether *ifa1* was recruited from floral meristems to branch meristems and thus responsible for the determinacy of the SPM and SM. An investigation of the expression and function of branch meristem determinacy genes in different grasses may answer such questions and lead to an understanding of the evolution of grass morphology.

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