

The control of maize spikelet meristem fate by the *APETALA2*-like gene *indeterminate spikelet1*

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The orderly production of meristems with specific fates is crucial for the proper elaboration of plant architecture. The maize inflorescence meristem branches several times to produce lateral meristems with determinate fates. The first meristem formed, the spikelet pair meristem, produces two spikelet meristems, each of which produces two floral meristems. We have identified a gene called *indeterminate spikelet1* (*ids1*) that specifies a determinate spikelet meristem fate and thereby limits the number of floral meristems produced. In the absence of *ids1* gene function, the spikelet meristem becomes indeterminate and produces additional florets. Members of the grass family vary in the number of florets within their spikelets, suggesting that *ids1* may play a role in inflorescence architecture in other grass species. *ids1* is a member of the *APETALA2* (*AP2*) gene family of transcription factors that has been implicated in a wide range of plant development roles. Expression of *ids1* was detected in many types of lateral organ primordia as well as spikelet meristems. Our analysis of the *ids1* mutant phenotype and expression pattern indicates that *ids1* specifies determinate fates by suppressing indeterminate growth within the spikelet meristem.

[Key Words: Meristem; spikelet; *indeterminate spikelet1*; *APETALA2*; determinacy]

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The development of the plant body is dependent on the activity of apical meristems, groups of indeterminate cells found at the growing tips. The ability of shoot apical meristems to remain indeterminate allows them to continually initiate the organs, tissues, and secondary meristems required for normal development. This property requires that the meristem set aside a population of cells to renew itself and replenish the cells lost to each lateral organ or secondary meristem. The failure of this process to occur results in the termination of the growing tip. Some meristems, such as floral meristems, are consumed in the production of lateral organs, and can be described as having a determinate fate. The inflorescence of maize is particularly useful for studying meristem determinacy as it undergoes several well defined branching events to produce meristems with increasingly restricted fates.

Grass florets are organized into units called spikelets, each consisting of a pair of sterile bracts called glumes enclosing a fixed number of florets (Clifford 1987). The regulation of floret number per spikelet is a prime determinant of spikelet architecture among members of the grass family. The spikelet of maize is determinate, pro-

ducing only two florets in defined positions on an axis called the rachilla (Weatherwax 1923). Relatives of maize are characterized by spikelets with an indeterminate number of florets, such as wheat, or contain only one floret per spikelet, as in barley. One classical criterion used to distinguish different grass species is whether their spikelets contain a determinate or indeterminate number of florets (Clifford and Watson 1977). The spikelet itself is but one component of the complex branched inflorescence of maize. In contrast to dicotyledonous plants such as *Arabidopsis* in which flowers are produced directly by the apical and lateral meristems (Hempel and Feldman 1994), there are at least two distinct inflorescence branching steps in maize before the spikelet meristem terminates in the production of two florets. These extra branching steps allow for greater morphological diversity among the grasses.

A number of mutants have been described in maize that result in an extra number of florets within the spikelet (Veit et al. 1993). In the *branched silkless* mutants, extra florets are initiated in male tassel spikelets (Kemp-ton 1934), although a more extreme transformation is seen in female spikelets of the ear in which florets are transformed into long indeterminate branches (Veit et al. 1993). Studies of the *Tasselseed6* mutation revealed that the spikelet meristem undergoes a delay in acquiring de-

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terminacy, allowing it to initiate florets for a longer period of time (Irish et al. 1994). The analysis of *Tassel-seed6* mutants led to a model (Irish 1997) in which the inflorescence meristem and its branch derivatives pass through an orderly, defined series of determinate developmental states, ending with the conversion of the terminal spikelet meristem into the upper floret. Similar branching mutants have also been described in other grass species and include the *multiflorous* mutation of barley (Bossinger et al. 1992) and the dominant *Naked* mutant of oats (Ougham et al. 1996).

An abundance of genetic and molecular studies have identified several genes important for floral development. One such gene, the *APETALA2* (*AP2*) homeotic gene of *Arabidopsis*, has several functions in flower, seed, and ovule development (Kunst et al. 1989; Jofuku et al. 1994; Modrusan et al. 1994). In addition to its role in determining floral organ identity, *AP2* affects the regulation of floral meristem identity. For example, double mutants of the weak *ap2-1* allele with floral meristem identity mutants such as *leafy* or *apetala1* produce more coflorescence side branches in the place of flowers (Bowman et al. 1993). Also, weak *ap2* alleles under short days cause the formation of tertiary floral shoots in the axils of transformed sepals (Schultz and Haughn 1993). The *AP2* gene belongs to a large gene family, 12 of which have been identified in *Arabidopsis* (Okamoto et al. 1997). Numerous homologs have been identified in both monocots and dicots (Jofuku et al. 1994; Ohme-Takagi and Shinshi 1995; Moose and Sisco 1997). Mutations in the *AP2*-like gene, *AINTEGUMENTA*, are defective in ovule development (Elliot et al. 1996; Klucher et al. 1996). The *glossy15* gene of maize has recently been shown to be an *AP2*-like gene that functions to repress adult leaf characters in juvenile leaves (Moose and Sisco 1997).

Here we describe a new branching mutant in maize, *indeterminate spikelet1* (*ids1*), that we obtained by analyzing the phenotype conditioned by loss of function of a maize *AP2*-like gene. *ids1* mutants have an indeterminate spikelet in which several florets are produced instead of the two found in wild-type maize. *ids1* expression was observed in a variety of lateral organs as well as the spikelet pair and spikelet meristems. Our analysis indicates that the *ids1* gene is critical for the regulation of spikelet meristem determinacy in maize.

Results

Isolation of the ids1 gene

The *AP2* gene from *Arabidopsis* was used to screen two maize cDNA libraries at low stringency, one prepared from immature ears and the other from vegetative meristems. The same class of cDNA was isolated from each library. The longest clone of this class was 1967 nucleotides and contained an ORF of 433 amino acids (Fig. 1) with several domains showing striking amino acid similarity to the *AP2* gene of *Arabidopsis* (Jofuku et al. 1994). Two tandemly repeated 68 amino acid motifs were found

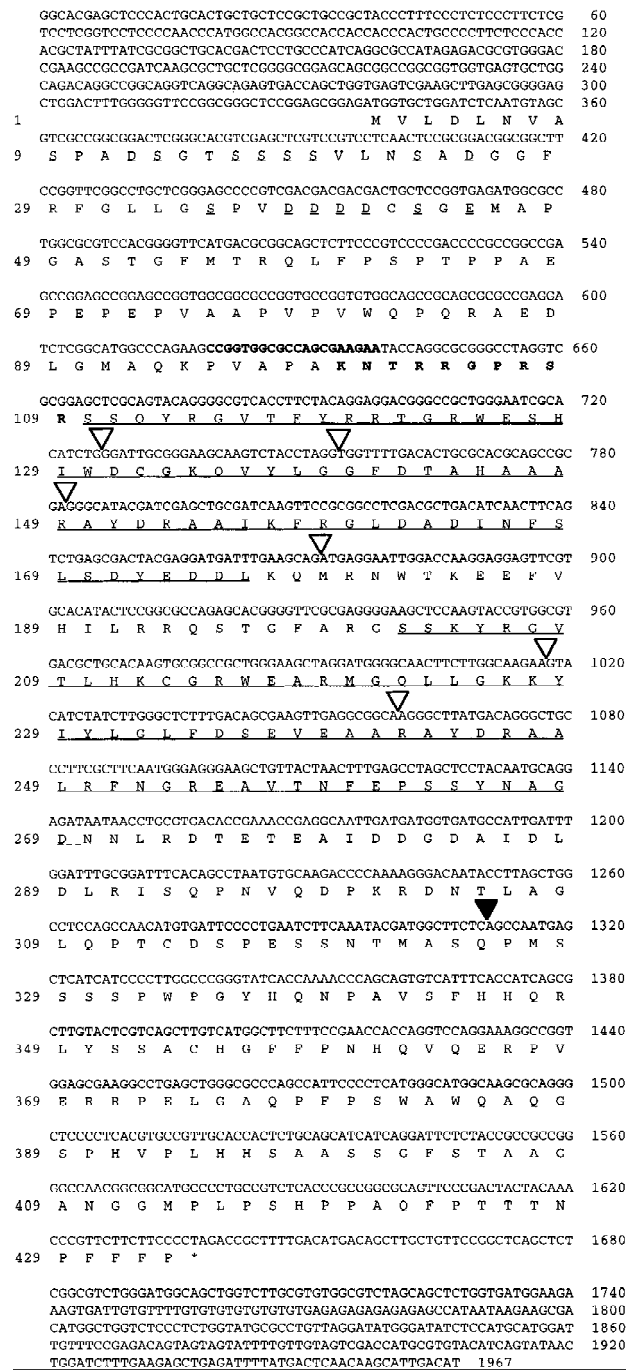
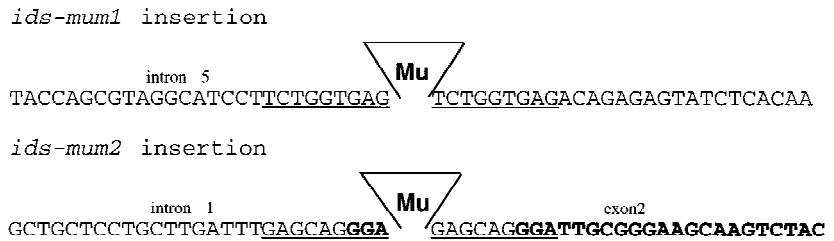


Figure 1. Nucleotide and deduced amino acid sequence of *IDS1*. Amino acid numbers are shown to the left; nucleotides are numbered on the right. Underlined amino acid sequences represent the two *AP2* domains (Jofuku et al. 1994). Serines and acidic amino acids at the amino terminus are indicated with short underlines. Amino acid sequences in bold represent the basic region with putative nuclear localization activity. Bold nucleotide sequences correspond to the *ZAP2-1* primer used for the PCR screen. (▽) The six intron positions conserved between *IDS*, *AP2*, and *GL15*; (▼) the other intron.

that share 86% amino acid identity with the *AP2* domain of the *Arabidopsis* *AP2* protein. The *AP2* gene fam-

Figure 3. Localization of the *Mu* elements in *ids1-mum1* and *ids1-mum2*. *ids1* intron and exon sequences are shown in normal and bold-face lettering, respectively. Intron and exon numbers are shown above the DNA sequences. The underlined sequences represent the characteristic 9-bp duplication associated with *Mu* insertions.



lets often contain more than one silk located at ectopic positions (Fig. 4D). These silks, however, usually do not develop normally; they are often unfused and fail to elongate to their proper length. If the developing ear is manually opened and pollinated, a few kernels develop, suggesting that all other aspects of fertilization and fruit development are normal. When grown to maturity, these kernels display *ids1-mum* homozygous phenotypes, and so do not represent germinal excisions of the *Mu* elements. The *ids1-mum2* allele appears more severe, in that most of the extra florets fail to develop fully formed lateral organs (Fig. 4E, left). Approximately 5% of the *ids1-mum2* female spikelets show an elongating rachilla that emerges from the spikelet (Fig. 4F). In such cases, it can be observed that the rachilla does not terminate in the uppermost floret further confirming its indeterminate nature. No obvious and consistent phenotypes were seen in the leaves and roots of *ids1* mutants despite the fact that *ids1* is expressed in those organs.

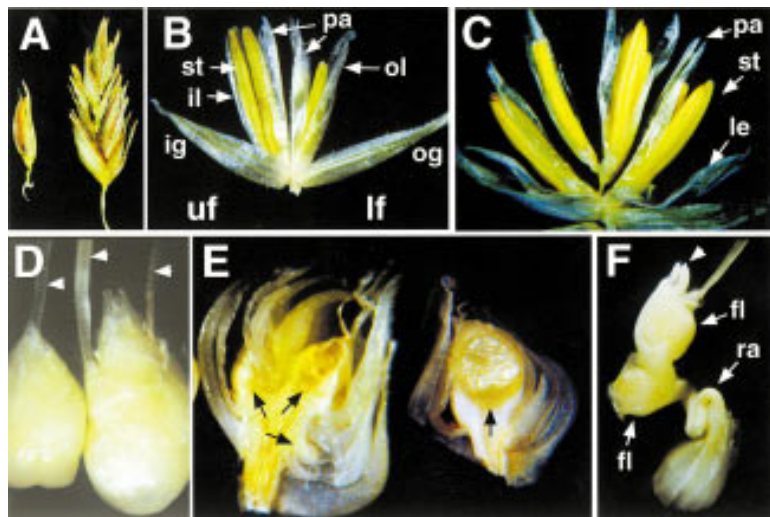
Scanning electron microscopy analysis

Scanning electron microscopy was used to determine the point at which *ids1-mum* spikelet meristem development diverges from that of wild type. The inflorescence meristem normally initiates spikelet pair meristems acropetally, which, in turn, branch to form two spikelet meristems (Fig. 5A) (Cheng et al. 1983). The spikelet me-

ristems also branch to form the lower and upper florets. These florets initiate in a distichous pattern, that is, alternating 180 degrees from one another (Fig. 5B). Although the lower floral meristem initiates first, lateral organ development occurs in the upper floret much earlier than in the lower floret (Fig. 5C,D). The palea is the first lateral organ to differentiate, and the three stamens develop soon after (Cheng et al. 1983). Sexual differentiation occurs at later stages in which the gynoecium aborts in male florets and the stamens abort in female florets (Dellaporta and Calderon-Urrea 1993).

ids1-mum mutants show normal development until the stage at which the spikelet meristem begins to branch. At this point, the *ids1-mum* spikelet meristems appear slightly elongated (Fig. 5E, top). In slightly older spikelet meristems (Fig. 5E, middle), two distinct branching events can be seen as shown by the presence of two lemmas (arrows). Floral meristems then form in the axils of these lemmas (Fig. 5E, bottom, and F). These branching events occur on opposite sides of the spikelet meristem in a distichous pattern. The spikelet meristem persists after each branching event and remains in between the two last formed florets (Fig. 5G,H). This residual spikelet meristem continues to initiate extra lemmas and florets distichously (Fig. 5F,G). The rate of lateral organ development between the first two *ids1-mum* florets is similar (Fig. 5H), in contrast to wild type in which the organs in the upper floret develop first.

Figure 4. Phenotypes in *ids1-mum* male and female spikelets. (A) Normal A632 tassel spikelet (left) and *ids1-mum2* tassel spikelet (right). (B) Dissected normal A632 tassel spikelet showing lateral organs of upper and lower florets. (C) Dissected *ids1-mum2* tassel spikelet showing four florets instead of two. Each floret has three stamens surrounded by palea and lemma as in A632. (D) Normal A632 ear spikelet (left) and *ids1-mum1* ear spikelet (right). Arrowheads point to silks. (E) Median longitudinal sections through unfertilized 25-day-old *ids1-mum2* ear spikelet (left) and A632 ear spikelet (right). Black arrows point to florets. A632 spikelets have a single floret with an enlarged nucellus; *ids1-mum2* ear spikelets have at least three florets with greatly reduced lateral organ development. (F) *ids1-mum2* ear spikelet with an elongating rachilla containing two florets. The single arrowhead points to the tip of the rachilla. (st) Stamen; (il) inner lemma; (pa) palea; (ol) outer lemma; (ig) inner glume; (og) outer glume; (uf) upper floret; (lf) lower floret; (ra) rachilla; (fl) floret.



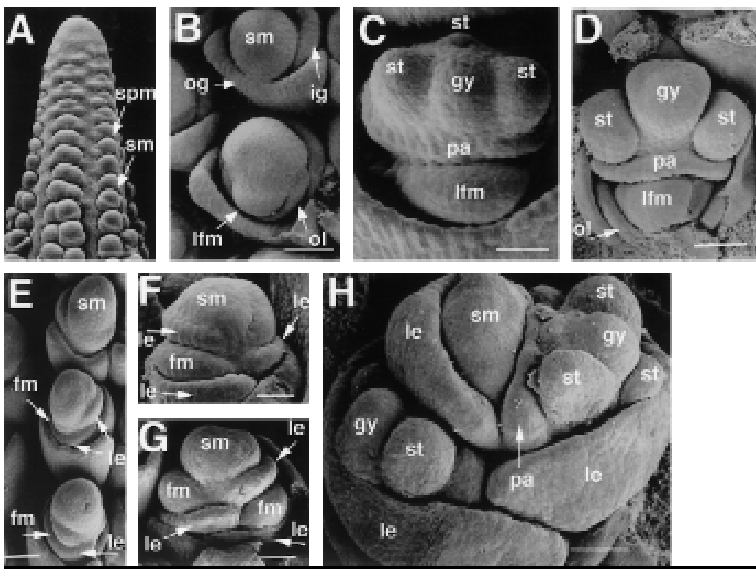


Figure 5. Scanning electron microscopy of normal and *ids1-mum2* ears. (A) A632 ear primordium with initiating spikelet pair primordia and spikelet primordia. Bar, 300 μm . (B) A632 unbranched spikelet meristem (*top*) and slightly older branched spikelet meristem (*bottom*). The older spikelet meristem undergoes lateral branching to initiate the lower floret. Bar, 102 μm . (C) Development of lateral organs in A632 ear spikelets. The lateral organs of the upper floret develop first. Bar, 80 μm . (D) Maturing lateral organs of the upper floret of A632 ear spikelets with initiating gynoecial ridge. Bar, 104 μm . (E) Branching *ids1-mum2* spikelet meristems. The uppermost spikelet meristem has not branched yet and appears elongated. Simultaneous floret branching and lemma initiation occurs on opposite sides of the middle and lower spikelet meristems. Bar, 156 μm . (F) Further development of *ids1-mum2* spikelet meristems with glumes removed showing development of the floral meristems within the axils of the lemmas. The spikelet meristem persists after each branching event. Bar, 58 μm . (G) Older *ids1-mum2* spikelet meristem with glumes removed. The residual spikelet meristem elongates to initiate another floret. Bar, 60 μm . (H) *ids1-mum2* spikelet showing maturing lateral organs. There is no distinction between upper and lower floret in terms of lateral organ development. The youngest floret branching from the spikelet meristem is obscured by the lemma. Bar, 171 μm . (spm) Spikelet pair meristem; (sm) spikelet meristem; (lfm) lower floral meristem; (gy) gynoecium; (fm) floral meristem; (le) lemma.

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We used the *knotted1* (*kn1*) homeobox gene of maize, which is expressed in meristems and not in determinate lateral organs, to follow the branching events in *ids1-mum* spikelets. *kn1* is expressed in the spikelet and floral meristems of maize (Fig. 6A,B) (Jackson et al. 1994). Hybridization with a *kn1* anti-sense probe on *ids1-mum2* spikelet meristems showed normal expression within the spikelet meristem (Fig. 6C). However, as soon as the *ids1-mum2* spikelet meristem branches to form florets, the spikelet meristem becomes larger, and consequently, the domain of *kn1* expression expands (Fig. 6D). Strong *kn1* expression is observed within the extra florets initiating from the spikelet meristem at later stages (Fig. 6E, arrowheads) confirming the meristematic origins of the extra florets. All other aspects of *kn1* expression within the florets, including absence from the lateral organs and the L1 layer, appear normal.

Analysis of *ids1* expression

An initial characterization of *ids1* expression by use of RNA gel blots indicated that the gene was expressed in both vegetative and floral tissues. An RNA gel blot probed with the 3' portion of the *ids1* cDNA shows that *ids1* transcript is present in all tissues tested (Fig. 7A). The lowest amount of expression was seen in embryo tissue and the highest in inflorescence tissue.

To determine the levels of *ids1* transcript in *ids1-mum* mutants, poly(A)⁺ RNA was isolated from mutant ears and compared with RNA from wild-type ears. As shown in Figure 8A, no wild-type size transcript was found in either *ids1-mum* allele. Instead, faint, higher molecular weight transcripts were observed that may be the result of chimeric or alternatively spliced transcripts.

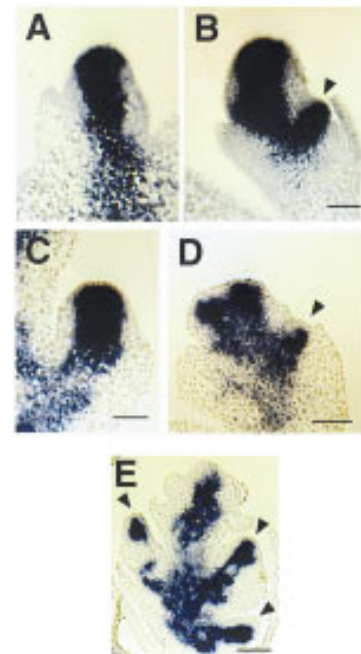


Figure 6. In situ localization of *kn1* within A632 and *ids1-mum2* spikelet meristems. (A) *kn1* expression within an unbranched A632 spikelet meristem. Bar, 50 μm . (B) *kn1* expression within a branching A632 spikelet meristem. Expression can be seen in the initiating lower floret. Bar, 50 μm . (C) *kn1* expression within an unbranched *ids1-mum2* spikelet meristem. Bar, 50 μm . (D) *kn1* expression within an early branching *ids1-mum2* spikelet meristem. Initiating florets can be seen on both sides of the spikelet meristem. Bar, 60 μm . (E) *kn1* expression within an older *ids1-mum2* spikelet. Strong expression persists within the developing florets. Bar, 80 μm . Arrowheads point to initiating florets in B, D, and E.

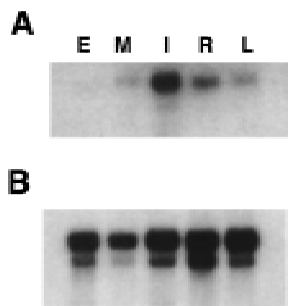


Figure 7. Analysis of *ids1* expression in different organs of B73 maize. (A) An RNA gel blot containing 1 μ g of poly(A)⁺ RNA isolated from embryos 17 days postpollination (E), shoot meristems including unexpanded stem and the youngest leaf primordia (M), ear inflorescence primordia up to 2 cm long (I), primary roots from seeds germinated in humid air (R), and the blade portion of fully expanded juvenile leaves (L) was blotted and hybridized to the 3' portion of the *ids1* cDNA that does not include the AP2 domain. (B) Control hybridization. The blot in A was probed with the maize ubiquitin cDNA to show relative loading.

This result demonstrates that the *Mu* element insertions into *ids1* are not spliced out, and that both *ids1-mum1* and *ids1-mum2* may represent loss-of-function alleles.

To localize the temporal and spatial pattern of *ids1* expression more precisely, we used in situ hybridization. Figure 9A shows longitudinal sections of a wild-type vegetative shoot apex probed with digoxigenin labeled anti-sense RNA made from the 3' end of the *ids1* clone. The positions of leaves within the maize shoot have been described in terms of the plastochron index (Lamoreaux et al. 1978) in which the youngest leaf is referred to as plastochron 1 or P₁ and successive leaves as P₂, P₃, etc. The position of the incipient leaf in the meristem is referred to as P₀. *ids1* expression is detected on the flanks of the meristem in a discrete zone (Fig. 9A). From the position of the youngest leaf (labeled as P₁), we assume this zone of meristem expression is in the position in which the next leaf will initiate (labeled as P₀). Expression is also seen in the tips of the next two older leaves, P₁ and P₂.

In longitudinal sections of floral tissue, *ids1* expression is detected in several different lateral organ primordia as well as the spikelet pair and spikelet meristems. In

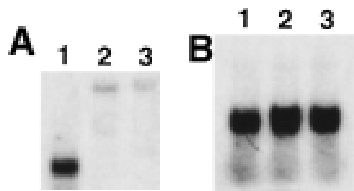


Figure 8. Analysis of *ids1* expression in *ids1-mum* mutant ears. (A) An RNA gel containing 1 μ g of poly(A)⁺ RNA isolated from 2-cm ears from A632 (1), *ids1-mum2* (2), and *ids1-mum1* (3) was blotted and hybridized to the 3' portion of the *ids1* cDNA. (B) Control hybridization. The blot in A was probed with the maize ubiquitin cDNA to show relative loading.

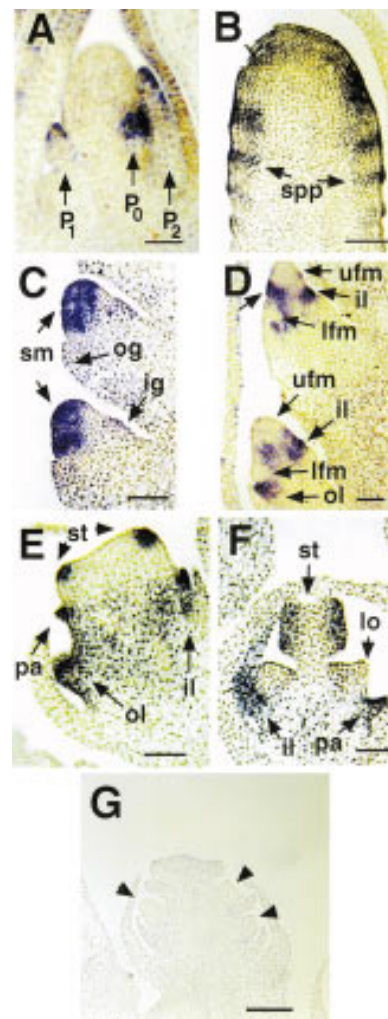


Figure 9. In situ localization in B73 vegetative and floral apices by use of the 3' portion of the *ids1* cDNA. (A) Shoot apical meristem with young leaf primordia (P₁ and P₂) and incipient leaf primordia (P₀). Bar, 65 μ m. (B) Young ear inflorescence. *ids1* expression can be seen in the acropetally initiating spikelet pair primordia. Bar, 70 μ m. (C) Unbranched spikelet meristems. Expression can be seen in the spikelet meristem but not in the glumes. Bar, 36 μ m. (D) Branching spikelet meristems. Strong expression can be seen in the inner and outer lemmas as well as the zone in between the initiating upper and lower florets (unlabeled arrow). This zone of expression gradually disappears in the older spikelet below. No expression is detected in the initiating floral meristems. Bar, 70 μ m. (E) Upper floret with initiating stamens. Discrete areas of *ids1* expression can be observed in the initiating stamen primordia and palea. *ids1* expression persists in the expanding inner and outer lemmas. Bar, 70 μ m. (F) Older floret with lodicules. Stamen expression now localizes to the locule chambers of the anther. No expression can be seen in the lodicules (lo). Bar, 70 μ m. (G) *ids1-mum2* spikelet probed with *ids1*. Little or no *ids1* transcript can be detected. Arrowheads point to florets. Bar, 130 μ m.

Figure 9B, longitudinal sections of early inflorescence meristems probed with *ids1* show stripes of expression in a repeating pattern that evidently corresponds to spikelet pair primordia. At a later stage, after the pedi-

cellate and sessile spikelets form, *ids1* expression is found throughout the spikelet meristem but not in the first two lateral organ primordia formed from the spikelet meristem, the outer and inner glumes (Fig. 9C). Soon after floral meristems initiate (Fig. 9D), *ids1* expression is present in the initiating inner and outer lemma bract leaves subtending the florets, but is absent from the floral meristem itself. In addition, a strong band of expression is seen in the region between the upper and lower florets (Fig. 9D, unlabeled arrow). In slightly older spikelets as shown at the bottom of Figure 9D, this band of expression begins to disappear. A scanning electron micrograph of spikelets at equivalent stages is shown in Figure 5B. In more mature tassel florets (Fig. 9E), discrete groups of cells of the floral meristem that are positioned to form the stamens and palea express *ids1*. Expression persists at later stages in the older expanded palea and lemma and also the locule chambers of the anther (Fig. 9F). Little or no expression was seen in the lodicules or the gynoceium of the ear (Fig. 9F; data not shown). As shown in Figure 9G, when an anti-sense *ids1* probe is used on *ids1-mum* spikelets, no *ids1* expression is detected.

Discussion

Regulating the number of florets initiated by the spikelet meristem is a critical step in the determination of spikelet morphology. Mutations within the *ids1* gene affect spikelet meristem determinacy. Instead of producing only an upper and lower floret, the *ids1-mum* spikelet meristem continues to initiate additional florets in a distichous phyllotaxy. Understanding how *ids1* promotes spikelet meristem determinacy requires an examination of different models for maize floret initiation.

One model for floret initiation suggests that the lower floret is initiated laterally whereas the upper floret is terminal, produced by the transformation of the spikelet meristem into the upper floret (Fig. 10A) (Irish 1997). In some grass species such as *anthoxanthum*, the spikelet terminates in a flower with the ovule being the terminal structure (Clifford 1987). If the upper floret is a terminal structure in normal maize development, the IDS1 protein may function to promote the transformation of the spikelet meristem into the upper floret. IDS1 may do this alone, or through the activation of floral meristem identity genes such as orthologs of *LEAFY* and *APETELA1* that can transform inflorescence meristems into floral meristems (Mandel and Yanofsky 1995; Weigel and Nilsson 1995). If this were the case, we would speculate that the transformation of spikelet meristem to floral meristem is delayed in *ids1-mum* mutants, allowing the spikelet meristem to persist and initiate more florets. Such an interpretation has been invoked to explain the phenotype of the dominant *Ts6* mutants of maize in which extra florets form (Irish 1997). *Ts6* mutants differ from *ids1-mum* mutants, however, in that they also affect sex determination and cause an absence of carpel suppression within the tassel, but not in the ear.

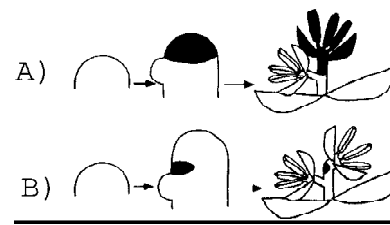


Figure 10. Models for maize floret initiation. (A) Terminal upper floret model. The spikelet meristem branches once laterally to form the lower floret. The residual spikelet meristem (shown in black) is then transformed into the upper floret (black). (B) Lateral branching model. The spikelet meristem undergoes lateral branching to form the lower and then upper florets. The residual spikelet meristem (shown in black) is found in a small region in between the florets. A rudiment of the spikelet meristem is found in the rachilla in the mature spikelet (black).

An alternative model would suggest that both upper and lower florets are lateral products of the spikelet meristem. The two florets of maize have been interpreted as lateral branches of the rachilla with little or no remnant of the axis left in between the florets (Bonnett 1953). If upper floret branching is lateral, then a greatly reduced rudiment of the spikelet meristem might be found in the rachilla after upper floret initiation (Fig. 10B). According to this model, IDS1 would participate in suppression of indeterminate growth within the spikelet meristem, thus preventing the spikelet meristem from regenerating after upper floret branching. In *ids1-mum* mutants the residual spikelet meristem proliferates and continues to initiate florets.

On the basis of the expression pattern of *ids1* and the timing of the *ids1-mum* defects, we favor the second model and hypothesize that IDS1 functions to suppress indeterminate growth within the spikelet meristem. According to the first model, the switch in spikelet meristem fate occurs only after the lower floret is initiated. However, defects in *ids1-mum* spikelet meristems were first observed in the spikelet meristem prior to lower floret initiation (Fig. 5E). The timing of the defect suggests that the change in fate determination occurs earlier, in agreement with the *ids1* expression observed in the spikelet meristem before floret branching occurs (Fig. 9C). The first model also suggests that the entire spikelet meristem becomes transformed into an upper floret, leaving no residual spikelet meristem. Yet, *ids1* expression was detected in a zone between the upper and lower florets in wild type (Fig. 9D) in which the residual spikelet meristem would be expected to be located. Additional evidence that this region represents the residual spikelet meristem comes from the observation that it regenerates after floret branching in *ids1-mum* mutants (Fig. 5F,G). Furthermore, an expectation of the first model is that *ids1* would be expressed throughout the future upper floret to promote its transformation to floral identity. Instead, as shown in Figure 9D, we see no *ids1* expression in either the upper or lower floral meristems. Finally, if determinacy of the spikelet meristem

were simply being delayed in *ids1-mum* mutants in accordance with the first model, one would expect to see the rachilla eventually terminate in a floret. This was not observed in either male and female *ids1-mum* spikelets (Fig. 4F; data not shown).

The development of a critical meristem size in maize is thought to provide the cue for branching to occur (Sundberg and Orr 1996). One mechanism by which IDS1 may suppress indeterminate growth is to restrict the size of the residual spikelet meristem such that it can no longer initiate florets. In *ids1-mum* mutants, the size of the residual spikelet meristem may be deregulated and expand to larger than normal. A larger spikelet meristem could potentially allow greater numbers of branching events to produce extra florets. In support of this idea, the *ids1-mum* spikelet meristem appears more elongated than normal before florets are initiated (Fig. 5E). Also, a larger spikelet meristem diameter is observed soon after the first floret is initiated in *ids1-mum* mutants (Fig. 6D).

It is important to recognize, however, that meristem fate is not always dependent on meristem size. For example, the upper floral meristem is much larger than the lower floral meristem at the time of initiation (Fig. 5B), and yet both are equally determined to make florets. Thus, having a larger spikelet meristem would not necessarily endow it with greater indeterminacy. An equally important component of the distinction between determinate and indeterminate spikelet meristem fates is the ability to regenerate after floret initiation. Meristems with indeterminate fates may show a greater ability at self-regeneration regardless of their initial size. The altered morphology seen in the spikelet meristems of the *ids1-mum* mutants may simply be a reflection of this ability.

It is possible that one way IDS1 functions to maintain spikelet meristem determinacy is to repress those factors necessary for maintaining indeterminacy. The *kn1* homeobox gene is expressed in several types of indeterminate meristematic tissues and not in determinate organs such as leaves (Smith et al. 1992; Jackson et al. 1994). Loss-of-function mutants of *kn1* in maize show reduced tassel branching and fewer spikelets. This phenotype has been postulated to result from a loss of indeterminate inflorescence cells which, in the absence of *kn1*, have adopted determinate fates (Kerstetter et al. 1997). If *kn1* is in fact maintaining meristem indeterminacy, then *kn1* expression should persist within the *ids1-mum* spikelet meristem and show an expanded domain. Although this was observed (Fig. 6D), it is important to note that *kn1* is normally expressed in both spikelet and floral meristems (Fig. 6A,B). Thus, the fact that the domain of *kn1* expression is expanded in *ids1-mum* mutants may simply reflect extra areas of floret initiation.

The *ids1* gene was cloned by homology to the *AP2* gene of *Arabidopsis* and shown to contain two repeats of the highly conserved AP2 domain. Although the IDS1 protein shows greater similarity to *AP2* compared with all other *AP2*-like genes cloned thus far, the *ids1* gene may not represent the actual *AP2* ortholog of maize. The

amino acid homology between IDS1 and *AP2* does not extend outside of the *AP2* domain. Furthermore, the expression of *ids1* was not detected in carpels, as is found for *AP2* (Jofuku et al. 1994). Finally, preliminary experiments show that the overexpression of the *ids1* cDNA in *Arabidopsis* does not complement the *ap2-1* mutant phenotype (G. Chuck unpubl.). Additional *AP2* gene family members are likely to be present in the maize genome, because low stringency hybridizations with the *ids1* *AP2* domain as a probe on maize Southern blots show several hybridizing bands (data not shown). In addition, several expressed sequence tags from maize cDNA libraries have been shown to have *AP2* domains (Klucher et al. 1996).

Even though *ids1* is expressed in vegetative and floral lateral organ primordia, no obvious mutant phenotypes were detected in those organs. A certain level of genetic redundancy may operate in these organs, which can only be uncovered through the analysis of double mutants. For example, double mutants of *ap2* and *aintegumenta* show floral organ phenotypes not seen in either single mutant in *Arabidopsis* (Elliot et al. 1996). Considering the large number of *AP2*-like genes present in the genome of *Arabidopsis* (Okamuro et al. 1997), it would not be surprising to find such functional redundancy occurring in maize. Recently, it has been suggested that two closely related MADS box genes, *zag1* and *zmm2*, play redundant roles in specifying carpel and stamen identity in maize (Mena et al. 1996).

Although floral organs of *ids1-mum* tassel florets are fully functional and resemble those of wild type, floral organ defects were observed in *ids1-mum* female spikelets. A possible explanation for this defect may lie in the fact that the lower floret aborts in normal female spikelets. If the distinction between upper and lower florets is lost in *ids1-mum* mutants, then all the florets may receive the abortion signal that is normally only present in the lower floret in wild type. Double-mutant analysis with the *tasseled 2* mutant, in which lower floret abortion does not occur (DeLong et al. 1993) will be useful to test this model.

In light of the fact that many members of the grass family possess indeterminate spikelets, it is tempting to speculate that *AP2*-like genes play a role in regulating floret number in other grasses. Spikelets with many florets are assumed to be an ancient trait given that derived species have almost universally evolved a reduction in floret number (Stebbins 1987). A possible mechanism for such a reduction may have involved a change in the function or domain of expression of the *ids1* gene to include the spikelet meristem in addition to the various lateral organs where it is normally expressed. The analysis of *ids1* expression in both modern and ancient grasses will be interesting to analyze in this regard.

Materials and methods

Cloning the *ids1* gene

Forty thousand plaques of a B73 cDNA library constructed from 1- to 3-cm inflorescence ears (Jackson et al. 1994) were probed at

reduced stringency at 55°C in 50% formamide (Schmidt et al. 1993) with the full-length AP2 cDNA from *Arabidopsis* (Jofuku et al. 1994). Approximately 75 positive clones were isolated. Only the strongest hybridizing class was analyzed further. A representative clone from this class was used to probe a B73 vegetative meristem cDNA library (Lambda ZapII, Stratagene) from which a full-length 1.9-kb cDNA was isolated. The cDNA was subcloned into pSK- and both strands sequenced with an ABI sequencer. Sequence analysis was done by use of GCG (Wisconsin Genetics Group) and the National Center for Biotechnology Information Blast E-MAIL server.

RNA gel blots and in situ hybridization

RNA was isolated from embryos, ears, vegetative meristems, and roots as described previously (Kerstetter et al. 1994). In situ hybridization was performed as described by Jackson et al. (1994) by use of a 3' end probe between nucleotides 1330 and 1835 outside the conserved AP2 domain. This same DNA fragment was used to probe all RNA gel blots. A single band is observed to hybridize to this probe on DNA gel blots (data not shown), therefore, cross hybridization with other AP2 family members is unlikely.

Isolation of recessive *ids1* alleles by PCR

Approximately 42,000 F₁ plants from crosses between stocks containing active *Mu* transposable elements were grown and screened by PCR by Pioneer Hi-Bred International for insertion into *ids1* (Bensen et al. 1995) with the ZAP2-1 primer (5'-CCGGTGGCGCCAGCGAAGAA-3') and Mu-9242 (5'-CCCTGAGCTCTTCGTC(CT)ATAATGGCAATTATCTC-3'), a degenerate primer that binds to the terminal inverted repeat of *Mu*. PCR reactions were run on gels, blotted, and probed with the *ids1* cDNA. PCR products that hybridize to the *ids1* cDNA identified individuals that likely have *Mu* insertions into the *ids1* gene. Such a screen identified nine candidates by use of the ZAP2-1 primer. DNA gel blot analysis of digested DNA made from self-pollinated progeny of each candidate revealed polymorphisms for only two of the nine families when probed with the *ids1* cDNA. These same two families were the only ones that generated *ids1* PCR products by use of the ZAP2-1 and Mu 9242 primers. It is likely that the seven other families that showed no polymorphisms represent somatic insertion events of *Mu* into *ids1* that did not transmit into the germ line. Each *ids1*-*mum* allele was generated from a cross between a *Mu* active line and the A632 inbred line. Both alleles were backcrossed to A632 once and self-pollinated to observe phenotypes in the homozygotes. The phenotypes were also observed after a second backcross to A632 and a backcross to inbred W23.

Scanning electron microscopy

Tissue was fixed in FAA (50% ethanol, 5% acetic acid, 3.7% formaldehyde) at 4°C overnight and dehydrated in a graded ethanol series to 100%. The samples were then critical point dried and sputter coated with palladium. Samples were viewed on an ISI 30 SEM at 10 kV accelerating voltage.

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