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The Dominant Developmental Mutants of Tomato, *Mouse-ear* and *Curl*, Are Associated with Distinct Modes of Abnormal Transcriptional Regulation of a *Knotted* Gene

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The *Curl* (*Cu*) and *Mouse-ear* (*Me*) mutations of tomato cause two seemingly unrelated developmental syndromes with a wide range of pleiotropic phenotypes. Yet, the distinct morphogenic alterations in shoots, leaves, and inflorescences conferred by the two mutations appear to be caused by unchecked meristematic activity that characterizes dominant mutations in *Knotted1* (*Kn1*)-like genes of monocot plants. We have been unable to separate the two closely linked *Cu* and *Me* mutations, and they may lie in the same gene. A homeobox-containing class I *Kn1*-like gene, *TKn2*, also maps to the same location. Significantly, the dominant mutations are associated with two aberrant modes of *TKn2* transcription. Overexpression of the two in-frame wild-type transcripts of *TKn2* is associated with the *Cu* mutation, whereas misexpression of an abundant and oversized fusion mRNA is associated with the *Me* mutation. Available molecular evidence strongly suggests that the defective *Me–TKn2* transcript is generated via a novel splicing event that merges transcripts of two closely linked genes. The translated fusion product is comprised of most of the 5′ end of the adjacent PPi-dependent fructose 6-phosphate phosphotransferase (*PFP*) transcript spliced in-frame to coding position 64 of the *TKn2* transcript, leaving the *TKn2* homeobox intact. We suggest that class I *Kn1*-like genes were selected early during evolution to regulate basic programs of aerial meristems and that subtle alterations in their function may be the basis for the wide diversity in growth parameters of shoot systems, leaves, and inflorescences among plant species.

INTRODUCTION

The final morphology of all plant organs is shaped by early developmental events in their meristems (Sussex, 1988). In generating the immense variation and developmental flexibility observed in shoots, it appears that all plant species must use homologous regulatory systems in their meristems. It follows, therefore, that to dissect plant morphogenesis, it is essential to study the mechanisms by which a subtle variation in the operation of a given meristematic gene results in gross morphological alterations. Such an opportunity is provided by members of the meristem-specific homeobox-containing *Knotted1* (*Kn1*) gene family.

The major reason for considering class I *Kn1*-like genes (hereafter *Kn1* genes) as legitimate meristematic regulators is that they constitute the only group of genes that incite meristematic activity when ectopically expressed outside of the apical meristems (Hake et al., 1995). Another reason is that they are expressed in the relevant meristems throughout development, as opposed to floral homeotic genes of the MADS box family, for example, which operate only in floral meristems and only in the context of flower differentiation.

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The *kn1* gene was cloned by transposon tagging and was the first plant gene shown to contain a conserved homeobox (Hake et al., 1989; Vollbrecht et al., 1991). Kerstetter et al. (1994) classified the 13 different members of the *Kn1* family of maize into two classes based on sequence homology. In addition, *Kn1* genes of class I are expressed mainly in vegetative shoot apical meristems, inflorescence meristems, and stems, but not in root meristems or differentiated leaves and flowers, whereas genes of the second class may be differentially expressed in all organs.

Dominant mutations in class I *Kn1* genes have been identified in the monocots maize and barley. They were caused either by gene duplication or by insertion elements that alter gene expression (Veit et al., 1990; Greene et al., 1994; Muller et al., 1995). Misexpression of the *kn1* gene in the lateral vasculature of the maize leaf results in typical outgrowths designated as "knots." Ligulelike tissues appear in distal sites, the proportions of the blade are altered, and there is an overall retardation of growth (Freeling and Hake, 1985; Smith et al., 1992; Jackson et al., 1994; Sinha and Hake, 1994). Dominant mutations in the *rough sheath1* and *liguleless3* genes of maize, which affect the more proximal domain of the leaf, were identified with overexpression and misexpression of class I *Kn1* genes (Schneeberger et al.,

1995; Sylvester et al., 1996). Likewise, Muller et al. (1995) discovered that the appearance of extra flowers on the lemmas of the barley *Hooded* mutant florets is also associated with an overexpression of a *kn1* homolog.

Overexpression of the *kn1* gene or its Arabidopsis ortholog in dicot species, such as tobacco, Arabidopsis, or tomato, results in leaf malformations, epiphyllic shoots, loss of apical dominance, and extreme growth retardation (Sinha et al., 1993; Chuck et al., 1996; Hareven et al., 1996). We have shown that although simple leaves of Arabidopsis, tobacco, or tomato become lobed and rumpled when *kn1* is overexpressed, their architecture remains simple. Compound leaves of tomato, in contrast, respond to *kn1* overexpression by forming ramified supercompound structures (Figures 1B and 1C; Hareven et al., 1996).

The observation that diverse pleiotropic effects—such as growth retardation and loss of apical dominance on the one hand and leaf ramification or ectopic apices on the other—are caused by unregulated expression of *Kn1*-like genes suggests that finely tuned spatial and temporal expression of these meristem-specific genes may be a critical mechanism by which plants regulate their growth habit and organ morphogenesis.

Tomato is particularly useful for this type of study because its major meristematic programs are modified in comparison to the major model dicot system, Arabidopsis, and therefore permit a different range of mutant phenotypes. The tomato shoot is sympodial; its vegetative and reproductive cycles alternate regularly; the inflorescence meristems are determinate; and leaf architecture is compound. We took advantage of the immense developmental diversity in tomato to investigate the role of *Kn1*-like genes in shaping organ and plant architecture in dicot species.

Two vastly different plant architectures, leaf morphologies, and inflorescence organizations are conditioned by the dominant *Curl* (*Cu*) and *Mouse-ear* (*Me*) mutations. However, the two syndromes share the basic features of extreme growth retardation, formation of supercompound leaves, ectopic meristem activity, and loss of apical dominance that are common to *Kn1* overexpressing plants. We show here that the two phenotypic features of *Cu* and *Me* are correlated with overexpression and misexpression of the newly isolated tomato *Kn2* (*TKn2*) gene. The differences in phenotype most likely reflect the type of gene product, an upregulated normal transcript in the case of *Cu*, and misexpression of an oversized fusion messenger that involves a neighboring gene transcript in the case of *Me*.

RESULTS

The *Cu* and *Me* Syndromes

The *Me* phenotype was detected in a single plant of the Rutgers cultivar that was heterozygous for the mutation (Harrison, 1955). The dominant *Cu* mutation originated in a branch of a chimeric plant of the Stocksdale cultivar (Young, 1955). The two loci were independently mapped to positions 48 and 49 of chromosome 2, respectively, on the genetic map (Stevens and Rick, 1986). The two dominant mutations, named after their peculiar leaf phenotypes, condition two vastly different morphogenic syndromes, yet most of their pleiotropic effects can be attributed to exaggerated or ectopic meristematic activity that is characteristic of dominant mutations in *Kn1*-like genes. In this study, we first describe the morphological features of *Me* and *Cu* mutant phenotypes that are relevant to this working hypothesis and thus are important later for the interpretation of the molecular and developmental observations.

The Cu *Syndrome*

The phenotypes associated with the *Cu* mutation are presented in Table 1. *Cu* is characterized by compact foliage structures that are nevertheless formed in the correct phyllotactic pattern. They consist of compound ramified leaves with wrinkled, curled blades and an extremely corrugated leaf surface, presumably as a result of intercalary disproportionate growth (Figure 1D) and diminutive, unexpanded axillary branches. In addition, epiphyllic shoots of all types emerge from the adaxial surface of the supercompound leaves (Figures 1F and 1G). The growth of *Cu/Cu* plants is greatly retarded: internode length is approximately one-quarter that of the wild-type length, and leaves and immature fruits develop extreme dark green pigmentation.

The first inflorescence in *Cu* plants appears relatively late, after ~12 to 18 internodes. The sympodial nature of the shoot is maintained, but inflorescences appear at a variable spacing of three to eight nodes rather than at the regular three nodes (Figure 1A). Whereas flowers set fertile fruits, the relative size of their organs is altered. The most dramatic phenotype, indicative of a possible involvement of a *Kn1*-like gene, is the appearance of ectopic shoots, up to 1 cm long, on distal adaxial sites of the short sepals (Figure 1E).

As summarized in Table 1, the phenotype of *Cu/+* plants is similar to but milder than that of *Cu/Cu*. The lamina is slightly more expanded in *Cu/+* plants than in *Cu/Cu* plants, but the leaves remain supercompound, curled, sessile, and dark green. Sepals of *Cu/+* plants also form ectopic shoots, but less often than do *Cu/Cu* sepals.

The Me *Syndrome*

The primary shoot apex of the *Me* plant takes one of three different developmental fates. The shoot meristem may terminate after two to six leaves (i.e., internodes) with an unusual inflorescence shoot; it may be consumed after only two to four leaves, whereby subsequent growth continues in the form of lateral or ectopic "inflorescence" shoots (Figures

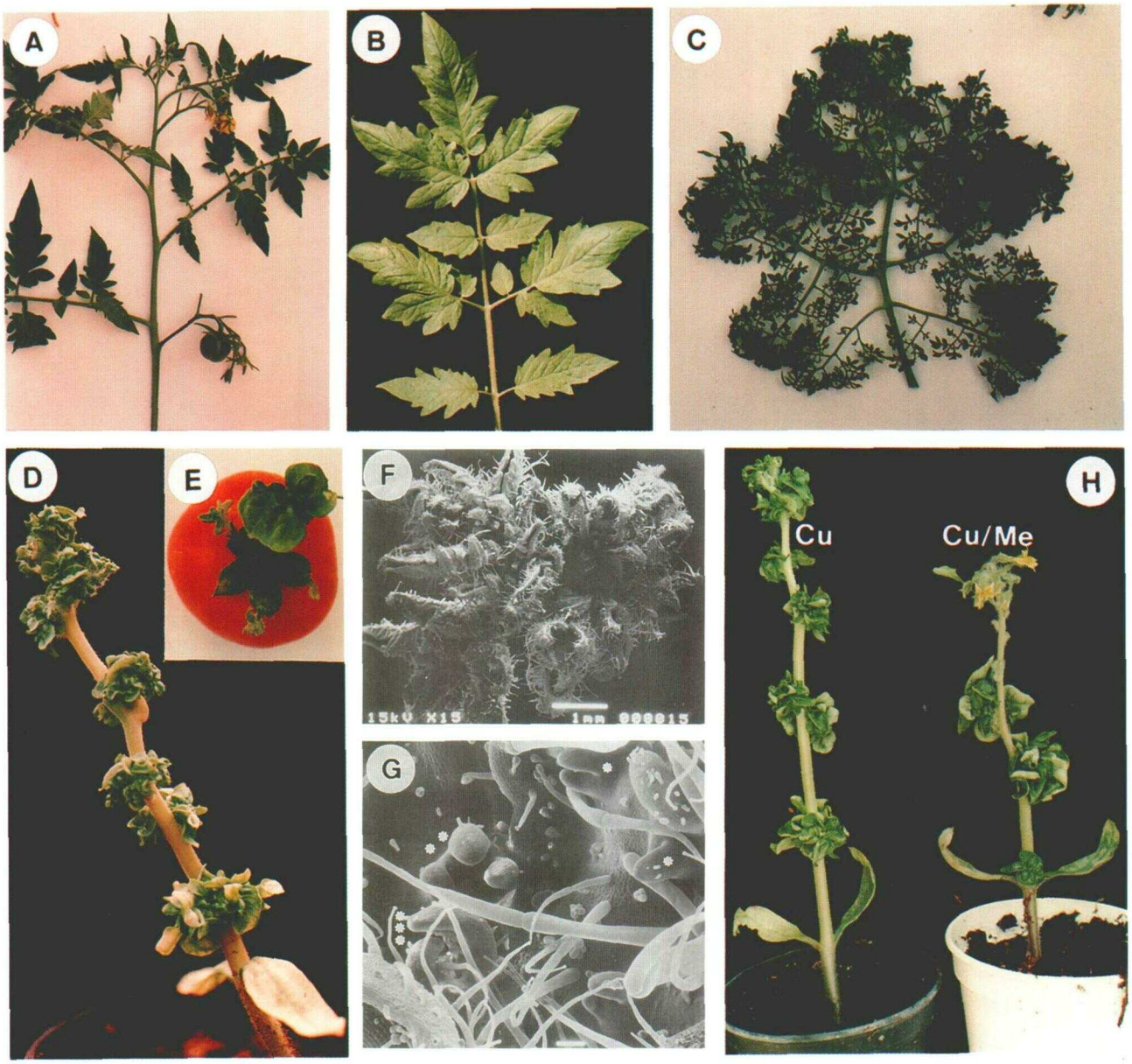

Figure 1. Wild-Type Tomato and the *Cu* Syndrome.

(A) Part of a wild-type, indeterminate tomato shoot. The shoot is sympodial, with each section composed of three nodes and terminal inflorescence. Growth continues from a bud in the axil of the leaf just below the terminal inflorescence.

(B) Typical wild-type compound leaf. One terminal leaflet, three to five pairs of lateral leaflets, and several intercalated folials are shown.

(C) Supercompound leaf of tomato plant expressing the 35S::*kn1* gene of maize (reprinted with permission from Hareven et al. [1996]. Cell **84,** 735–744; Figure 1B, page 736).

(D) *Cu* shoot; homozygous plant. The compact foliage structure contains axillary shoots and ectopic shoots of all kinds (see Figures 5A and 6), but the leaves are arranged in the normal phyllotaxis.

(E) Ectopic shoots on sepals of *Cu* fruit. Note the *Cu* phenotype of the miniature ectopic leaf.

(F) *Cu* leaf. Scanning electron microscopy of a ramified unexpanded leaf from the top of the shoot shown in Figure 1E.

(G) Ectopic primordia on the adaxial (upper) side of the *Cu* leaf. Primordia of unidentified, vegetative, and floral buds are marked with one, two, or three asterisks, respectively.

(H) *Cu/Cu* (left) and *Cu/Me* (right) shoots of 6-week-old plants (see text).

Table 1. Summary of Shoot Phenotypes Associated with the *Cu* and *Me* Mutations

Genotype	Leaf Morphology	Leaf Compoundness[a]	Ectopic Shoots[a]	Apical Dominance[a]	Growth Habit	Inflorescence Architecture	Flower Modifications
Wild type	Petiole, compound with nine leaflets	+	None	+++	Wild type	Wild type	None
Cu/Cu	Petioleless, curled, miniature	+++	+++	+	Extended sympodial sections	Reduced complexity, short	++
Cu/+	Same as *Cu/Cu* but less extreme	++	++	+	Like *Cu/Cu*	*Cu/Cu*–like, less extreme	+
Me/Me	Petiolated expanded lamina, irregular architecture or filamentous	++	None	Abolished	Modified	Fasciated, ramified	++
Me/+	Supercompound, irregular architecture	+++	None	+++	Wild type	Wild type	None
Me/Cu	*Cu*-like	++	++	+	*Me/Me*–like	*Me/Me*–like	*Cu/Cu*–like

[a] +++, ++, and +: high, moderate, and low penetration, respectively, of the indicated phenotypes.

2A and 2B); or, rarely, it may terminate after one to three leaves, with no additional growth ensuing (data not shown). The first two to six leaves are supercompound; however, their leaflets, which display distorted proportions, emerge in irregular pattern along the more distant part of the slightly rolled, convoluted midrib (Figure 2B).

The apex of the *Me* inflorescence shoot in its early "vegetative" stage (Figure 2E) is different from that of the wild type shown in Figure 2D. In addition to the much expanded apex, there is inward curving of leaf primordia, which also characterizes tomato plants expressing the maize *kn1* gene (Hareven et al., 1996). Apices of the inflorescence shoots are usually terminated by a complex and fasciated truss (Figure 2C). Leaves that emerge on the flanks of such shoots become progressively filamentous, and almost all of their axillary buds develop shoots, suggesting a complete loss of apical dominance (Figures 2B and 2C). Fasciated organs, ramified leaves, and release from apical dominance characterize tomato plants overexpressing the maize *kn1* gene (Hareven et al., 1996). Most flowers of *Me* plants abort and display extremely elongated sepals but reduced petals and stamens, and may also be fasciated. The carpels tend to form parthenocarpic compartments in the otherwise semifertile fruit.

In contrast to *Cu*, all pleiotropic effects displayed by *Me/Me* plants, except for leaf architecture, are recessive and rescued in *Me/+* plants (Table 1). Growth habit, flowers, and inflorescences as well as nodal length of *Me/+* plants are indistinguishable from those of the wild type. *Me/+* leaves, however, are subdivided to a higher order than are those of *Me/Me* plants, and the heart-shaped leaflets still appear in an irregular rather than a pairwise pattern. The leaves, however, are never filamentous (compare homozygous and heterozygous *Me* leaves in Figures 2A and 2B [*Me/Me*] with those in Figure 2F [*Me/+*]).

Interestingly, *Cu/Me* heterozygous plants display abnormal shoots that appear to be a developmental blend of each of the corresponding dominant homozygotes (Table 1). Initially, the primary shoots display the *Cu* phenotype, including growth retardation, node size, and the morphology of the leaves and axillary branches. Growth rate of the *Cu/Me* plants is at least as retarded as that of *Cu/Cu* plants. The *Cu*-like shoots of *Cu/Me* plants terminate, after the production of up to six leaves, with an inflorescence shoot of the *Me*-type (Figure 1H). *Cu/Me* flowers, however, are similar to the *Cu* type in that they have sepals with ectopic shoots and dark green *Cu*-type fruits.

The *Me* and *Cu* genes were reported, by independent experiments, to locate at a distance of 1 centimorgan (cM) from each other (Stevens and Rick, 1986). For a more direct estimate of genetic distance, we scored 2032 F_2 progeny of selfed *Cu/Me* heterozygotes but found no *Me/+* or *Cu/+* recombinant plant, suggesting that *Me* and *Cu* are <0.1 cM apart.

Molecular Organization of the *TKn2* Gene

The homeobox of *TKn1* (Hareven et al., 1996) was used to screen cDNA libraries constructed from mRNA of flowers, floral meristems, and shoot apices under relaxed conditions. Four new *Kn1*-like genes were identified (*TKn2*, *TKn3*, *TKn4*, and *TKn5*; GenBank accession numbers U76407 to U76410, respectively) and mapped using restriction fragment length polymorphism markers. *TKn2* was found to be tightly linked with marker TG454 (Tanksley et al., 1992) on chromosome 2. Previously, we had also mapped the *Me* gene to the same chromosomal location (see Methods for details).

To explore the possibility that *Cu* and *Me* may represent two different dominant mutations in the same gene and, in particular, *TKn2*, we investigated the molecular organization and transcription pattern of the *TKn2* gene. The complete amino acid sequence of the *TKn2* gene product is shown in Figure 3A. The homeodomain of *TKn2* retains all features of class I *Kn1* genes, as defined by Kerstetter et al. (1994). A detailed comparison of the homeodomains of six *Kn1* genes

from various species (Figure 3C) indicates, however, that *TKn2* along with the Arabidopsis *SHOOTMERISTEMLESS* (*STM*) gene and the *soybean homeobox1* (*SBH1*) gene constitute a distinguishable subgroup of the class I *Kn1* genes. Comparison of sequences of members of class I indicates that the two subgroups are distinguished by homology outside of the homeodomain as well (data not shown). In addition, the *TKn2* genomic clone, schematically depicted in Figure 3B, contains only three introns, as does *STM* (Long et al., 1996); the genes that we grouped in the other *kn1* subgroup have four introns.

Two types of wild-type *TKn2* cDNA clones were identified. The type A cDNA is shown in Figure 3A. The type B cDNA clone contains two additional amino acids; the glycine and valine residues are inserted between positions 205 and 206 (arrow in Figure 3A), respectively, of the class A sequence.

To investigate the possible developmental significance of this putative cryptic splicing event, we looked for the presence

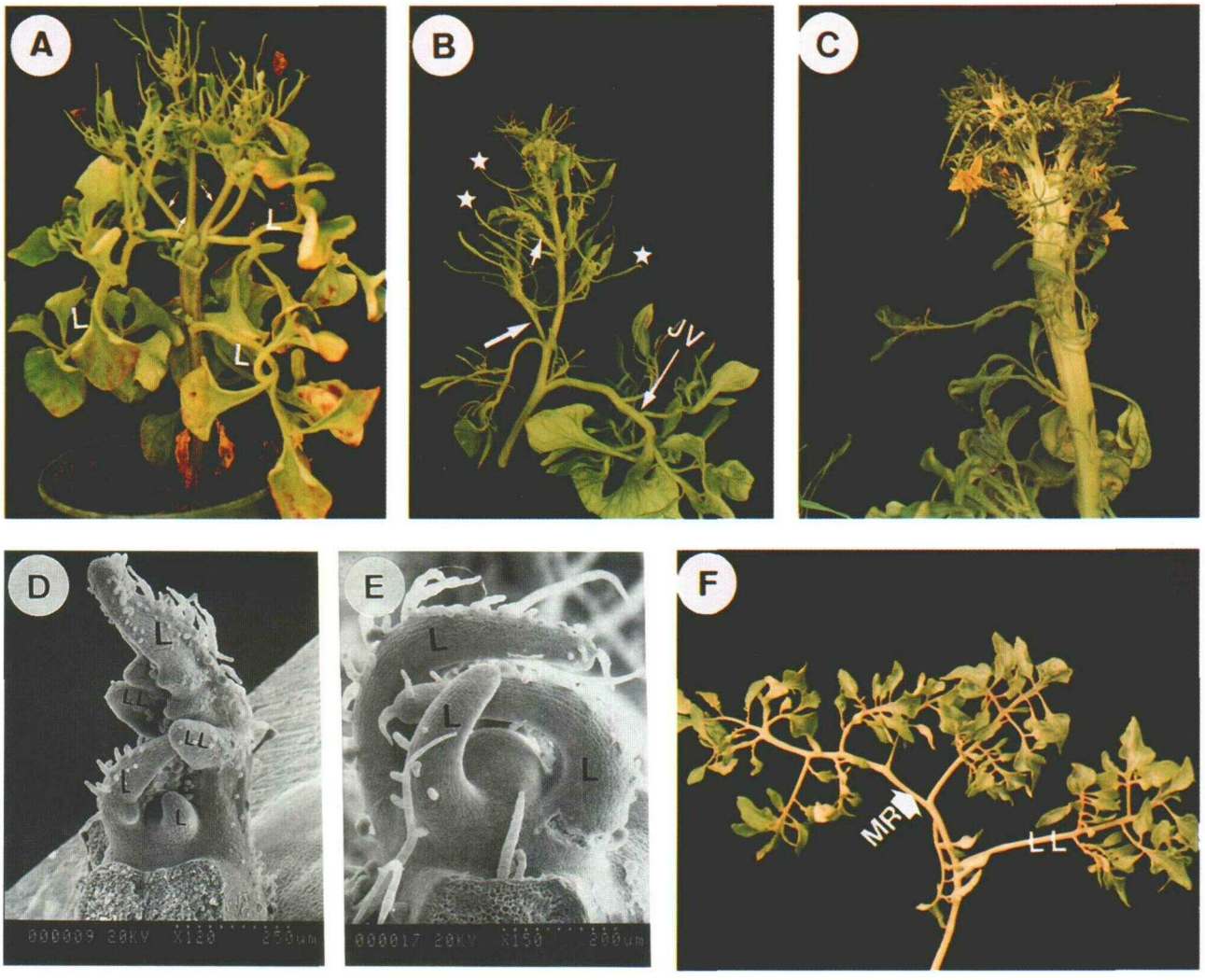

Figure 2. Essential Features of the *Me* Syndrome.

(A) to **(C)** Vegetative and reproductive phases of *Me* plants. In **(A)**, a young *Me* seedling that is 1 month old, with three leaves (L) and three inflorescence shoots (arrows), is shown. The upper shoot is part of the primary axis, and the second one is axillary. In **(B)**, the advanced growth of an *Me* inflorescence shoot with a typical first *Me/Me* juvenile leaf (JV) displaying complete loss of apical dominance (arrows) and distal filamentous leaves (stars) is illustrated. In **(C)**, the terminal portion of a fasciated inflorescence shoot of an *Me/Me* plant is shown.

(D) and **(E)** Scanning electron microscopy of a wild-type shoot apex **(D)** and early vegetative *Me/Me* inflorescence apex **(E)**. Note the inward orientation of the *Me* leaf primordia and the absence of lateral leaflets (LL). The youngest three leaves (L) are shown in each apex.

(F) A heterozygous *Me/+* ramified leaf with irregular branching pattern and altered shape of the single blades. MR, midrib.

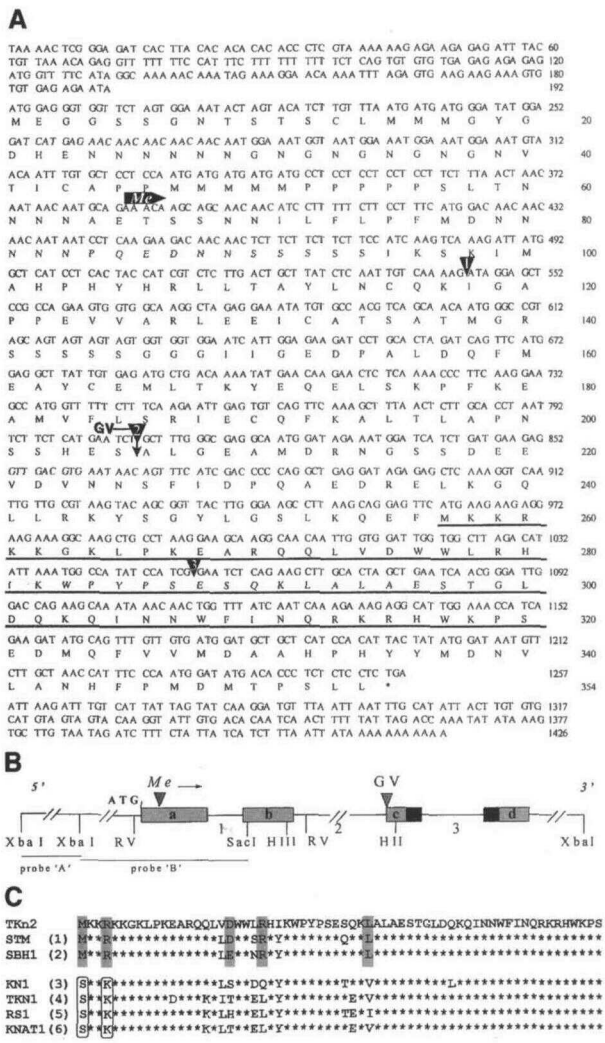

Figure 3. Molecular Characterization of the *TKn2* Gene Product.

(A) Nucleotide and deduced amino acid sequence of the *TKn2* cDNA clone. The homeodomain is underlined; the sites of the three introns are identified by arrowheads. The fusion site of the *PFP–TKn2* transcripts in *Me* and the position of cryptic splicing are also indicated.

(B) Organization of the *TKn2/1* genomic clone. Major indicative restriction sites and the four exons (a, b, c, and d) are displayed. Introns 1, 2, and 3 are 242, 2734, and 417 bp, respectively. Black regions of exons c and d identify the homeobox. Arrowheads mark the position of the *PFP–TKn2* splice site (*Me*) and the insertion point of glycine and valine in the type B cDNA clone (GV). Probes 'A' and 'B' were used in DNA gel blot analysis of *Cu* genomic DNA.

(C) Subdivision of class I *Kn1* genes. Amino acid comparison of homeodomains of seven class I *Kn1* gene products is shown. Conserved positions for the *STM/TKn2* subclass are shaded. Sequence (1) is from Long et al. (1996); sequence (2), Ma et al. (1994); sequence 3, Vollbrecht et al. (1991); sequence 4, Hareven et al. (1996); sequence 5, Schneeberger et al. (1995); and sequence 6, Lincoln et al. (1994).

of types A and B transcripts in mRNA populations from several plant organs: shoot apices, leaves, and flowers of wild-type plants and floral meristems of the *anantha* plant. As shown in Figures 4A and 4B, the two types of mRNA of *TKn2* were detected in transcripts of all wild-type organs studied.

Cu and *Me* Are Characterized by Two Different Transcription Defects in *TKn2*

RNA gel blot analysis of *TKn2* transcripts in wild-type plants revealed a low level of transcripts in shoot apices, upper

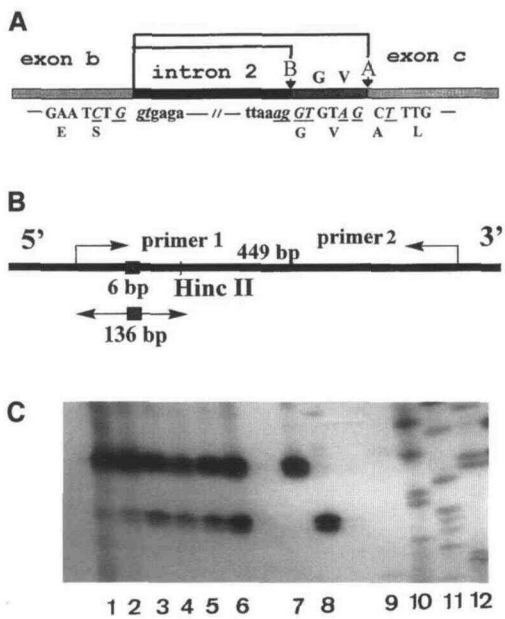

Figure 4. Cryptic 5′ Splicing Site of Intron 2 of the *TKn2* Gene: Occurrence of Types A and B Transcripts in Wild-Type Organs and Mutants.

(A) Sequence organization of the 3′ cryptic splice site of intron 2. Alternative splice sites are indicated by arrows. 5′ and 3′ consensus base pairs are underlined and indicated in italics. Lowercase letters indicate the base pairs at the ends of intron 2. The addition of 6 bp of GTGTAG results in the insertion of G and V amino acids between the S and A residues.

(B) Scheme of the experiment. Primers 1 and 2 flanking the site of interest were used to generate ^{32}P-labeled reverse transcriptase–polymerase chain reaction products from RNA of different organs or tomato lines. cDNA products subsequently were digested with HincII, leaving 130- or 136-bp fragments that were fractionated on a sequencing gel along with appropriate size markers.

(C) Lane 1 contains fractionated wild-type cDNA fragments from young leaves; lane 2, growing leaves; lane 3, flowers; lane 4, shoot apices; lane 5, *Me/Me* flowers; lane 6, *Cu* shoot apices; lanes 7 and 8, size markers prepared from type A and B cDNA clones, respectively; and lanes 9 to 12, sequencing reaction products solely to show the 6-bp difference.

parts of stems, early floral buds, and carpels (Figure 5A). Much higher levels of steady state mRNA were found in the floral meristems of the *anantha* mutant inflorescences (Figure 5A, lane 9). *anantha* inflorescences (Helm, 1951) serve as an excellent internal standard for the calibration of RNA levels because they provide a relatively homogeneous material that is rich in meristematic tissue and characterized by high expression levels of meristematic markers (Pri-Hadash et al., 1992), including *Kn1* genes (Hareven et al., 1996). The wild-type expression pattern was dramatically altered qualitatively and quantitatively in *Me* and *Cu* plants. In *Cu* plants, there was at least fivefold overexpression of the 1.6-kb *TKn2* transcript in leaves, shoot apices, and young floral buds but not in mature petals, anthers, or carpels, suggesting differential upregulation of *TKn2* in *Cu* plants (Figure 5B). We failed to obtain undegraded RNA from *Cu* sepals. Dot spot analysis indicates, however, that in comparison with the wild type, *TKn2* is upregulated two- to threefold in *Cu* sepals.

In *Me*, the gene was upregulated in all tested organs, including young leaves and flowers. Most significant, however, is the fact that the majority of the *TKn2* transcripts in *Me* plants are ~2.7 kb, as compared with ~1.6 kb in the wild type (Figure 6A). Separate experiments verified that the large transcript of *Me* is polyadenylated and is not recognized by any other *Kn1* gene probes (data not shown). Thus, the *Me* and *Cu* syndromes are apparently identified by transcriptional defects at the very same gene, *TKn2*.

In situ localization of *TKn2* mRNA in wild-type plant organs revealed a pattern very similar to that of *TKn1* (Hareven et al., 1996). The gene was expressed in vegetative apices (Figure 7A), in provascular tissues and growing points of leaf primordia (Figures 7B and 7C), and in floral meristems and floral primordia (Figures 7E and 7F). It was also expressed along the vascular strands of all developing organs.

The unequal growth of the lamina attracted our attention to the localization of *TKn2* transcripts in *Cu* leaves. As shown in Figure 7D, almost all of the signals were localized to the spongy mesophyll cells. No overexpression was detected in the adaxial palisade (columnar) tissue of the leaf or along the vascular strands, where the wild-type gene is normally expressed (compare with Figures 7B and 7C).

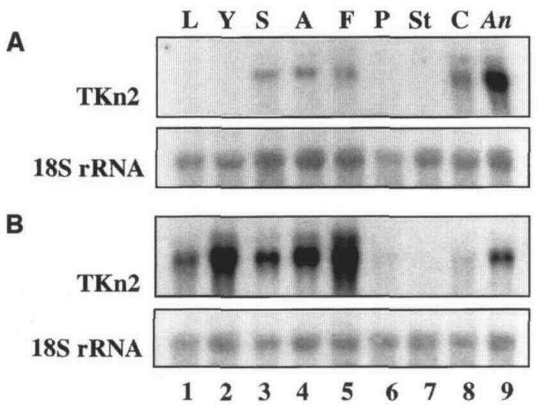

Figure 5. *TKn2* Is Overexpressed in Green Aerial Organs of *Cu* Plants.

(A) Expression level of *TKn2* in wild-type organs. Lane 1 contains growing leaves (L); lane 2, young leaves (Y); lane 3, stems (S); lane 4, apices (A); lane 5, floral buds (F); lane 6, petals (P); lane 7, stamens (St); lane 8, carpels (C); and lane 9, *anantha* floral meristems (*An*). Growing leaves are 2 to 3 cm long, and young leaves are 3 to 6 mm long. Stems represent the proximal 1-cm portion of the epicotyls from seedlings with four or five true leaves. Apices were taken from the same seedlings, but they include the apical meristems with their primordia. Floral buds are 2 to 5 mm long, whereas floral organs were dissected from 1-mm-long flowers. Ten micrograms of total RNA was loaded for each lane. In this and subsequent blots, *TKn2* wild-type transcripts in *anantha* inflorescences provide an internal reference. A 500-bp *TKn2*-specific fragment was used as a probe. The 18S rRNA served as a control to show that approximately equal amounts of total RNA were loaded in each lane.

(B) Expression of *TKn2* mRNA in *Cu* mutant plants. The order of the samples is as given in **(A)**. The blot was rehybridized with the 18S rRNA probe.

Analysis of Transcripts and Coding Regions of *TKn2* in *Cu* and *Me* Plants

Sequence analysis of amplified *Cu–TKn2* transcripts verified that they are identical in every respect to the wild-type gene. The size of the amplified but not yet sequenced segments of all three introns was identical with that of the wild type (see the legend to Figure 3B). Genomic DNA gel blots, containing digests with eight different restriction enzymes, were probed separately with the *TKn2* cDNA clone and genomic flanking sequences A and B, as shown in Figure 3B, and did not reveal any genomic alterations. Moreover, the nucleotide sequence of 1450 bp 5′ to the translation initiation site of the *Cu–TKn2* gene was also normal.

To characterize the long *TKn2* transcripts found in *Me/Me* plants, we prepared a cDNA library from floral RNA by using the 5′ end of the wild-type *TKn2* cDNA clone as a probe. The longest of these clones, *Me–TKn2/7* (2644 bp), contains a 5′ sequence of 1615 bp that is not recognized by the *TKn2* probe. Shorter versions of this sequence were found in most other isolated clones. In the complete *Me–TKn2/7* clone, an untranslated region followed by a reading frame potentially coding for 358 amino acids was spliced to a truncated version of the *TKn2* gene missing the first 64 N-terminal residues. The 5′ 1.6 kb of foreign sequence in *Me–TKn2/7* is homologous to the β subunit of pyrophosphate fructose 6-phosphate 1-phosphotransferase (*PFP*; EC 2.7.1.90) of potato (Carlisle et al., 1990), which catalyzes, in plants, the reversible transfer of a phosphate group from PPi to fructose 6-phosphate to yield fructose 1,6-bisphosphate. On the assumption that the tomato and potato genes are similar, the *Me–TKn2/7* clone consists of all of the translated N-terminal

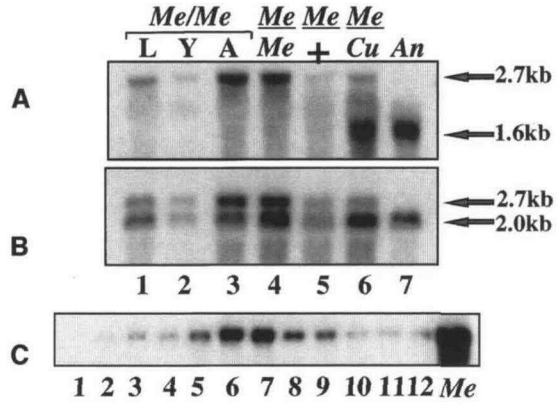

Figure 6. Altered Size and Distribution of *TKn2* Transcripts in *Me* Plants.

(A) Expression and size distribution of *TKn2* transcripts in *Me* plants. Lane 1 contains *Me/Me* leaves (L); lane 2, *Me/Me* young leaves (Y); lane 3, *Me/Me* apices (A); lane 4, *Me/Me* flowers; lane 5, *Me/+* flowers; lane 6, *Me/Cu* flowers; and lane 7, *anantha* (*An*) floral meristems. The arrows indicate the positions of the fusion and wild-type *TKn2* transcripts.

(B) Expression and size distribution of *PFP* transcripts in *Me* plants. The order of the samples is as given in **(A)**. The 1.6-kb *PFP* part of the *Me–TKn2/7* clone was used as a probe. The arrows indicate the positions of the fusion and wild-type *PFP* transcripts.

(C) The *PFP–TKn2* transcript from *Me* flowers is associated with polysomal fractions.

Polysomal fractions were prepared from *Me* flowers according to the method of Barkan (1993). The gradients were fractionated into 12 fractions, fraction 1 being collected from the top and fraction 12 from the bottom of the sucrose gradient. The fractions were further separated on regular RNA denaturing gels, and membranes were probed with ^{32}P-labeled *TKn2* DNA. Simultaneously, control gradients containing EDTA were fractionated, and the signal was concentrated in the two lighter sucrose fractions.

region of the *PFP* gene but is missing the 45 C-terminal residues of the wild-type PFP protein.

The splice site of the *TKn2* gene resides within an exon, but as illustrated in Figure 8, the flanking sequence agrees well with the plant consensus sequence for 3′ splice sites. The 5′ (*PFP*) splice site is a part of legitimate exon–intron border, most probably intron 13 if the organization of the tomato gene is identical to that of the castor bean gene (Todd et al., 1995). We have also verified that base sequences of the splice site and the flanking exon–intron regions are identical in *Me* and wild-type plants. The splice site of the *PFP–TKn2* transcript is not related to that of the cryptic site in wild-type transcripts, and type A and B transcripts are found in *Me* and *Cu* plants as well (Figure 4C, lanes 5 and 6).

Although the majority of the *TKn2* transcripts detected in *Me* plants were large, we suspected that transcripts of the wild-type size were also present. To verify the existence of these presumptive transcripts, we searched among the isolated *Me–TKn2* cDNA clones for those that were not recognized by the *PFP* probe. One of 12 *Me–TKn2* cDNA clones consisted of a complete, normal *TKn2* wild-type sequence. Moreover, reverse transcriptase–polymerase chain reaction with appropriate primers successfully amplified both fusion and wild-type cDNA forms from *Me* flowers, but only wild-type cDNA was amplified from wild-type tissue. Evidently, the formation of aberrant fusion transcripts is not the only option available for *TKn2* in the *Me* genomic domain. As shown in Figure 6B, transcripts of two sizes were found for the *PFP* gene as well—2 kb of the wild-type size and 2.7 kb of the fusion *PFP–TKn2* size. Significantly, unlike *TKn2* of *Me* plants, only up to 40% of the *PFP* transcripts are of the large size.

Because the majority of *TKn2* mRNA is fused to that of *PFP*, its distribution in leaves and flowers of *Me* plants, as shown in Figures 7G and 7H, respectively, reflects that of the fusion transcript. The expression pattern of *PFP*, as detected by a specific antibody for the potato protein (gift of B. Plaxton, Queen's University, Kingston, Ontario, Canada), indicates that PFP is most abundant in regions of the leaves (Figure 7I), floral buds (Figure 7J), and *Me* floral buds (Figure 7K) overlapping the *TKn2* expression domains. Longer exposure as well as the RNA gel blots shown in Figure 6B suggest that *PFP*, as expected of a housekeeping gene, is constitutively expressed in all organs and tissues.

The *Kn1*-like aspects of *Me* imply that the chimeric *PFP–TKn2* mRNA is translated and that the protein product is localized to the nucleus. Analysis of polysomal fractions of *Me* flowers shown in Figure 6C suggests that the 2.7-kb fusion transcripts are indeed associated with polysomes. In pilot experiments, we have also fractionated crude cytosolic and nuclear protein preparations from wild-type, *Me/Me*, and *Me/+* plants and have probed these with the PFP antibody. A novel protein of the predicted size (~90 kD) was detected only in *Me/Me* and *Me/+* but not in the wild-type or *Cu* nuclear protein preparations or in any of the cytosolic samples (data not shown). These preliminary results are consistent with the possibility that the translation product of the *PFP–TKn2* transcripts is localized to the nucleus. The nuclear localization signals that exist within and near the homeodomain of Kn-like proteins (Meisel and Lam, 1996) are retained in the fusion transcript.

Overexpression of *TKn2* in Tomato and Tobacco Plants Induces Specific Morphogenic Alterations

Misexpression of the *TKn2* gene in *Cu* and *Me* mutants (Figures 1D, 2B, 2C, and 2F) and of *kn1* in transgenic tomato plants (Hareven et al., 1996; Figure 1C) results in different phenotypes. The *kn1* gene was overexpressed under the control of the ubiquitous cauliflower mosaic virus 35S promoter, whereas expression of the *TKn2* genes in *Me* and *Cu* plants is obviously driven by other regulatory regimes. For a more reliable comparison of *TKn2* and *kn1*, we have gener-

ated tomato and tobacco transgenic plants expressing *TKn2* under the control of the 35S promoter.

Twenty-six tobacco 35S::*TKn2* T_1 plants were studied. Like *kn1*-expressing plants, growth was generally retarded, internodes were shorter, and leaves were sessile (Sinha et al., 1993). Leaf shape of tobacco 35S::*TKn2* plants was consistently different from that of 35S::*kn1*–expressing plants (Sinha et al., 1993). The first three to six leaves of T_1 plants were only slightly lobed and had curled margins. Leaves of mid-axis internodes, however, were petioleless and deeply incised to the extent of being nearly palmated. Leaves of distal internodes had an elongated lanceolate-like shape and were very reduced in size (Figures 9A to 9D). Surprisingly, leaves of axillary branches, irrespective of their position along the primary stem, were only slightly lobed and mostly lanceolate. The flowers were normal and fertile.

Sixteen 35S::*TKn2* transgenic tomato plants were obtained. All but two could not be transferred to soil and were constantly propagated in culture vessels from cuttings. Three types of leaves were recorded: leaves that are subdivided to the third order with leaflets emerging in irregular patterns reminiscence of *Me*/+ plants (Figure 9G); leaves with reduced lateral leaflets, some being as filamentous as late leaves of *Me/Me* plants; and regular compound leaves with excessive but quite regular comblike proliferation of appendages along the margins of their leaflets (Figures 9E and 9F).

The interaction between the *Me–TKn2*—defective gene and *kn1* of maize was followed in *Me/Me* plants expressing the 35S::*kn1* gene. None of the parental genotypes ever gave rise to epiphyllic shoots on midribs, as was reported for *kn1*-expressing plants in tobacco and Arabidopsis or that was observed in *Cu* mutant leaves. Nevertheless, multiple ectopic shoots of the type shown in Figure 1G appeared on midribs of the much retarded *Me/Me*;35S::*Kn1* plants (data not shown). This observation is important because it means that the truncated *TKn2–Me* gene contributes to the accelerated appearance of a classical *Kn1* effect (see Discussion) in homozygous plants.

Other interactions of *Me* or *Cu* with *Lanceolate*, *trifoliate*, and *entire* are also additive, suggesting that *Me* and *Cu*, as does *kn1*, exert their effect within the context of the other mutations. To illustrate this point further, comparison of the interactions of *Me* and *kn1* with *entire* is shown in Figures 9H and 9I. Note how similar the phenotypic expression of *kn1* (maize) in transgenic *entire* leaves is to that of *TKn2* in some of the transgenic wild-type leaves.

DISCUSSION

The Genetic Nature of the *Me* and *Cu* Mutations

The *Me* and *Cu* genes are tightly linked to each other as well as to the homeobox-containing gene *TKn2*. Both mutations incite ectopic meristematic activity that results in ramification of the compound leaf, suppression of apical dominance, and retardation of growth. The formation of ectopic shoots on the adaxial surface of *Cu* leaves and sepals and the fasciated inflorescence shoots in *Me* plants also suggest ectopic meristematic activity that is characteristic of dominant *Kn1* genes (Hake et al., 1995). These observations strongly imply that the two defective modes of transcription that are associated with the *TKn2* gene of *Cu* and *Me* are responsible for the phenotypes of the two mutants.

Interpretation of the *Cu* phenotype, with respect to the upregulation of *TKn2*, is formally simple because all features are dose dependent. The leaf defects are correlated with the organ-specific overexpression of *TKn2* (Figure 7D). However, the ectopic shoots, emerging from only one specific adaxial site of the sepals, may represent yet another alley by which *Kn1* genes can regulate development. It is possible that this intriguing phenomenon reflects differential sensitivity of the sepals to an overall change in the balance of hormones. It has been noted already that misexpression of *Kn1* genes mimics in many ways responses to hormonal applications (Hake et al., 1995). These include uncoupling of flowering and apical dominance as in *Cu-*, *Me-*, and *kn1*-expressing plants, growth retardation and alteration of growth habit in *Cu* and *Me*, and extreme green pigmentation of leaves and fruits in *Cu-* and *kn1*-expressing plants. All of these features, just like site-specific shoots on sepals, do not necessarily correlate with local autonomous or even with local and restricted nonautonomous (Lucas et al., 1995) overexpression of *Kn1* genes. They may indicate that plant hormones thus might play a critical role in mediating the developmental functions of *Kn1* genes.

The interpretation of the *Me* phenotype is more complicated. The major product of the *TKn2* gene is a chimeric *PFP–TKn2* transcript, and the normal mRNA represents only a minority of the steady state *TKn2* transcripts. The formation and distribution of this transcript are regulated by a different regime and not by the legitimate *TKn2* promoter. Finally, some of the *Me* defects are not dose dependent and can be rescued by one copy of the wild-type *TKn2* gene (i.e., *Me*/+) but not by the overexpressed *Cu–TKn2* allele (i.e., in *Cu/Me*).

The following observations suggest that the truncated form of the *Me–TKn2* product is upregulated, translated, and functioning, *Kn* style, in *Me* plants. *Me*/+ leaves undergo additional rounds of subdivision as do *Cu-* and *kn1*-expressing plants (Figures 1 and 2). The inward orientation of leaf primordia in the *Me* apex (Figures 2D and 2E), the suppression of apical dominance, the fasciated inflorescence shoots (Figure 2C), and the additive interaction with *kn1* also imply, in the context of the *Kn1* genes, overexpression and misexpression rather than a loss of function.

It is possible, of course, that the suppression of vegetative apices and the appearance of determinate inflorescence shoots in *Me/Me* (but not in *Me*/+) plants are due to a partial loss of *TKn2* function by the truncated *PFP–TKn2* factor. This possibility is made more likely because *TKn2* is closely

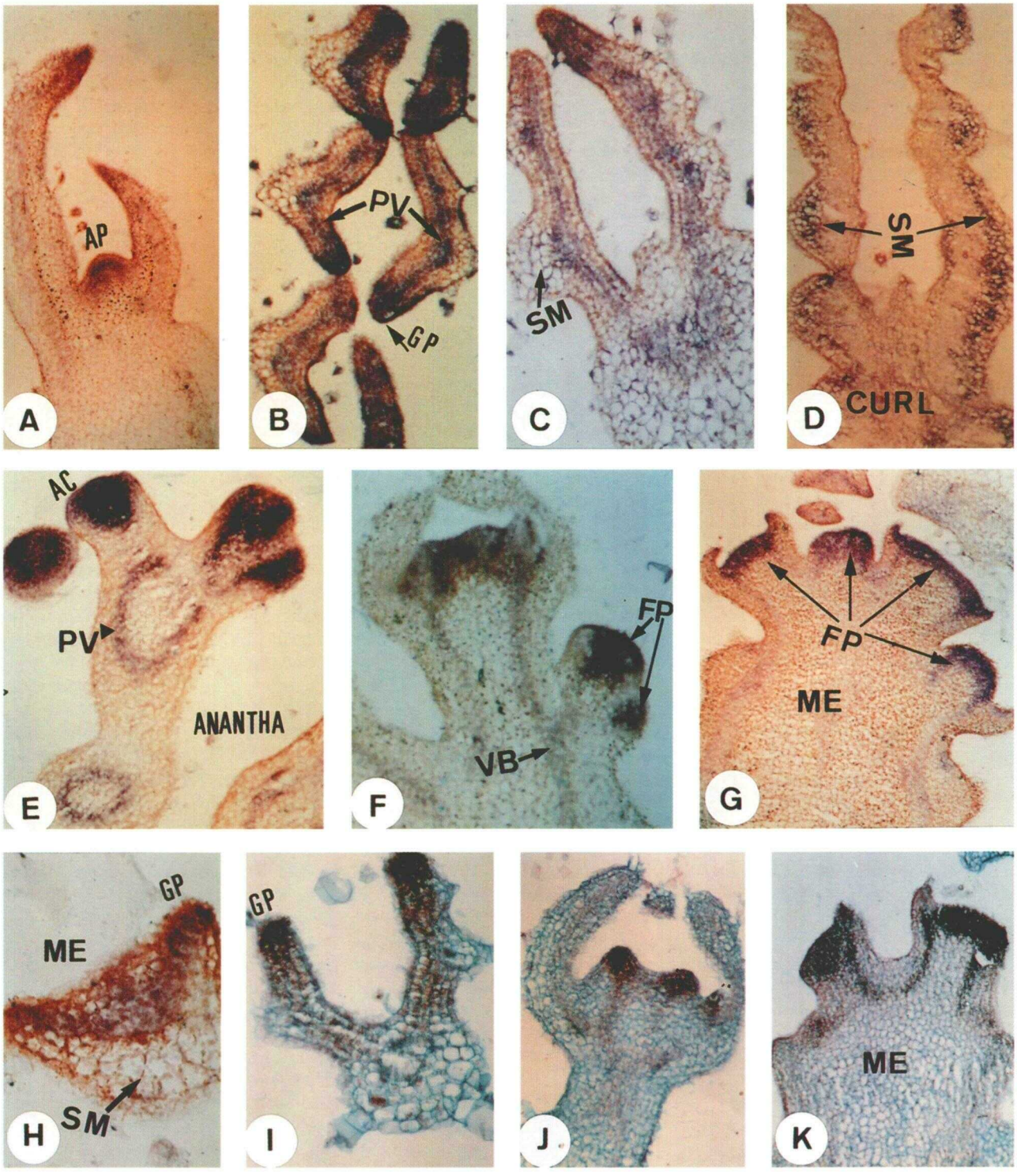

Figure 7. In Situ Localization of *TKn2* Transcripts and *PFP* Antigen in Wild-Type and Mutant Organs.

(A) to **(D)** Localization of *TKn2* transcript in apical meristems and in leaf primordia. In **(A)**, shoot apical meristems (AP) of a wild-type plant are shown. Signals are in L2 and L3 cell layers of the apex but are only marginally detected in L1 (future epidermal) cells. In **(B)** and **(C)**, cross-sections in young **(B)** and mature **(C)** wild-type leaves are shown. *TKn2* is expressed in the growing tips and in a relatively wide region around the vascular tissues. **(D)** is part of a cross-section in a convoluted *Cu* leaf. Note the concentration of signals in the spongy (bottom) mesophyll. Compare with **(C)**.

related to the *STM* gene of Arabidopsis in which recessive mutations result in lethal apical meristems (Long et al., 1996). If the recessive *Me* phenotypes result from a partial loss of *TKn2* function, then these lost functions are rescued by the wild-type allele in *Me*/+ genotypes but not by *Cu* in *Me*/*Cu* plants. Presumably, the *Cu–TKn2* gene fails to provide the right cells at the right time with the *TKn2* gene product. Alternatively, although misexpression of *Me–TKn2* incites meristematic activity ectopically, it also suppresses it in the early vegetative apex in the same way that it supports ramification in early leaves of *Me*/*Me* but suppresses lamina development in late leaves.

The Molecular Nature of the *Me* and *Cu* Mutations

All dominant mutations in *Kn1*-like genes recorded to date were found in monocot species and are associated with subgroup 1 (Figure 3C) of the class I *Kn1* genes (*kn1*, *rough sheath1*, and *hooded*). *Cu* and *Me* are the first dominant mutations reported in dicot species that are associated with the *STM*/*SBH1*/*TKn2* subgroup 2 of class I *Kn1* genes (Figure 3C). The regulation of the *Cu–TKn2* gene was compromised in the abaxial spongy mesophyll of the leaf but not in the palisade tissue or in the three inner whorls of the flowers. The abnormal expression patterns were not due to the gross genomic alterations within the coding region, introns, or immediate flanking sequences of the *Cu–TKn2* gene. The most likely possibility is that a minor sequence alteration was introduced in a putative vegetative silencer in the long intron 2. Mutations in remote flanking sequences that also have the potential of disrupting regulatory genetic modus operandi or even epigenetic changes cannot be excluded. Linkage studies suggest, however, that such a putative mutation should reside within 0.1 cM of the *Cu* mutation (50 to 100 kb on average).

In their recent analysis of the *TKn2* gene in the *Me* plants, Chen et al. (1997) convincingly showed that the *TKn2* gene (referred to as LeT6 in their study) of *Me* is fused at a position 1630 bp 5′ to a 15-kb genomic duplication of the *PFP* gene. This result is consistent with a readthrough transcript

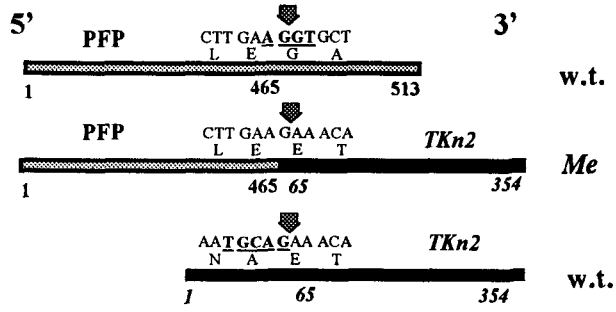

Figure 8. Schematic Representation of Splice Sites in the *PFP–TKn2* Transcripts.

Sequences of 5′ splice sites of the *PFP* gene are identical in tomato and potato mRNAs. Plant consensus sequences of 5′ and 3′ splice sites are presented in boldface and are underlined. Arrows indicate splice sites. Amino acids flanking splice sites are also given. w.t., wild type.

originating in the duplicated *Me* promoter and misspliced at the indicated positions (Figure 8; Chen et al., 1997). It is also in agreement with the presence of regular-sized *PFP* transcripts and the scarcity of intact *TKn2* transcripts. The latter observation also suggests that in agreement with the in situ hybridization results (Figure 7), the expression domains of the two genes do indeed overlap. It is not easy to determine why a 3′ splice site was chosen in the middle of the first *Me–TKn2* exon. Presumably, other more legitimate downstream 3′ sites of any of the *TKn2* introns result in a complete loss of function.

Different *Kn1* Class I Genes Have Different Meristematic Functions

Functional differences between *Kn1*-like genes may help to dissect elusive intrinsic meristematic programs. The sequence of *TKn2* is more similar to *STM* of Arabidopsis than it is to *TKn1* or *TKn3* of tomato, and *KNAT1* (*Kn1*-like from

Figure 7. (continued).

(E) Floral meristems of *anantha* plants.

(F) Floral primordia of wild-type plants. Three floral primordia (arrows) are shown.

(G) and **(H)** Distribution of *TKn2* transcripts in *Me* floral primordia **(G)** and young leaf **(H)**. The fasciated inflorescence primordia is fated to form the structure shown in Figure 2C. Note the wider distribution of signals in the primordial apices in comparison with the wild type. Tissues below the apex were also decorated after longer exposure.

(I) and **(J)** Immunolocalization of the PFP antigen in leaves **(I)** and floral primordia **(J)** of wild-type plants.

(K) Immunolocalization of the PFP antigen in fasciated inflorescence primordia of *Me*/*Me* plants.

A 500-bp *TKn2*-specific fragment was used to prepare digoxigenin-labeled antisense and sense RNA probes. Sense probes gave no hybridization signals. The specific anti-PFP antibody for the potato protein was kindly supplied by B. Plaxton (see Carlisle et al., 1990). AC, apical cells; AP, apex; FP, floral primordia; GP, growing tips; PV, provascular strand; SM, spongy mesophyll; VB, vascular bundles.

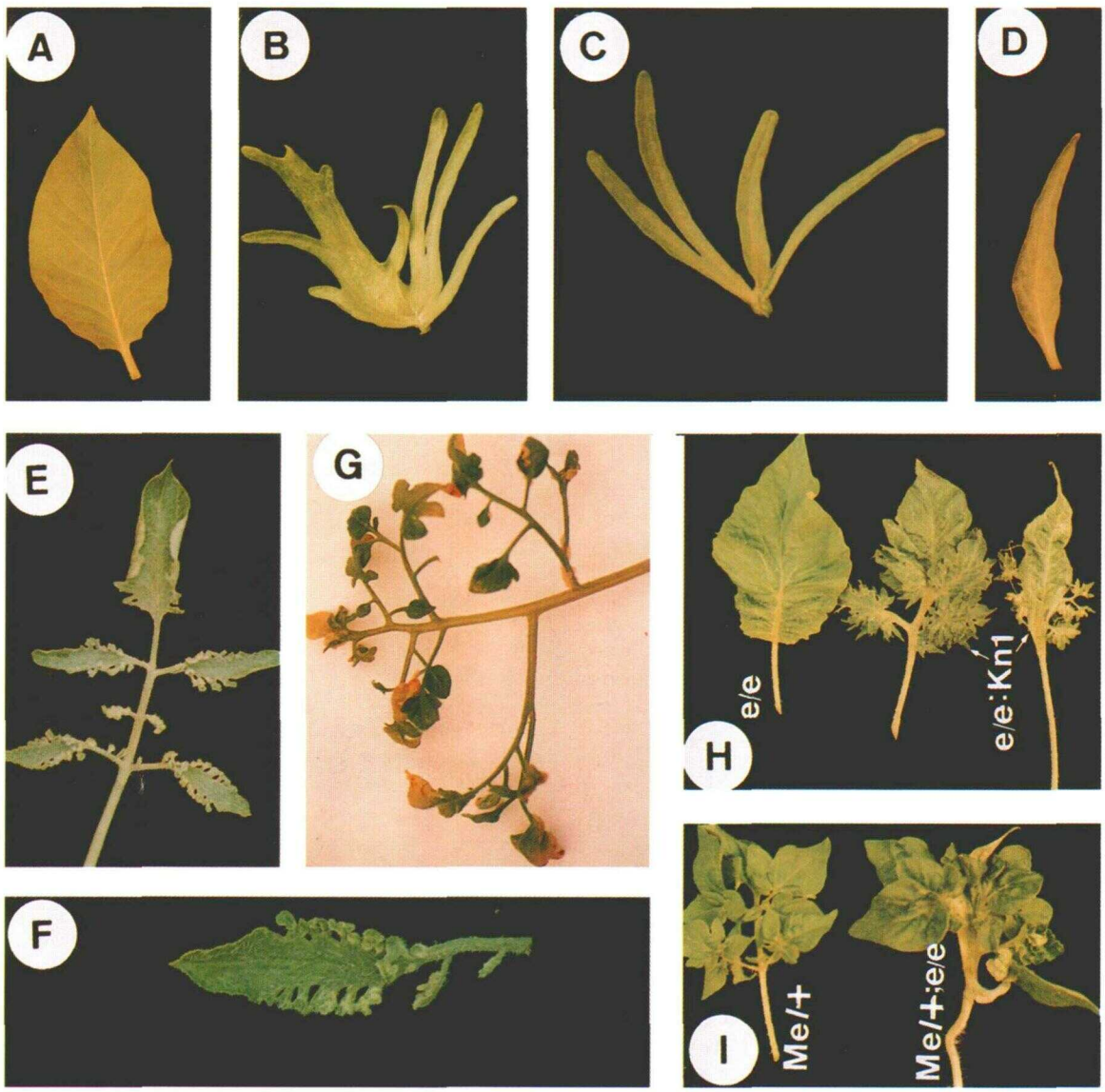

Figure 9. Leaf Shapes and Architecture in Transgenic Plants and Double Mutant Combinations.

(A) to **(D)** Transgenic tobacco plants expressing the 35S::*TKn2* gene. **(A)** shows a wild-type tobacco leaf. **(B)** to **(D)** show lower, middle, and upper leaves, respectively, of tobacco plants expressing the 35S::*TKn2* gene. Note the palmate architecture of the leaf in **(C)** and the lanceolated shape of upper leaves in **(D)**.

(E) to **(G)** Phenotypic expression of *TKn2* in tomato transgenic plants. Two different leaf phenotypes of two independent transgenic plants are shown. Comb-like leaflets in a compound leaf of 35S::*TKn2* transgenic plant are shown in **(E)** and **(F)**. Note the similarity with respect to marginal outgrowth between this type of transgenic plant and *e/e*;35S::*kn1* plants shown in **(H)**. In **(G)**, *Me*-like arrangement of leaflets in another tomato plant expressing the 35S::*TKn2* gene is shown. Compare with Figure 2F.

(H) Interaction between *entire* and the maize *kn1* gene in homozygous *entire* plants carrying one dose of the 35S::*kn1* gene.

(I) Leaf architecture in *Me/+*;*e/e* plants. Note that the ramification site of the *entire* leaf is restricted to its distal region in both *e/e:kn1* **(H)** and *Me/+:e/e* plants.

Arabidopsis thaliana) is more closely related to *kn1* and *TKn1* than it is to *STM*, suggesting that functional differences among *Kn1*-like genes were established before these species diverged. Functional classification has been hindered, presumably for lack of appropriate criteria. Misexpression of all *Kn1*-like genes elicits ectopic meristematic activity, growth retardation, and loss of apical dominance, and it is also likely that null alleles in many class I *Kn1* genes will have detrimental effects on meristems, similar to *STM* and *rough sheath1*. The morphogenic differences induced by the two *Kn1* genes in the same organs, for example, *kn1* and *Cu* in leaves, are not indicative of different functions, because the two genes are expressed under different spatial and temporal controls. One approach therefore is to compare the effects of different *Kn1* genes in the same species and under the control of the same regulatory sequences.

In tomato, 35S::*kn1* induces extreme but regular subdivision of the leaf, but no ectopic shoots are formed. 35S::*TKn2* confers much milder, irregular subdivision, similar to that of *Me*/+ leaves. The 35S::*TKn1* transgene conditions growth retardation so extreme that it permits shoots of only 10 to 15 cm, unexpanded leaves of only 1 cm in length, and unusual early flowering that results in abortive diminutive buds. In tobacco, 35S::*kn1* induces lobed and rumpled leaves with occasional epiphyllic shoots (Sinha et al., 1993), and 35S::*TKn2* induces lobed, dissected, and lanceolate leaves (Figure 7), whereas most extreme growth retardation and rounded leaves with miniature epiphyllic shoots, corrugated surface, and dark pigmentation—all reminiscent of tomato *Cu* leaflets—are induced by *TKn1* (E. Lifschitz, unpublished data). The unique phenotypes caused by three different *Kn1*-like genes in the same species and under the same promoter are consistent with the idea that each *Kn1* gene controls at least some distinct developmental program.

The Relationship between Expression Domains of *Kn1* Genes and the Initiation and Architecture of Leaves

Initiation of Leaves

Suppression of *Kn1* expression in the future initiation sites of leaves on the flanks of maize and Arabidopsis apices invoked the inference that such a downregulation is a prerequisite for leaf initiation (reviewed in Hake et al., 1995). It follows that ubiquitous expression of *Kn1* genes alters phyllotaxis. This prediction has not yet been confirmed. In plants bearing dominant overexpressing mutations such as *Kn1*, *rough sheath1*, *Cu*, or *Me*, or in transgenic tobacco, Arabidopsis, and tomato plants expressing *Kn1*-like genes, phyllotaxis was never altered. In addition, we have shown here (Figure 7) and elsewhere (Hareven et al., 1996) that in contrast to maize and Arabidopsis, the tomato *TKn1* and *TKn2* genes are expressed across the meristematic domains of the apex.

It is possible, albeit unlikely, that *Kn1* expression marks leaf initiation only in species with simple leaves, whereas in species with compound leaves, even of the same family, a completely different mechanism is evolved. Alternatively, mRNA levels alone may be the wrong parameter, and post-transcriptional but otherwise species-specific regulation operates in all plants to eliminate unwanted *Kn1* gene products. It is also possible that we are looking at a secondary phenomenon, that other unknown factors are of primary importance, and that correlation alone may sometimes be misleading.

Leaf Architecture

The tomato *TKn1* and *TKn2* genes are expressed in leaf and floral primordia of tomato (Figure 7; Hareven et al., 1996) but not in the corresponding organs of maize and Arabidopsis (Jackson et al., 1994; Lincoln et al., 1994; Hake et al., 1995). We previously considered the possibility that *Kn1* genes are required to promote compoundness, with the provision that the observed differential response of simple and compound leaves to *kn1* misexpression may also be due to different developmental potentials at a critical developmental window (Hareven et al., 1996). *Me* is informative in this regard because ramified, simple, and even filamentous leaves are formed on the same plant (Figures 2A to 2C) and because *Me*/+ leaves are more compound than are *Me*/*Me* leaves and their leaflets are never laminaless. Restriction of lamina expansion is thought to be a precondition for increased compoundness and has also been observed in ramified leaves of extreme *kn1* phenotypes (Hareven et al., 1996) and along the palmated veins in 35S::*TKn2*–expressing tobacco plants as well (Figures 9B to 9D). Thus, in addition to the route leading to simple leaves that is exemplified by the double mutant combination of the recessive *trifoliate* and *potato leaf* genes (Stettler and Imber, 1966), modified expression of *Kn1* genes (i.e., *Me*) may represent another route. The dominant *Lanceolate* gene (Dengler, 1984) may well be involved in this second alley.

Kn1 Genes and Growth Habit

The shape and architecture of a plant and consequently its economical success depend primarily on growth habit—the pattern in which vegetative and reproductive organs alternate and in which side branches emerge. Growth habit is determined in apical meristems in which *Kn1*-like genes are expressed. In most cases, the upregulation of *Kn1* genes in their legitimate expression domains does not lead to morphogenic alterations (Hake et al., 1995). Consequently, their role in regulating growth habit was neglected. Yet, common to all plants overexpressing *Kn1* genes is the enhancement of branching, for example, uncoupling of apical dominance and flowering. *Me* and *Cu* alter the pattern of reproductive development by terminating vegetative growth prematurely

and provoking reproductive development in *Me* or extending it in the *Cu* sympodial shoot and by permitting extensive proliferation of the inflorescence in *Me* but suppressing floral meristems in *Cu*. The formation of ectopic flowers in barley *Hooded* mutants (Muller et al., 1995) occurs in already determined reproductive organs. Although it is not clear to what extent the Hooded phenotype is different from the appearance of epiphyllic inflorescence meristems on transgenic tobacco leaves, it is nevertheless in agreement with a role of *Kn1*-like genes in regulating vegetative to reproductive transitions.

The study of the *TKn2* gene in tomato and tobacco plants suggests that the very same meristematic regulatory gene operating under the same promoter can generate a range of variations in leaves—from supercompound to filamentous in *Me* or from lanceolate to palmated in 35S::*TKn2* tobacco. Under different expression regimes, as in *Me* and *Cu*, *TKn2* confers extreme variations in morphogenesis and growth habit in the same plant species.

It follows that subtle alterations in the activity of *TKn2* and of a relatively small group of *Kn1* genes may support a wide range of variations in inflorescence complexity, leaf morphology, and growth habit in tomato plants. Transcription factors of the MADS box family were selected early during evolution of plants (Ma et al., 1991; Pnueli et al., 1991) to regulate the fate and development of flowers and floral organs. Likewise, it is possible that *Kn1* class I homeodomains were adopted as the most suitable regulators of growth mechanisms that are common to all aerial meristems.

METHODS

Plant Material

Seeds of *anantha* (LA536), *Curl* (*Cu*; LA325), *entire* (*e*; LA922), *Mouse-ear* (*Me*; LA324), *trifoliate* (*tf*; LA579), and *Lycopersicon pennellii* (lA716) were obtained from C.M. Rick (University of California, Davis). For tomato transformation, we used TK9/8. Generation of transgenic tomato plants and the line used for transformation were as described by Pnueli et al. (1994). Transformation of tobacco plants (Samsun 5) was done according to the method of Horsch et al. (1985). For the *Me/+;e/e*, and *e/e;kn1* double mutant lines shown in Figures 9H and 9I, transgenic line M_1, expressing the 35S::*kn1* gene construct, was used as the male parent to pollinate *Me* or *e* homozygous plants. Appropriate segregants were selected from the F_2 generation.

Mapping Procedure

L. pennellii was crossed to *Me/Me* plants, and F_2 *Me* segregants were analyzed for linkage with chromosome 2 restriction length polymorphism markers. No recombinants were found between *Me* and TG454 among 83 F_2 *Me* plants. The tomato *Knotted2* (*TKn2*) and PPi-dependent fructose 6-phosphate phosphotransferase (PFP) clones were mapped using the population of an *L. pennellii* introgression line (Eshed and Zamir, 1995). Both clones revealed the *L. pennellii* polymorphism when probing DNA of IL2-2 and IL2-4, which share an overlapping introgressed region marked by TG454 and TG353 and were assigned to the same site on the restriction fragment length polymorphism map (Tanksley et al., 1992).

Nucleic Acid Procedures

cDNA libraries were prepared in the λZAP II vector (Stratagene, La Jolla, CA) from poly(A)$^+$ RNA of shoot apices, floral buds, and *anantha* floral meristems. A genomic library of wild-type tomato line (93-137) was prepared in the λFIX vector (Stratagene), according to the manufacturer's protocol. The polysome fractions were analyzed according to the method of Barkan (1993). Other nucleic acid procedures were performed as described by Maniatis et al. (1982) or Ausubel et al. (1988).

Primers

The following primers were used for the polymerase chain reaction procedures reported in the text: primer 1, 5′-CTTCAAGGAAGCCATGG-3′; primer 2, 5′-CCATCGATGGGCACACAAGTAATATGC-3′; primer 3, 5′-ATGGAGGGTGGTTCTAGTGGAAATACTAGT-3′; and primer 4, 5′-CTTACTCCCCAGTGCCTTGAGCTC-3′. Primers 1 and 2 were used for the experiment reported in Figure 4B and for amplification of intron 2 from *Cu* DNA. Primers for the reverse transcriptase–polymerase chain reaction isolation of *TKn2* cDNA from *Cu* were 2 and 3. Primers 2 and 4 were used in the attempt to isolate a fusion transcript from *Me* and wild-type RNA.

Cytological Procedures

In situ hybridization using digoxigenin-labeled RNA probes (Jackson, 1991) was performed as described by Hareven et al. (1996). Preparation of tissues for scanning electron microscopy was according to the the method of Smyth et al. (1990). Immunogold labeling was as described by Shahar et al. (1992) and Samach et al. (1995).

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