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Interactions between *jointless* and Wild-Type Tomato Tissues during Development of the Pedicel Abscission Zone and the Inflorescence Meristem

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The *jointless* mutation of tomato results in the formation of flower pedicels that lack an abscission zone and inflorescence meristems that revert to vegetative growth. We have analyzed periclinal chimeras and mericlinal sectors of *jointless* and wild-type tissue to determine how cells in different meristem layers (L1, L2, and L3) and their derivatives interact during these two developmental processes. Cells in the inner meristem layer, L3, alone determined whether the meristem maintained the inflorescence state or reverted to vegetative growth. Moreover, L3 derivatives determined whether a functional pedicel abscission zone formed. Limited and disorganized autonomous development of wild-type L2-derived cells occurred when they overlay mutant tissue. Adjacent mutant and wild-type L3-derived tissues in pedicels developed autonomously, indicating little or no lateral communication. Only the outermost L3-derived cells within the pedicel were capable of orchestrating normal pedicel development in overlying tissues, revealing the special status of those cells as coordinators of development for L1- and L2-derived cells, whereas the innermost L3-derived cells developed autonomously but did not influence the development of other cells.

INTRODUCTION

Analyses of genetically mosaic plants have provided abundant evidence that cells act in a coordinated manner during developmental processes occurring in shoot meristems (Szymkowiak and Sussex, 1996). For example, an organ is not initiated from a single cell that proliferates to form a multicellular primordium but from populations of founder cells already present in the meristem (Poethig and Sussex, 1985a, 1985b). To replace the cells that are lost to newly forming primordia, the remaining cells of a meristem undergo divisions so that a functional meristem is maintained. To do this accurately, cells within the meristem must somehow monitor the size of the remaining population of meristem cells and divide only if additional divisions are needed. In addition, major developmental changes involve the entire meristem. The transition from vegetative to reproductive growth, in which the type and placement of primordia formed change and patterns of organ initiation switch from repetitive to sequential, is one such example. Thus, there is a variety of developmentally critical processes for which the cells of the meristem must act in an integrated manner.

The cells of shoot meristems of most dicots, including tomato, are arranged in three persistent cell layers, namely, L1 (layer 1), L2, and L3 (Satina et al., 1940). This arrangement is due to a restriction in the planes of cell division in the outer two cell layers, resulting in meristems composed of three distinct cell lineages whose cellular derivatives coordinate their activities during developmental processes. This arrangement of cells permits the generation of a specific type of genetically mosaic plants, a periclinal chimera, in which cells of one meristem layer are genetically different from the other cells of the meristem. Mericlinal chimeras have cells of different genotypes adjacent to each other within a single meristem layer. The phenotypes of plants having genetically different cells juxtaposed in the meristem in various arrangements can provide information about the cellular integration of the meristem. We have used periclinal chimeras previously to investigate the process of cell/tissue coordination during two types of developmental processes that occur in the meristem: the process of organ initiation (Szymkowiak and Sussex, 1993) and the regulation of organ number and meristem size (Szymkowiak and Sussex, 1992). We found that cells in L3 of the meristem controlled these processes, and L1 and L2 cells responded to L3 cells, regardless of their own genotypes.

Those chimeras provided strong evidence that at least in processes occurring within the tomato meristem, the position of a particular cell dictated whether it directed developmental processes or responded to other cells. Thus, interactions among meristem cells do occur, and signaling is directional: from the inside out. In other systems, different patterns of signaling have been found, such as in snapdragon chimeras involving *floricaula* (*flo*), in which *FLO* in any meristem layer was sufficient to induce determinacy in reproductive meristems (Carpenter and Coen, 1995; Hantke

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et al., 1995). In *flo* chimeras, information flows bidirectionally. The different signaling patterns raise questions concerning how common certain signaling patterns are, whether there are species-specific differences in patterns, and whether a given pattern is associated with a particular type of developmental process.

To expand the types of processes analyzed with genetically mosaic plants as well as to gain insights into the process of pedicel abscission zone development, we have generated a set of tomato chimeras from the *jointless* mutant and wild-type plants. The *jointless* mutation is pleiotropic, with effects on developmental processes in the meristem itself and in structures derived from the meristem. As a wild-type tomato plant grows, the main shoot axis terminates in the inflorescence. Here, the inflorescence meristem initiates several flowers, with the number depending on the genetic background. After those flowers have been initiated, meristem activity ceases. Because tomato plants exhibit a sympodial pattern of growth, subsequent growth of the shoot is continued by a precociously developing meristem in the axil of the uppermost leaf (Sawhney and Greyson, 1972; Pneuli et al., 1998). *jointless* inflorescence meristems, like the wild type, initiate flowers but, unlike the wild type, revert to vigorous vegetative growth after forming one to three floral meristems (Rick and Sawant, 1955). That is, the inflorescence meristem reverts to the formation of leaves and remains indeterminate rather than continuing to undergo cycles of forming a floral meristem and a new inflorescence meristem (Sawhney and Greyson, 1972). As a result, the main axis is not terminated once flowering has begun, and a leafy inflorescence phenotype is produced. *Jointless*$^+$ is therefore involved in the maintenance of the inflorescence meristem state.

The second phenotypic effect of the *jointless* mutation is the formation of flower pedicels that lack an abscission zone (Butler, 1936). The tomato pedicel abscission zone is a relatively simple structure consisting of a band of anatomically distinct cells in which cell separation occurs for the shedding of flowers or fruits (Kendell, 1918). Abscission is an active process involving ethylene. Ethylene stimulates the production of enzymes that degrade the middle lamella between cells in the abscission zone (Jensen and Valdovinos, 1968; Valdovinos and Jensen, 1968; van Doorn and Stead, 1997). The presence (or absence) of the pedicel abscission zone is an agronomically important trait in many species. In tomato plants, fruits harvested from wild-type plants retain the calyx and the distal end of the pedicel, whereas *jointless* fruits are "stemless" when harvested. This characteristic is highly desirable for mechanically harvested fruit. The *jointless* mutation does not affect other abscission zones, such as those of leaves, style, or corolla.

Much is known about the physiological process of abscission (Sexton and Roberts, 1982; van Doorn and Stead, 1997), but far less is known about the formation of the structure, the abscission zone, where abscission occurs (Osborne, 1989). *jointless* is interesting because it provides a means of perturbing two distinct developmental processes: a change in state of the meristem and formation of a simple structure by cells that are derived from all meristem layers. The pleiotropy of *jointless* also raises questions about the relationship of the pedicel abscission zone to the inflorescence meristem. Finally, the *jointless* locus has become molecularly accessible through map-based cloning (Wing et al., 1994; Zhang et al., 1994); this advance will make possible a molecular analysis of the function of *jointless* during development as well as addressing a potential role in cell–cell interactions.

RESULTS

Autonomous and Nonautonomous Development of the Pedicel Abscission Zone

Wild-type tomato plants develop an abscission zone at the midpoint of flower pedicels. Abscission zones are completely lacking in pedicels of *jointless* mutants; flower pedicels from a wild-type plant, showing abscission, and from a *jointless* plant are shown in Figure 1A. To investigate the patterns of cell and tissue coordination that occur during pedicel abscission zone differentiation, we generated periclinal chimeras from *jointless* and wild-type plants. Because tomato meristems have three persistent cells layers, there are six possible periclinal chimeric arrangements of two different genotypes. All six periclinal chimeras between *jointless* and the wild type were generated in vivo and in vitro by using grafting and shoot regeneration from *jointless*/wild-type mixed tissues. These techniques were previously described (Szymkowiak and Sussex, 1992, 1993). The genotypic identity of each cell layer of a chimera was determined by the presence or absence of cell layer autonomous, mutant phenotypic markers that differed between the wild-type and *jointless* stocks, such as those affecting trichome and chloroplast characters, as shown in Figures 1B and 1C. *jointless* tissues were marked with *Xanthophyllic-2* (*Xa-2*), *hairless* (*h*), and *anthocyanin gainer* (*ag*). Wild-type tissues were also wild type for those marker genes. The periclinal chimeras were vegetatively propagated, and many individual clones of each were grown through multiple flowering cycles.

The formation of the pedicel abscission zone in each chimera was examined. These results are summarized in Table 1. Macroscopically, a tomato pedicel abscission zone appears as a pronounced groove flanked by slightly thicker tissue. This groove is located halfway between the calyx and the peduncle and is where abscission takes place (Kendell, 1918). Chimeras $j++$ (L1 is *jointless*, *j*; L2 and L3 are wild type, $+$), $jj+$, and $+j+$ had a wild-type abscission zone phenotype. Each of these chimeras had wild-type L3-derived cells in the pedicel. Abscission zones become evident early in pedicel development. They appear first as an epidermal

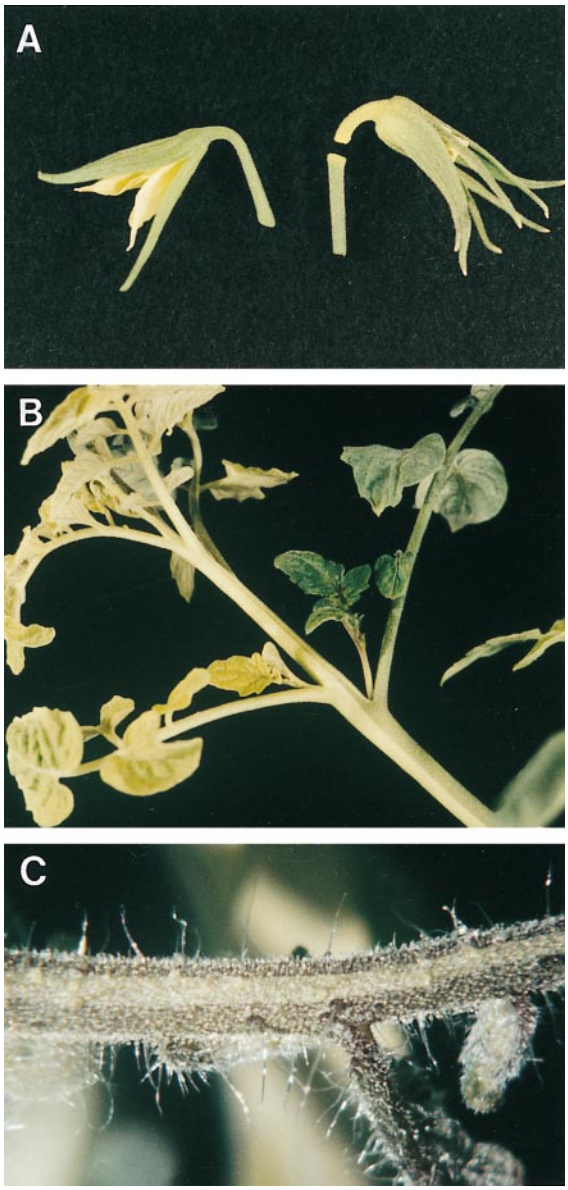

Figure 1. Normal and *jointless* Tomato Pedicels and Cell Layer Markers.

(A) At right is a pedicel from a *j*++ chimera, which has a normal abscission zone and shows a clear separation between the distal and proximal portions of the pedicel after abscission. *jointless* pedicels (left) fail to differentiate an abscission zone.

(B) Generation of periclinal chimera *j*+*j*, visible as a green branch contained within a sector on a mericlinal chimera. The adjacent tissue has the arrangement *jjj*. *jointless* L1-derived tissue was marked by *h*, and *jointless* internal tissues were marked by *Xa-2*, which confers yellow rather than green pigmentation.

(C) Stem of a mericlinal chimera, showing wild-type trichomes in an L1-derived epidermis, marking L1 as the wild type. A sector of *Xa*-2 *ag* subepidermal tissue is evident. The sector had the arrangement +*jj* in an otherwise ++*j* stem.

region lacking trichomes. This is followed by elongation and thickening of the pedicel distal and proximal to the abscission zone. Figure 2A shows an early-stage pedicel of chimera *jj*+ in which morphological differentiation of the abscission zone is already apparent. Pedicels of wild-type (+++) and chimeras *j*++, *jj*+, and +*j*+ exhibited an identical pattern of development. In addition to having a normal appearance, these chimeras also exhibited normal abscission, in which cell separation for shedding flowers or fruits occurred in the abscission zone (Figure 1). In contrast to the chimeras with wild-type L3 cells, chimeras ++*j*, +*jj*, and *j*+*j*, which all have *jointless* cells in L3, developed pedicels that had neither abscission zone morphology nor the ability to undergo cell separation. Figure 2B shows developing pedicels from a *jointless* plant.

Pedicels of the chimeras were analyzed histologically by longitudinal sectioning of fresh or fixed and embedded tissue and compared with nonchimeric pedicels of wild-type and *jointless* plants. Abscission zones are comprised of ~10 to 15 tiers of nonelongated, small cells and so form a groove in the pedicel (Roberts et al., 1984; Figures 2C and 2F). It is among these small cells that cell separation takes place during abscission. The epidermis of the pedicel, including the abscission zone, was derived from L1 of the shoot meristem. Expression of cell layer markers in the pedicels and/or in the distal sepals of chimeras indicated that at least a two-cell thickness of subepidermal pedicel tissue whose cells contain plastids is derived from L2. L2 may contribute some additional internal cells to the pedicel. L3-derived cells formed the remaining internal tissue of the pedicel. Thus, all three meristem layers contribute to the pedicel abscission zone.

Derivatives of each layer in the abscission zone are anatomically distinct from pedicel cells distal and proximal to the abscission zone. The L1-derived epidermis of abscission zones has nonelongated cells and lacks trichomes. The L2-derived subepidermal pedicel tissue is pigmented as a result of numerous chloroplasts and develops intercellular air spaces similar to spongy mesophyll of leaves. In contrast, L2-derived cells in the abscission zone lack chloroplasts, and intercellular air spaces are absent (Figures 2C and 2E). L3-derived cells in the pedicel, including the vascular cylinder and internal tissues, are small and rounded in the middle of the abscission zone and are flanked by elongated and swollen cortical cells immediately distal and proximal. Outside the abscission zone, L3-derived cells are uniformly elongated.

jointless pedicels are uniform in anatomy along their length (Figure 2B), with no regions of subepidermal tissue lacking chloroplasts or intercellular air spaces or exhibiting smaller or larger cells (Figure 2D). Chimeras *j*++, *jj*+, and +*j*+ had normal abscission zone anatomy in all tissues of the pedicel, even though some of the tissue was composed of genotypically *jointless* cells (Figure 2C). The L2-derived tissue of pedicels of chimeras *jj*+ and +*j*+ was mutant yet did not undergo chloroplast differentiation or form air spaces

Table 1. Pedicel and Inflorescence Development in Periclinal Chimeras

L1, L2, and L3	Pedicel	Inflorescence
Nonchimeric plants		
+++	Normal abscission zone	Determinate
jjj	Jointless	Leafy
Periclinal chimeras		
jj+	Normal abscission zone	Determinate
+*j*+	Normal abscission zone	Determinate
j++	Normal abscission zone	Determinate
++*j*	Jointless[a]	Leafy
j+*j*	Jointless[a]	Leafy
+*jj*	Jointless	Leafy

[a]Pedicels have patches of raised tissue.

in the abscission zone, typical of cells in those locations in wild-type pedicels. Similarly, the L1-derived pedicel epidermal cells of chimeras *j*++ and *jj*+ were genetically *jointless* yet were small and lacked trichomes in the region of the abscission zone, as is found in wild-type plants.

Histological and scanning electron microscopic examination of pedicels of chimeras ++*j*, +*jj*, and *j*+*j*, which have *jointless* L3-derived cells, in general showed a typical *jointless* anatomy and morphology, despite the presence of wild-type cells in the pedicel. Slight differences could be detected in how pedicels of these three chimeras differentiated. The pedicels of chimera +*jj*, having wild-type cells in the epidermis only, were indistinguishable from those of nonchimeric *jointless* plants (Figure 2D). In contrast, the pedicels of chimeras *j*+*j* and ++*j*, although having an overall appearance of a *jointless* pedicel, often showed slight surface irregularities, as shown in Figure 3. The irregularities consisted of bumps or raised patches of small, nonelongated cells (Figures 3A to 3D). Both of these chimeras had wild-type L2-derived cells overlying *jointless* L3-derived cells.

The cells in the raised patches were not aligned in the smooth files characteristic of pedicels and abscission zones. No cell separation was observed in the region of the bumps. Both L1- and L2-derived cells in chimeras *j*+*j* and ++*j* showed some characteristics typical of cells in those locations in abscission zones, such as small size and lack of plastids in the region of the bumps (Figure 3D). The outermost L3-derived cells and/or innermost L2-derived cells of the chimeric pedicels were abnormally enlarged and elongated longitudinally (Figure 3D), reminiscent of the swollen tissue at the edges of an abscission zone. These large cells, as well as an apparent increased number of epidermal cells, were most likely responsible for the raised tissue.

The bumps first became visible much later in pedicel development than abscission zones appear on normal pedicels (cf. Figure 3A with Figure 2A). The bumps were found midpedicel at the approximate location where an abscission zone normally would form. They were arranged in a discontinuous spiral around the pedicel (Figure 3B). Often the bumps were very sparse or even absent, so that in these chimeras the bulk of many pedicels, including cells that were genetically wild type, had typical *jointless* characteristics, such as plastids and air spaces in the L2-derived tissue.

No Lateral Interactions in Sectored Pedicels of Mericlinal Chimeras

In periclinal chimeras, all cells in one meristem layer are different from the remaining cells in the meristem and thus provide information about interactions among cells that occur in a radial dimension within the meristem. Because derivatives of each of the meristem layers retain their relative positioning in the developing pedicel, periclinal chimeras also provide information about radial interactions during pedicel development. Sectored or mericlinal chimeras have a fraction of cells in one meristem layer that are different from the other cells within the same layer. We used mericlinal chimeras to address whether lateral interactions occur during abscission zone development. Mericlinal chimeric meristems arose both during our initial generation of chimeras before they became stabilized as periclinal chimeras and from layer rearrangements that occurred during shoot regeneration from tissue culture of a preexisting chimera. Occasionally, a mericlinal arrangement persisted into the inflorescence meristem and yielded sectored pedicels. In such cases, a fraction of the pedicel, including the abscission zone, was formed from a chimeric portion of the meristem. The remaining pedicel tissue was formed from a nonchimeric or differently chimeric portion of the meristem. Unlike periclinal chimeras, mericlinal sectors are not persistent, and these usually terminated in a flower whose subtending pedicel was sectored. Although each sector originated independently, examination of multiple occurrences of each type of sectored pedicel provided valuable information about interactions between adjacent, side-by-side cells that occurred either early, in the meristem, or in its derivatives at any later point during abscission zone development.

A sectored pedicel in which one portion was *j*++ derived and the other was *jj*+ derived (designated as having a *j*++/*jj*+ "constitution," indicating that it came from a mericlinal *j*++/*jj*+ meristem) had a normal, functional abscission zone, as shown in Figure 4A. The abscission zone was continuous across the sector boundary, and the pedicel was indistinguishable from those of chimeras *j*++ or *jj*+ or nonchimeric wild-type plants. This phenotype was consistent with those of the periclinal chimeras that had wild-type cells in L3, because the L3-derived cells were uniformly wild type in the sectored pedicel. There was no visible effect of having adjacent wild-type and *jointless* L2-derived cells within the pedicel on abscission zone development.

A second type of sectored pedicel in which the genotype of L3-derived cells differed arose multiple times. In sectors with the constitution *jj*+/*jjj*, as indicated by the cell layer

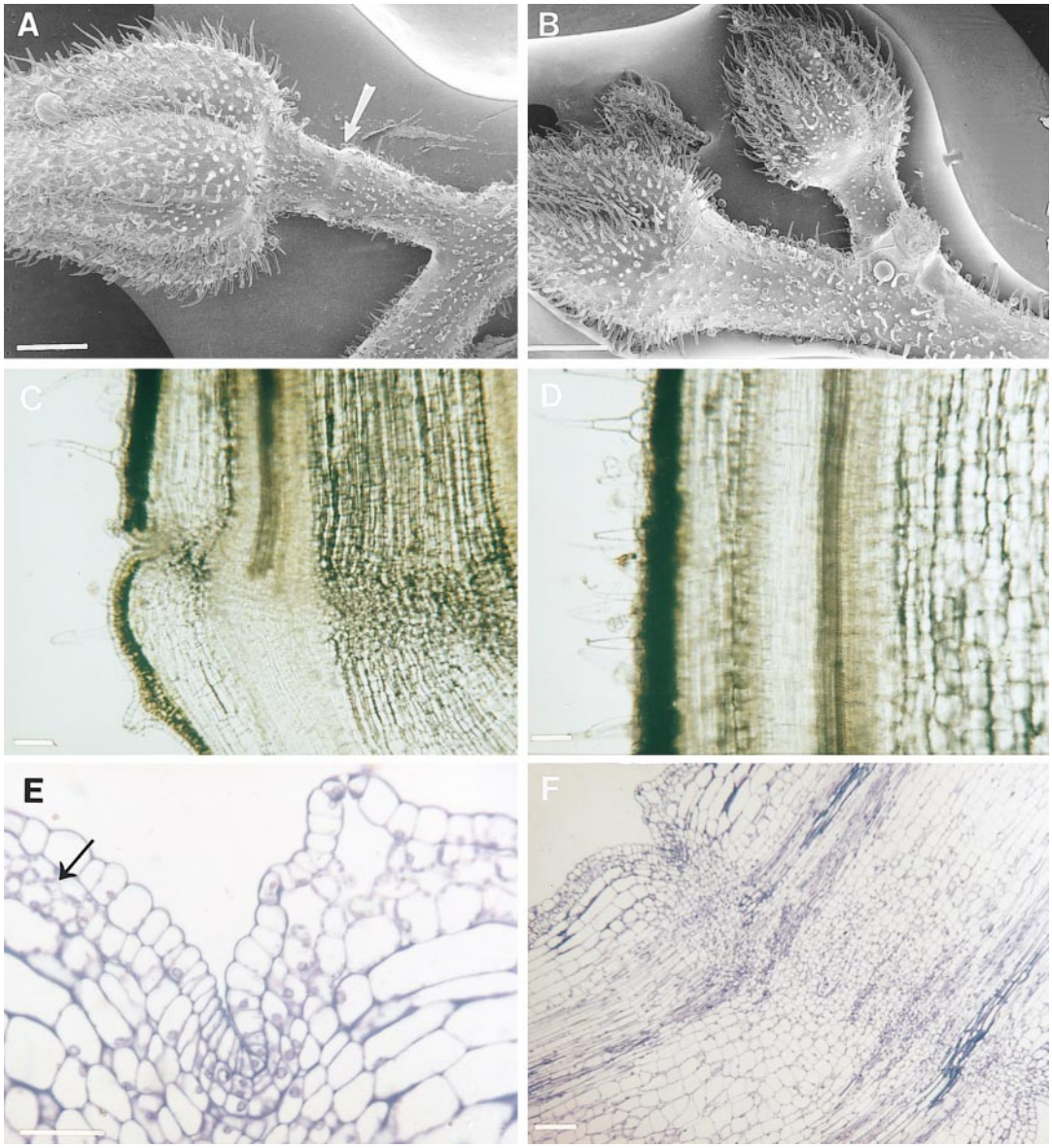

Figure 2. Pedicel Abscission Zone Development.

(A) Scanning electron microscopy of a developing flower and pedicel of chimera *jj*+, showing a developing groove (arrow) where the abscission zone will differentiate. Bar = 500 μm.
(B) Scanning electron microscopy of an inflorescence from a *jointless* plant, showing two different stages in development of a *jointless* pedicel. Note the complete absence of a groove. Bar = 500 μm.
(C) A longitudinal, freehand-cut section through the pedicel of chimera *jj*+, which has normal abscission zones, at the abscission zone. Note the green coloration due to chloroplasts in the L2-derived subepidermal cells of the pedicel, except in the abscission zone as is found in the wild type, even though those cells are genotypically *jointless.* Bar = 100 μm.
(D) A longitudinal, freehand-cut section through the midpoint of a *jointless* pedicel. Note the uniform files of cells throughout the pedicel as well as chloroplast development throughout the subepidermal tissue. Bar = 100 μm.
(E) A section of a plastic-embedded wild-type pedicel showing the groove and small cells associated with a normal abscission zone. Note the plastids and intercellular spaces (arrow) in L2-derived subepidermal tissue, except in the region of the abscission zone. Bar = 100 μm.
(F) A section of a plastic-embedded wild-type abscission zone showing multiple tiers of small cells traversing the pedicel. Note the large cortical cells that flank the abscission zone. Bar = 100 μm.

markers, pedicel tissue that was *jj+* developed a groove in the normal location typical of abscission zones, whereas adjacent pedicel tissue that was *jjj* did not develop a groove (Figures 4B to 4F). Cell separation occurred in the groove but not in the nongrooved portion of the pedicels. We observed this separation in pedicels in which as little as one-fifth was *jjj*, with the remainder being *jj+* (Figures 4E and 4F), or in pedicels in which as much as four-fifths of the pedicel was *jjj* and one-fifth was *jj+* (Figure 4D). Sectored pedicels that had the constitution *+j+/+jj*, as shown in Figure 4G, showed the same sharp boundary as did *jj+/jjj* pedicels, differing from the latter only in the genotype of the L1-derived cells, which was wild type. Cell separation always occurred within these sectored abscission zones. In both of these types of sectored pedicels, the genotype of L3-derived cells differed, being either wild-type or *jointless*, and there was a clear demarcation of abscission zone/no abscission zone that corresponded to the sector boundaries.

A third type of sectored pedicel had the constitution *+jj/++j*, with the difference being among L2-derived cells overlying *jointless* L3-derived cells. These pedicels lacked abscission zones; however, in the region that was *++j*, raised discontinuous patches developed that were identical to those formed on pedicels of chimera *++j.*

All of these mericlinal chimeras indicate that whereas communication between cells occurs to coordinate development in chimeric pedicels, there was no lateral communication during abscission zone development detectable in this study. If lateral communication occurs, it does so over a very short distance and has very little effect on the overall development of the abscission zone.

Autonomous Development of Deep Pedicel Tissue

Each of the six periclinal chimeras was generated on more than one occasion, and the phenotypes of plants having a particular periclinal arrangement of cells did not vary. An exceptional case occurred in the generation of different *+jj* chimeras. Most periclinal shoots with the arrangement *+jj*

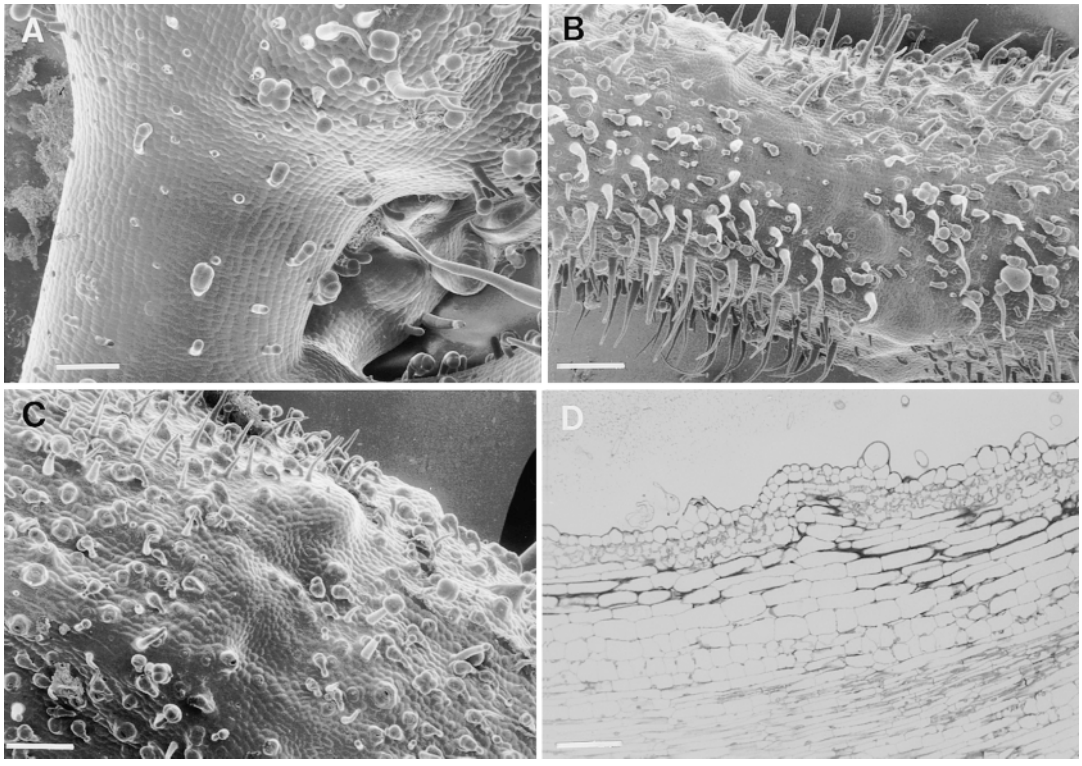

Figure 3. Pedicels of Chimeras *j+j* and *++j.*

(A) to **(C)** Scanning electron microscopy of pedicels. **(A)** shows developing pedicel of chimera *j+j.* Note the absence of a developing groove of an abscission zone. A mature pedicel of chimera *j+j*, which had severe expression of raised patches of tissue, is shown in **(B)**. Note the diagonal zone of raised patches of tissue. A mature pedicel of chimera *++j* is shown in **(C)**. Bar in **(A)** = 100 μm. Bars in **(B)** and **(C)** = 200 μm.
(D) Longitudinal section of plastic-embedded pedicel of chimera *++j* through a patch of raised tissue. Bar = 100 μm.

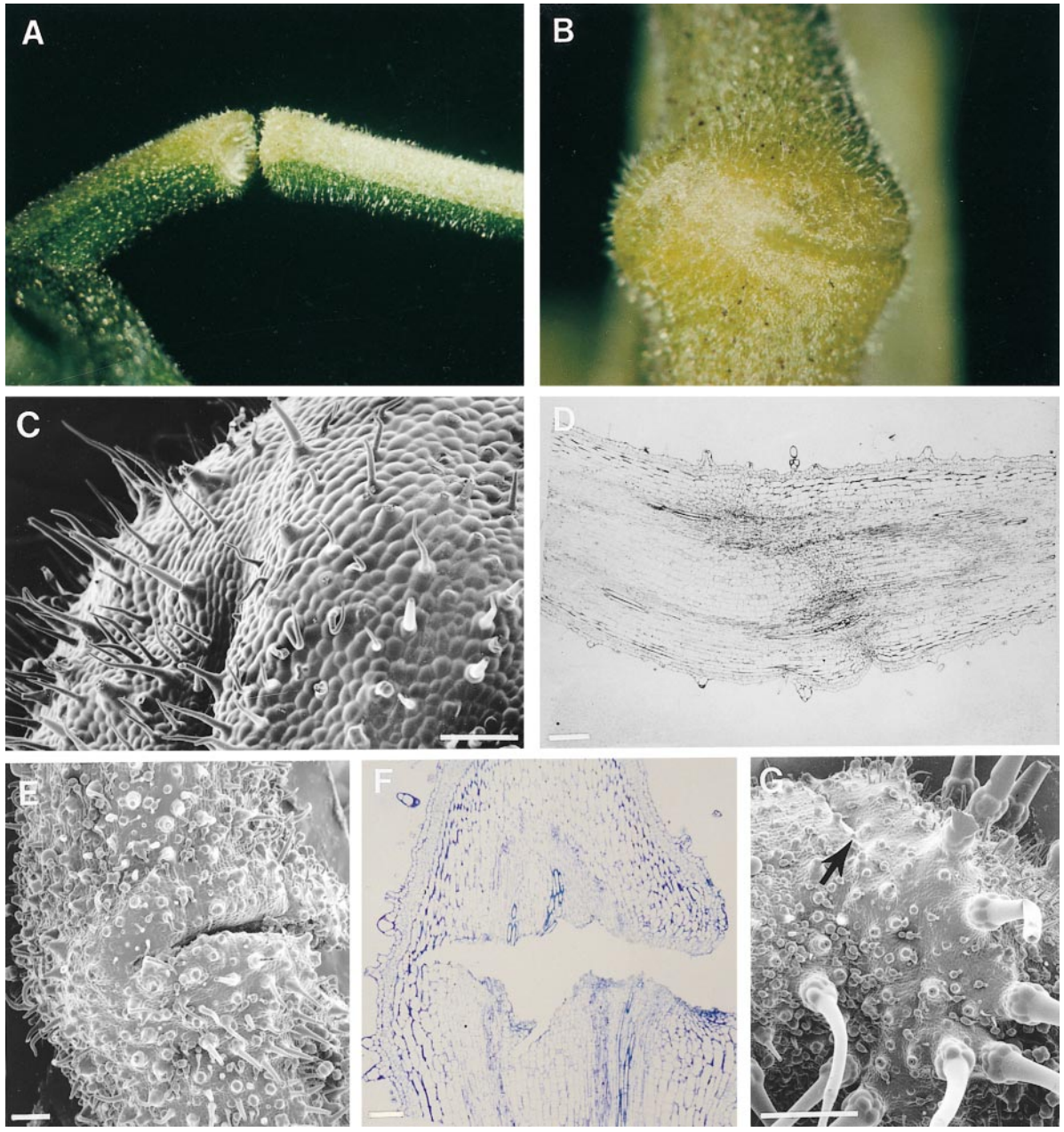

Figure 4. Sectored Pedicels.

(A) A sectored pedicel of the constitution *j++/jj+*. The yellow (*Xa*-2) portion of the pedicel has *jointless* L2-derived cells, and the green portion has wild-type L2-derived cells. All L3-derived cells were wild type, and abscission occurred uniformly across the pedicel.
(B) A sectored pedicel with the constitution *jj+/jjj*, showing normal abscission zone morphology on the *jj+* portion of the pedicel and no abscission zone on the *jjj* portion.
(C) Scanning electron microscopy of the pedicel shown in **(B)** at the border between the regions with and without abscission zone development. Bar = 200 μm.
(D) Longitudinal section of a plastic-embedded *jj+/jjj* split pedicel. Bar = 200 μm.
(E) Scanning electron microscopy of a split pedicel, similar to that shown in **(D)**, in which four-fifths was *jj+* and the rest was *jjj*. Bar = 200 μm.
(F) Longitudinal section through the abscission zone region of the four-fifths *jj+*, one-fifth *jjj* pedicel shown in **(E)**. Cell separation was limited to the regions having wild-type L3-derived tissue. Note plastid development in subepidermal tissue in the *jjj* portion of the pedicel, as would be found in a *jointless* pedicel. Bar = 200 μm.
(G) Scanning electron microscopy of a sectored pedicel of constitution *+j+/+jj*. The arrow indicates the boundary of the sector. Bar = 500 μm.

produced pedicels with a normal *jointless*-conferred phenotype. One event gave rise to a +*jj* shoot that, based on its combination of cell layer markers, should have been identical to the other +*jj* shoots. Instead, that shoot bore pedicels that showed unusual swelling at the midpoint of the pedicel even though there was no abscission zone. Such swellings were never observed in pedicels of the other +*jj* shoots.

The floral pedicels on the first inflorescence that arose on the unusual +*jj* shoot showed a gradation of phenotypes. The pedicel of the first flower had the most severe phenotype, with pronounced swelling at the site of the abscission zone, as shown in Figure 5A, but had no abscission zone–type groove or ability to abscise. The second-formed flower pedicel on that inflorescence (Figure 5C) exhibited milder swelling, and the third pedicel had a normal *jointless*-conferred phenotype with no swelling (Figure 5E). The inflorescence meristem reverted to vegetative growth, as is typical of the *jointless* plants (see section below). The swollen pedicel phenotype persisted for four sympodial cycles (in which the shoot meristem forms several leaves and converts to an inflorescence, and vegetative growth of the shoot is continued by reiteration of this pattern by the uppermost axillary meristem). The swollen pedicel phenotype also was observed in the first few inflorescences derived from axillary branches from this shoot. In later-formed inflorescences the swelling phenotype was no longer evident, and this shoot became like other +*jj* plants with typical *jointless* pedicels and leafy inflorescences.

Histological examination of the swollen pedicels showed a novel anatomy. The superficial layers of the pedicel in the region of the swelling had characteristics of cells in those positions in a *jointless* pedicel (Figure 5B). Internally, there were small cells typical of those found at the center of an abscission zone. Immediately external to the small cells were cells that were slightly larger than normal. There was a progressive decrease in the amount of abscission zone–type tissue in the cores of sequentially formed pedicels. The most severely swollen pedicels had the largest regions of small cells in the core (Figures 5B, 5D, and 5F). None of these pedicels abscised. We have not determined whether cell separation can occur among these small cells, but they may not have abscised simply as a result of the physical constraint of being confined by *jointless* pedicel tissues. In later-formed pedicels that had no detectable swelling, that is, had a typical *jointless* morphology, no small cells were present in the center.

The unusual swollen but jointless-pediceled shoot originated from a mericlinal shoot having the arrangement +*jj*/+++. That shoot eventually yielded three stable periclinal chimeras: +*jj*, ++*j*, and +*j*+. The other +*jj* shoots without the swollen pedicels arose from sectors that were of the constitution +*jj*/*jjj*. The difference between the two +*jj* pedicel phenotypes was most likely a result of their origins from different types of mericlinal meristems, with one having a portion composed of solid wild-type tissue, and the other with solid *jointless* tissue. To understand this difference, it is helpful to review the fates in the shoot of cells in different positions in the meristem.

The three persistent meristem layers (L1, L2, and L3) differ in the extent and complexity of their contribution to the meristem and the shoot. L1 and L2 constitute the tunica, which is made up of sheaths of cells that cover the corpus of the meristem. L3 generates a solid core of cells consisting of multiple nested lineages of cells. Some are near the surface, and others are deep within the meristem, but all contribute to the shoot. Cells deep within the meristem contribute very little if any tissue to organ primordia (Poethig, 1984) but contribute a substantial amount to stem tissue. Meristems in which the superficial lineages derived from L3 differ from the central core of cells can produce shoots in which the internal tissues of stems and similar structures, such as pedicels, are composed of cells of different genotypes. Eventually, all of the L3 will become uniform as derivatives of the L3 initials replenish the corpus through cell divisions in apical initials and their daughters.

It is likely that the swollen pedicel shoots originated from a meristem that had in its center a mixture of *jointless* and wild-type cells. These cells had been present as a mixed population during the initial recruitment of cells to form a new meristem in shoot regeneration from chimeric tissue (Figures 6A to 6E). Initially, in the sectored shoot that produced the unusual periclinal +*jj* shoot, the larger circumferential fraction of the sectored shoot, which gave rise to the +*jj* shoot, was uniformly made up of wild-type cells, whereas the smaller sector, +*jj*, was external to a core of wild-type cells. These two arrangements are designated +++[+] and +*jj*[+], referring to concentric tiers of cells forming the pedicel and not to stable meristem cell layers, because, it must be emphasized, there is no stable L4. The exact boundaries of the internal pedicel tissues of different genotypes from the mosaic meristems, unfortunately, cannot be distinguished with the markers used in this study; however, the progression of phenotypes associated with the process of layer rearrangement is consistent with the explanation that there is a shifting makeup of cells deep within the meristem.

A second case of autonomous development in the central tissue of the pedicel was found. In this instance, L3 was wild type, overlying a core of *jointless* cells in the meristem. Here, a shoot of chimera ++*j*, which does not form an abscission zone, produced an inflorescence whose pedicels had normal abscission zones. This atypical phenotype most likely arose as the result of a periclinal division in L2 of the ++*j* meristem and the consequent replacement of *jointless* cells with wild-type cells in L3. This replacement resulted in the formation of abscission zones that had a central core of *jointless* tissue. Histological examination of the pedicel formed during the transition from ++*j* derived to +++ derived, designated +++[*j*], showed large cells in the core characteristic of *jointless* pedicels, as shown in Figure 7A. Abscission occurred in the +++[*j*] pedicels, but it was not possible to determine whether cell separation took place in the central

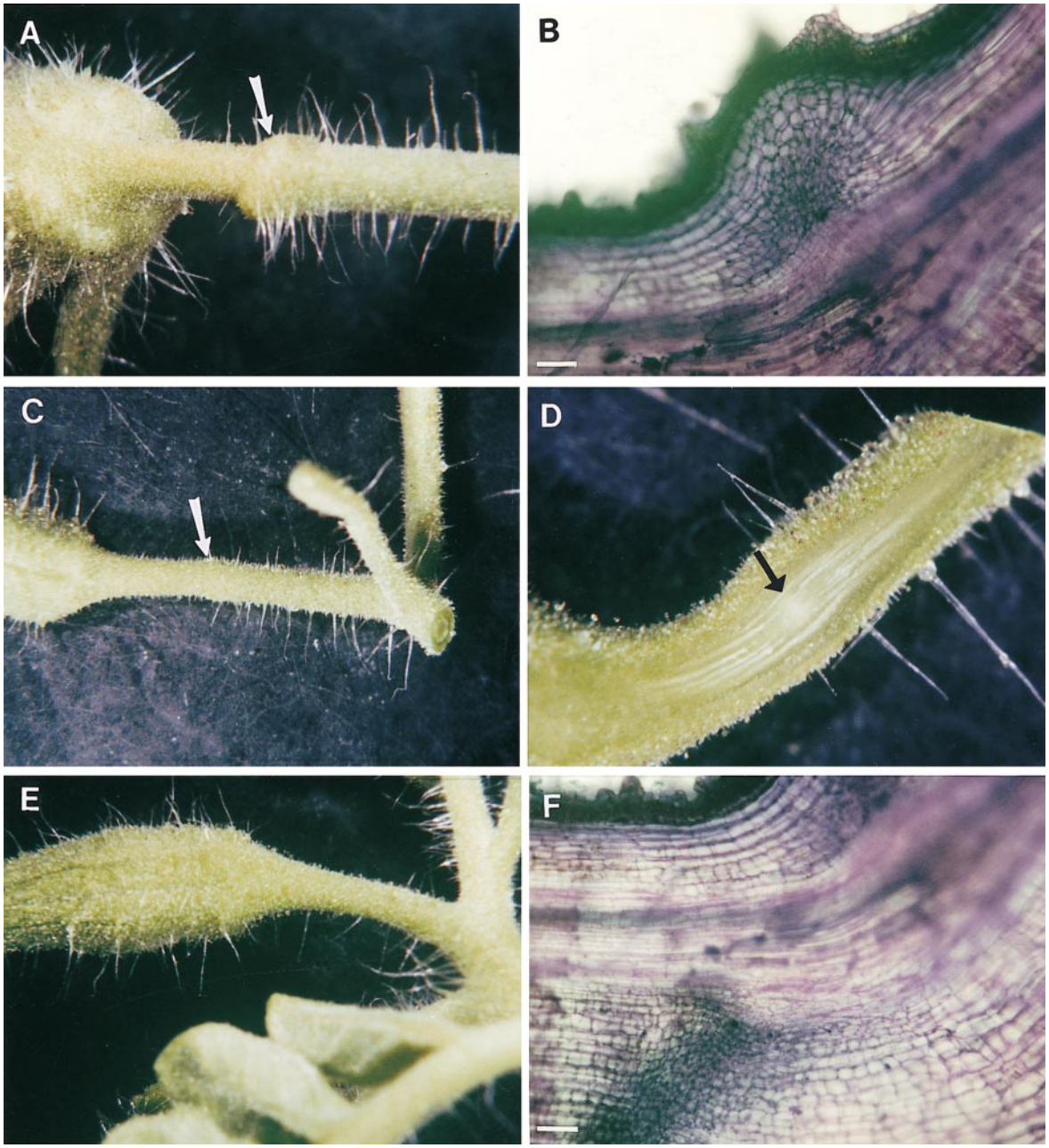

Figure 5. Autonomous Development of Deep Wild-Type Tissue in a *jointless* Pedicel: Chimera +*jj* from +*jj*/+++ Sector.

(A) and **(B)** First flower of inflorescence (+*jj*). In **(A)**, a mature pedicel with pronounced swelling (arrow) in the region of the abscission zone is shown. In **(B)**, a freehand-cut longitudinal section stained with toluidine blue O is shown in the region of swelling of the pedicel shown in **(A)**. Note that the normal abscission zone–type tissue extends almost to the outer cortex, whereas L1- and L2-derived tissues have a *jointless*-conferred phenotype. Bar in **(B)** = 100 μm.

(C) to **(F)** Second flower of inflorescence (+*jj*). In **(C)**, a mature pedicel shows slight swelling (arrow) in the region of abscission zone. In **(D)** is a freehand-cut longitudinal section in the region of swelling of the pedicel shown in **(C)** showing the core of abscission-like differentiation (arrow). In **(F)** is a freehand-cut longitudinal section of pedicel shown in **(C)** in the region of swelling, stained with toluidine blue O and showing abscission zone–type anatomy only within the vascular cylinder. **(E)** shows the third flower of inflorescence (+*jj*). It has a normal *jointless*-conferred phenotype. Bar in **(F)** = 100 μm.

tissue or whether the tissue simply ripped when the external tissues separated. In this case, the mixed internal tissue did not persist beyond the first inflorescence, and thus only three pedicels of this type have been observed.

A third case in which deep L3-derived cells contributed to and developed autonomously within the pedicel was a sectored shoot with the constitution *++j/+jj.* This shoot had both wild-type and *jointless* cells in the core of the meristem (*++j*[*j*]/*+jj*[+]). This sector is shown in Figure 7B. Pedicels that arose from the green (wild-type L2-derived cells) side of the shoot (*++j*[*j*]) showed no swelling and had a normal *jointless*-conferred phenotype. In contrast, pedicels arising on the yellow (*jointless* L2-derived cells) side of the shoot exhibited swelling similar to that found in the *+jj*[+] sector described above. The center of the pedicel, on the side that was *+jj*[+], had small cells in the abscission zone region (Figure 7C).

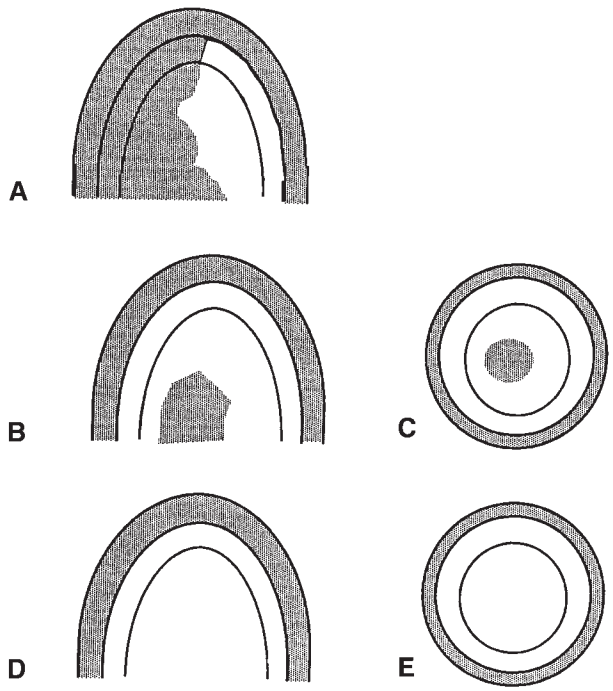

Figure 6. Schematic of an Example of a Transition in Meristem Core Composition.

A change in the identity of cells in L3 results in a mixed meristem core. Pedicels formed by mixed-core meristems may have central tissue that is different from outer L3-derived tissue. In this example, shaded is wild type and unshaded is *jointless.*

(A) A mericlinal chimeric meristem in which all of L1 is wild type and a fraction of L2 and L3 is wild type.

(B) A mericlinal chimeric meristem that could be derived as an axillary bud arising on the right side of a shoot whose meristem has the arrangement in **(A)**. The L1 is wild type, L2 is completely *jointless*, and L3 is *jointless*, enclosing a core of tissue that is mixed *jointless* and wild type.

(C) The pedicel tissue identities derived from the meristem shown in **(B)**.

(D) A stable meristem arrangement derived from the meristem shown in **(B)**.

(E) The pedicel tissue identities derived from the meristem shown in **(D)**.

The Genotype of L3 Cells Determines Inflorescence Meristem Activity

Coordination of *jointless* and wild-type cells within the meristem to maintain the inflorescence meristem state was examined in the six periclinal chimeras. Wild-type tomatoes form flowers in cymes. In this type of inflorescence, a predictable, line-dependent number of flowers are produced sequentially by the inflorescence meristem, which then ceases activity. In contrast, *jointless* plants have "leafy" inflorescences, which have one or two flowers at the base and continue with a vegetative, indeterminate shoot, forming leaves, stems, and axillary buds. Although some lines of tomato that have the wild-type *Jointless*$^+$ allele occasionally develop a leaf in an inflorescence, this is very different from the *jointless* leafy inflorescences, whose shoots contribute substantially to the mass of the plant. Examination of the phenotypes of the six periclinal chimeras revealed that the genotype of L3 cells determined the pattern of inflorescence activity (Table 1). The inflorescence phenotypes are shown in Figures 8A to 8H. Chimeras *j++*, *jj+*, and *+j+* had normal, determinate cymes like nonchimeric wild-type plants, whereas chimeras *++j*, *+jj*, and *j+j* had leafy inflorescences that were identical to those of *jointless* plants.

Development of inflorescences in chimeras was compared with those of nonchimeric wild-type and *jointless* plants by using scanning electron microscopy. In tomato, when reproductive development begins, the shoot apical meristem ceases initiating leaves and then can be identified as an inflorescence meristem. Morphologically, an inflorescence meristem first enlarges, followed by the formation of a furrow. This results in the formation of two meristems of different sizes. The larger meristem becomes a floral meristem and begins to initiate sepals, whereas the smaller meristem retains inflorescence meristem identity and enlarges, forms a furrow, and repeats this process. *jointless* inflorescence meristems undergo one or two cycles of floral meristem plus inflorescence meristem formation but then revert to a vegetative state. As shown in Figure 9, in wild-type plants (Figure 9D) and in chimeras with a wild-type L3 (Figure 9E), developing inflorescences showed a succession of less and less advanced floral meristems that eventually terminated with the inflorescence meristem. Floral meristems could be recognized by their whorls of floral organ primordia. Scanning electron microscopy of *jointless* inflorescences showed that they bore one or two floral meristems and terminated with a vegetative bud (Figures 9A to 9C). Vegetative buds could be recognized by the presence of leaf primordia arranged in a

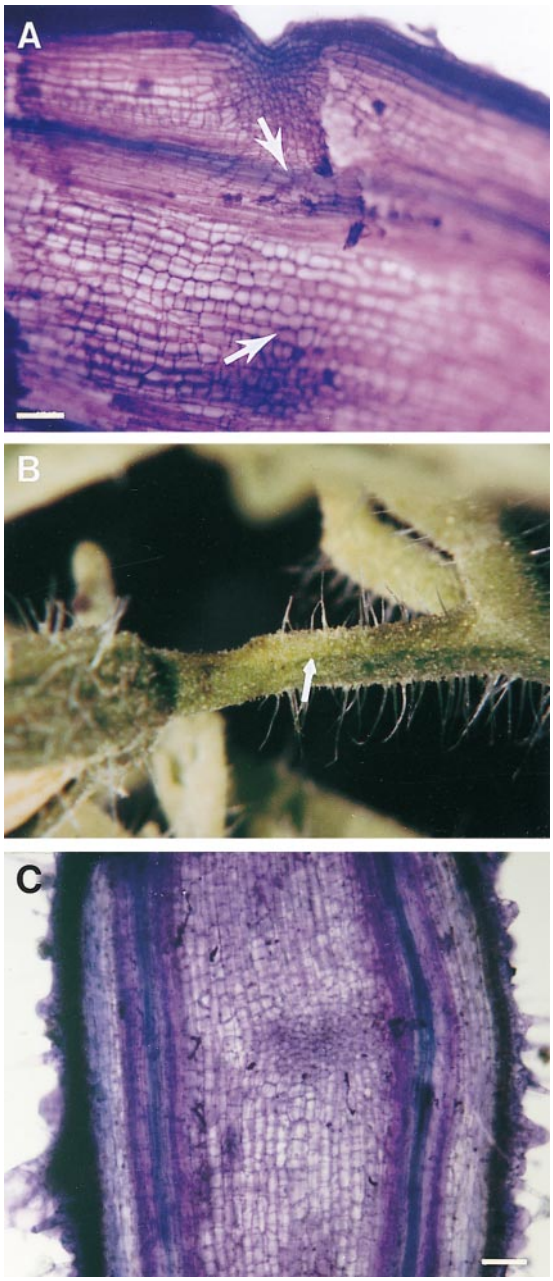

Figure 7. Autonomous Development of Deep Pedicel Tissue.

(A) Zone of large cells, typical of *jointless* pedicels, in the center of the abscission zone (boundaries indicated by arrows) from a pedicel with the constitution $+++[j]$. Bar = 100 μm.

(B) Mature pedicel. The green tissue has the constitution $++j[j]$ and lacks abscission zone–type tissue in the core. The yellow tissue has the constitution $+jj[+]$. The arrow indicates a sector boundry.

(C) Freehand-cut longitudinal section of the pedicel shown in **(B)**. Note the region of small cells characteristic of an abscission zone in the core of the pedicel on the right side. Bar = 100 μm.

spiral around the meristem. All chimeras with a *jointless* L3, $j+j$ (Figure 9F), $++j$, and $+jj$, developed identically to *jointless.*

Sectored inflorescences in which L3 was composed of a mixture of wild-type and *jointless* cells, as described above, produced flowers that clearly had maintained that mixture of cells. These flowers were normal and did not have vegetative sectors. None of the sectored inflorescences reverted to vegetative growth. This suggests that only a subset of the L3 cells needs be wild type to maintain the inflorescence meristem state and that maintenance of a meristem state involves a more global integration than does pedicel development. In inflorescences with the constitution $+jj[+]$, the deep wild-type tissue was lost before the third flower was produced, at which point the internal tissue was uniformly *jointless.* These inflorescences reverted to vegetative growth, like other plants with a *jointless* L3.

Position, and Not Clonal Origin, Determines Function

In four of the six periclinal chimeric arrangements, displacement of L3 cells by periclinal divisions in L2 resulted in a change from a wild-type to a *jointless*-conferred phenotype or vice versa. These are listed in Table 2. Each of these rearrangments, although uncommon, has been observed over the course of 6 years. In every case, the role of the newly located L3 cells changed from responding to underlying tissue to affecting overlying tissues, even though they were clonally related to L2 cells. For example, in chimera $j+j$, genotypically wild-type L2 cells behaved as if they were *jointless* in response to underlying *jointless* cells. Once this chimera rearranged to form $j++$ via a periclinal division that placed a cell clonally related to L2 in L3, that cell and its derivatives were then able both to behave according to their own genotypes and to signal overlying cells to behave in that same way. Thus, position and not lineage determined both how a cell differentiated and whether it was a signaler or a responder.

DISCUSSION

Coordination of Development by L3 Cells

Development of a complex, multicellular organism requires the harmonious functioning of groups of generative cells to produce its final component structures. Much of this integration may be accomplished by cell–cell signaling, in which one cell or group of cells provides information to neighboring cells to coordinate all of their activities. To understand the role of cell–cell signaling in plant development, one must first determine where, when, and in what

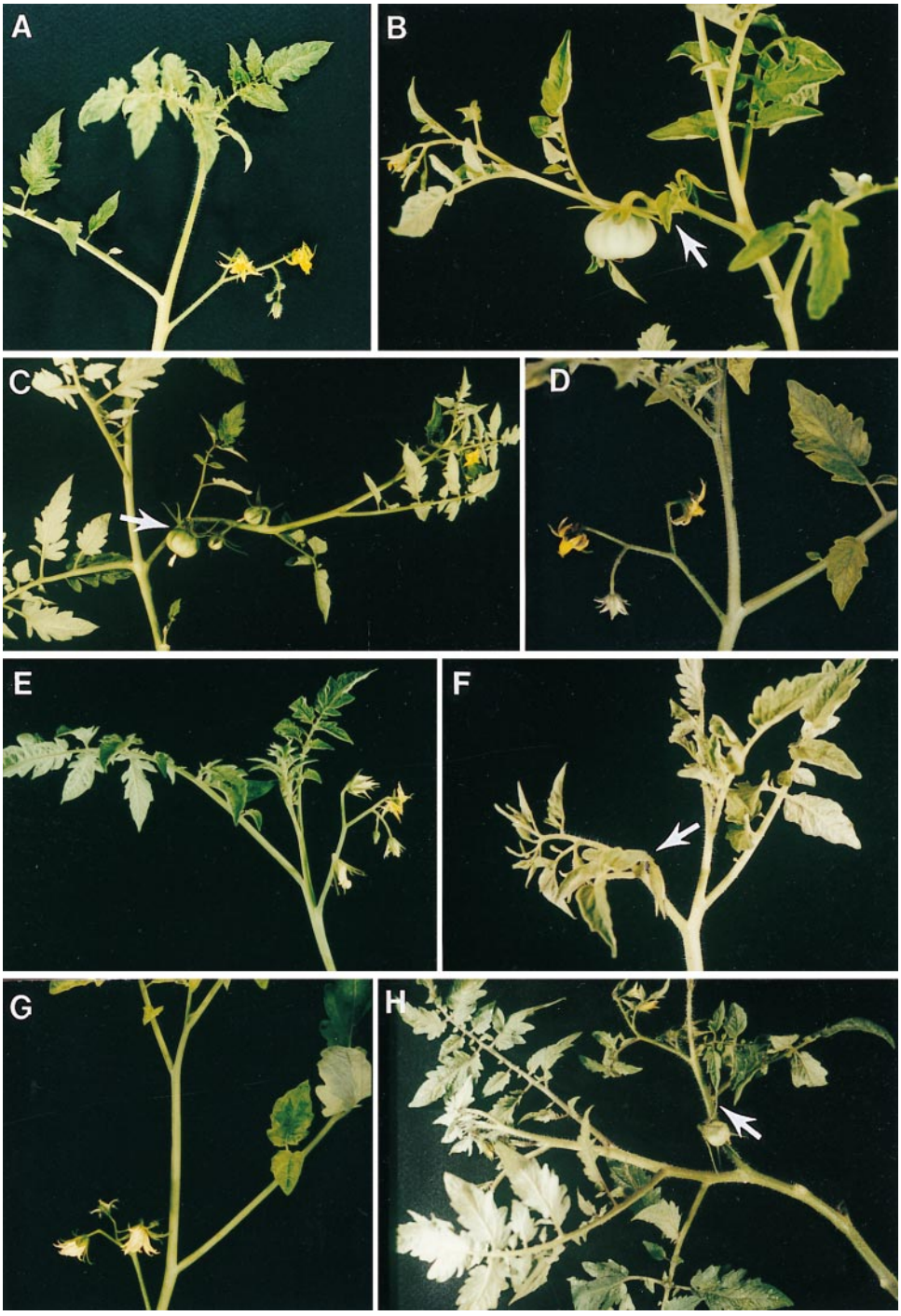

Figure 8. Inflorescences of Wild-Type, *jointless*, and Periclinal Chimeras.

(A) Wild type (+++).
(B) *jointless* (*jjj*). Note the leafy inflorescence (arrow).
(C) Chimera *j*+*j*. Note the leafy inflorescence (arrow).
(D) Chimera +*j*+.
(E) Chimera *j*++.
(F) Chimera +*jj*. Note the leafy inflorescence (arrow).
(G) Chimera *jj*+.
(H) Chimera ++*j*. Note the leafy inflorescence (arrow).

pattern signaling occurs. Chimeras in which cells differ for the manner in which some developmental process is conducted are extremely useful in determining these patterns.

In this study, we used periclinal chimeras and the *jointless* mutation to examine how genetically different cells interact in two developmental processes. One occurs within the meristem and maintains the inflorescence meristem state, and the other results in the formation of a mature structure, the integrated tissues of a pedicel abscission zone. Shoot meristems of *jointless* mutant plants, like wild-type shoot meristems, can complete the switch from vegetative to reproductive growth and subsequently form floral meristems. However, rather than continuing to form floral meristems, inflorescence meristems revert to a vegetative pattern of development. In the six periclinal chimeras generated between wild-type and *jointless* plants, a very clear correlation of phenotype with genotype of L3 cells was observed. Cells in L1 and L2 either maintained the inflorescence pattern of meristem function or reverted to vegetative development, according to whether *jointless* or wild-type cells constituted L3 and not according to their own genotypes. This result is identical to our previous results with tomato. In one study, we examined whether an organ primordium was initiated in chimeras with *lateral suppressor* (Szymkowiak and Sussex, 1993). In another study, we examined how cells interact within the meristem to maintain meristem size relative to the number of organs initiated in floral organ whorls in chimeras with *faciated* (Szymkowiak and Sussex, 1992). The consistent finding that L3 cells regulated events in the meristem suggests a general pattern of cell coordination within the tomato meristem in which L1 and L2 cells display extreme plasticity relative to their genotype; that is, how they develop is determined solely by underlying L3 cells.

The shoot meristem has a simple and relatively stable organization of sheets of cells overlying a central solid core of cells. Organs derived from the shoot meristem have a more complex arrangement of cells with respect to their origins from the three meristem lineages as a result of proliferation of cells to form organs that are much larger than the meristem. In addition, the pattern of proliferation from each lineage can vary in amount and pattern, even among different individual organs of the same organ type on the same plant (Szymkowiak, 1996). The chimeras generated in this study allowed us to examine cell interactions that occur in the formation of a relatively simple, shoot meristem–derived organ, the floral pedicel, and its associated abscission zone. The chimeras showed that in the pedicel, the genotype of L3 cells determined the presence or absence of a functional abscission zone. Thus, in *jointless* chimeras, L3 cells determined how development proceeded in a meristem, and L3 or L3 derivatives determined how development proceeded in a differentiated organ. Whether signaling that induces abscission zone development occurs early, while the cells that will give rise to it are still part of the floral meristem, or later, during pedicel development among derivatives of the meristem layers, is not known.

Partial Autonomous Development of Wild-Type L2-Derived Cells

The chimeric pedicels developed like either wild-type or *jointless* pedicels, except for chimeras in which wild-type L2-derived cells overlay *jointless* L3-derived cells, which resulted in a novel pedicel phenotype. In such pedicels, a variable number of small raised patches of L1- and L2-derived cells that had some qualities of wild-type abscission zone tissue was observed; however, these cells were not organized as an abscission zone, and cell separation associated with abscission was not observed in these tissues. It appeared that wild-type L2-derived cells were able to influence the development of L1-derived cells to participate in abscission zone–like development. They may or may not have affected L3-derived cell differentiation, depending on how many tissue layers of the pedicel are L2 derived; the cell layer markers used here did not allow us to make that determination. Therefore, it is possible that wild-type L2-derived cells perceive and respond to developmental information in a partially autonomous manner. Because wild-type L2-derived cells alone were not able to coordinate a normal abscission zone, even in the outer region of the pedicel, it is possible that some other factor, which would require a functional *Jointless*$^{+}$ allele for its production, might need to be provided by underlying cells. This factor would then act in concert with the *Jointless*$^{+}$ product to induce normal abscission zone development. Such a requirement for joint activity of two genes in chimeras has been demonstrated in snapdragon floral organ differentiation (Perbal et al., 1996). In periclinal chimeras in which L1 was genetically *deficiens* (*def*), neither *DEF* nor *GLOBOSA* (*GLO*) mRNA was detected in epidermis, because the DEF–GLO heterodimer is required to stabilize the expression of both genes. Nonetheless, the presence of *DEF*$^{+}$ cells in L2 and L3 resulted in the occurrence of both DEF and GLO in epidermal cells as well as the induction of petaloid epidermal differentiation. Microinjection studies have confirmed that DEF and GLO can traffic through plasmodesmata (Mezitt and Lucas, 1996).

An alternative explanation for the pedicel bumps is that L2 cells contributed to deeper tissue of the pedicel in small patches that our markers did not allow us to discern. These deeper, wild-type cells would then have been isolated islands of tissues in a position that enabled them to coordinate abscission zone development in L1- and L2-derived tissues. If divisions resulting in wild-type, L2-derived cells becoming located deeper occurred at a time later than would be needed for complete wild-type function, such patches of disorganized tissues might be expected.

Mericlinal chimeras provided information about lateral cell–cell signaling during development. Certain sectored pedicels had dramatic sharp delineations between normal abscission zone tissue and *jointless* tissue. These sectored pedicels derived from mericlinal meristems showed that, unlike the radial coordination by wild-type L3-derived tissues on outer L1- and L2-derived tissue during pedicel development,

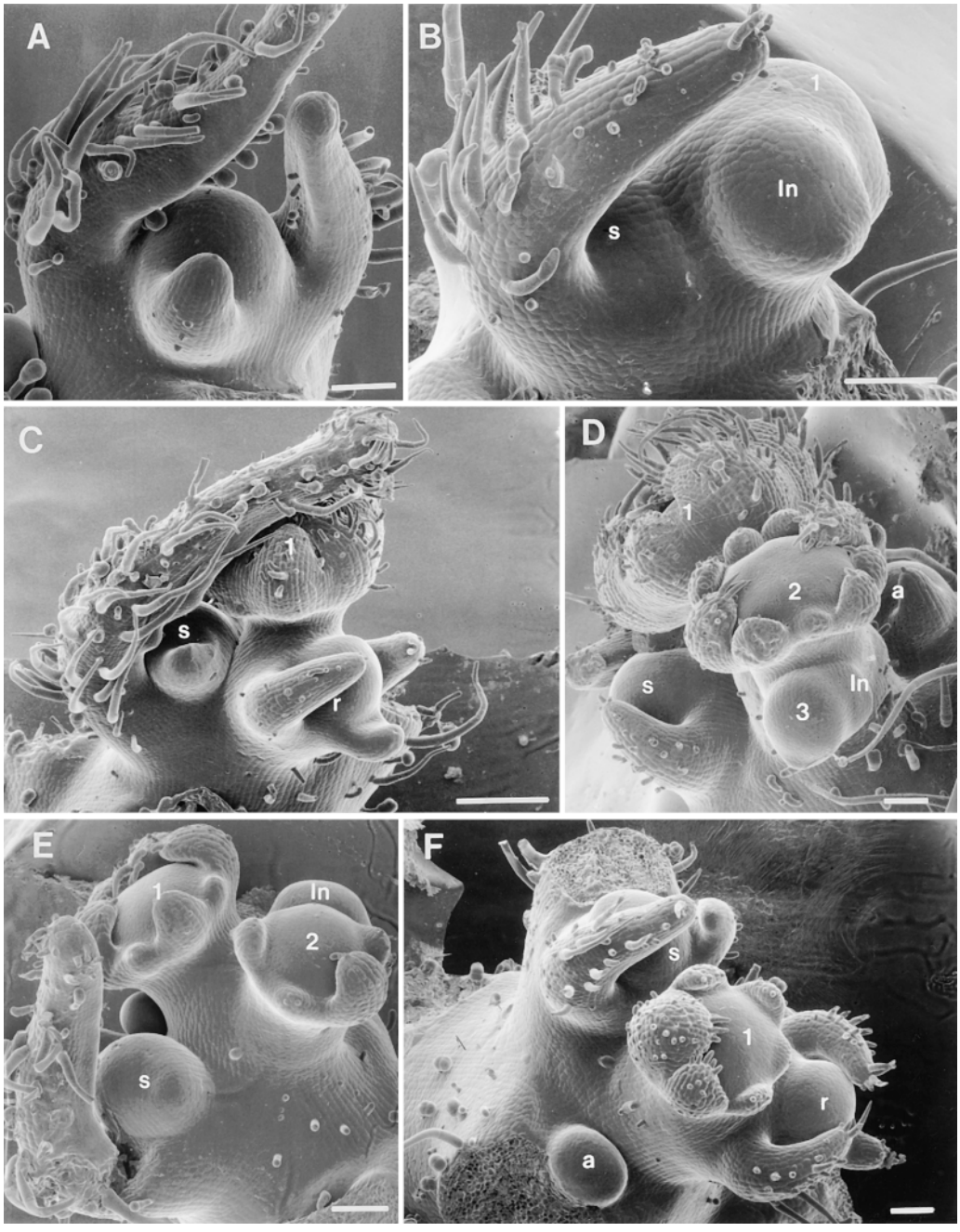

Figure 9. Scanning Electron Microscopy of Developing Inflorescences.

(A) to **(C)** *jointless.* **(A)** shows a vegetative shoot apex from a *jointless* plant showing the meristem surrounded by its three youngest leaf primordia. Wild-type shoot apices have identical morphology at this stage. In **(B)**, a reproductive shoot apex from a *jointless* plant shows the last-formed leaf primordium with the sympodial meristem in its axil and the incipient inflorescence, composed of the inflorescence meristem and a floral meristem. Wild-type shoot apices have identical morphology at this stage. The inflorescence meristem at this stage may form another floral meristem. **(C)** shows a later-staged *jointless* inflorescence, with the last-formed leaf and in its axil the sympodial meristem plus its first leaf primordium, and a single developing flower in the inflorescence. At the inflorescence terminus is the reverted meristem, surrounded by three leaf primordia.

(D) Wild-type inflorescence, with two developing flowers, a floral meristem, and the inflorescence meristem.

(E) Chimera *jj*+, showing a wild-type phenotype.

(F) Chimera *j*+*j*, showing a *jointless*-conferred phenotype.

1, first flower of inflorescence; 2, second flower of inflorescence; 3, third flower of inflorescence; a, axillary meristem; In, inflorescence meristem; r, reverted meristem; s, sympodial shoot. Bars = 100 μm.

there was no or very little influence laterally from L3-derived to adjacent L3-derived tissue or obliquely out to other L2- and L1-derived tissue in the adjacent sector. Although we focused here on the formation of the abscission zone and not on the physiology of abscission, it is remarkable how precisely morphology and function corresponded in these sectors. Unlike the clean splits between mutant pedicel and wild-type abscission zone tissues, however, no split flower/vegetative shoots were observed at the tips of sectored inflorescences at the position of expected vegetative reversion. Although we cannot rule out the possibility that such sectors present at the correct developmental time did not occur during the course of this experiment, these observations suggest that functionally split meristems cannot occur. Because a split meristem, in which one fraction retains the inflorescence state and the other reverts to a vegetative state, is likely to be dysfunctional, the plant may employ a mechanism that integrates the function of sectored meristems. It also may be that the flowering signal is diffusible within L3, unlike the signaling during abscission zone development, so that as long as any L3 cell in the inflorescence meristem is wild type, floral meristems will continue to be formed.

Outermost L3-Derived Cells Coordinate Development

One of the more interesting findings in these chimeras is the clearly autonomous development of the very deep tissue of pedicels in rare chimeras where this tissue is different from the outer L3-derived tissue. In these cases, the behavior of the outer L3-derived tissue and L1- and L2-derived tissue was identical to the stable chimeras of the same combination; only the inner L3-derived tissues both developed autonomously and did not influence the development of adjacent tissues. Without knowing when the information for induction of the abscission zone during development is provided and responded to, it is difficult to explain this autonomous development in the deep pedicel tissue. The swelling that occurs in these pedicels in the region of the abscission zone may be related directly to abscission zone formation or alternatively could be a consequence of the resolution of differential expansion of independently differentiating cells within the developing chimeric pedicel.

A clonal analysis of *jointless* and wild-type pedicels to determine whether the pedicels develop in the same manner, originating from equivalent proliferation patterns in pedicel founder populations, may provide insight into the basis for distorted pedicels. It is clear, however, that for a perfect abscission zone to form, wild-type *jointless* function must be present in this internal core tissue, although it alone is not sufficient for abscission zone development. This indicates a special role of the outermost L3-derived tissue in induction of abscission zone development: those cells must function normally for L1- and L2-derived cells to be coordinated to form an abscission zone, and when those cells are *jointless*, the pedicel is unable to differentiate an abscission zone.

Table 2. Periclinal Layer Rearrangements Change Roles of Cells from Responder to Signaler

Original Arrangement	New Arrangement	Original Phenotype	New Phenotype
jj+	*jjj*	Wild type	Jointless
j+*j*	*j*++	Jointless	Wild type
++*j*	+++	Jointless	Wild type
+*j*+	++*j*	Wild type	Jointless

Common Mechanisms of Coordinating Development

Are there multiple coordinating mechanisms in plants, one operating for each developmental process? We have found that for a number of developmental processes that occur within the meristem, development proceeds according to L3 genotype (Szymkowiak and Sussex, 1992, 1993; this study). This is in contrast to results with snapdragon periclinal chimeras having a single meristem layer with a functional *FLO* allele, in which wild-type function in any of the layers was sufficient for flower rather than inflorescence branch development, and in which the most normal phenotype was associated with *FLO* in L1 only (Carpenter and Coen, 1995; Hantke et al., 1995). Those results show that signaling directed by the *FLO* gene, although not strictly polar, preferentially occurs in the direction opposite that found in our tomato chimeras and in *def* chimeras of snapdragon (Perbal et al., 1996).

In cases of organ differentiation, such as in flowers of camellias (Stewart et al., 1972) and Arabidopsis (Bouhidel and Irish, 1996), L1-derived tissues determined the pattern of differentiation, although in neither case has the extent of L2 and L3 contribution to these organs been established. The margins of second whorl organs of snapdragon, which were most likely entirely composed of L1-derived tissues, in snapdragon *def* chimeras showed differentiation patterns according to their own genotype, even though in the center of those organs, L1-derived cells differentiated according to the genotype of the underlying L2- and L3-derived cells (Perbal et al., 1996). The novel floral phenotypes of all of the *flo* chimeras also show that tissues derived from the different meristem layers had a limited ability to undergo autonomous development during the process of floral organ differentiation. In the meristem-derived tissue of the tomato pedicel, we found evidence for some autonomous activity of L2-derived subepidermal tissue (Figure 3) and deep L3-derived tissue (Figures 5 and 7). It may be that events that occur within the meristem and very early in the development of a lateral organ are under greater developmental restrictions that require the meristem to function coordinately. As an organ progresses through differentiation, the restrictions may be loosened, with the result that autonomous development can be permitted. More examples of different developmental processes in a variety of model systems need to be examined to determine whether a certain pattern of signaling is associated with a type of process.

METHODS

Seed Stocks

Seed stocks of *Lycopersicon esculentum* lines carrying the mutations used here were provided courtesy of C.M. Rick and R.T. Chetelat (Tomato Genetics Resource Center, Department of Vegetable Crops, University of California, Davis).

Abscission Assay

Functional abscission zones were assayed by wounding a flower or by preventing fertilization through removal of the anther cone (Kendell, 1918). Otherwise, if fruits formed, a functional abscission zone allowed a clean break in the middle of the pedicel at harvest. Wounded or unfertilized flowers of *jointless* plants do not abscise, and *jointless* fruits when harvested separate from the calyx, not the pedicel.

Chimera Generation

Chimeras were generated incorporating autonomous cell layer markers as decribed previously (Szymkowiak and Sussex, 1992). *hairless* (*h*) was used to mark epidermal derivatives of the L1. *Xanthophyllic-2* (*Xa-2*) was used to mark L2 and L3 derivatives in leaves, stems, sepals, and pedicels. *anthocyanin gainer* (*ag*) was used to mark L2 derivatives in the stem. Although all of the markers showed up clearly in leaves and sepals and thus the chimeric arrangement of a plant could readily be established, L3 identity in stems and pedicels could not be determined directly from expression of markers.

Chimeras were generated directly from grafts, as described previously (Szymkowiak and Sussex, 1992), and from rare meristem layer rearrangements that occurred in preexisting chimeras, such as that shown in Figure 1B. These arose relatively frequently as sectors on chimeras that had been subjected to severe pruning, which stimulated rapid cycles of axillary bud outgrowth (in vivo chimera generation). For example, such treatment of chimera *jj+* yielded numerous *jj+/jjj* sectors. Sectors also were generated in new shoots arising from cultured leaf discs from periclinal chimeras (in vitro chimera generation).

Chimera identity was confirmed as described previously (Szymkowiak and Sussex, 1992), using the phenotype of self-pollination progeny to confirm the identity of L2 and regeneration of nonchimeric shoots through tissue culture to determine that no spontaneous mutations occurred that were responsible for the phenotype of the chimeras.

Tissue Culture

Additional chimeras with different cell layer combinations were obtained from the original chimeras by regenerating shoots in tissue culture, as described previously (Szymkowiak and Sussex, 1992). Expanding leaves were sterilized by soaking in 15% Clorox for 15 min and then were rinsed in two changes of sterile distilled water. Leaf discs or stem explants were placed in Petri dishes containing regeneration medium consisting of Murashige and Skoog salt base (JRH Biosciences, Lenexa, KS), 1 mg/L thiamine HCl, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine, 3 μM indoleacetic acid–aspartic acid, 5 μM zeatin riboside, 30 g/L sucrose, and 9.5 g/L agar. The medium was sterilized after the hormones were added. Plates were placed in a plant growth chamber that provided 16 hr of light per day. Shoots obtained from the cultured leaf discs or stem explants were transferred directly to the mist chamber and rooted in soil, where they were screened for chimeric sectors. Regeneration of chimeric shoots from stem explants was very effective.

Chimeras were stored as shoot tips on basal medium (regeneration medium without hormones) and passed to fresh medium every 4 months.

Scanning Electron Microscopy

Shoot tips and pedicels were examined using scanning electron microscopy with the replica cast technique of Sylvester et al. (1990). In this way, a sample could be examined by scanning electron microscopy yet be allowed to continue to grow. The replica cast technique also provided the option of examining the same sample both by scanning electron microscopy and by sectioning. Molds of the specimens were made using the dental impression material Extrude (Medium; Kerr Manufacturing, Romulus, MI). Molds were then filled with 2-Ton Epoxy (Devcon Corp., Des Plaines, IL) cement, which was allowed to set overnight. The casts were mounted on stubs, sputter-coated for 2 min with palladium, and viewed with a scanning electron microscope (model S-4000; Hitachi, Tokyo, Japan) at 5 kV accelerating voltage.

Histology

Pedicels were fixed in formalin:alcohol:acetic acid, embedded in plastic resin, sectioned by using a microtome, and stained with a 0.5% aqueous solution of toluidine blue O. Fresh samples were cut with a razor blade and stained with toluidine blue O.

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