REVIEW

Cell–cell interactions during plant development

Sarah Hake[1] and Bharat R. Char

Plant Gene Expression Center, United States Department of Agriculture–Agriculture Research Station and the University of California at Berkeley, Albany, California, 94710 USA

The large body of information that has come from genome sequencing projects has demonstrated that plants and animals have many sequences in common, suggesting that they have either co-opted the same molecules for different purposes, or that they utilize similar mechanisms (Reuzeau and Pont-Lezica 1995; Clark 1996). One might expect many housekeeping tasks, such as regulation of the cell cycle, to be similar in plants and animals, but what of the multicellular tasks, the developmental processes that coordinate growth and differentiation of cells? Unlike animal cells, plant cells never move, thus induction by cell movement such as occurs during gastrulation in animal embryo development, is not likely to occur. Lineage-based development would be convenient in plants as they are forever attached to their parent cell; however, with few exceptions (Sachs 1978), plant cells do not appear to differentiate based on lineage. How, then do plant cells find their cues?

A growing body of clonal analysis supports the statement that plant cells differentiate according to position (Steffensen 1968; Johri and Coe 1983; Jegla and Sussex 1989; Irish and Sussex 1992; Bossinger and Smyth 1996). Initial cells are as likely to contribute to somatic tissues such as leaves as they are to the eggs and sperm embedded in the flower; in other words, there are no sequestered germ cells. The cells of the epidermis, or outer layer, are clonally related, but if a cell divides such that a daughter cell is pushed into the internal tissues, that daughter cell takes on the fates of internal cells. Similarly, the internal cells that find themselves pushed into the epidermis differentiate as epidermis (Stewart 1978). Differentiation of cell types in the *Arabidopsis* root appears to follow specific lineages (Dolan et al. 1993, 1994; Scheres et al. 1994), however, laser ablation experiments have shown that ablated cells are replaced by cells from another lineage which differentiate according to their new position (van den Berg 1995). These experiments suggest that the apparent lineage of the root is a consequence of the simple and regular cellular arrangements. Positional information has also been shown to play a role in the differentiation of photosynthetic cell types in maize leaves (Langdale et al. 1989), as well as the spacing of hairs (trichomes) on the epidermis of *Arabidopsis* leaves (Larkin et al. 1996).

If development and differentiation are based on positional information in plants, we can then refine the question to ask how positional information is assessed, and how it is transferred between cells, that is, how do plant cells communicate. Although cell–cell interactions are also utilized in a plant's response to the environment and during sexual reproduction, we will focus our review on development and refer the reader to recent reviews on plant responses to wounding or disease (Hammond-Kosack and Jones 1996; Schaller and Ryan 1996) and pollination strategies as they relate to cell–cell interactions (Cheung 1995; Dodds et al. 1996).

Clonal analysis to study cell–cell interactions

Plants initiate new organs from meristems, totipotent stem cells that replenish themselves while producing determinate organs in a predictable pattern. Meristem maintenance occurs in the cells found toward the center of the meristem whereas organ primordia arise at the periphery. Angiosperm shoot meristems are constructed in layers. Depending on the species, one or two outer layers of cells divide as in a sheet with walls laid down perpendicular to the surface of the meristem. Below these outer layers, the new walls of the inner cells are laid down in all directions. The outer layer, L1, contributes to the epidermis of the plant, and the L2 and L3 contribute to the body of the plant in proportions that vary in different organs (Fig. 1) (Huala and Sussex 1993). The L2 usually contributes to the germ cells (Stewart 1978).

Clonal analysis has been used to investigate the interactions between genetically distinct cell types. A number of different techniques can be used to generate sectors in plants. In one method, two different genotypes or species are grafted together. Callus tissue at the site of the graft union produces adventitious shoots and occasionally a shoot forms that contains cells from both genotypes. Periclinal chimeras, in which two different genotypes contribute one or more of the layers, are stable, and can be propagated indefinitely from cuttings (Tian and Marcotrigiano 1993). Another method takes

[1]Corresponding author.
E-MAIL maizesh@nature.berkeley.edu; FAX (510) 559-5678.

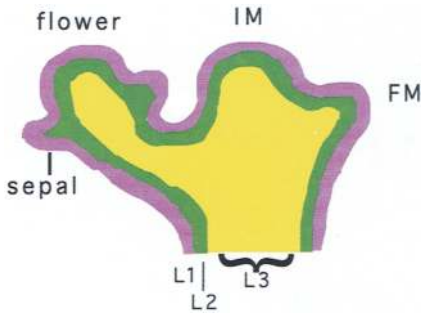

Figure 1. Cell layers in an Angiosperm floral apex. The inflorescence meristem (IM) bears floral meristems (FM) on its flanks which give rise to flowers. The L1 layer remains contiguous throughout development and organ formation, whereas the L2 and L3 layers contribute to organs in varying proportions.

advantage of transposon excision and has been used to generate sectors in maize (Dawe and Freeling 1990; Bossinger et al. 1992), *Antirrhinum* (Vincent et al. 1995), and *Arabidopsis* (Dolan et al. 1994; Bossinger and Smyth 1996; Larkin et al. 1996). The *Antirrhinum* transposon *Tam3* has the advantage of temperature-dependent excision, thus the amount or timing of sector formation can be controlled somewhat. A third method relies on the fact that plants tolerate hemizygosity. Plants that are heterozygous for a visible marker are irradiated as seeds or seedlings. Cells in which the dominant allele is lost following chromosome breakage produce clones of cells distinguishable from surrounding cells. A recent method takes advantage of transposon-induced suppressible alleles that are coordinately regulated (Martienssen and Baron 1994; Fowler et al. 1996; Martienssen 1996). Regardless of the method chosen to generate sectors, the end result is the same; genetically distinct cells are found adjacent to one another.

The boundary between genetically distinct cells is identified by cell autonomous traits, most of which involve the differentiation of single cells. The presence or absence of trichomes, wax deposition, color pigments, reporter genes such as β-glucuronidase, and functional chloroplasts all provide useful cell-autonomous markers. More recently, RNA expression patterns have also been used. The clonal sectors identified by these cell autonomous traits allow the investigator to determine the effect of gene products involved in processes such as meristem size or organ identity.

Cell interactions during maize leaf development

Maize leaves are characterized by the presence of three domains: blade, auricle, and sheath. The blade is wide and photosynthetic; the auricle consists of two wedge-shaped hinges that allow the blade to tilt out from the stem, and the sheath wraps around the stem. At the position between the auricle and blade an epidermal fringe called the ligule forms (Sharman 1942; Sylvester et al. 1990). Two genes are known to be required for normal ligule development, *liguleless1* (*lg1*) and *liguleless2* (*lg2*). Genetic analysis suggests they function in the same pathway, and phenotypic analysis shows that the mutant phenotypes are superficially similar, with a lack of auricle and ligule fringe (Harper and Freeling 1996).

Clonal analysis was performed using X-ray induced chromosome breakage in which mutant *lg1* cells were marked with a linked albino gene. Because of the nature in which maize leaves grow, sectors extended the length of the leaf. Sectors that were confined to the epidermis, that is, mutant epidermal cells overlying wild-type internal cells, were liguleless with normal auricles. Sectors with wild-type epidermis overlying mutant subepidermal tissue had a normal ligule but lacked auricle tissue (Becraft et al. 1990). The albino sectors that included all layers of the leaf lacked ligules and auricles. Thus, LG1 is needed in the epidermis to make ligule and in the subepidermal tissue to make auricle.

The borders of these sectors appeared sharp, suggesting that the adjacent wild-type tissue did not influence the liguleless sector. However, the ligule of the adjacent wild-type tissue was displaced downward in 40% of the sectors. These results suggested to Becraft and Freeling (1991) that a signal spreads from the midrib toward the margins in the normal propagation of ligule. If LG1 is required for appropriate transmissal of the signal, the sector of liguleless tissue not only prevents ligule formation but interferes with the propagation of the signal. In contrast to *lg1*, clonal analysis showed that *lg2* acts non-autonomously suggesting that it regulates a diffusible ligand (Harper and Freeling 1996).

A number of dominant mutants in maize disrupt the sheath and blade domains. *Knotted1* (*Kn1*) mutants are characterized by alterations to the cells along the veins of the blade. Foci of cells grow inappropriately compared to the surrounding cells, resulting in the production of hollow outpockets of tissue or knots (Fig. 2A) (Gelinas et al. 1969; Freeling and Hake 1985). Other cells along the veins have characteristics of sheath, auricle, or ligule cells (Sinha and Hake 1994). All layers of the leaf are affected. Clonal analysis of the *Kn1-N* allele showed that the genotype of the epidermis is irrelevant (Hake and Freeling 1986) and that the internal cells are responsible for the phenotype (Sinha and Hake 1990). In fact, a knot will form even if only a few of the cells surrounding the vein carry the dominant mutant allele (Fig. 2B). Thus, a product made by internal cells induces adjacent cells to divide and differentiate abnormally. More distant cells, such as those in the adjacent vein, are not influenced by the *kn1* gene product.

kn1 is a member of a homeobox gene family (Vollbrecht et al. 1991). Other members of this family, such as *roughsheath* (*rs1*), *liguleless3* (*lg3*), and *liguleless4* (*lg4*), are also expressed in the meristem or subdomains of the meristem (Jackson et al. 1994; Kerstetter et al. 1994) and produce dominant leaf phenotypes when ectopically expressed (Freeling 1992; Schneeberger et al. 1995; Fowler and Freeling 1996). The *Lg3-O* mutation transforms blade, auricle, and ligule cells near the midrib into sheath-like cells (Fowler and Freeling 1996). Similar to

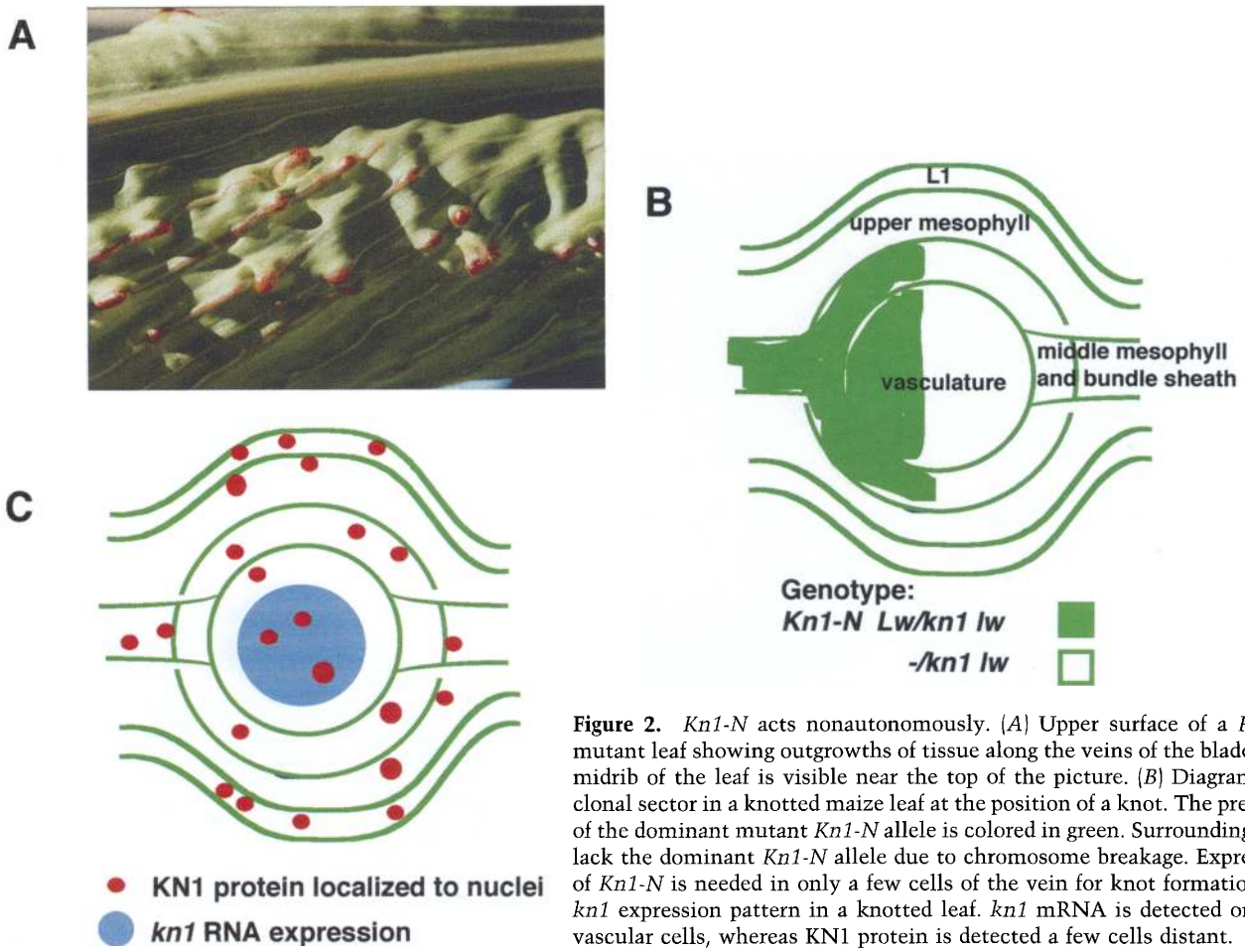

Figure 2. *Kn1-N* acts nonautonomously. (*A*) Upper surface of a *Kn1-N* mutant leaf showing outgrowths of tissue along the veins of the blade. The midrib of the leaf is visible near the top of the picture. (*B*) Diagram of a clonal sector in a knotted maize leaf at the position of a knot. The presence of the dominant mutant *Kn1-N* allele is colored in green. Surrounding cells lack the dominant *Kn1-N* allele due to chromosome breakage. Expression of *Kn1-N* is needed in only a few cells of the vein for knot formation. (*C*) *kn1* expression pattern in a knotted leaf. *kn1* mRNA is detected only in vascular cells, whereas KN1 protein is detected a few cells distant.

the findings with *Kn1-N, Lg3-O* internal cells alter the fates of wild-type epidermal cells, but not vice versa. Unlike the results with *Kn1-N* where the lateral signaling was confined to adjacent cells of the same vein, mutant *Lg3-O* had an effect on cells many veins distant (Fowler et al. 1996). *Rs1-O* mutants, which have disorganized ligular regions with sheath-like tissue at the base of the blade, showed an even greater degree of nonautonomy (Becraft and Freeling 1994). A lateral nonautonomous effect of *Rs1-O* was seen in almost all sectors that extended the length of the leaf (Becraft and Freeling 1994).

A potential reason for the difference between *Kn1-N* and the other two dominant mutants could be the tissue affected. Both *Rs1-O* and *Lg3-O* affect the ligule region, whereas *Kn1-N* affects cells surrounding the vasculature of the blade. Ectopic ligule is not seen in any of these dominant mutants in a *lg1* background, although all other aspects of the dominant mutant phenotypes are unaffected, suggesting that the dominant mutations ectopically induce *lg1* (Freeling and Hake 1985; Becraft and Freeling 1994; Fowler and Freeling 1996). Although it is not known whether *Rs1-O* and *Lg3-O* are capable of ectopically inducing *lg2*, such a result is quite likely. A possible explanation for the long distance effect of *Rs1-O* and *Lg3-O* may be their induction of *lg2*, which then acts nonautonomously to influence adjacent sectors of wild-type tissue.

Cell–cell interactions during flower development

The shoot apical meristem undergoes a transition from a vegetative meristem, which initiates leaves, to an inflorescence meristem which initiates floral meristems from its flanks (for review, see Huala and Sussex 1993). Typical dicotyledonous flowers develop with four whorls of organs. The outer whorl contains the leaf-like sepals, the second whorl contains petals, the third whorl is stamens, and the inner whorl is carpels. A number of mutants have been studied in tomato, *Antirrhinum*, and *Arabidopsis* that affect the number of organs or identity of organs (Coen 1991). Periclinal chimeras are beginning to help unravel the cell–cell interactions involved in floral organ development.

fasciated (*fas*) tomato mutants have a larger meristem that results in an increased number of flower organs; there are approximately twice as many petals, three times as many stamens, and four times as many carpels (Szymkowiak and Sussex 1992). Two different periclinal chimeras were identified that were wild type for *fas* in either the L1 only or the L1 and L2 (Szymkowiak and

Sussex 1992). The L1 chimera had organ numbers similar to the *fas* mutants, whereas the L1L2 chimera produced flowers with 1.5 times as many petals and stamens and 3 times as many carpels. Examination of the cells in the L1 of both chimeras showed that there was an increase in cell number. Thus, the underlying *fas* tissue was able to induce extra cell divisions in the wild-type layer(s) and, by the same token, the wild-type layer(s) were not able to correct the *fas* deficiency. By its recessive nature we assume that the wild-type FAS protein normally prevents the meristem from overexpansion, possibly by repressing a diffusible signal. The difference between the two chimeras may simply be due to the amount of *fas* tissue, that is, amount of diffusible signal emanating from the mutant L2 and L3 or just the mutant L3. What influence a *fas* L1 would have on floral organ number remains to be determined.

Mutants have been described in *Antirrhinum* and *Arabidopsis* that are blocked in the transition from inflorescence meristem to floral meristem (Coen and Meyerowitz 1991). The *Antirrhinum* mutant, *floricaula* (*flo*), produces inflorescence branches in place of flowers. The *flo* gene, cloned by transposon tagging, encodes a novel protein of 396 amino acids that is expressed in floral meristems (Coen et al. 1990). Expression disappears from the center of the floral meristem as floral organs initiate but returns in sepal, petal, and carpel primordia (Coen et al. 1990; Hantke et al. 1995). Owing to the instability of the transposon allele, revertant branches were found that could be maintained indefinitely from cuttings (Carpenter and Coen 1995). Analysis of *flo* RNA expression in floral meristems of revertant branches revealed three different types of periclinal chimeras (Hantke et al. 1995). The L1 periclinal chimeras, in which *flo* RNA was only detected in the L1 layer, produced almost normal flowers. The L3 chimeras produced abnormal flowers and flowers from L2 chimeras were of intermediate appearance (Fig. 3). These results suggested that the presence of *flo* in one layer was sufficient to activate flower development in the other layers.

The effect of *flo* gene expression in the periclinal chimeras was also studied by expression analysis of *deficiens* (*def*), a MADS box gene that is regulated by *flo* (Hantke et al. 1995). *def* is required for stamen and petal identity and is expressed in these organ primordia (Sommer et al. 1990). In the absence of *flo*, *def* expression was not detected, however, *def* expression was detected in all three layers of *flo* L1 and L3 periclinal chimeras. The timing of *def* expression was delayed relative to wild type and the domain of expression was reduced (Hantke et al. 1995). These results clearly demonstrate that *flo* activates gene expression across cell layers. Whether the *flo* gene product itself moves from layer to layer or an intermediate molecule moves to transmit the *flo* signal is yet to be determined.

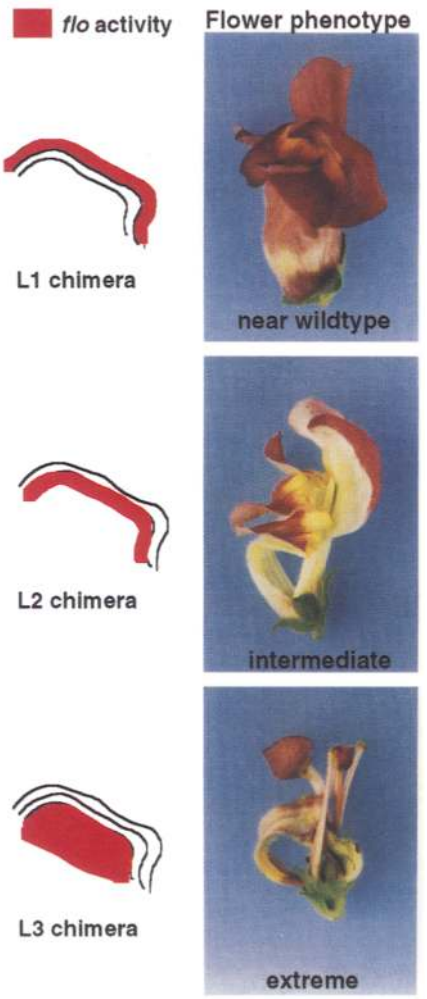

Figure 3. The presence of *flo* in one cell layer can affect flower development in other layers. L1 chimeras, which have *flo* gene activity in the epidermis only, produce normal-looking flowers (*top*). L2 chimeras produce flowers that are somewhat affected (*middle*), whereas L3 chimeras give rise to abnormal flowers (*bottom*) (Carpenter and Coen 1995; Hantke et al. 1995). In the absence of all *flo* activity, the flowers are transformed into inflorescences (Coen et al. 1990). (Flower photos reprinted, with permission, from Carpenter and Coen 1995).

Plasmodesmata create cytoplasmic continuity between cells

What mechanisms are available for plant cell interactions? Plant cells share cytoplasmic continuity between cells through pores called plasmodesmata. Plasmodesmata are membrane-lined channels across the cell wall that allow electrical continuity and the flow of small molecules. In the middle of the plasmodesmata is a strand of appressed endoplasmic reticulum (Fig. 4). The cytoplasm connecting the cells runs between the plasma membrane and the endoplasmic reticulum (ER). Protein bodies are embedded in both these membranes when visualized by transmission electron microscopy (Ding et al. 1992). Thus, plasmodesmata differ quite dramatically in structure from the counterpart pore in animal cells, the gap junction, although their basal size exclusion limits, around 1000 kD, are similar (for review, see Gunning

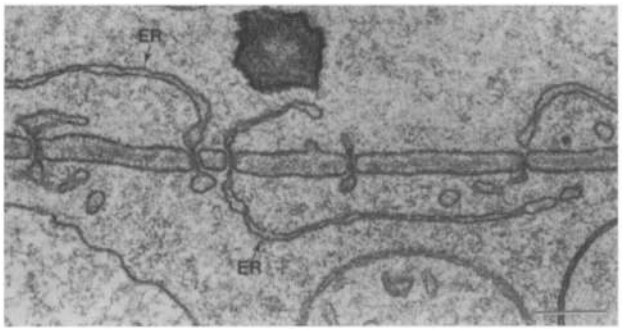

Figure 4. Electron micrograph of primary plasmodesmata from *Zea mays* root tip. Strands of appressed endoplasmic reticulum (ER) extend through the plasmodesmata. (Micrograph reprinted, with permission, from Lucas et al. 1993).

and Robards 1976; Robards and Lucas 1990; Lucas et al. 1993). More significantly, plasmodesmata differ from gap junctions in the ability of the plant to regulate the size of the pore, as will be discussed later.

Primary plasmodesmata form during the wall formation that accompanies cell division and secondary plasmodesmata form through existing walls. Primary walls arise by fusion of vesicles at the phragmoplast, a specialized structure that contains microtubules oriented perpendicular to the developing new wall (Staehelin and Hepler 1996). Plasmodesmata form due to the interruption of this growing wall by perpendicularly positioned ER. Exactly how secondary plasmodesmata form is not known, but clearly, enzymatic digestion of the wall must occur. Detailed structural information has come from studies of secondary plasmodesmata formation at a graft union (Kollman and Glockmann 1991). These investigators were able to document concerted wall thinning that allowed the plasmalemma and endoplasmic reticulum of the genetically different cells to fuse. How the two cells coordinate wall thinning is not known. They also documented many half plasmodesmata, suggesting that coordination between the cells did not always occur.

Mechanisms for cell–cell interactions: viral movement proteins as a paradigm

Plant viruses have evolved clever ways to move around in plants. A large volume of data has demonstrated clearly that many viruses encode movement proteins that facilitate the movement of infectious material through plasmodesmata (Citovsky and Zambryski 1991; Citovsky 1993; Mezitt and Lucas 1996). Movement proteins have been detected immunologically in plasmodesmata of virus-infected plants and transgenic plants overexpressing movement proteins (Tomenius et al. 1987; Wolf et al. 1989; Atkins et al. 1991; Moore et al. 1992). That movement proteins are capable of increasing the size exclusion limit (SEL) of plasmodesmata has been shown by measuring the SEL of transgenic plants overexpressing movement proteins (Wolf et al. 1989) and by injecting fluorescently labeled dextrans into intact plant cells along with the movement protein (Waigman et al. 1994). In both types of experiments, the SEL increases from 1 kD to 10–20 kD. Injection of fluorescently labeled movement proteins into leaf cells has shown that the protein itself can rapidly move from cell to cell (Fujiwara et al. 1993; Nouiery et al. 1994; Waigman et al. 1994; Ding et al. 1995). Movement proteins have been shown to bind nucleic acids in vitro (Citovsky 1993 and references therein) and facilitate the movement of labeled nucleic acids when co-injected (Fujiwara et al. 1993; Nouiery et al. 1994; Ding et al. 1995). Virus movement proteins seem to show selectivity for the type of nucleic acid they transport but not the sequence (Mezitt and Lucas 1996). For example the Red Clover Necrotic Mosaic Virus (RCNMV) movement protein of this RNA virus traffics RCNMV RNA as well as nonspecific RNAs, but not single- or double-stranded DNA (Fujiwara et al. 1993).

The association of microtubules with the TMV P30 movement protein (Heinlein et al. 1995) suggests that viral movement proteins use the cytoskeleton to move viral RNA to the plasmodesmata (Oparka et al. 1996). Similar conclusions were reached by antibody colocalization of the P30 movement protein and microtubule proteins in protoplasts, as well as by in vitro binding studies (McLean et al. 1995). Colocalization of actin and P30 was also documented, but to a lesser degree (McLean et al. 1995). Support for an association of actin with plasmodesmata comes from the finding that the SEL is increased following injections of actin inhibitors such as cytochalasin D (Ding et al. 1996). Deletion and alanine-scanning mutagenesis have identified functional domains for a number of movement proteins (Giesman-Cookmeyer and Lommel 1993 and references therein), but as yet, no common structural domains have emerged (Mezitt and Lucas 1996). Determining exactly how movement proteins function in intercellular trafficking may require the isolation of the endogenous plant proteins with which they interact.

Do transcription factors traffic between plant cells?

Clonal analysis has shown that many gene products, including transcription factors, function nonautonomously to influence adjacent cells. Such experiments, however, do not address by what means the influence takes place. KN1 localizes to the nuclei of meristems and the stem, and is normally absent from leaves (Smith et al. 1992). Comparison of *kn1* RNA localization and protein has shown some discrepancy in the expression patterns. In leaves carrying the dominant mutation, RNA is tightly restricted to three or four cells in the center of a vein and the protein is found distributed further, including the epidermis (Fig. 2C). Examination of expression in the meristem, where *kn1* functions in wild type, showed protein in the L1 but not RNA (Jackson et al. 1994). These results imply that either differential RNA degradation occurs or the protein moves into adjacent cells.

Experiments by Lucas and his colleagues have shown that KN1 protein can move between plasmodesmata in tobacco and maize leaves (Lucas et al. 1995). Similar to viral movement proteins, the SEL is increased to 20 kD, and other proteins traffic when co-injected. A series of mutations were made in the KN1 protein. Most mutations decreased the rate of movement, but a mutation in a putative nuclear localization signal (Meisel and Lam 1996) blocked movement altogether. Surprisingly, KN1 was also able to traffic its own RNA, but unlike TMV movement protein, it did not traffic other tested RNAs (Lucas et al. 1995). Whether KN1 also moves in the meristem and whether endogenously encoded *kn1* RNA traffics remains to be determined. A recent observation of sucrose transporter mRNA localized to enucleate sieve elements as well as the companion cells, which are cytoplasmically connected via the plasmodesmata, supports the ability of RNAs to traffic between cells (Kühn et al. 1997).

Schwarz-Sommer and colleagues recently presented more direct evidence that plant proteins can move from cell to cell using periclinal chimeras (Perbal et al. 1996). Both *def* and *globosa* (*glo*) are required for the identity of floral organs in the second and third whorl floral organs; petals are replaced by sepals and stamens have carpel identity in *def* or *glo* mutant plants (Sommer et al. 1990; Schwarz-Sommer et al. 1992; Tröbner et al. 1992). *glo* and *def* are induced independently but require each other for upregulation; in the absence of one protein, the other protein is missing (Schwarz-Sommer et al. 1992; Tröbner et al. 1992).

In the petals of *Antirrhinum* flowers, the L1 contributes the epidermis and the internal tissues at the margins of the petal, whereas the L2 contributes the internal tissue except at the margins. L1 *def* chimeras, in which only the L1 was wild type, had a normal petal epidermis and normal margins (Fig. 5). The internal tissue, except at the margin, had sepal identity, that is, was mutant. Petals of L2 chimeras, on the other hand, were fairly normal, including the epidermis. Only the margins showed mutant characteristics (Fig. 5). These results suggest that DEF acts autonomously in the L1, but nonautonomously in the L2.

def and *glo* RNA and protein were examined in these chimeras. In the L1 chimeras, *def* RNA and protein were confined to the L1 as was GLO protein. GLO RNA and protein did not accumulate in the L2, presumably owing to the lack of DEF. In the L2 chimera, *def* RNA was confined to the L2 of *def* chimeras, but GLO RNA and protein were detected in all layers. Because sustained *glo* transcription requires *def*, the presence of GLO protein in the L1 indicated that DEF somehow upregulated *glo* transcription across cell layers. Examination of DEF protein in these chimeras showed the presence of DEF in the L1, at least later in flower development. This result strongly supports the movement of DEF protein from L2 to L1 and suggests that direct movement of DEF is responsible for the activation of *glo* in the L1 layer and thus the presence of normal-appearing epidermal cells.

Although these chimeras suggest that polar communication between cell layers occurs, some degree of cell autonomy exists within layers. For example, sectors of wild-type epidermal cells were surrounded by mutant cells in unstable *def* mutants (Carpenter and Coen 1990), and the margins of the *def* L2 chimera petals, which are L1-derived, were not rescued by adjacent wild-type L2 cells. Similarly, sectors that bisect *flo* flowers longitudinally produced half normal and half abnormal flowers (Carpenter and Coen 1995). These results may speak to the difference between the primary plasmodesmata within layers and the secondary plasmodesmata between layers, or to a simple problem in distance. The interdermal distances are one to two cells in the meristem. If intradermal communication occurred, the sector boundary might change by one or two cells and not be detected.

Many genes have been shown to function nonautonomously (Poethig 1988; Carpenter and Coen 1990; Dudley and Poethig 1993; Becraft and Freeling 1994; Bouhidel and Irish 1996). Whether direct movement of the gene product between cells can explain other examples of nonautonomy remains to be determined.

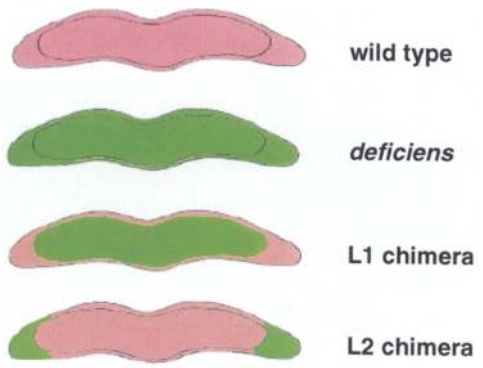

Figure 5. Schematic diagram of chimeric petal cross sections. The L1 contributes to the epidermis and internal tissue at the margins; the L2 contributes to the bulk of the internal petal tissue. Wild-type tissue is shown in pink, *def* tissue in green. In L1 chimeras, the petals have a normal epidermis, but mutant internal tissue. L2 chimeras, however, have normal internal tissue as well as a normal epidermis, except at the margins. DEF and GLO protein were detected in the wild-type tissue (pink). These results suggest that DEF protein is capable of trafficking from the L2 to the L1, but not vice versa (Perbal et al. 1996).

Mechanisms to regulate cell–cell communication

If it is true that numerous regulatory proteins are capable of moving from cell to cell, what are the mechanisms that prevent total chaos? Microinjection experiments demonstrate that the SEL can be regulated during development and can differ between cell types. Histological analysis has suggested that the number of plasmodesmata is often correlated with developmental processes. We discuss a few of the experiments that have been carried out to investigate these potential mechanisms.

Regulating the SEL

Certain types of differentiated cells sever their cytoplasmic connections to neighboring cells. The epidermis of the *Arabidopsis* root is characterized by files of two different cell types, hair cells and nonhair cells. The hair cell file is separated by one to two nonhair cell files (Dolan and Roberts 1995). To determine the SEL of cells within files and between files, low-molecular-weight probes were microinjected into the cytoplasm of epidermal cells and the movement of the fluorescent probe followed. Cells in the growing tip showed movement of the dye within cell files, between files, and into the cortex. However, when cells that had begun to differentiate were injected, the dye remained in the injected cell (Duckett et al. 1994). Thus, the cytoplasmic connection between root cells was shut off as the root hairs began to differentiate. Another example is stomatal guard cells (Palevitz and Hepler 1985); injection experiments have shown that the precursor cells to guard cells allow dye movement, but mature guard cells do not. The cells surrounding differentiated guard cells still permit movement through the plasmodesmata.

Each cell type may respond to endogenous movement proteins in its own fashion, for example, trichome hair cells of tobacco leaves have a SEL of 7 kD, in comparison to the 1 kD SEL of mesophyll cells. Differences are also found between trichome and mesophyll cells in the ability of movement protein to traffic other molecules following microinjection (Waigman and Zambryski 1995).

The role of Ca^{2+} in SEL regulation

The mechanisms by which the SEL could be lowered are unknown, however, elevated levels of Ca^{2+} are correlated with a decrease in SEL (Erwee and Goodwin 1983; Tucker 1990). In animal cells, calcium waves are transmitted via functional gap junctions. The unidirectionality of the wave is achieved by means of a transient closing of the gap junction after the signal has been transmitted. This gap junction closure is effected by intracellular Ca^{2+} (Sanderson 1995). In plants, small, hydrophilic, and anionic molecules readily pass through plasmodesmata, but large cationic ones fail to do so (Tucker and Tucker 1993). Inositol-1,4,5,-triphosphate (IP_3 and Ca^{2+} are representative of the two such molecules, respectively, and both effect blockage of cell–cell diffusion of fluorescent markers through plasmodesmata (Tucker 1990).

Tucker and Boss (1996) have directly investigated the role of Ca^{2+} fluxes and their effect on plasmodesmata closure. The staminal hairs of the flowering plant *Setcreasea purpurea* are formed by a chain of cells in which diffusion of small molecules through plasmodesmata can be monitored. When a cell at the tip of a hair was injected with IP_3 or mastoparan (a peptide that activates G proteins and increases IP_3 and Ca^{2+} in plant cells), a rapid increase in intracellular levels of Ca^{2+} occurred along with a simultaneous closure of plasmodesmata between the injected cell and the adjacent one, resulting in a blockage in movement of the fluorescent marker, carboxyfluorescein. After a short period of time, the plasmodesmata reopened as the Ca^{2+} wave dissipated. The effect of mastoparan was negated in the presence of a Ca^{2+} channel blocker, showing that the effect was Ca^{2+}-mediated.

The authors hypothesize that the IP_3 in the injected cell causes a release of Ca^{2+} from intracellular stores as it rapidly diffuses through plasmodesmata to the adjacent cell where it repeats the process. The initial Ca^{2+} increase leads to the closure of plasmodesmata possibly via a protein phosphorylation, and the subsequent decrease of Ca^{2+} permits the reopening. Thus, it appears that plasmodesmata function in a similar fashion to gap junctions in propagating Ca^{2+} waves. Although this mechanism of signal transmission may not occur in all plant tissues, it may play a major role where precise transmission over a few cell layers is required.

Other mechanisms for regulating the SEL also operate. 1-3-β-glucan synthase, thought to be located in the plasma membrane, can either constrict the plasmodesmata or close it all together with the production of callose (Lucas et al. 1993). This enzyme is highly dependent on cytoplasmic Ca^{2+} levels, thereby making plasmodesmata closure dependent on Ca^{2+} levels. The closure is reversible by activation of 1-3-β-D-glucanase.

Regulating the number of plasmodesmata

A possible mechanism to regulate plasmodesmata may be to simply control their number. A classic study by Tilney et al. (1990) examined the number and position of plasmodesmata in developing gametophytes of the fern *Onoclea sensibilis*. The gametophyte grows as a file of cells in the dark. Given a light stimulus, the apical cell develops into a two-dimensional structure that is one-cell thick. With each division, the number of plasmodesmata increased at the apical cell. The apical cell always retained the greatest density of plasmodesmata and the number of plasmodesmata in any given cell declined as the cell was pushed away from the apical cell by further division. No secondary plasmodesmata were formed. By this method, the plasmodesmata network would be able to produce a gradient of a diffusible substance with differences between the apical cell and cells furthest away.

The production of secondary plasmodesmata has been correlated with certain environmental or developmental stimuli. For example, the formation of secondary plasmodesmata between photosynthetic bundle sheath cells and vein cells increases ~10-fold in squash plants at the time when photoassimilates are transported out of leaves (Volk et al. 1996). Whether this method is utilized may depend on how quickly a response is needed.

Boundaries defined by regulatory genes

A combination of expression patterns and mutant phenotypes has suggested that certain floral genes may play a role in limiting cell–cell communication in flower development. *fimbriata* (*fim*) is expressed in the junction

between whorls 1 and 2 and whorls 2 and 3. *fim* mutants have homeotic conversions and chimeric organs in whorls 2 and 3 (Simon et al. 1994). *unusual floral organs* (*ufo*) is considered to be the cognate ortholog of *fim* in *Arabidopsis* (Ingram et al. 1995). *ufo* mutants have highly abnormal flowers and inflorescences; defects include number and size of organs, organs fusions, mosaic organs, and timing of organ initiation (Wilkinson and Haughn 1995; Levin and Meyerowitz 1995). These phenotypes suggest that *fim* and *ufo* may establish a boundary to prevent cells in different whorls from interacting. The *superman* mutation results in the transformation of carpels into stamens. Genes that are normally expressed in stamens and restricted from carpel expression are expressed in the carpel whorl in *superman* mutants (Sakai et al. 1995). From following the timing of expression patterns, *superman* is thought to act after a boundary has been established (Sakai et al. 1995). Boundary initiation or maintenance could be accomplished by regulating cell division orientations, by suppressing cell division, by restricting plasmodesmata, or by repressing organ identity expression.

Signaling through walls

What do cells do when their plasmodesmata are closed or nonexistent and they still want to talk? Small molecules such as ethylene (Ecker 1995) diffuse through cell walls and kinases extend their extracellular domains into the cell wall, either contacting diffusible ligands or reaching across walls to the extracellular domains of other proteins.

Over the last few years it has become apparent that plant cells possess proteins that resemble transmembrane receptors like those found in animal cells, although specific ligand-induced activation of plant receptor-like proteins and subsequent downstream transmission of the signal has yet to be demonstrated. Plant transmembrane receptors identified so far are of the serine/threonine kinase type and are called receptor-like kinases (RLKs). The RLKs fall into at least three classes according to the type of extracellular domain they possess. The S-domain class was the earliest identified, and is best characterized in *Brassica* where it functions in self incompatibility (Nasrallah and Nasrallah 1993). Members have been characterized in maize (Walker and Zhang 1990), *Arabidopsis* (Walker 1993), and rice (Zhao et al. 1994). In *Brassica oleracea*, the S-locus contains at least two genes, encoding the S Receptor Kinase (SRK) and the S locus glycoprotein (SLG). SRK has an extracellular domain that resembles the cysteine-rich "S"-domain of SLG, and an intracellular domain that has similarity to protein kinases of the serine/threonine type (Nasrallah and Nasrallah 1993). SLG is a glycoprotein with a signal peptide. The two proteins are postulated to act together to transduce a signal originating from pollen. A second class of RLKs is the leucine-rich repeat (LRR) type. The LRR motif is quite variable and is found in a wide variety of proteins that perform diverse cellular functions. This motif is believed to participate in protein–protein interactions, and the variability is thought to contribute to the specificity of the interaction. Recently, a number of genes conferring resistance to specific pathogens have been found to contain potential extracellular domains with LRR motifs (Jones et al. 1994; Song et al. 1995). The third type of RLKs is the epidermal growth factor (EGF)-like repeat class. Presently this class has a single member, PRO25 of *Arabidopsis* (Kohorn et al. 1992), which was identified as a protein that interacts with a chlorophyll-a/b-binding protein.

The recent isolation of the maize *crinkly4* (*cr4*) gene demonstrates the existence of a plant transmembrane receptor involved in epidermal differentiation. Recessive *cr4* mutants show defects in epidermal histology in leaves and floral organs but not roots. The epidermal cells are unusually large and irregular in shape, and the cell walls fail to interlock with neighboring cells. The epidermis can be several layers thick and occasionally fuses with the epidermis of neighboring organs, producing plants with crinkled, adhered leaves. In kernels, differentiation of the outer layer of the endosperm, or aleurone, is progressively affected. *cr4* encodes a receptor kinase protein with an extracellular domain similar to the ligand binding domain of tumor necrosis factor receptors (TNFRs) (Becraft et al. 1996), providing an exciting opportunity to identify upstream components of this signal transduction pathway.

Future experiments

Both surgical and genetic studies have demonstrated the ability of plant cells to coordinate their activites; perturbations that might cause abnormal growth or tumors in animals fail to affect normal plant morphogenesis. A bisected meristem will produce two meristems of equal ability (Pilkington 1929). Overexpression of the *Arabidopsis* cell cycle gene, *cyc1At*, which is expressed in dividing root cells immediately before cell division, increases the root mass of *Arabidopsis* but does not change the morphology or organization of the meristem (Doerner et al. 1996). The *tangled* mutation in maize, which fails to execute normal longitudinal cell divisions, is normal in morphology (Smith et al. 1996). The recent discoveries of plasmodesmata SEL regulation and transmembrane receptors provide possible mechanisms for this supracellular control. These clues, however, generate as many questions as they answer. The transcription factors discussed, KN1, FLO, and DEF, all have discrete domains of expression; clearly, mechanisms must be in place that permit movement between certain cells and not between others. The identification of intragenic mutants that no longer move and extragenic mutants that restrict movement will be important in determining the significance of transcription factor trafficking. Other approaches include finding mutant phenotypes for proteins that have been immunolocalized to plasmodesmata (Epel et al. 1996) and identifying the genes for mutations that affect plasmodesmata (Russin et al. 1996). Identifying the ligands that activate receptor-like kinases will also be extremely valuable. We can then fashion chime-

ras in which adjacent cells differ in the presence or absence of a ligand to test its ability to communicate across cell walls. Hopefully, a combination of genetics and cell biology will continue to open the doors toward a better understanding of cell interactions in plants.

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