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A Role for the Stele in Intertissue Signaling in the Initiation of Abscission in Bean Leaves (*Phaseolus vulgaris* L.)[1]

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A combination of microdissection and viscometric endo-β-1,4-glucanhydrolase assays was used to investigate if the early appearance of the abscission-related isoelectric point-9.5 endo-β-1,4-glucanhydrolase in the stele of the pulvinus and abscission zone of the foliar abscission zone of *Phaseolus vulgaris* L. prior to cell separation (reported by E. del Campillo, P.D. Reid, R. Sexton, L.N. Lewis [1990] Plant Cell 2: 245–254) indicates that the vascular tissue of this region has a specific role in abscission. We find that no endo-β-1,4-glucanhydrolase activity or cell separation is detectable in the abscission zone cortex if the abscission zone cortex is separated from the stele tissue. If the stele is separated from the abscission zone cortex after a lag period but again before any endo-β-1,4-glucanhydrolase activity is present in the abscission zone cortex, then the enzyme is produced in the cortex and abscission ensues. We conclude that the cortex of the abscission zone is able to abscind independently of the vascular tissue only after the vascular tissue has begun to respond to abscission-promoting signals. We suggest that ethylene promotes formation of an abscission-permitting signal in the stele of the abscission zone and pulvinus, and that this signal is an essential elicitor for the synthesis of cell separation enzymes in the target cells of the abscission zone cortex.

Abscission is the process of shedding multicellular organs by plants. In higher plants this is achieved by dissolution of the middle lamellae of a specific group of positionally differentiated cells called "abscission cells," and the region of layers of abscission cells at this point constitutes an abscission zone (Osborne, 1989). In dicotyledonous plants abscission is accelerated by ethylene and inhibited by auxin. ABA also advances abscission, but this may be caused by increased ethylene synthesis due to promotion of senescence, and not by a direct effect of ABA on the cells of the abscission zone (Jackson and Osborne, 1970, 1972). Sagee et al. (1980) prevented abscission in *Citrus sinensis* L. by treating abscission-zone explants with the ethylene-biosynthesis inhibitor aminoethoxyvinyl-glycine. Treatment with ABA did not reverse aminoethoxyvinyl-glycine inhibition of abscission, but ethylene treatment did. However, Suttle and Hultstrand (1993) prevented abscission of cotyledons of *Gossypium hirsutum* by growing the plants on a medium that contained norfluorazon, an inhibitor of ABA synthesis; ABA treatment alone restored abscission in this system. It seems probable, therefore, that ABA and ethylene are both required for abscission to occur under certain conditions.

The specific and partial cell-wall dissolution involved in abscission has focused attention on the role of cell-wall glycosidases. Correlations have been reported between polygalacturonase activity and abscission (e.g. Morré, 1968; Taylor et al., 1990; Pandita and Jindal, 1991), and with an ethylene-induced endo-β-1,4-glucanhydrolase first reported by Horton and Osborne (1967). The latter, purified by Koehler et al. in 1981, is a protein with a molecular mass of 51 kD and a pI of 9.5. Antiserum prepared against this protein has no affinity for the constitutive acidic pI-4.5 *Phaseolus vulgaris* cellulase, and abscission was prevented in *P. vulgaris* abscission zones that had been injected with the antiserum (Sexton et al., 1980). Until recently it was believed that this enzyme was induced only in the abscission zone itself, although an early paper by Sexton et al. (1981), using immunocytochemical techniques and enzymic assays, demonstrated that the pI-9.5 endo-β-1,4-glucanhydrolase was also present in the stele of the adjacent pulvinus. At the time this observation was attributed to a diffusion of the enzyme from the induced cells of the abscission zone. In 1990, del Campillo et al. showed that the pI-9.5 endo-β-1,4-glucanhydrolase was, in fact, induced in the stele regions of the pulvinus and abscission zone before the enzyme was detectable in the abscission zone cortex. Because tissue printing does not provide a quantitative transfer of protein to the blot, as Taylor et al. (1993) have shown, it is possible that the result of del Campillo et al. (1990) was due to a tissue-specific transfer of antigen and not to a tissue-specific protein distribution.

Confirmation that both cortex and stele synthesize the specific abscission pI-9.5 endo-β-1,4-glucanhydrolase was provided by in vitro hybridization studies. Using tissue sections as well as tissue prints, Tucker et al. (1991) demonstrated that mRNA for the abscission glucanhydrolase was

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Abbreviations: CMC, carboxy-methyl-cellulose; mwco, molecular weight cut off; pI, isoelectric point.

present in both the stele of the pulvinus and the petiole tissue adjacent to the abscission zone.

These results led us to inquire if the abscission zone and pulvinar vascular system could play a vital part in the process of abscission. In some plants the first cell separation in abscission is adjacent to stelar tissue, although the vascular bundles are usually the last tissue to separate (*Olea europea*, Polito and Lavee [1980]; *Impatiens sultani*, Sexton [1976, 1979]). In many plants, the vascular tissue of the abscission zone has a different configuration than that in the adjacent tissue. Examples are the fusion of vascular traces to form a single stele in the pulvinus of the distal foliar abscission zone of *P. vulgaris* and the division of the vascular bundles of *Sambucus nigra* rachis at the junction with the leaflet vascular system. In this paper we will present evidence that in *P. vulgaris* the stele of the abscission zone is indeed involved in the coordination of abscission, probably via the production of a specific signal produced in or around the vascular tissue of the abscission zone in response to ethylene.

MATERIALS AND METHODS

Plant Material

Phaseolus vulgaris L. (var Masterpiece) was grown under glass for 18 to 21 d. Explants 1.5 cm in length, including the distal abscission zone and pulvinus, were excised from the petioles of the primary leaves with a sharp razor blade. The explants were placed in sealable 500-mL glass containers on perspex racks partly embedded into 1.5% agar (w/v) and maintained in the dark.

Microdissection

All of the microdissections described here were conducted using a hand-held scalpel blade. The exact surgical treatments used are described with the corresponding results. However, it should be noted that these procedures are quite difficult, especially with small plants. It was essential to ensure that all vascular tissue at the zone region was removed, because otherwise cell separation would occur. The presence or absence of vascular tissue was determined by staining for lignin with phloroglucinol-HCl (McLean and Cook, 1958) and observing the tissue by light microscopy.

Hormone Treatments

A volume of ethylene necessary to give the required treatment concentration was injected into the sealed containers through a suba-seal vaccine cap using a 1-mL syringe and hypodermic needle. The containers were opened for 5 min every 24 h to aerate the explants. ABA solutions were prepared by neutralizing (±)-*cis, trans*-ABA (Sigma, Poole, UK) with an equal number of moles of 0.1 N NaOH and diluting to the required concentration. The solution was applied to the distal cut surface of each pulvinus as a 1-μL droplet.

Endo-β-1,4-glucanhydrolase Assay

Tissue for assay was ground in a chilled, glass, hand-held homogenizer in cold extraction buffer (200 mM Na acetate, pH 4.5, containing 400 mM NaCl and 5 mM DTT, 3 μL mg^{-1} fresh weight). The resultant homogenate was centrifuged in a Beckman Microfuge B at 8740*g*. The clear supernatant was removed and kept at 4°C. Endo-β-1,4-glucanhydrolase activity was assayed by the reduction in viscosity of a solution of CMC (cellulose powder type 7HF, Hercules Inc., Wilmington, DE). Ten microliters of the enzyme extract were added to 240 μL of 1.25% CMC (w/v) in distilled water. The final assay solution was therefore 8 mM acetate buffer (pH 4.5), 12 mM NaCl, and 1.2% CMC. The viscosity of the solution was determined from the rate of flow through a marked pipette (Vitrex 100-μL precalibrated, disposable; Camlab, Cambridge, UK). Assays were conducted at room temperature.

IEF and Western Blot

The salt and buffer concentrations of extracts prepared for endo-β-1,4-glucanhydrolase assay were reduced by a factor of 100 by diluting the extracts with distilled water. High mol wt components of the extract were reconcentrated by addition of coarse Sephadex G-25 beads (Sigma, Poole, UK). The extracts were diluted in two steps (10× dilution at each step). After each dilution 0.4 g mL^{-1} extract of Sephadex G-25 was added and the concentrated solution was separated from the Sephadex by centrifugation.

Four microliters of the reduced-salt protein extracts were loaded onto Pharmacia IEF pI-3 to -9 Phastgels and fractionated by IEF using a Pharmacia Phast electrophoresis system. Western blots of the IEF gels were prepared by placing a piece of nitrocellulose membrane (Schleicher & Schuell, BA 85, 0.45 μm; Anderman & Co. Ltd., London), which had been moistened with 10 mM Na acetate buffer (pH 4.5) on the gel for 10 min. While the membrane was prepared at room temperature, prechilling the gel to 4°C reduced the tendency of the gels to adhere to the nitrocellulose. The blots were probed with antiserum to the *P. vulgaris* pI-9.5 endo-β-1,4-glucanhydrolase, which was prepared using the procedure of Koehler et al. (1981) and very kindly provided by Dr. R. Sexton. Antiserum binding on the western blots was detected by conventional development procedures using an immunoconjugated peroxidase (GAR/IgG[H+L]PO from Nordic Immunological Labs Ltd., Tilburg, The Netherlands) as second antibody with 3,3-diamino-benzidine/H_2O_2 to provide an insoluble, colored enzyme product.

ELISA

Detection of the presence of pI-9.5 endo-β-1,4-glucanhydrolase in the dilute extracts of the plant material was carried out by antigen recognition with the *P. vulgaris* pI-9.5 antiserum using conventional ELISA plate procedures with the immunoconjugated peroxidase GAR/IgG(H+L)PO and *o*-diphenylamine/H_2O_2 to provide a soluble enzyme product. Product development was measured at 495 nm in a microplate analyzer (Microplate Autoreader EL 311, Bio-Tek Instruments, Winooski, VT) after different times of incubation in the dark at 24°C. Values for Table I are given after 10 min of incubation, those for Table II are for 20 min.

Table I. *Endo-β-1,4-glucanhydrolase activity in extracts of isolated pulvini and pulvini of complete abscission zone explants after incubation at 25°C for 40 h under air or under 2.5 μL L⁻¹ ethylene, and pI-9.5 endo-β-1,4-glucanhydrolase immunorecognition in ELISAs of extracts made from isolated pulvini incubated for 72 h*

Tissue	Viscosity Decrease	
	Isolated pulvinus	Explant pulvinus
	% mg^{-1} *fresh wt* h^{-1}	
Fresh pulvinus	0.0 ± 0.0	0.0 ± 0.0
Pulvinus after 40 h in air	0.5 ± 0.2	0.7 ± 0.7
Pulvinus after 40 h in ethylene	8.3 ± 0.1	7.8 ± 0.3
	ELISA	
	A_{495}	
Fresh pulvinus	0.000 ± 0	
Pulvinus after 72 h in air	0.022 ± 0.001	
Pulvinus after 72 h in ethylene	0.076 ± 0.003	

[a] Incubation 10 min.

Ethylene Assays

Ethylene production by excised pulvini was determined by GC against ethylene standards as described by Ward et al. (1978). Samples of 10 pulvini were enclosed in 10-mL vials stoppered with a suba-seal vaccine cap. Samples (1 mL) of the internal gas phase were removed by hypodermic syringe after a 1- to 3-h interval and injected directly into a gas chromatograph (Pye 104, ATI Unicam Ltd., Cambridge, UK) fitted with an F1 alumina column.

RESULTS

Induction of pI-9.5 Endo-β-1,4-glucanhydrolase in the Distal Pulvinus

In these experiments whole *P. vulgaris* distal pulvini were excised, ensuring that the experimental tissue did not include any of the abscission zone or leaf, and incubated under air or 2.5 μL L^{-1} ethylene at 25°C. Treatment with ethylene induced an endo-β-1,4-glucanhydrolase activity in the isolated pulvini as seen from the viscometric assays, and this activity was associated with positive immunorecognition of the pI-9.5 endo-β-1,4-glucanhydrolase in ELISA (Table I).

In other experiments pulvini incubated in air were treated with either 2 × 1 μL L^{-1} drops of 1 mM ABA or water. ABA treatment advances senescence (Osborne, 1968), and a senescence-associated increase in ethylene synthesis in the pulvinus occurred (Table II). The endo-β-1,4-glucanhydrolase activity of extracts of the incubated and fresh pulvini was determined by the reduction of viscosity in the solutions of CMC. The extracts were also either run on IEF gels and western blots of the IEF gels prepared and probed with antiserum to pI-9.5 endo-β-1,4-glucanhydrolase, or the original extracts were assayed for binding with the pI-9.5 endo-β-1,4-glucanhydrolase by ELISA on microplates.

As can be seen from Table II, ABA induced elevated endo-β-1,4-glucanhydrolase activity in pulvini, even though there was no abscission zone tissue attached to the pulvinus during the treatment. The positive recognition of the pI-9.5 enzyme by ELISA and the detection of a protein of pI ≥9 by antiserum raised against *P. vulgaris* pI-9.5 endo-β-1,4-glucanhydrolase in the western blots of fractionated extracts of 40-h ABA-treated pulvini indicates that the elevated CMCase activity observed in this material is due to an induction of pI-9.5 endo-β-1,4-glucanhydrolase outside the abscission zone.

Removal of the Abscission Zone Stele Prevents the Appearance of Endo-β-1,4-glucanhydrolase in the Abscission Zone

To determine if the presence of the abscission zone vascular system is necessary for abscission to be achieved and for endo-β-1,4-glucanhydrolase induction in the abscission zone to occur, explants were prepared with the vascular system of the abscission zone, pulvinus, and 2 mm of the petiole removed. The required surgery was performed with a hand-held scalpel blade and the removed tissue is indicated in Figure 1. Similar cuts were made in control explants but without removing any of the stele.

Endo-β-1,4-glucanhydrolase activities of the abscission zone were determined immediately after the explants had been incubated for 72 h at 25°C under 20 μL L^{-1} ethylene. A high concentration of ethylene was added to eliminate any effects of differences in wound ethylene synthesis between the two groups of explants. It is apparent from Table III that cell separation and the appearance of endo-β-1,4-glucanhydrolase activity in the abscission zone were prevented by removal of the abscission zone and pulvinus vascular system.

Table II. *Endo-β-1,4-glucanhydrolase activity in excised pulvini and pI-9.5 endo-β-1,4-glucanhydrolase immunorecognition by ELISA*

Pulvini were treated with 2 × 1 μL drops of water or 0.5 mM ABA. After 40 h, pulvini were extracted for endo-β-1,4-glucanhydrolase activity and ELISA assay. Samples of the extracts were desalted and fractionated on IEF gels. After western blotting the membranes were probed with antiserum to the pI-9.5 endo-β-1,4-glucanhydrolase. Other pulvini, incubated for 48 h, were monitored for ethylene production and extracted and assayed for endo-β-1,4-glucanhydrolase activity.

Tissue	Viscosity Decrease	ELISA
	% mg^{-1} *fresh wt* h^{-1}	A_{495nm}
Fresh pulvinus	0.5 ± 0.5	
Pulvinus after 40 h treated with water	1.5 ± 0.2	0.110 ± 0.030
Pulvinus after 40 h treated with ABA	4.0 ± 0.0[b]	0.312 ± 0.082
		Ethylene Production
		nL g^{-1} *fresh wt* h^{-1}
Pulvinus after 48 h treated with water	2.0 ± 0.3	0.2 ± 0.1
Pulvinus after 48 h treated with ABA	15.0 ± 0.3	3.8 ± 0.3

[a] Incubation 20 min. [b] Indicates binding to a protein band with a pI of ≥9.5 on IEF gels.

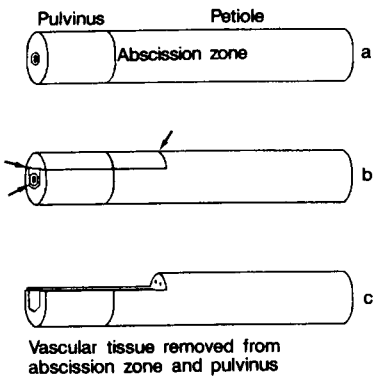

Figure 1. Preparation of *P. vulgaris* explants (a) lacking abscission zone or pulvinus stele (c). The vascular tissue was removed by making the indicated incisions (b) using a hand-held scalpel blade. Most of the vascular bundles fuse, forming a ring near the center of the petiole as they pass through the abscission zone. However, two small bundles pass through the abscission zone at the top of the petiole. It may be significant that abscission often begins near these small bundles.

Table III. *Endo-β-1,4-glucanhydrolase induction and abscission in explant abscission zones with and without stele*

The abscission zone and pulvinus stele was removed from one group of explants. Control explants were cut but without removing the stele. Both groups of explants were then incubated for 72 h under 20 μL L^{-1} ethylene. The proportion of explants that had abscinded in each group were recorded at 40 and 72 h. After 72 h the abscission-zone endo-β-1,4-glucanhydrolase activities of both groups of explants were assayed.

Tissue	Viscosity Decrease
	% mg^{-1} *fresh wt* h^{-1}
Fresh abscission zone	0.0 ± 0.0
Abscission zone (−stele) after 72 h under ethylene	0.0 ± 0.0
Abscission zone (+stele) after 72 h under ethylene	16.0 ± 1.0

	Abscission	
	After 40 h	After 72 h
	%	
Abscission zone (−stele)	0	5
Abscission zone (+stele)	20	91

If only a small portion of the abscission zone cortex was parted from the vascular system by a small excision (again made with a hand-held scalpel blade) after 66 h under 20 μL L^{-1} ethylene, cell separation was confined to that part of the abscission zone cortex within 1 mm of the stele and was connected to it by uncut tissue. Separate CMCase assays of "connected" separated abscission zone tissue and "parted" unseparated abscission zone cortex showed that the occurrence or absence of cell separation was reflected in differences in the endo-β-1,4-glucanhydrolase activity (see Fig. 2).

Demonstration of an Intertissue Signal in the Regulation of Abscission

These results showed that the presence of the stele is necessary for abscission to proceed. The detection of pI-9.5 endo-β-1,4-glucanhydrolase mRNA in the abscission zone cortex of *P. vulgaris* explants by Tucker et al. (1991) indicates that the abscission zone cortex as well as the stele can synthesize pI-9.5 endo-β-1,4-glucanhydrolase. Therefore, the stele must be involved in the regulation of the pI-9.5 endo-β-1,4-glucanhydrolase expression in the abscission zone cortex. This hypothesis was tested by preparing explants and incubating them under 5 μL L^{-1} ethylene for a total of 96 h at 25°C. The pulvinar, abscission zone, and upper petiolar vascular system were excised from groups of explants after 0, 24, and 48 h of incubation under ethylene. The endo-β-1,4-glucanhydrolase activities of the removed steles and of the abscission zone cortex of groups of the explants were determined immediately after the tissue was excised. The endo-β-1,4-glucanhydrolase activity of the abscission zone cortex of the remainder of the explants was determined when the explants had received a total of 96 h of ethylene treatment.

The results of one such experiment are presented in Table IV, which shows that the abscission zone cortex acquires the ability to separate and to synthesize endo-β-1,4-glucanhydrolase, provided that it remains attached to stelar tissue for a period of 24 h. At 24 h there was no detectable endo-β-1,4-glucanhydrolase activity in the abscission zone cortex. No activity arises in the cortex of explants desteled at 0 h, nor does any cell separation occur at the abscission zone. This suggested that a signal, necessary for the separation of cortical cells, passes from the abscission zone vascular system

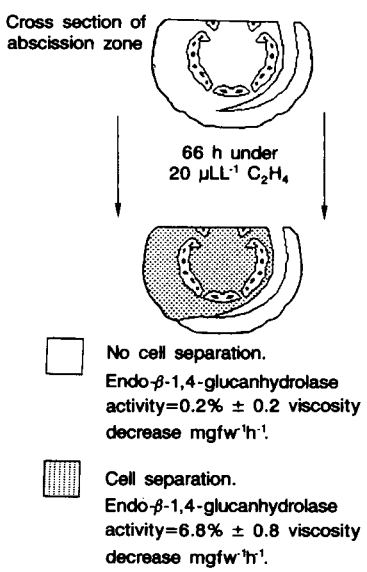

Figure 2. In explants in which part of the abscission zone cortex was separated from the vascular system by an incision, treatment with 20 μL L^{-1} ethylene for 66 h did not induce abscission or endo-β-1,4-glucanhydrolase activity in that part of the cortex separated from the stele, although both were induced in cortical tissue still attached to the stele.

Table IV. *Explants were prepared and incubated under 5 μL L^{-1} ethylene for a total of 96 h*

The vascular tissue was removed from the abscission zone and pulvinus of groups of explants after 0, 24, and 48 h under ethylene. Endo-β-1,4-glucanhydrolase activities of the removed steles and of the abscission zone cortex of half of the explants in the group were assayed when the steles were removed. The endo-β-1,4-glucanhydrolase activities of the abscission zone cortex of the remainder of the explants were determined after the explants had been incubated under ethylene for a total of 96 h.

Tissue	Endo-β-1,4-glucanhydrolase Activity			
	After 0 h under C_2H_4	After 24 h under C_2H_4	After 48 h under C_2H_4	After 96 h under C_2H_4
	% *viscosity decrease* mg^{-1} *fresh wt* h^{-1}			
Abscission zone stele of explants desteled at 0 h	0.0 ± 0.0			
Abscission zone cortex of explants desteled at 0 h	0.0 ± 0.0			2.1 ± 0.3
Abscission zone stele of explants desteled at 24 h		0.6 ± 0.6		
Abscission zone cortex of explants desteled at 24 h		0.2 ± 0.2		14.6 ± 0.5[a]
Abscission zone stele of explants desteled at 48 h			17.5 ± 0.5	
Abscission zone cortex of explants desteled at 48 h			15.3 ± 0.0	6.5 ± 0.5[a]

[a] Tissue in which abscission takes place by 96 h.

to the cortex at some point in the first 24 h of the abscission process.

We have had limited success in restoring the capacity of the abscission zone cortex of desteled explants to abscind. Separating the abscission zone and pulvinus cortex from the stele but leaving the stele still attached to the petiolar vascular system prevents abscission. However, abscission can be restored in 39% (±6%) of these explants by binding the abscission zone with cotton thread to restore tissue contact. Cellulose ester membrane (mwco 2000, Spectra/Por CE, Medicell International Ltd., London, UK) and pre-prepared dialysis membrane (mwco 12,000–14,000, Pierce and Warriner, UK Ltd., Chester, UK) inserted between the cortex and stele of such "bound" explants prevented abscission. If small incisions were made in the membranes, then cell separation (as determined by eye using a dissecting microscope) took place in small patches of abscission-zone cortex tissue, which lay directly beneath the membrane incisions (see Fig. 3), indicating a limited transport of the signal factor.

DISCUSSION

The results described suggest that the abscission zone is more complex than previously believed. In the *P. vulgaris* distal foliar abscission zone the stele appears to have an essential coordinating role in the process of abscission. In this system we show that the abscission zone cortex requires a signal from the vascular bundles before the cells of the cortex can fully respond to defined abscission-promoting events or to hormonal treatments. While the nature of this signal is presently unknown, it appears to operate over a very limited number of cells relative to conventional plant hormones (≥0.5 mm). Although Sexton (1979) reported that small pieces of foliar abscission zone tissue of *Impatiens sultani* retained the ability to separate after dissection, in that study the cortical tissue was not specifically separated from the associated vascular tissue. Additionally, not all of the tissue pieces showed equal speed of abscission; however, the tissue pieces showing slowest cell separation did appear to be the pieces most distant from the vascular bundles.

It was surprising that the mwco 12,000 to 14,000 dialysis membrane prevented abscission; a plant hormone with a mol wt of greater than 12,000 would be almost unprecedented. It is more probable that the signal molecule binds to the membrane. A similar observation has been reported by Verbeke (1989) for a signal involved in carpel fusion. It is possible that the signal is an oligosaccharin, perhaps released by the actions of a polygalacturonase. Certainly in apple fruit abscission zones, polygalacturonase activity is induced in and around the abscission zone much earlier than endo-β-1,4-glucanhydrolase activity (Pandita and Jindal, 1991). It is also possible that very low levels of endo-β-1,4-glucanhydrolase activity induced in and around the vascular bundles are sufficient to release a signaling cytoplasmic or cell-wall fragment. This would explain the report of Sexton et al. (1980) stating that injecting *P. vulgaris* foliar abscission zones with antiserum raised against the pI-9.5 endo-β-1,4-glucanhydrolase prevented abscission. It seems unlikely that injected antibodies would be mobile enough to inactivate enzyme throughout the abscission zone, but if release of a biologically active fragment was prevented, the localized inactivation of the enzyme might then be sufficient to prevent abscission.

The discovery that the pI-9.5 endo-β-1,4-glucanhydrolase is not absolutely confined to separating tissue suggests that differentiation of the abscission zone "target" cells involves and requires the synthesis of a specific cell-wall structure,

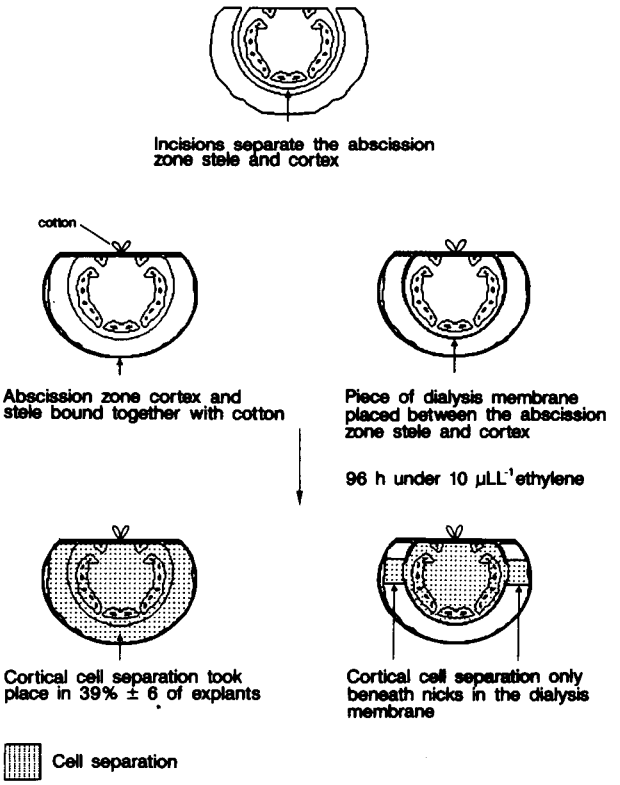

Figure 3. In explants in which the abscission zone and pulvinus stele was cut away from the abscission zone cortex but remained attached to the petiole vascular bundles, cortical abscission was partially restored by binding the abscission zone cortex to the stele with cotton. Cortical cell separation was prevented by insertion of an intact dialysis membrane between the abscission zone cortex and stele, but cell separation occurred where the membrane was nicked.

since there is now no known cell-wall hydrolytic enzyme that is confined to abscinding tissue alone. Such restricted differentiation could allow a coordinating role for cell-wall fragments derived from other specific cell-wall structures from a wider region than that of the final target cells of the zone itself. Perhaps abscission is mediated by the interaction of endo-glucanhydrolases and glycosidases with abscission zone stele-specific polysaccharide breakdown products that either directly interact with the cell-wall enzymes or induce new gene expression in the target cells. The behavior of the saccharide fragment XG-9 (XXXG by the nomenclature of Fry et al., 1993) might provide a model for such a system of regulation. In *Pisum sativum* epicotyl segments, XG-9 has an anti-auxin effect at low concentrations, apparently acting via cellular signal transduction pathways, whereas at higher concentrations of XG-9, the oligosaccharide partially replaces long-chain xyloglucan polymers as a substrate for the endogenous xyloglucan endotransglycosylase. Xyloglucan endotransglycosylase cuts xyloglucan molecules and rejoins the new reducing end so generated to another xyloglucan molecule. When XG-9 is present it can be joined to the new reducing end instead of another long-chain xyloglucan molecule, and so a much shorter polymer can result. This is believed to reduce the degree of cross-linking between cellulose microfibrils, promoting cell elongation (McDougall and Fry, 1988, 1990; Passioura and Fry, 1992). Maclachlan and Brady (1992) have also extracted a xyloglucanase from ripening tomato fruit pericarp, which requires xyloglucan oligosaccharides to reduce xyloglucan chain length and is implicated in tissue softening.

Every cell is responsive to particular signals. Therefore, cells can be considered as target cells for those signals to which they are competent to respond. Abscission zone cells have been considered as a specialized type of ethylene-responsive target cell, which are not only immunologically distinct from their neighbors (McManus and Osborne, 1990a, 1990b, 1991) but are also ones in which critical levels of ethylene induce cell enlargement (Wright and Osborne, 1974), a specific endo-β-1,4-glucanhydrolase, and a subsequent precisely restricted cell separation. This concept of a single type of ethylene-responsive target cell at the zone must now be considered too simplistic an analysis of the abscission process, because as the experiments described here show, the cortical cells do not respond to ethylene alone. Instead, to separate, they require a product of stelar cells that is induced by ethylene but is produced in a wider region of tissue than that of the separation locus itself. There are, therefore, at least two target cell types in the abscission zone complex. One type (as yet unidentified) is present in the stele. These cells respond to ethylene by producing a signal that, when perceived by the cortical cells of the zone, leads to the synthesis by them of the pI-9.5 endo-β-1,4-glucanhydrolase and subsequent cell separation.

From the intertissue dependence we have described for the attainment of abscission in *P. vulgaris*, we propose that a molecular specificity must exist either in an ethylene-induced cell-wall fragment released by the abscission zone stele or in the cell walls of the abscission zone target cells. Such separation-permissive cell or cell-wall specificity must be one component of each competent ethylene-responsive abscission zone for the precise positional coordination of the cell separation events of abscission to be achieved.

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