Delayed cell separation historically was one of the first agricultural traits selected for by man. Successful collection of fruits and seeds of crops such as wheat (Triticum monococcum), rice (Oryza sativa), and a variety of legumes was only possible due to the selection for delayed fruit abscission or pod shatter in which seeds were retained on the stalk rather than rapidly shed. This selection pressure was further imposed as human beings began to harvest by sickle rather than collection in baskets because only the grains that remained longer on the plant were harvested and propagated for future use. A glance at some of the wild relatives of wheat and barley (Hordeum vulgare) shows the presence of brittle, easily shed, seed-bearing stalks rather than the tougher, seed-retaining stalks of some of today’s cultivars. In crops like amaranth (Amaranth caudatus) and rice, there is still a strong emphasis on additional selection for delayed seed shatter because major crop loss occurs.

Although plant breeders have selected for nonabscising or early abscission traits for centuries, it is only in the last 50 years that scientists have begun to understand the processes regulating abscission. Scientists initially identified abscisic acid (ABA) as the primary substance responsible for abscission of leaves and fruits. The primary role of ABA in regulating seed dormancy and stomatal opening and closing subsequently was recognized. Although the initial name, ABA, has been retained, the role of ABA in regulating abscission is minor. Furthermore, the identification of ethylene as the gas responsible for leaf abscission and senescence associated with gas lighting nearly 100 years ago by Anton Nikolovich Neljubov, led researchers to focus on ethylene as a primary regulator of abscission. In addition, the elucidation of the biosynthetic pathway of ethylene synthesis by Yang and Hoffman (1984) provided additional methods to understand ethylene’s involvement in abscission.

Abscission is an active process and has a variety of roles during plant development. Plant parts such as pollen, fruits, seeds, and leaflets may be shed in response to developmental cues to guarantee efficient dispersal or propagation of the plant. Unwanted organs such as flower petals, sepals, and filaments alternatively may be shed when they no longer serve a functional role to the plant. Damaged or infected organs may be rapidly shed as a mechanism of defense.

Early studies clearly define the anatomy of the abscission zone using bean (Phaseolus vulgaris), tomato (Lycopersicum esculentum), and Sambucus nigra as model systems (Jensen and Valdovinos, 1967; Addicott, 1982; Osborne, 1989). To speak generally, the abscission zone encompasses several layers of small densely cytoplasmic cells at the juncture of the organ and the main body of the plant. These cells are predetermined at an early stage and proceed through a series of morphological changes associated with the developmental position or stage of the organ being shed. They are characterized by increased rough endoplasmic reticulum associated with the plasma membrane and Golgi. Accumulation of microbodies and invaginations of the plasma membrane are also observed. Associated with this invagination is swelling of cells on either the proximal or distal side of the abscission layer. Irregular cellulose microfibril rearrangement has also been observed in cells within the abscission zone. Delineating the timing of these structural changes is a goal of today’s researchers.

In the past, many biochemical changes within the cells of the abscission zone were measured in association with the process of abscission. Modifications of the elemental composition of the cells, changes in hormones, and increased expression of cell wall hydrolytic enzymes are some of the most frequent observations. To be specific, lower levels of calcium have been observed in active abscission zones in correlation with the conversion of insoluble pectins to soluble pectic acids. Changes in the levels of pectin methylsterases and pectate lyases are thought to be involved in demethylation of the pectins, and thus the breakdown of the middle lamella. Other cell wall hydrolytic enzymes that have been demonstrated to be up-regulated in correlation with abscission include glucanases, xyl glucan hydrolyses, and pecti galacturonases (PGs; Hadfield and Bennett, 1998; Roberts et al., 2000). In some cases, the genes coding for these enzymes are represented by very large families and the identification of the specific genes involved in the abscission process will be much more difficult. Last, hormones such as ethylene and auxin have long been associated with regulating abscission, and levels of ethylene have been shown to be higher within abscission zone tissues. The addition of ethylene or ethylene analogs to many plants similarly has been shown to accelerate the abscission process, whereas auxin and auxin analogs delay abscission.
Up-regulation of cell wall hydrolytic enzymes such as β-1,4-endo-glucanases (EGases) and PGs specifically is observed following ethylene treatment, whereas auxin treatment can suppress these increases in enzyme activity. It is unfortunate that many of these biochemical changes are also observed in other developmental processes or in other tissues, and thus their specific role in abscission is still unclear.

Although researchers working with Arabidopsis are only beginning to understand the processes regulating abscission, there have been many significant findings in the last several years. Arabidopsis does not display leaf or fruit abscission; however, it does have programmed floral organ abscission. In 1997, Bleecker and Patterson showed that Arabidopsis floral organ abscission was similar to some of the characteristics of abscission in bean and tomato, and thus could be used as a model system to study this process (Bleecker and Patterson, 1997; Van Doorn and Stead, 1997). In addition, pod dehiscence in Arabidopsis, another programmed process of cell separation, has recently been the focus of Yanofsky and coworkers (Ferrandiz et al., 2000; Liljegren et al., 2000). Many scientists are observing unique patterns of gene expression localized to either the abscission or dehiscence zone and generating both cell wall-related antisense plants and overexpression plants that are altering the cell separation process. Especially exciting is the ability to identify knockouts in specific genes, thus enabling researchers to look for direct effects of several of the cell wall hydrolytic enzymes and other genes associated with the abscission zone. With the rapid development of new techniques and the completion of the sequencing of the Arabidopsis genome, we anticipate the ability to ask new questions and to approach abscission and dehiscence in new ways.

**MODEL OF ABSCISSION**

A working model for abscission proposes four major steps in the abscission pathway (Fig. 1). The ontogeny of the abscission zone (Fig. 1A) has been recognized as the first step up to now, as there have been no observations on abscission occurring without this initial formation. In the next steps (Fig. 1, B and C), I have tried to distinguish between competence to respond to abscission signals (Fig. 1B) and the activation of the abscission process (Fig. 1C). I have represented the activation of abscission as potentially following two paths during step C, because it is unclear whether elongation or expansion of cells is a consequence of cell separation or an essential component of the pathway. I have also eliminated a step illustrating cell division as an integral component of abscission because abscission in Arabidopsis does not involve cell division. Last, we recognize the differentiation of a protective layer (Fig. 1D) as the last step in the abscission pathway.

**MORPHOLOGY AND ANATOMY OF ABSCISSION**

Anatomical characterization of the floral organ abscission zone in Arabidopsis using a variety of techniques including light microscopy, scanning electron microscopy, and transmission electron microscopy supports this model of abscission. In Figure 2A, the scanning electron micrograph illustrates an Arabidopsis flower with attached sepals, petals, and filaments, whereas the micrograph in Figure 2B shows that these organs have detached. Figure 2C illustrates the progression of changes in the fracture plane of the petal abscission zone in wild-type Arabidopsis at positions 2, 3, and 9. Position one identifies the youngest flower on the inflorescence having visible petals and anthesis, and is also designated as stage 13.
by Smyth et al. (1990). Flowers at position 1 are just beginning to display the white tips of the petals. Flowers in later positions are older chronologically and basal to position 1. Broken cells are revealed at position 2 (Fig. 2C, top) in which the petal has been forcibly removed to uncover the abscission zone. At position 3 (Fig. 2C, middle), a flattened fracture plane is observed, and at position 9 (Fig. 2C, bottom), the cells are fully rounded. (For a more detailed look at wild type and several additional mutants, see Bleecker and Patterson, 1997.)

**DELAYED ABSCISSION IN ARABIDOPSIS**

One of the most significant contributions to understanding abscission will result from the identification and characterization of delayed abscission mutants. Delayed floral organ abscission has been observed in several different genetic mutants of Arabidopsis (Fig. 2E). Whereas many of the delayed abscission mutants code for unknown or novel gene products, others can be grouped into several general classes including hormone response mutants, pathogen response mutants, cell wall-associated mutants, and MADS-box associated mutants. In wild-type Arabidopsis, ecotype Columbia, the petals, filaments, and sepal are detached from the receptacle at position 6 (Fig. 2E, i). The ecotypes Wassilewskija and Landsberg erecta display a similar pattern of abscission showing organ loss at positions 7 and 6, respectively. Illustrations of lines with delayed abscission include the ethylene response mutant *etr1-1*, which demonstrates abscission at positions 10 or 11 (Fig. 2E, ii), the novel, delayed abscission mutant *dab3-3* with abscission at position 16 (Fig. 2E, iii), and the 35S AGL-15 overexpression line, which results in delayed abscission beyond position 20 (Fig. 2E, iv).

**HORMONE RESPONSES REGULATING ABSCISSION**

Ethylene’s role in accelerating abscission has been recognized for decades. However, only recently have researchers begun to address whether ethylene is absolutely essential for abscission (Addicott, 1982; Abeles et al., 1992). Ethylene response mutants in Arabidopsis provide a marvelous tool to address this question. Changes in the progression of floral organ

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**Figure 2.** Floral organ abscission in Arabidopsis. A and B, Scanning electron micrographs of an Arabidopsis flower showing the stamen, petal, and sepal abscission zones. C, Illustrates the fracture plane of the petal abscission zone at positions 2, 3, and 9 (top to bottom). Note the broken cells at position 2, the flattened fracture plane at position 3, and the fully rounded cells at position 9. D, Illustrates pattern of β-glucuronidase (GUS) expression in the floral organ abscission zones for chitinase-GUS and glucanase-GUS. E, Provides examples of different inflorescences. Note the delay in abscission in the mutants contrasted with wild type. White arrow in i shows the location of abscission at position six. In the mutants, petal abscission has not yet occurred on the flowers pictured in the inflorescence. i, Wild type (WS). ii, *etr1-1*. iii, *dab3-3*. iv, 35SAGL15.
Abscission and Dehiscence in Arabidopsis

Abscission have been observed in several ethylene response mutants, including etr1, ein2, ein3, and ers2 (Bleecker and Patterson, 1997; Chao et al., 1997; A. Hall, personal communication). In most cases, floral organ abscission is delayed to later developmental stages, but once initiated, abscission still proceeds normally. The ability to detect changes (or lack of changes) at the different steps in the abscission process will help determine which stages of abscission can proceed independently of ethylene perception. Genes involved in the synthesis of ethylene have also contributed to our understanding of ethylene’s role in abscission. Antisense plants of 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase) have been generated in Arabidopsis (Ecker and Theologis, 1994). These plants have been shown to have decreased ethylene synthesis, and this decrease in ethylene synthesis delays abscission as well as ripening and senescence. In addition, anatomical and morphological analysis of GUS expression driven by ACC synthase promoter shows localized expression within the abscission zone (Ecker and Theologis, 1994). Changes in expression patterns of these genes in novel delayed abscission mutants will further contribute to our understanding of the involvement of ethylene in the different stages of abscission.

Although little attention has been directed toward the role of auxins in abscission using Arabidopsis, the involvement of auxin was reported in bean almost 40 years ago (Abeles and Rubinstein, 1964). More than 20 auxin-regulated genes have been identified in Arabidopsis, and these mutants provide yet another approach to studying the processes regulating abscission (Nagpal et al., 2000). To be specific, we know already that auxin can inhibit or minimize responses to ethylene, thus providing a developmentally wider window to observe anatomical and biochemical changes associated with each step in the abscission pathway. In addition, there may be unexpected regulation of changes in the abscission zone. The study of these auxin response mutants presents exciting new opportunities to clarify the role of ethylene and auxin in abscission and the possibility to identify new auxin responses. Changes in the regulation of abscission have been reported in other plant species in response to gibberellins, ABA, and cytokinins. Although it is generally thought that these changes are due to interactions of these growth regulators with auxins or ethylene rather than as direct responses, there are many of these hormone mutants in Arabidopsis that could be utilized to further characterize the involvement of these growth regulators in abscission.

MADS-BOX GENES ASSOCIATED WITH ABSCISSION AND DEHISCENCE

Some of the most exciting work on cell separation has evolved from studies on MADS-box genes SHATTERPROOF 1 and 2 (SHP1 and SHP2), AGL-15, and the JOINTLESS gene in tomato (Fernandez et al., 2000; Ferrandiz et al., 2000; Liljegren et al., 2000; Mao et al., 2000). The regulation of abscission and dehiscence by MADS-box genes was not anticipated and the discovery of these interactions has been quite elucidating. Liljegren and others showed that SHP1 and SHP2 act together to regulate dehiscence zone differentiation constitutively, and are essential for normal pod dehiscence. The four layers of cells that normally form the valve margin show reduced lignification and do not develop the entire valve margin throughout the siliqua. The regulation of the SHP genes is further explained by the identification of FRUITFUL, which has been shown to be a negative regulator of SHP gene expression. The SHP mutants are also interesting in that they provide an example of the importance of differentiation of unique cells within the region of cell separation (dehiscence or abscission; Fig. 1, step A). Also of interest is the
observation that overexpression of AGL-15 severely delays floral organ abscission. It seems possible that AGL-15 could be interacting with other MADS-box genes, or that it is directly involved in regulating floral organ abscission. As proposed by Liljegren, additional understanding of the interaction between other MADS-box genes awaits identification of loss of function alleles and the characterization of double, triple, and quadruple mutants.

CELL WALL HYDROLYTIC ENZYMES ASSOCIATED WITH ABSCISSION

Dissolution of the middle lamella or shared cell wall in the separation layer is a fundamental step in the abscission process. Enzymes associated with disassembly and modification of the cell wall include PGs, EGases, pectin methyltransferases, pectate lyases, and expansins. Much of the earlier work on these enzymes has focused on other crops and only recently have researchers begun to study Arabidopsis. A current review on cell separation in all plants by Roberts et al. (2000) suggests that the lack of leaf abscission in Arabidopsis and the size limitations of the floral organ abscission zone have discouraged biochemical and molecular analyses. However, despite the small physical size and biochemical limitations of the floral organ abscission zone, the numerous genetic mutants, sequence information, microarray tools, and tagged mutant populations all make Arabidopsis an excellent system to develop new approaches for studying these cell wall hydrolytic enzymes.

Increases in PG activity have been measured in several plant species. The sequencing of Arabidopsis presents an excellent opportunity to identify many of these genes. More than 40 family members can be identified by BLAST searches, and more than a dozen of these are closely related to abscission-associated PGs from tomato (Hong and Tucker, 1998). Although it is unlikely that each of these PGs has an independent function, it remains for researchers to determine the role of each of these genes. These enzymes traditionally have been associated with fruit ripening, fruit softening, pollen dehiscence, and abscission. Since then, PGs have been found expressed throughout the plant and function in a wider range of developmental processes than originally predicted (Hadfield and Bennett, 1998). One can group the PGs into three major clades by alignment of amino acid sequences. Although there are sequence differences between clade A and B in respect to the presence or absence of a prosequence, these two clades cannot be distinguished by patterns of expression, and abscission-related expression is reported in both clade A and B. As a consequence, researchers have their work laid out for them in regards to identification of PG-associated abscission genes. The Biotechnology Group (Frederiksborg, Denmark), in collaboration with researchers at the Long Ashton Research Station (Bristol, UK) as well as groups at the University of Nottingham (UK), Crop and Food Research (Palmerston North, New Zealand), the University of Maryland (College Park), the University of California (Davis), and the University of Wisconsin (Madison) are all studying functions of these genes. A few genes are being identified, and Gonzalez-Carranza and Roberts (2000) reported the identification of an abscission-related PG (PGAZAT) that is up-regulated in the floral organ abscission zone in response to ethylene. Interactions between these genes and others, as well as identification of knockouts, will provide valuable new information. Gonzalez-Carranza is establishing a Web site to promote the exchange of mutant lines and ideas (http://ibis.nott.ac.uk/pgmg/).

Significant efforts have also been directed toward cloning and characterization of EGases because these enzymes are directly involved in hydrolysis of β-1,4 linkages. Similar to the PGs, the EGases represent a large gene family and have been associated traditionally with a variety of cell wall hydrolytic responses including abscission. A membrane-anchored member of this family, KORRIGAN, has been recently cloned and shown to be involved in cell wall elongation and cytokinesis, but not in abscission (Nicol et al., 1999; Zuo et al., 2000). Difficulties confounding the identification of EGases involved in abscission are similar to those concerning PGs because there are also more than two dozen EGase family members identified by BLAST searches in Arabidopsis. For a recent review on EGases in Arabidopsis, see del Campillo (1999).

The expansins represent another class of cell wall-associated enzymes being studied for their role in abscission as well as in other cell separation processes. Although these genes were originally identified by their involvement in cell elongation and elasticity, expression at the base of the leaf petiole and the pedicel has led Cho and Cosgrove (2000) to conclude that they are associated with abscission. To be specific, expansin 10 (AtEXP10, GenBank accession no. AF229431) was reported to be expressed in the abscission zone at the base of the leaf petiole and the base of young pedicels. In Arabidopsis, these regions lack morphologically distinct abscission zones and do not abscise under normal developmental conditions. In addition, the timing of expression does not correlate with the process of abscission. An alternative interpretation could be that expression at the base of the petiole and pedicel is involved in formation of these organs rather than abscission. This confusion emphasizes the need to clarify the role of expansins in abscission and dehiscence.

NOVEL GENES ASSOCIATED WITH ABSCISSION

Several new mutants that are associated with delayed abscission do not fall into any of the previously discussed classes that have recently been characterized. Inflorescence deficient abscission (ida) and delayed

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Abscission (dab1, dab2, dab3, dab4, and dab5) are new mutants reported last summer at the Eleventh Arabidopsis Meeting in Madison, WI (Butenko et al., 2000; Patterson and Bleecker, 2000). All six of these mutants demonstrate normal ethylene responses yet delayed abscission. Walker and coworkers concurrently reported regulation of abscission as the function for RLK5, a Leu-rich repeat receptor-like kinase (Jinn et al., 2000). Walker has renamed RLK5 as HAESA, from the Latin haesa (hae) meaning to cling or adhere. Plants containing HAESA-GUS constructs showed expression in the floral organ abscission zone and the vestigial abscission zone at the base of the leaf petiole. In addition, plants with intermediate levels of HAESA antisense expression exhibited delayed abscission, and those with strong antisense expression failed to abscise. There are many additional reports of gene expression associated with the abscission zone, but these mutants need to be characterized to demonstrate an association with abscission. In fact, 15% (1,781 lines) of the enhancer trap T-DNA collection generated by Tom Jack (Dartmouth College, Hanover, NH) was reported to show staining in the abscission zone (http://www.dartmouth.edu/~tjack/).

SUMMARY

Recent work on Arabidopsis has contributed some additional knowledge on abscission, but our understanding of this important developmental process is still in its infancy. There are several new delayed abscission mutants that have been identified whose genes are currently being cloned. Identification and characterization of these genes will undoubtedly provide new insights. The role of hydrolytic enzymes in regulating abscission remains somewhat elusive, but the current efforts directed toward these questions are promising. With all of the recently identified hormone mutants, the ability to carefully look at the involvement of these other hormones in abscission now becomes possible. In addition, there are many genes that are expressed within the abscission zone with no understood function. It remains for us to look at the interactions of these genes and others to begin to develop or propose a genetic pathway. Many of the genes expressed in the abscission zone do not directly affect abscission. Rather, they are general housekeeping genes or genes that are expressed during basic plant processes. The process of abscission may trigger major changes that ultimately affect the expression or suppression of hundreds of genes. In conclusion, researchers in Arabidopsis are using multiple approaches combining physiological, anatomical, biochemical, and molecular techniques to study abscission. Continued efforts in these directions are extremely promising and will contribute to our basic understanding of abscission in Arabidopsis. This knowledge ultimately will lead to improved control of abscission and dehiscence in many crop plants.

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