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Regulation of fruit dehiscence in *Arabidopsis*

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Abstract

Fruit dehiscence is a strategy that many fruits adopt to achieve seed dispersal. The dehiscence process involves the differentiation of specialized cell types and a tight co-ordination of molecular and biochemical events that eventually lead to a cell separation process that frees the seeds once they have matured. In the last few years, great progress has been made in identifying the molecular mechanisms underlying fruit dehiscence in the model plant *Arabidopsis thaliana*. In this review, a summary of our current knowledge is presented, and possible future explorations are suggested.

Key words: *Arabidopsis*, fruit dehiscence, regulation.

Introduction

Fruits have evolved to mediate the maturation and dispersal of seeds. For this purpose, a multitude of mechanisms can be found. Essentially, the fruit is a mature ovary, which may include additional parts of the flower, forming a complex structure that can be arranged in a great variety of ways. Among these, many dry simple fruits undergo a process of dehiscence to achieve seed dispersal. This process is accomplished through a carefully orchestrated event that occurs late in fruit development, and involves the differentiation of specialized cell types that must allow cell separation at some point.

Since the manipulation of dehiscence could improve crop yield, both plant breeders and scientists have focused their attention on this process. Physiological studies from the 1970s focused on fruits such as cotton and pecan (Lipe and Morgan, 1972), while in the last decade, *Brassica napus* became the focus for the morphological and molecular characterizations of the dehiscence process (Meakin and Roberts, 1990*a*, *b*; Roberts *et al*., 2000). Comparative anatomical and physiological studies in the model plant *Arabidopsis thaliana* revealed that dehiscence is very similar to the same process in *Brassica* (Spence *et al*., 1996). The use of *Arabidopsis*, for which extensive genetic and molecular tools are available, has allowed a rapid advance in understanding how this process is regulated. In this review, the focus will be on the genetic mechanisms underlying the regulation of pod dehiscence in this model plant.

The dehiscence process

The *Arabidopsis* fruit develops from a gynoecium composed of two fused carpels, which, upon fertilization, grows to become a typical silique that contains the developing seeds. After seed maturation, dehiscence takes place, and valves detach from the central septum freeing the seeds (Bowman *et al*., 1999; Ferrándiz *et al*., 1999; Fig. 1)

The dehiscence zone (DZ) is a region that is no more than a few cells wide extending the entire length of the fruit at the boundaries between the valves and the replum (i.e. external septum). At maturity, the DZ can be considered to consist of a non-lignified separation layer (SL), placed between a region of lignified cells in the valve and the lignified vasculature of the replum (Fig. 1).

The differentiation of specialized tissues in the fruit is apparent from floral developmental stages 11/12 in *Arabidopsis* (according to Smyth *et al*., 1990), which is shortly before fertilization of the ovules. After fertilization (stage 13), longitudinal creases, corresponding to SL differentiation, clearly delimit the valves from the replum. Cells at the SL are morphologically different from adjacent non-separating cells. The SL cells differentiate early during silique development as small, isodiametric cells with dense cytoplasm and many plasmodesmatal connec-

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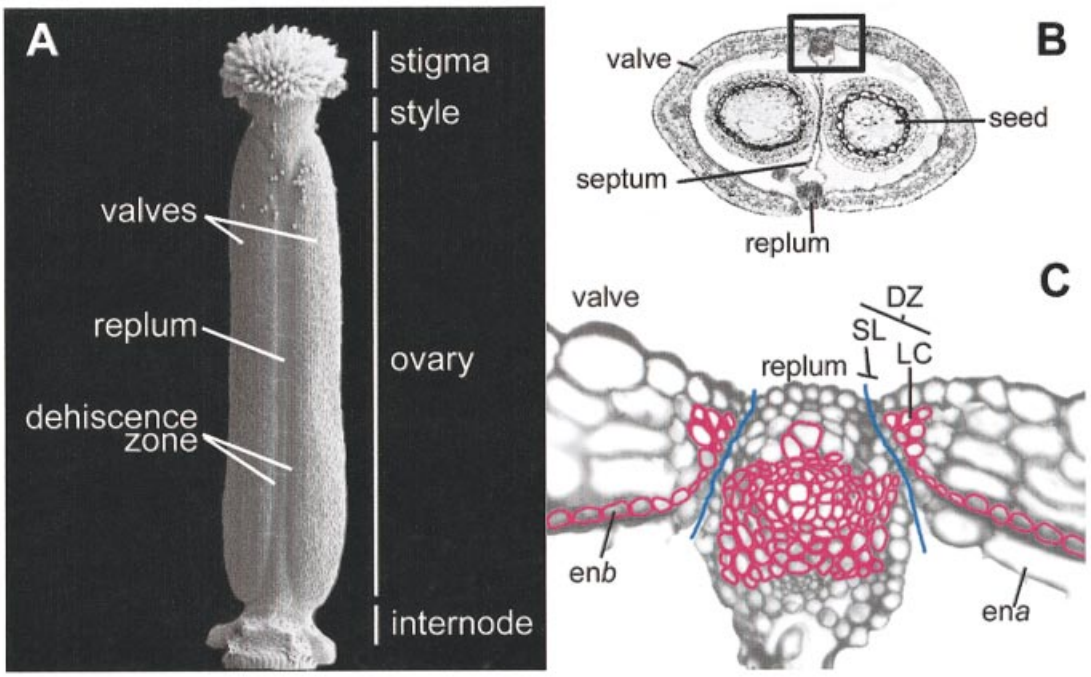

Fig. 1. The *Arabidopsis* fruit. Scanning micrograph of a silique shortly after pollination (stage 14, according to Smyth *et al.*, 1990). The different parts of the fruit are indicated. Transverse section of the ovary of a mature silique (stage 17B). A close-up of the boxed area is shown in (C). Section of the dehiscence zone of a stage 17B silique. Lignified cell walls have been traced in pink for clarity, and the fracture surface in the separation layer is noted by a blue line. DZ, dehiscence zone; SL, separation layer; LC, lignified cells at the valve margin mesocarp; en*a,* endocarp*a,* already disintegrating in the left valve; en*b,* endocarp*b.*

tions, and appear to remain in a state of arrested development while the adjacent cells continue to differentiate, expand, and vacuolate. Cells of the inner epidermis of the valves (en*a*, according to Spence *et al*., 1996) expand and remain thin-walled, whereas cells of the inner subepidermal layer (en*b*) develop thickened walls (stage 17A, Ferrándiz *et al*., 1999), which subsequently lignify (stage 17B). Patches of a few mesocarp cells adjacent to the SL also become lignified (stage 17B). Simultaneously, the main vascular strands that run along the replum enlarge and show much additional lignification. Yellowing and desiccation of all tissues occurs, and cells of en*a* finally disintegrate (stage 18). During the final stages of fruit development, detachment of the valves from the replum proceeds (stage 19). Cell separation takes place by degradation of the middle lamella at the fracture surface of the SL. Spence *et al*. (1996) describe the cells at the SL as remaining intact. However, studies in *Brassica napus* by Meakin and Roberts (1990*b*) and the more recent work from Rajani and Sundaresan (2001) in *Arabidopsis*, support the idea that autolysis of SL cell protoplasts also takes place and could be needed for the release of cell-wall-degrading enzymes. Pod shatter appears to occur by a combination of cell wall loosening at the SL and the tensions established by the differential mechanical properties of the lignified tissues at the en*b* layer and the valve margin patches, and of the drying exocarp and mesocarp cells (Meakin and Roberts, 1990*a*; Spence *et al*., 1996).

The genetics of pod shattering

In the last few years, many genes have been identified that are involved in DZ differentiation and in the associated biochemical and physiological changes prior to cell separation. Genes have been isolated through several different approaches. The identification of mutants both through classical mutagenesis and reverse genetics has led to the molecular cloning of genes that direct DZ cell differentiation (Liljegren *et al*., 2000*a*; Rajani and Sundaresan, 2001). An alternative strategy with valuable results has been the identification of genes specifically expressed in the DZ. Given the anatomical and physiological specificity of this tissue, genes that are specifically expressed in these cells and not in the valves are likely to be involved in dehiscence. In *Brassica*, DZ genes have been identified mainly by isolating mRNAs expressed in the valve margins (Coupe *et al*., 1993, 1994; Petersen *et al*., 1996; Whitelaw *et al*., 1999). This direct isolation is more difficult in *Arabidopsis* due to the smaller size of the silique, but the identification of *Arabidopsis* sequences related to *Brassica* DZ genes has revealed that sequence homology often correlates with expression pattern similarity (Jenkins *et al*., 1999; Sander *et al*., 2001). In the last

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few years, different laboratories have generated collections of *Arabidopsis* enhancer or gene trap lines. These experiments are based on the random insertion of reporter genes in the *Arabidopsis* genome, so that when inserted in the proximity of an enhancer or within a gene, the expression of the reporter is driven by the neighbouring regulatory sequences (Sundaresan *et al*., 1995). Several of these lines showing reporter expression in the valve margins have allowed the cloning of new DZ-related genes and the identification of different cell identities within the valve margin.

A variety of activities are required for pod shattering to occur. First, early regulators of cell differentiation must act to mediate cell fate specification. Once the different cell types are determined, then several enzymatic activities must work to accomplish the associated processes, such as changes in cell wall composition, lignification, and disintegration of the middle lamella in the separation layer. This programme of differentiation and the downstream enzymatic activities must be tightly regulated; therefore the signalling mechanisms are important to ensure a perfect co-ordination of events.

The regulation of DZ cell differentiation

The first transcription factors shown to participate in DZ specification were the MADS-box genes *SHATTERPROOF1 (SHP1)* and *SHATTERPROOF2 (SHP2). SHP1* and *SHP2* are required for the proper specification of the different cell types within the valve margin and the DZ. They encode redundant activities, as the single mutants show no phenotype and only the double *shp1 shp2* mutant siliques are unable to shatter after fruit desiccation. In *shp1 shp2* siliques, lignification is reduced at the valve margins and the SL does not develop properly (Liljegren *et al*., 2000*a*). *SHP1* and *SHP2* probably represent the top of the hierarchy regulating DZ formation. They appear to be direct targets of regulation by *AGAMOUS*, a carpel identity gene, and their identical patterns of expression are restricted to cells that mark the DZ formation from very early stages in carpel development (Flanagan *et al*., 1996; Savidge *et al*., 1995).

Another MADS-box gene involved in dehiscence zone formation is *FRUITFULL (FUL). FUL* expression appears at the inception of the carpel primordia, and soon after becomes restricted to the cells that will give rise to the valves, a pattern that is complementary to that of the *SHP* genes (Gu *et al*., 1998; Mandel and Yanofsky, 1995). In *ful* mutants, the valves fail to elongate and differentiate after fertilization, the mesocarp cells lignify ectopically, and the valves usually tear open in what could be interpreted as the development of an ectopic DZ cell fate throughout the valve (Gu *et al*., 1998; Liljegren *et al*., 2000*a*). *FUL* negatively regulates the *SHP* genes in the valves, as the *SHP* genes become ectopically expressed in the valves of *ful* loss-of-function mutants. Conversely, fruits from plants constitutively expressing *FUL* are indehiscent due to a complete lack of DZ differentiation with conversion of all cells into a valve cell identity (Ferrándiz *et al*., 2000). This phenotype is somehow more severe that the *shp1 shp2* phenotype, suggesting that *FUL* is probably not only acting through *SHP* repression, but also able to regulate other factors involved in DZ cell fate specification. It is interesting to note that, in addition to the *SHP* genes and *FUL*, other MADS-box genes appear to be involved in related processes of cell separation. For example, preliminary results indicate that the *SEEDSTICK (STK)* MADS-box gene is required for normal funiculus development and seed abscission and acts redundantly with *SHP1* and *SHP2* in repressing *FUL* in ovules and seeds (Pinyopich *et al*., 2001). Other examples are *JOINTLESS*, a tomato MADS-box gene required for the differentiation of the floral pedicel abscission zone (Mao *et al*., 2000; Szymkowiak and Irish, 1999), or *AGL15*, whose constitutive expression is able to prevent floral organ abscission (Fernandez *et al*., 2000).

A new element in DZ differentiation was identified by a gene trap line, GT140, that showed specific reporter expression in the valve margins of developing siliques (Sundaresan *et al*., 1995). GT140 reporter expression in the DZ was shown to be positively regulated by the *SHP* genes, and negatively regulated by *FUL* (Ferrándiz *et al*., 2000; Liljegren *et al*., 2000*a*). GT140 corresponds to a bHLH transcription factor, *INDEHISCENT1 (IND1). IND1* is required for DZ differentiation, as loss-of-function *ind1* mutant siliques lack the lignified patches at the valve margins and are unable to shatter. *IND1* ectopic expression in *ful* mutant valves appears to be responsible for most of the *ful* phenotypes in the fruit, since *ind1* mutations are largely able to suppress these phenotypes (Liljegren *et al*., 2000*b*; CF Yanofsky, M Yanofsky, unpublished results).

Recently, a new indehiscent mutant, *alcatraz (alc)* has been isolated. In *alc* mutants both lignification patterns and external morphology of DZ are normal, but close inspection reveals that *alc* SL fails to define a clear fracture layer. In the final stages of fruit development, lignified cells form a bridge between the en*b* layer and the vascular bundle of the replum, blocking valve detachment after middle lamella disintegration. *ALC* corresponds to another bHLH transcription factor and it is expressed preferentially in the valve margin, albeit with a dynamic pattern, becoming progressively restricted to the cells that form this lignified bridge in later stages (Rajani and Sundaresan, 2001). It is still unclear how *ALC* is regulated. *SHP1, SHP2* and *FUL* levels of expression appear unchanged in the *alc* background, suggesting that *ALC* could be a downstream target of these genes, although further analysis needs to be done. *alc ful* double mutants show a partial reduction of *ful* phenotypes in the valves, suggesting that *ALC* might be repressed by *FUL* in this tissue, in a similar

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way to that observed for the *SHP* genes or *IND1* (Rajani and Sundaresan, 2001).

Other candidate factors appear to be involved in DZ differentiation. One of these could be the gene corresponding to another gene trap line, YJ161. The YJ161 reporter is expressed in the inner and outer epidermis of the valve margin. This expression pattern is different from those of the *SHP* or *IND1* genes, which span all cell layers of the valve margin, or the dynamic pattern of the *ALC* gene. The YJ161 valve margin expression is not regulated by *SHP*, but is negatively regulated by *FUL*. The YJ161 marker corresponds to the expression pattern of a putative zinc finger protein, for which future identification of loss-of-function mutants could provide new insights on DZ specific cell type differentiation (Roeder *et al*., 2001).

The downstream effectors

Breakdown of the cell wall and, in particular, of the middle lamella, is a basic event in the process of dehiscence (see above), and a shared feature with other processes such as abscission or senescence (Patterson, 2001; Roberts *et al*., 2000). Cell wall loosening proceeds through the action of extracellular enzymes such as cellulases and pectinases. Several of these enzymatic activities can be recognized in *Arabidopsis*, and it appears that some of those involved in dehiscence are specific to this process.

The first gene cloned that has been shown to be related to pod shattering encodes a polygalacturonase (PG), an enzyme responsible for degradation of the major component of pectin, expressed in the DZ of *Brassica napus* pods (*SAC66*, Jenkins *et al*., 1996; *RDPG1*, Petersen *et al*., 1996). A homologous gene in *Arabidopsis* was isolated by these two groups, based on high sequence similarity in both the regulatory and the coding regions, (*SAC70*, Jenkins *et al*., 1999; *ADPG1*, Sander *et al*., 2001). Detailed expression pattern analyses have been performed in heterologous systems, both by examining *RDPG1* promoter activity in *Arabidopsis* transgenic plants (Sander *et al*., 2001), and *SAC70* promoter activity in *Brassica* (Jenkins *et al*., 1999). In *Brassica*, the *SAC70* promoter drives reporter expression in cells of the DZ that will undergo separation. Interestingly, *SAC70* expression was also detected in regions where two other processes of cell separation occur: in the junction between the seed and the funiculus prior to seed abscission, and in cells adjacent to the anther dehiscence site. Similar results were observed when SAC70 promoter activity was tested in *Arabidopsis* (Roberts *et al*., 2002). In addition to transcriptional regulation, a post-translational control of PG activity has been proposed. RDPG1, and other PGs expressed in fruit, dehiscence and abscission zones, have a cleavable N-terminal domain. The presence of this N-terminal peptide appears to block PG secretion into the cell wall, and only upon its removal, triggered by an unknown signal, is the mature protein directed to the extracellular compartment to exert its function (Dal Degan *et al*., 2001).

An increase in β-1,4-glucanase (cellulase) activity has been reported during *Brassica napus* pod dehiscence, although the corresponding gene has yet to be identified (Meakin and Roberts, 1990*b*). In plants, β-1,4-glucanases have tissue-specific patterns of expression associated with many developmental processes such as tissue expansion, fruit ripening or organ abscission (Brummell and Harpster, 2001). In the *Arabidopsis* genome, several putative cellulases can be recognized (Henrissat *et al*., 2001). Whether the β-1,4-glucanase activity involved in dehiscence is specific to this process or not needs to be clarified and the corresponding gene(s) identified.

Another enzymatic activity related to wall loosening is xyloglucan endotransglycosylase (XET) (Fry *et al*., 1992). An XET-encoding gene is specifically up-regulated in the DZ of *Brassica napus* at the final stages of pod development (Roberts *et al*., 2000). In *Arabidopsis*, an enhancer trap line was identified (YJ8) which has a T-DNA inserted ~3 kb upstream of a gene encoding a XET-related protein. YJ8 shows GUS-expression in 3–4 cells of the valve margin inner epidermis and at the sepal and petal abscission zones, and appears to be positively regulated by *SHP1/2* (Østergaard *et al*., 2001).

Many other enzymatic activities are probably required to achieve pod shatter, such as those related to lignin synthesis, and others that still need to be proved to be related to dehiscence. With the completion of the *Arabidopsis* genome sequence and the comprehensive analysis of expression patterns and metabolic pathways currently underway, it is likely that a wealth of information will arise soon. It will be interesting to learn which other activities are needed and how to integrate this knowledge to get deeper insights into the physiology of dehiscence.

Signalling and co-ordination

By contrast to the increasing number of transcription factors that have been related to DZ specification, little is known about the signalling mechanisms that must exist to ensure the co-ordination of events leading to pod shatter. The identification and characterization of regulatory elements in the promoters of the genes encoding the dehiscence-related enzymes listed above, and of putative targets of post-translational regulation in the encoded proteins could provide some insights as to how the process is co-ordinated. Such enzymatic activities are probably downstream in the regulatory cascade that originates with the transcription factors involved in DZ formation, but there are no consistent data on the nature and components of this cascade. So far, neither mechanisms of cell-to-cell communication nor signalling molecules have been identified as unequivocally related to this process. However,

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some candidates could be proposed based on diverse evidence.

Two putative membrane-bound proteins identified by the enhancer trap strategy could be suggested as participants based on sequence similarity and expression pattern analyses, although no functional data are reported yet. YJ80 shows reporter expression at the valve margin and in the seed abscission zone. The YJ80 T-DNA is inserted close to a gene encoding a protein with weak similarity to mammalian ankyrins, and which appears to be genetically downstream of *SHP1/SHP2* and *IND1* (Østergaard *et al*., 2001). The YJ115 T-DNA insertion is upstream of a gene of unknown function that contains a putative transmembrane domain. YJ115 shows reporter expression in the abaxial replum and the valve margins, and is regulated by the *SHP* genes (Roeder *et al*., 2001).

It has recently been reported that *DEFENSE, NO DEATH1* (*DND1*), a gene encoding a cyclic nucleotide-gated ion channel (CNGC), is expressed in senescing organs and in the DZ of *Arabidopsis* siliques (Köhler *et al*., 2001). The *dnd1* mutation was first recognized for producing a defective response to pathogen infection. *dnd1* mutants are dwarf and, upon infection by avirulent pathogens, are unable to undergo the characteristic programmed cell death associated with the hypersensitive response (Yu *et al*., 1998). The CNGC encoded by *DND1* is a membrane-protein able to conduct K^+ and Ca^{2+} ions, a shared functional characteristic with other animal CNGCs involved in signal transduction (Leng *et al*., 1999). Although possible defects in dehiscence have not been studied in these mutants, it is tempting to speculate that the process of cell separation in the DZ could be affected by a failure in inducing specific programmed cell death.

As pointed out before, dehiscence and abscission are related processes. A leucine-rich repeat receptor-like protein kinase (LRR-RLK), HAESA, was shown to be involved in the regulation of floral organ abscission in *Arabidopsis* (Jinn *et al*., 2000). LRR-RKs are a large family in *Arabidopsis*, and some of their members have been related to different developmental processes based mainly on mutant phenotypes. Whether there is any LRR-RLK involved in fruit dehiscence regulation is still unknown, although it would be interesting to explore this possibility. SAC29 is an mRNA specifically up-regulated in *Brassica napus* DZ during late pod development. SAC29 encodes a protein with homology to the receiver domain of response regulator proteins (Whitelaw *et al*., 1999). In plants, several of these systems, organized in diverse modular arrangements, have been implicated in the response to different stimuli such as ethylene or cytokinin signals (reviewed in (D'Agostino and Kieber, 1999). Although no functional role has been assigned to SAC29, it represents an exciting starting point to study signal transduction mechanisms related to dehiscence.

Clearly, much work needs to be done to begin to clarify how fruit dehiscence is co-ordinated and which are the signals and signalling cascades involved. The study of the functional significance of some of the data presented above should be complemented with new efforts in this direction.

Is there hormonal control of dehiscence?

Abscission processes have been linked by considerable evidence to the opposing action of the plant hormones ethylene and auxin (reviewed in González-Carranza *et al*., 1998). Ethylene works by promoting abscission, while auxins act to delay it. Together, the balance between these two hormones appears to be an important factor regulating the timing of abscission. The hormonal induction of pod dehiscence is less well characterized, although some studies have been reported suggesting similar responses. In studies on fruit development of *Brassica* parthenocarpic pods, a slightly delayed shattering was correlated with a reduced ethylene production, and normal timing of cell separation could be restored by the application of exogenous ethylene (Child *et al*., 1998). However, *Arabidopsis* mutants with non-functional ethylene receptors exhibit a normal time-course of silique dehiscence, suggesting that ethylene might have a minor role in shatter induction.

By contrast, auxins are likely to play a dominant role in dehiscence regulation. In *Brassica*, an increase in cellulase activity in the DZ was shown to correlate with a specific decrease in auxin content in those cells. In addition, the exogenous application of auxin analogues to *Brassica* developing pods significantly delayed, although it did not prevent, DZ cell separation (Chauvaux *et al*., 1997). It was observed that these auxin analogues inhibited the increase in cellulase activity and, interestingly, also affected RDPG1 pectinase activity by blocking its secretion into the cell wall (see above, Dal Degan *et al*., 2001).

Related to the possible role of auxin in dehiscence regulation the pattern of expression reported for the bHLH transcription factor *SPATULA (SPT)* is suggestive. In *spt* mutants the formation of the gynoecium apical tissues and the septum is reduced and the fertility is low. *SPT* is required for the development of carpel tissues specialized in pollen tube growth: stigma, style and transmitting tract (Alvarez and Smyth, 1999). However, *SPT* is not only expressed in the pollen tract tissues, but it shows a complex pattern of expression in many different tissues and developmental stages, indicating that SPT may act redundantly with other factors to control tissue growth. Especially intriguing is *SPT* expression related to cell separation: *SPT* mRNA is found in the anther stomiun, the DZ of grown siliques, and in the seed abscission zone (Heisler *et al*., 2001). Diverse evidence indicates that *SPT* is responsive to auxin: it has been shown that *ETTIN*, an Auxin Response Factor (ARF), represses *SPT* in some

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carpel tissues (Sessions *et al*., 1997; Heisler *et al*., 2001); several Auxin Response Elements, the binding motifs for ARFs, are found in the *SPT* promoter, and it appears that, together with repression by ETT, other ARFs could activate *SPT* expression (Heisler *et al*., 2001); finally, inhibitors of auxin polar transport suppress *spt* carpel phenotypes (Nemhauser *et al*., 2000). It would be interesting to explore whether SPT could be a link between cell differentiation and auxin action related to DZ formation, and other separation processes such as anther dehiscence or seed abscission.

Pod dehiscence and seed abscission are likely to be co-ordinated, and they could share common regulatory signals. Meakin and Roberts (1991) confirmed this correlation in infection-induced pod dehiscence of *Brassica* fruits. In addition, some of the genes described above, including *RDPG1,* YJ80-reported gene*,* and *SPT*, are expressed in both dehiscence and seed abscission zones and a functional relationship has been established between DZ genes (*SHP,* Pinyopich *et al*., 2001). What could be those common signals? So far, little evidence exists to propose such a mechanism, but abscisic acid may be suggested as a possible co-ordinating signal: its role in seed development is well established, and it has been related to the regulation of programmed cell death in processes like cereal seed germination (Young and Gallie, 2000). A parallel can be proposed between pod shatter and anther dehiscence. These are analogous processes of cell separation that share a number of specifically expressed genes (*RDPG1*, *SPT*, see above). Jasmonic acid (JA) has been related to anther dehiscence, and mutants defective in JA synthesis show an altered timing of anther opening (Ishiguro *et al*., 2001; Sanders *et al*., 2000). So far, there are no reports of a possible role for JA in pod shattering, but the search for a common signalling factor between anther and pod dehiscence is still far from extensive.

It appears that hormone action could have a role in both DZ differentiation and the co-ordination of physiological events leading to cell separation. Few studies have directly addressed these questions so far, but a large collection of *Arabidopsis* mutants affected in different hormone synthetic pathways or hormone responses is available that could be used for further characterization of the role of these likely regulators.

Summary

The study of dehiscence regulation has important biotechnological potential and could be of great applicability in manipulating seed dispersal in many crops. DZ formation also provides a great system to study tissue specification from a basic science point of view. From these studies, it appears that regulation of cell differentiation involves many factors that interact in a complex network (Fig. 2). The characterization of novel genes acting at this level and the analyses of the regulatory interactions among all of them could be the key to understanding how the different cell fates are established and the physiological events co-ordinated both spatially and temporally. The identification of the signalling cascade(s) involved in co-ordinating the process is still a major issue and much work needs to be done in this direction, but there are already some indications of the paths that should be explored. Solving the many questions posed is an attractive challenge already undertaken by several researchers, and could open the door to new insights in cell differentiation issues, not only those directly related to dehiscence, but also with a broader perspective.

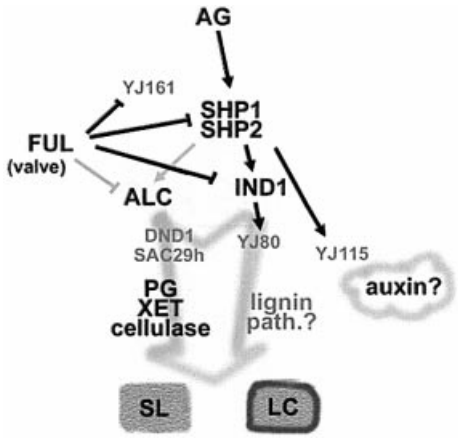

Fig. 2. A model for differentiation and regulation of dehiscence. Genes/activities/relationships backed up by direct evidence are shown in black. Proposed activities and interactions are noted in grey. PG, polygalacturonase; XET, xyloglucan endotransglycosylase; Sl, separation layer cell; LC, lignified cell at the valve margin mesocarp.

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References

Alvarez J, Smyth D. 1999. *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS*. *Development* **126,** 2377–2386.

Bowman J, Baum S, Eshed Y, Putterill J, Alvarez J. 1999. Molecular genetics of gynoecium development in *Arabidopsis*. *Current Topics in Developmental Biology* **45,** 155–205.

Brummell D, Harpster M. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology* **47,** 311–340.

Chauvaux N, Child R, John K, Ulvskov P, Borkhardt B, Prinsen E, Van Onckelen H. 1997. The role of auxin in cell separation in the dehiscence zone of oilseed rape pods. *Journal of Experimental Botany* **48,** 1423–1429.

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Child R, Chauvaux N, John K, Ulvskov P, Onckelen H. 1998. Ethylene biosynthesis in oilseed rape pods in relation to pod shatter. *Journal of Experimental Botany* **49,** 829–838.

Coupe S, Taylor J, Isaac P, Roberts J. 1993. Identification and characterization of a proline-rich mRNA that accumulates during pod-development in oilseed rape (*Brassica napus* L.). *Plant Molecular Biology* **23,** 1223–1232.

Coupe S, Taylor J, Isaac P, Roberts J. 1994. Characterization of an mRNA that accumulates during development of oilseed rape pods. *Plant Molecular Biology* **24,** 223–227.

D'Agostino IB, Kieber JJ. 1999. Phosphorelay signal transduction: the emerging family of plant response regulators. *Trends in Biochemical Sciences* **24,** 452–456.

Dal Degan F, Child R, Svendsen I, Ulvskov P. 2001. The cleavable N-terminal domain of plant endopolygalacturonases from clade B may be involved in a regulated secretion mechanism. *Journal of Biological Chemistry* **276,** 35297–35304.

Fernandez DE, Heck GR, Perry SE, Patterson SE, Bleecker AB, Fang SC. 2000. The embryo MADS domain factor *AGL15* acts post-embryonically. Inhibition of perianth senescence and abscission via constitutive expression. *The Plant Cell* **12,** 183–98.

Ferrándiz C, Liljegren S, Yanofsky M. 2000. *FRUITFULL* negatively regulates the *SHATTERPROOF* genes during *Arabidopsis* fruit development. *Science* **289,** 436–438.

Ferrándiz C, Pelaz S, Yanofsky MF. 1999. Control of carpel and fruit development in *Arabidopsis*. *Annual Reviews in Biochemistry* **68,** 321–354.

Flanagan CA, Hu Y, Ma H. 1996. Specific expression of the *AGL1* MADS-box gene suggests regulatory functions in *Arabidopsis* gynoecium and ovule development. *The Plant Journal* **10,** 343–353.

Fry S, Smith R, Renwick K, Martin D, Hodge S, Matthews K. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochemical Journal* **282,** 821–828.

González-Carranza Z, Lozoya-Gloria E, Roberts J. 1998. Recent developments in abscission: shedding light on the shedding process. *Trends in Plant Science* **3,** 10–14.

Gu Q, Ferrándiz C, Yanofsky MF, Martienssen R. 1998. The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. *Development* **125,** 1509–1517.

Heisler M, Atkinson A, Bylstra Y, Walsh R, Smyth D. 2001. *SPATULA*, a gene that controls development of carpel margin tissues in *Arabidopsis*, encodes a bHLH protein. *Development* **128,** 1089–1098.

Henrissat B, Coutinho P, Davies G. 2001. A census of carbohydrate-active enzymes in the genome of *Arabidopsis thaliana*. *Plant Molecular Biology* **47,** 55–72.

Ishiguro S, Kawai-Oda A, Ueda J, Nishida I, Okada K. 2001. The *DEFECTIVE IN ANTHER DEHISCENCE* gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis. The Plant Cell* **13,** 2191–2209.

Jenkins E, Paul W, Coupe S, Bell S, Davies E, Roberts J. 1996. Characterization of an mRNA encoding a polygalacturonase expressed during pod development in oilseed rape (*Brassica napus* L.). *Journal of Experimental Botany* **47,** 1111–1115.

Jenkins E, Paul W, Craze M, Whitelaw C, Weigand A, Roberts J. 1999. Dehiscence-related expression of an *Arabidopsis thaliana* gene encoding a polygalacturonase in transgenic plants of *Brassica napus*. *Plant, Cell and Environment* **22,** 159–167.

Jinn T, Stone J, Walker J. 2000. HAESA, an *Arabidopsis* leucine-rich repeat receptor kinase, controls floral organ abscission. *Genes and Development* **14,** 108–117.

Köhler C, Merkle T, Roby D, Neuhaus G. 2001. Developmentally regulated expression of a cyclic nucleotide-gated ion channel from *Arabidopsis* indicates its involvement in programmed cell death. *Planta* **213,** 327–332.

Leng Q, Mercier R, Yao W, Berkowitz G. 1999. Cloning and first functional characterization of a plant cyclic nucleotide cation channel. *Plant Physiology* **121,** 753–761.

Liljegren S, Ditta G, Eshed Y, Savidge B, Bowman J, Yanofsky M. 2000*a*. Control of fruit dehiscence in *Arabidopsis* by the *SHATTERPROOF* MADS-box genes. *Nature* **404,** 766–769.

Liljegren S, Kempin S, Chen A, Roeder A, Guimil S, Khammungkhune T, Yanofsky M. 2000*b*. A bHLH gene, *INDEHISCEN*T1, is required for fruit dehiscence and mediates the *fruitfull* phenotype. In: *11th International conference on* Arabidopsis *research*. Madison, WI**.** USA: University of Madison Press.

Lipe J, Morgan P. 1972. Ethylene: role in fruit abscission and dehiscence processes. *Plant Physiology* **50,** 759–764.

Mandel MA, Yanofsky MF. 1995. The *Arabidopsis AGL8* MADS box gene is expressed in inflorescence meristems and is negatively regulated by *APETALA1*. *The Plant Cell* **7,** 1763–1771.

Mao L, Begum D, Chung H, Budiman M, Szymkowiak E, Irish E, Wing R. 2000. *JOINTLESS* is a MADS box gene controlling flower abscission zone development. *Nature* **406,** 910–913.

Meakin P, Roberts J. 1990*a*. Dehiscence of fruit in oilseed rape. I. Anatomy of pod dehiscence. *Journal of Experimental Botany* **41,** 995–1002.

Meakin P, Roberts J. 1990*b*. Dehiscence of fruit in oilseed rape. II. The role of cell wall degrading enzymes. *Journal of Experimental Botany* **41,** 1003–1011.

Meakin P, Roberts J. 1991. Induction of oilseed rape pod dehiscence by *Dasineura brassica*. *Annals of Botany* **67,** 193–197.

Nemhauser J, Feldman L, Zambryski P. 2000. Auxin and ETTIN in *Arabidopsis* gynoecium morphogenesis. *Development* **127,** 3877–3888.

Østergaard L, Roeder A, Liljegren S, Eshed Y, Bowman J, Alonso J, Ecker J, Yanofsky, M. 2001. Enhancer trap lines with GUS expression in developing *Arabidopsis* fruits. In: *12th International conference on* Arabidopsis *research*. Madison, WI, USA: University of Wisconsin Press.

Patterson S. 2001. Cutting loose. Abscission and dehiscence in *Arabidopsis*. *Plant Physiology* **126,** 494–500.

Petersen M, Sander L, Child R, van Onckelen H, Ulvskov P, Borkhardt B. 1996. Isolation and characterization of a pod dehiscence zone-specific polygalacturonase from *Brassica napus*. *Plant Molecular Biology* **31,** 517–527.

Pinyopich A, Ditta G, Yanofsky M. 2001. Roles of *SEEDSTICK* MADS-box gene during ovule and seed development. In: *12th International conference on* Arabidopsis *research*. Madison, WI, USA: University of Wisconsin Press.

Rajani S, Sundaresan V. 2001. The *Arabidopsis* myc/bHLH gene *ALCATRAZ* enables cell separation in fruit dehiscence. *Current Biology* **11,** 1914–1922.

Roberts JA, Whitelaw CA, Gonzalez-Carranza ZH, McManus MT. 2000. Cell separation processes in plants: models, mechanisms and manipulation. *Annals of Botany* **86,** 223–235.

Roberts JA, Elliot KA, Gonzalez-Carranza ZA. 2002. Abscission, dehiscence, and other cell separation processes. *Annual Reviews in Plant Physiology and Plant Molecular Biology* **53,** 131–158.

Roeder AHK, Liljegren SJ, Eshed Y, Bowman JL, Alonso JM, Ecker JR, Yanofsky MF. 2001. Enhancer trap lines YJ161 and YJ115 are expressed in the developing fruit. In: *12th International conference on* Arabidopsis *research*. Madison, WI, USA: University of Wisconsin Press.

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Sander L, Child R, Ulvskov P, Albrechtsen M, Borkhardt B. 2001. Analysis of a dehiscence zone endo-polygalacturonase in oilseed rape (*Brassica napus*) and *Arabidopsis thaliana*: evidence for roles in cell separation in dehiscence and abscission zones, and in stylar tissues during pollen tube growth. *Plant Molecular Biology* **46,** 469–479.

Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, Weiler EW, Goldberg RB. 2000. The *Arabidopsis DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *The Plant Cell* **12,** 1041–1061.

Savidge B, Rounsley SD, Yanofsky MF. 1995. Temporal relationship between the transcription of two *Arabidopsis* MADS box genes and the floral organ identity genes. *The Plant Cell* **7,** 721–733.

Sessions A, Nemhauser J, McColl A, Roe J, Feldmann K, Zambryski P. 1997. *ETTIN* patterns the *Arabidopsis* floral meristem and reproductive organs. *Development* **124,** 4481–4491.

Smyth DR, Bowman JL. Meyerowitz EM. 1990. Early flower development in *Arabidopsis*. *The Plant Cell* **2,** 755–767.

Spence J, Vercher Y, Gates P, Harris N. 1996. 'Pod shatter' in *Arabidopsis thaliana, Brassica napus* and *B. juncea*. *Journal of Microscopy* **181,** 195–203.

Sundaresan V, Springer P, Volpe T, Haward S, Jones JD, Dean C, Ma H, Martienssen R. 1995. Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. *Genes and Development* **9,** 1797–1810.

Szymkowiak E, Irish E. 1999. Interactions between jointless and wild-type tomato tissues during development of the pedicel abscission zone and the inflorescence meristem. *The Plant Cell* **11,** 159–175.

Whitelaw C, Paul W, Jenkins E, Taylor V, Roberts J. 1999. An mRNA encoding a response regulator protein from *Brassica napus* is up-regulated during plant development. *Journal of Experimental Botany* **50,** 335–341.

Young T, Gallie D. 2000. Programmed cell death during endosperm development. *Plant Molecular Biology* **44,** 283–301.

Yu IC, Parker J, Bent AF. 1998. Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *Proceedings of the National Academy of Sciences, USA* **95,** 7819–7824.

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