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Invited Expert Review

Letting Go is Never Easy: Abscission and Receptor-Like Protein Kinases[F]

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Abstract

Abscission is the process by which plants discard organs in response to environmental cues/stressors, or as part of their normal development. Abscission has been studied throughout the history of the plant sciences and in numerous species. Although long studied at the anatomical and physiological levels, abscission has only been elucidated at the molecular and genetic levels within the last two decades, primarily with the use of the model plant *Arabidopsis thaliana*. This has led to the discovery of numerous genes involved at all steps of abscission, including key pathways involving receptor-like protein kinases (RLKs). This review covers the current knowledge of abscission research, highlighting the role of RLKs.

Keywords: Abscission; abscission zone; cell separation; cell wall remodeling; receptor-like protein kinase.

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Introduction

According to legend, Isaac Newton was inspired to think about gravity after observing an apple fall from a tree (McKie and De Beer 1951). Had Newton been a biologist, perhaps he would have thought about abscission instead of gravity. Abscission is the process by which plants shed (discard) entire organs, everything from fruits (such as Newton's apple) and seeds to petals and leaves. In some cases, abscission is a normal part of development, such as the abscission of cotyledons in some species of *Lupinus*, where it can be used to distinguish abscising species from non-abscising ones (Addicott 1982). Often, it is induced by environmental changes, such as light conditions and shading in soybeans (Heindl and Brun 1983), or as a defense response against pests (Williams and Whitham 1986). Depending on the context, abscission may or may not be desirable in agriculture. For example, farmers will sometimes spray apple orchards with naphthalene-1-acetic acid (NAA), a synthetic auxin, to induce abscission of excess fruits and increase fruit size of those that remain, but then spray the herbicide 2,4-D to prevent premature abscission during harvest (Cooper et al. 1968). Further understanding of abscission will be of practical use while contributing to the basic understanding of plant biology.

Abscission is a cell separation process. Physiologically, abscission occurs with the breakdown of the pectin-rich middle lamella that binds together the cell walls of two adjoining cells (Morre 1968). In this way, abscission is similar to other cell separation processes, such as dehiscence, lateral root emergence, and root cap sloughing. Understanding cell separation processes are of agricultural importance and have often been a trait of selection during domestication (Doebley et al. 2006; Gross and Olsen 2010).

Initial studies of abscission were conducted in a wide range of species, and focused primarily on anatomical and physiological changes during abscission. The effects of plant hormones were studied extensively (Jacobs 1962). Most of the emphasis has been on the roles of ethylene and auxin; ethylene is known to promote and accelerate abscission, and auxin appears to have an inhibitory effect (Addicott 1982; Sexton and Roberts 1982). Early hypotheses about abscission focused on the balance of ethylene and auxin or auxin gradients across the abscission zone (AZ) (Hall 1952; Addicott and Lynch 1955; Addicott et al. 1955). Abscisic acid (ABA), was first isolated from abscising cotton fruits and called abscisin II (Ohkuma et al. 1963; Addicott et al. 1964). However, the role of ABA in abscission has been uncertain due to reports of differing effects (Dale and Milford 1965; Cracker and Abeles 1969). The effect of ABA on abscission may be indirect, a result of ABA-induced ethylene production (Gomez-Cadenas et al. 1996).

Much of our recent genetic and molecular understanding of abscission has come from studies conducted on the model plant *Arabidopsis thaliana*, with significant contributions from studies on *Solanum lycopersicum* (tomato). These studies allow the process of abscission to be broken down into four broad sequential stages (**Figure 1**). The first stage is to potentiate abscission through the formation of an AZ. The second stage occurs when developmental or environmental cues induce abscission through a cascade of signals, which results in the third stage, the actual cell separation and abscission of the organ. The final stage includes all the post-abscission processes that culminate in the morphological changes to the AZ and formation of a protective scar layer over the abscission site. The most is known about the second and third steps of abscission where several receptor-like protein kinases (RLKs) are known to play a role.

Abscission Zone Formation

Actual cell separation is limited to an anatomically distinct cell layer, the AZ, at the base of the abscising organ (Sexton and Roberts 1982) (**Figure 1**). The morphology of the AZ layer is observed as a small, round, and cytoplasmically dense group of cells. Other characteristics associated with these cells include highly branched plasmodesmata, small intracellular spaces, starch deposits, and a lack of lignification (Sexton and Roberts 1982). In general, however, the fractural plane of separation is typically 1–5 cells wide and occurs at the pectin-rich middle lamella.

Whether or not AZ formation is an essential part of abscission is uncertain (Gawadi and Avery 1950; Sexton and Roberts 1982). A study in *Sambucus nigra* (elder) shows that abscission cannot be induced by signals, such as ethylene, until after the AZ is fully formed and differentiated (Osborne and Sargent 1976). Likewise, in *Arabidopsis* and tomato, improper AZ formation inhibits abscission (Mao et al. 2000; McKim et al. 2008). In contrast, Gawadi and Avery (1950) report that abscission without fully formed AZs could be induced in *Euphorbia pulcherrima* (poinsettia), *Gossypium hirsutum* (cotton), *Capsicum annuum* (pepper), and *Impatiens sultani* (impatiens).

Even less is known about the actual formation and differentiation of the AZ at the genetic level. In *Arabidopsis*, two transcription factors belonging to the NONEXPRESSOR OF

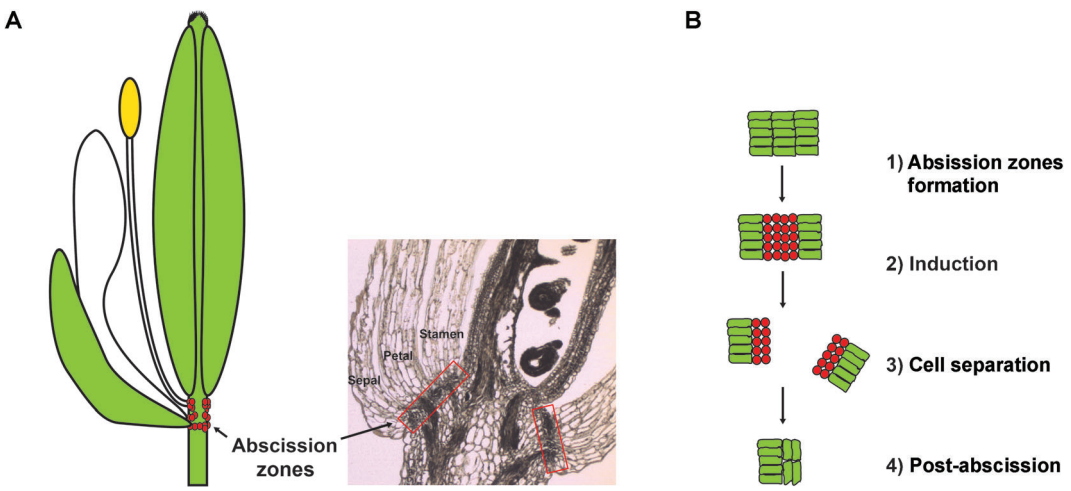

Figure 1. Abscission zones and stages of abscission.

(A) Location of floral organ abscission zones in *Arabidopsis*.
(B) The four stages of abscission. (1) The abscission zone first forms at the base of the abscising organ. (2) Abscission is induced by signaling pathways. (3) Break down of the middle lamella leads to cell separation. (4) Protective scar layers form over the abscission zone.

PR GENES 1 (NPR1) family, *BLADE-ON-PETIOLE 1* (*BOP1*) and *BOP2*, are involved in floral patterning (Hepworth et al. 2005). The *bop1 bop2* double mutant has multiple floral defects, including a failure to abscise and AZs that lack the typical small cytoplasmically dense appearance (McKim et al. 2008). Similarly, the *jointless* mutant in tomato does not develop a pedicel AZ and has long been known and selected for its advantages in mechanical harvesting (Butler 1936; Mao et al. 2000). *JOINTLESS* is a MADS-box transcription factor (Mao et al. 2000) that interacts with another MADS-box transcription factor, *MACOCALYX*, which is also essential for pedicel AZ formation in tomato (Nakano et al. 2012).

Induction of Abscission and HAE HSL2 Signaling

The RLK *HAESA* (*HAE*) (Jinn et al. 2000) has a role in floral organ abscission in *Arabidopsis*. Using *HAE* antisense transgenic lines, a loss of floral organ abscission is observed with decreasing levels of HAE protein. These lines, however, show no defect in ethylene response, indicating that *HAE* regulates abscission in an ethylene-independent manner. Interestingly, T-DNA mutants of *hae* have no observable phenotype (Cho et al. 2008). *HAE* belongs to a family of leucine-rich repeat? (LRR) RLKs that includes two paralogs, *HAESA-LIKE 1* (*HSL1*) and *HAESA-LIKE 2* (*HSL2*), so it seems likely that the phenotype of *HAE* antisense lines is due to targeting of multiple loci. In later stage flowers before abscission, expression levels of both *HAE* and *HSL2* are elevated, while expression levels of *HSL1* are reduced. Furthermore, *HAE* and *HSL2* promoter-β-glucuronidase (GUS) plants show expression specifically in the AZ. *hae hsl2* T-DNA double mutants are unable to abscise, showing that these two genes act redundantly to regulate floral organ abscission. Yet, they develop normal AZs. In contrast, *hae hsl1* and *hsl1 hsl2* double mutants have no abscission defect (Cho et al. 2008; Stenvik et al. 2008).

Multiple ethyl methanesulfonate (EMS) alleles of *hae* and *hsl2* have been isolated in screens conducted in the *hsl2-1* and *hae-1* T-DNA mutants in the Col-0 background, and additional *hae* mutants have been isolated in the Ler ecotype by EMS mutagenesis of the *hsl2-14* enhancer trap line (Sundaresan et al. 1995) (**Table 1**). These alleles are a valuable resource for future research. Having double mutants in both the Col-0 and Ler ecotypes makes it possible to map mutants in screens for suppressors of *hae hsl2*, and the use of transgenics will be improved by having a T-DNA-free background. These mutations are located in the extracellular LRR domains and the intracellular kinase domains (**Figure 2**), providing insight into potentially critical residues. For example, identical base pair changes, *hae-3*/*hae-6* and *hae-4*/*hae-11*, were isolated independently in the two ecotypes. Particularly interesting is the *hsl2-9* allele, which displays a much weaker phenotype than the other double mutants, indicating it is a partial loss of function allele.

Another gene with a role in the induction of abscission is *INFLORESCENCE DEFICIENT IN ABSCISSION* (*IDA*), which is the founding member of a family of genes predicted to encode small secreted peptides (Butenko et al. 2003). *IDA* possesses an N-terminal signal peptide that targets it for secretion, as well as a conserved C-terminal PIP motif that is functionally essential (Butenko et al. 2006; Stenvik et al. 2008). Like *hae hsl2* double mutants, *ida* mutants display no floral organ abscission but respond normally to ethylene treatment. Floral abscission can be induced in *ida* by exogenous application of synthetic peptides containing an extended-PIP (EPIP) motif to *ida* mutants (Stenvik et al. 2008). Constitutive expression of *IDA* using a CaMV 35S promoter results in premature abscission of flowers and disorganized AZs following abscission (Stenvik et al. 2006). This phenotype is blocked in *hae hsl2* double mutants (Cho et al. 2008; Stenvik et al. 2008), and exogenous application of the synthetic EPIP peptide cannot rescue *hae hsl2* mutants (Stenvik et al. 2008). Together, these observations suggest that *HAE* and *HSL2* are downstream of *IDA* and likely form a receptor-ligand pair (**Figure 3**). Biochemical evidence of receptor-peptide interaction, however, is needed to confirm this hypothesis.

Mitogen-activated protein (MAP) kinase cascades are a common signaling module found throughout eukaryotes and involved in many processes in plants (MAPK Group 2002). Typically, a MAP kinase cascade consists of three proteins that transmit a signal by sequential phosphorylation. A MAP KINASE KINASE KINASE (MAP3K) phosphorylates a MAP KINASE KINASE (MKK), which then phosphorylates a MAP KINASE (MPK). In *Arabidopsis*, a MAP kinase cascade consisting of *MKK4* and *MKK5* and their targets *MPK3* and *MPK6* is involved in abscission (Cho et al. 2008). A tandem RNAi construct targeting *MKK4* and *MKK5* has pleiotropic effects, including loss of floral abscission. Mutating the serine and threonine residues to aspartic acid in the activation loops of MKK4/5 can make constitutively active forms of the protein (Ren et al. 2002). When expressed in either a *hae hsl2* double mutant or an *ida* mutant, $MKK4^{DD}$ or $MKK5^{DD}$ restores abscission (Cho et al. 2008). MPK3 and MPK6 are known targets of MKK4/5 (Ren et al. 2002; Wang et al. 2007). While *mpk3 mpk6* double mutants are lethal, site-directed mutagenesis of key residues and MPK6 can have a dominant negative effect (Zhang and Liu 2001). When the mutated $MPK6^{KR}$ and $MPK6^{AF}$ transgenes are expressed in an *mpk3* mutant, the plants survive but display no floral organ abscission. Furthermore, MPK6 appears to have reduced protein kinase activity in *hae hsl2* double mutant flowers. It is still unknown what role MAP3K has in abscission. However, the findings so far suggest that HAE and HSL2 are part of a signaling cascade that is initiated by recognition of IDA and activates a downstream MAP kinase cascade that leads to abscission (**Figure 3**).

Table 1. Mutant alleles of *hae* and *hsl2*

Allele	Location (nt from start codon)	WT base	Mutant base	WT amino acid	Mutant amino acid	Ecotype
			Insertion alleles of *hae*			
hae-1	1,787		T-DNA Insertion SALK_105975			Col-0
hae-2	489		T-DNA Insertion SALK_015074			Col-0
			EMS alleles of *hae*			
hae-3	665	G	A	Cys	Tyr	Col-0
hae-4	2,802	G	A	Trp	Stop	Col-0
hae-5	1,566	G	A	Trp	Stop	Col-0
hae-6	665	G	A	Cys	Tyr	Ler
hae-7	765	G	A	Trp	Stop	Ler
hae-8	772	C	T	Glu	Stop	Ler
hae-9	2,788	G	Δ	Frameshift		Ler
hae-10	2,740	G	A	Glu	Lys	Ler
hae-11	2,802	G	A	Trp	Stop	Ler
hae-12	2,674	G	A	Intron-splicing?		Ler
hae-13	2,933	G	A	Arg	Lys	Ler
hae-14	1,123	G	A	Cys	Tyr	Ler
			Insertion alleles of *hsl2*			
hsl2-1	−205		T-DNA Insertion SALK_057117			Col-0
hsl2-2	1,968		T-DNA Insertion SALK_030520			Col-0
hsl2-15	639		Enhancer Trap GT15053.DS5.10.16.2004.jx94.603			Ler
			EMS alleles of *hsl2*			
hsl2-3	1,078	G	A	Gly	Arg	Col-0
hsl2-4	493	C	T	Gln	Stop	Col-0
hsl2-5	1,528	G	A	Glu	Lys	Col-0
hsl2-6	2,151	G	A	Trp	Stop	Col-0
hsl2-7	3,014	G	A	Arg	Lys	Col-0
hsl2-8	1,949	G	A	Trp	Stop	Col-0
hsl2-9	1,211	C	T	Pro	Leu	Col-0
hsl2-10	1,250	G	A	Arg	His	Col-0
hsl2-11	2,402	G	A	Gly	Glu	Col-0
hsl2-12	2,516	C	T	Ala	Val	Col-0
hsl2-13	2,090	C	T	Ser	Leu	Col-0
hsl2-14	2,224	C	T	His	Tyr	Col-0

WT, Wild type.

The class I knotted1-like homeobox transcription factor *BREVIPEDICELLUS/KNOTTED-LIKE FROM ARABIDOPSIS THALIANA1* (*BP/KNAT1*) is another potential downstream factor in HAE HSL2 signaling (**Figure 3**). *bp/knat1* mutants have abnormal AZs after abscission, similar to the phenotype observed with constitutive expression of *IDA* (Wang et al. 2006; Shi et al. 2011). When *bp/knat1* mutations are crossed with either *ida* or *hae hsl2* mutants, abscission is restored (Shi et al. 2011). *BP/KNAT1* regulates the expression of two other transcription factors from the same family, *KNAT2* and *KNAT6*, which appear to positively regulate abscission (**Figure 3**). The *knat2 knat6* double mutant displays an abscission defective phenotype, but when constitutively expressed in *ida* mutants, can restore abscission (Shi et al. 2011).

Other genes that may also be involved in the induction of abscission have been identified, although where they fit in the known pathways is unknown. RNA interference (RNAi)-mediated silencing of two nuclear actin-related proteins, ARP4 and ARP7, leads to loss of floral abscission but has no effect on AZ development (Kandasamy et al. 2005a,b). *ARP4* and *ARP5* are involved in the regulation of chromatin remodeling, suggesting a previously unknown mechanism involved in abscission. Five transcription factors also have been identified. These transcription factors appear to regulate abscission in a negative fashion, having been discovered by being constitutively expressed using

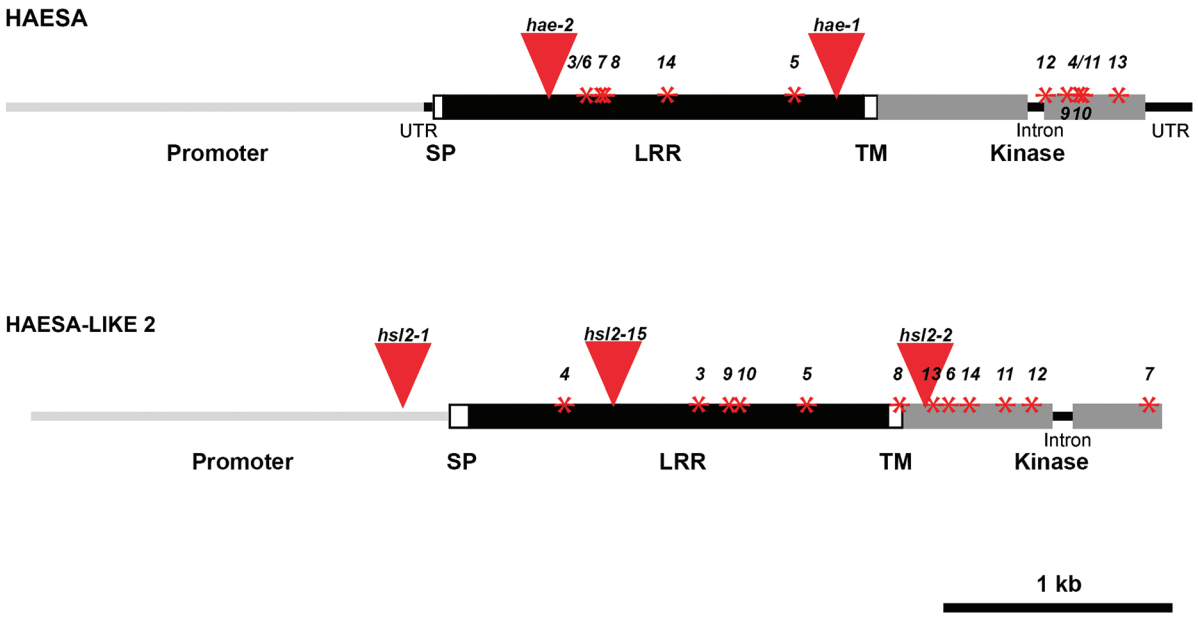

Figure 2. Mutant alleles of *hae* and *hsl2*.

Asterisks denote point mutations, triangles T-DNA insertions.

a CaMV 35S promoter. Among these transcription factors are the MADS-box transcription factors *AGAMOUS-LIKE 15* (*AGL15*), *AGL18*, *FOREVER YOUNG FLOWER* (*FYF*) (Fernandez et al. 2000; Adamczyk et al. 2007; Chen et al. 2011), the zinc finger protein *AtZFP2* (Cai and Lashbrook 2008), and the Dof family protein *AtDOF4.7* (Wei et al. 2010). Constitutive expression of *FYF* and fusion to transcriptional repressor or activation domains alters expression of *IDA* and *BOP2*, suggesting that *FYF* may be involved early in abscission before induction by *IDA* (Chen et al. 2011). Likewise, *AtDOF4.7* interacts with *AtZFP2* and can suppress expression of the polygalacturonase (PG) *ADPG2/PGAZAT*, which suggests a possible direct involvement in initiating abscission (Wei et al. 2010). However, because these phenotypes are observed only through constitutive expression, it is possible that these are indirect effects of mis-expression.

How plant hormones contribute to the induction or inhibition of abscission is still not fully understood. Ethylene is known to accelerate abscission, but it does not appear to be a requirement (Addicott 1982; Brown 1997). In *Arabidopsis*, plants with mutations in the ethylene receptor *ethylene-resistant1* (*etr1*) or the downstream *ethylene-insensitive2* (*ein2*) have significantly delayed floral organ abscission (Bleecker et al. 1988; Guzmán and Ecker 2002). Similarly, antisense lines with reduced expression of the tomato ethylene receptor *LeETR1* also have delayed petiole abscission (Whitelaw et al. 2002). That abscission still occurs suggests ethylene regulates the rate at which abscission occurs, rather than directly inducing it. On the other hand, *auxin response factor 1* (*arf1*) and *arf2* mutants, which are transcriptional repressors that are potentially negative regulators of auxin responses, have delayed abscission (Ellis et al. 2005). This is an effect which is synergistically increased when combined with *arf19* and *arf7* or *ein2* (Ellis et al. 2005). Further evidence for the role of auxin comes through the manipulation of auxin levels in AZs and its perception. Investigating mutants of the auxin influx facilitators *auxin resistant 1* (*aux1*), *like auxin resistant 1* (*lax1*), *lax2*, and *lax3*, there was a reduction in the force required to remove petals (Basu et al. 2013). By expressing two bacterial genes, *iaaM* and *iaaL*, under the promoter of *ADPG2/PGAZAT*, the levels of auxin in the AZ could be artificially increased and decreased (Basu et al. 2013). Increased levels of auxin delayed abscission, while decreased levels resulted in premature abscission. *AUXIN RESISTANT 3* (*AXR3*) is a transcriptional regulator that represses auxin inducible genes by expressing a semi-dominant mutant *axr3-1* under the *ADPG2/PGAZAT* promoter, and substantial delays of abscission were observed (Basu et al. 2013).

Abscisic acid was originally associated with abscission (van Steveninck 1959; Ohkuma et al. 1963), but was later thought to have only a minor role (Patterson 2001). More recent evidence has shown delayed floral abscission in ABA deficient *aba2* mutants in *Arabidopsis* (Ogawa et al. 2009). Jasmonic acid may even play a role as *allene oxide synthase* (*aos*) mutants that affect jasmonic acid biosynthesis also have delayed floral abscission (Ogawa et al. 2009).

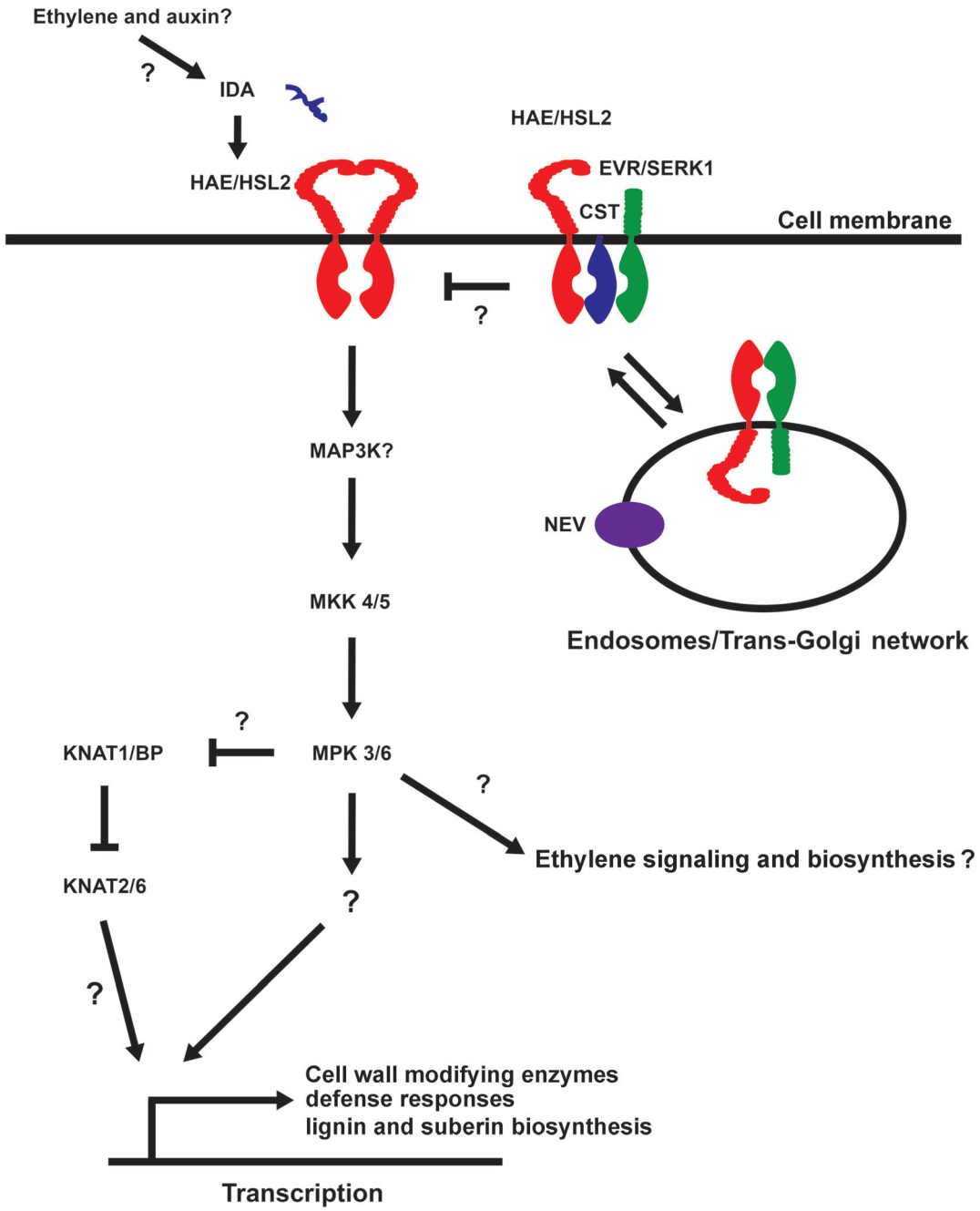

Figure 3. Model of receptor-like protein kinase signaling in abscission.

Perception of IDA by HAE or HSL2 leads to activation of MAP kinase cascade and transcription of cell wall modifying enzymes, defense responses, suberin, and lignin biosynthesis. EVR, SERK1 form receptor complexes with HAE and HSL2 mediated by CST leading to endocytosis. NVR mediates the recycling of HAE and HSL2 to the cell membrane.

Roles of Membrane Trafficking and RLKs

Membrane trafficking is essential to floral abscission, as observed in the *nevershed* (*nev*) mutants, which fail to abscise (Liljegren et al. 2009). *NEV* encodes an ARF-GAP protein. ARF-GAP proteins regulate vesicular trafficking and actin remodeling by hydrolyzing the GTP bound by ADP-ribosylation factors. *NEV* is specifically involved in the trans-Golgi network and recycling endosomes, and the *nev* mutant has a malformed Golgi. It is proposed that the abscission defects are the result of a failure to transport factors essential for abscission, such as HAE, to the cell membrane (**Figure 3**) (Liljegren et al. 2009).

Three suppressor mutations of *nev* have been identified: *evershed* (*evr*) (Leslie et al. 2010), *somatic embryogenesis receptor-like kinase1* (*serk1*) (Lewis et al. 2010), and *cast away* (*cst*) (Burr et al. 2011). All three suppressors encode protein kinases: *EVR* and *SERK1* belong to the LRR-RLK family, while *CST* is a membrane-associated receptor-like cytoplasmic protein kinase. *SERK1* is particularly intriguing as it belongs to the SERK family in subgroup II of the LRR-RLKs. This family consists of five members (Albrecht et al. 2008), the best known of which is *BAKI* (Li et al. 2002). BAK1 is known to interact with other LRR-RLKs, including BRI1 in brassinosteroid signaling and FLS2 in defense responses, where it may function in endocytosis of the receptors. SERK1 also interacts with BRI1 and is involved in brassinosteroid signaling (Albrecht et al. 2008; Karlova et al. 2006). SERKs functioning as co-receptors of other LRR-RLKs is an increasingly common theme, which has led to the hypothesis that SERK1 interacts with HAE and HSL2 in abscission (Lewis et al. 2010). Constitutive expression of *EVR* results in activation of cell death and defense responses (Gao et al. 2009). Split-YFP experiments have demonstrated that CST interacts with both *EVR* and *HAE* and may be involved in facilitating receptor complexes at the cell membrane (Burr et al. 2011). The current hypothesis is that *CST*, *SERK1*, and *EVR* mediate the availability of *HAE/HSL2* at the cell membrane, leading to their endocytosis (Liljegren 2012) (**Figure 3**).

Cell Separation

Abscission requires the breakdown of the pectin-rich middle lamella between two adjoining cells (Sexton 1976), making cell wall modifying enzymes likely targets of HAE HSL2 signaling. A cocktail of different cell wall modifying enzymes are expressed at different developmental stages of the AZ during the course of abscission, as has been shown in microarray studies of *Arabidopsis* stamen AZs (Cai and Lashbrook 2008; Lashbrook and Cai 2008). In closed flower buds and during anthesis, expansins and members of the glycosyl hydrolase family 17 are expressed along with pectolytic enzymes, which are not expressed during cell separation. During cell separation, members of the glycosyl hydrolase family 9 are expressed with a different set of pectolytic enzymes that include *QRT2* and *ADPG2/PGAZAT*. Various xyloglucan endotransglucosylase/hydrolases (XTHs) are expressed throughout all stages of cell separation (Lashbrook and Cai 2008).

Of particular importance are the PG enzymes. PG enzymes hydrolyze the glycosyl bonds of pectin, converting it to the water-soluble pectate. The role of PGs in abscission has been studied in elder (Taylor et al. 1993), *Citrus sinensis* (citrus) (Riov 1974), *Prunus persica* (peach) (Bonghi et al. 1992), *Elaeis guineensis* (oil palm) (Roongsattham et al. 2012), tomato (Taylor et al. 1991; Kalaitzis et al. 1995, 1997; Hong et al. 2000; Jiang et al. 2008), *Brassica napus* (rapeseed) (Sander et al. 2001; Gonzalez-Carranza et al. 2002; Wan et al. 2010), and *Arabidopsis* (Gonzalez-Carranza et al. 2002, 2007; Kim and Patterson 2006; Kim et al. 2006; Ogawa et al. 2009). Silencing the tomato PG gene, *TaPG1*, delays petiole abscission and increases the force required to remove a petiole (Jiang et al. 2008). In *Arabidopsis*, a double mutant of *adpg2/pgazat* and *quartet2* (*qrt2*) delays, but does not block, floral organ abscission (Ogawa et al. 2009). PGs are encoded by a large gene family. *Arabidopsis* has at least 72 PGs in the latest TAIR10 annotation, and it is likely multiple PGs are involved in abscission (Kim and Patterson 2006; Kim et al. 2006; Gonzalez-Carranza et al. 2007).

Pectin contains homogalacturonan polymers that are heavily methyl-esterified. Pectinesterase catalyzes the de-esterification of these polymers before abscission, as has been observed in poinsettia (Lee et al. 2008), making pectin accessible to other hydrolytic enzymes, including PGs, for further breakdown. Pectinesterase also has been detected in the AZ of citrus (Ratner et al. 1969), *Phaseolus vulgaris* (bean), and *Coleus blumei* (Lamotte et al. 1969; Moline et al. 1972).

It is unclear whether modifications to other parts of the cell wall are essential for abscission. Separation appears to be limited to the middle lamella in some species (e.g. *Arabidopsis*, *Rhus typhina*), while in other species (e.g. citrus) substantially more cell wall modifications have been observed (Lee 1911; Hodgson 1918; Addicott 1982; Sexton and Roberts 1982; Lee et al. 2008). However, other hydrolytic enzymes are associated with abscission and cell separation processes. Cellulase, for example, has been implicated in abscission in bean (Abeles 1969; Lewis and Varner 1970; Reid and Strong 1974; Del Campillo et al. 1988; Tucker et al. 1988; Del Campillo and Lewis 1992; Del Campillo et al. 2002), cotton (Mishra et al. 2008), tomato (Del Campillo and Bennett 1996), peach (Bonghi et al. 1992), soybean (Koehler et al. 1996; Kemmerer and Tucker 2002), pepper (Trainotti et al. 1998), and citrus (Ratner et al. 1969). However, although cellulase is widely expressed in the AZ, silencing of tomato *cel1* and *cel2* does not effect petiole abscission (Jiang et al. 2008). Other potential cell wall modifying enzymes may also be involved. For instance, XTH increases in expression upon

initiation of petal abscission in *Rosa bourboniana* (rose) (Singh et al. 2011). Interestingly, xylans and xyloglucans are not detected in poinsettia pedicel AZs until day 7, around the time of abscission, suggesting a possible structural change not directly involved in cell separation (Lee et al. 2008).

Many cell wall modifying enzymes have significantly lower expression in *hae hsl2* double mutants compared to wild type, indicating that they are potentially regulated by HAE HSL2 signaling (**Figure 3**) and part of the HAE HSL2-dependent abscission process (Niederhuth et al. 2013). Many of these same genes have reduced expression levels in *ida* mutants, as shown by quantitative reverse transcription polymerase chain reaction, lending further support to a model in which *IDA* and *HAE HSL2* are in the same pathway (Niederhuth et al. 2013). This model is further supported by promoter-GUS assays using the promoters of PGAZAT and XTR6 (an XTH) that lack GUS activity in *hae hsl2* flowers (Kumpf et al. 2013). Interestingly, *hae hsl2* and *ida* also may function in lateral root emergence, where PGs and other cell wall modifying enzymes also have reduced expression in *hae hsl2* and *ida* mutants (Kumpf et al. 2013).

Post-Abscission

The AZ after abscission is a potential point of infection, water loss, and nutrient loss. Thus, other physiological and molecular processes occur during and after abscission, the end result of which is the production of a protective scar layer over the AZ (Sexton and Roberts 1982). Changes in the AZ following abscission include, but are not limited to, increased expression of defense response genes, altered cell morphology, and modifications to the cell wall that includes the deposition of substances such as suberin and lignin.

Pathogenesis-related (PR) genes increase in expression as abscission progresses, possibly to avoid potential infection after abscission. Most notable of these PR genes is chitinase, which hydrolyzes chitin found in fungal pathogens (Del Campillo and Lewis 1992). Promoter-GUS assays using either the bean chitinase promoter (Patterson and Bleecker 2004) or the *Arabidopsis* chitinase promoter show strong and specific expression in *Arabidopsis* floral AZs (Chen and Bleecker 1995).

Abscission does not result in the breaking of AZ cells. On the contrary, these cells are still very much alive and have been observed to expand after abscission (Sexton and Redshaw 1981; Sexton 1976). Deposition of lignin occurs in poinsettia pedicel AZs (Lee et al. 2008) and in bean petiole AZs (Poovaiah 1974) during and after abscission. Both suberin and lignin accumulate in the AZ of *Lupinus augustifolius* during abscission (Clements and Atkins 2009). Lignin and suberin can act in a protective manner by creating a barrier over the AZ to prevent infection or water loss (Sexton and Roberts 1982). Similarly, callose also may be deposited during abscission, plugging the sieve elements, possibly to prevent water loss (Poovaiah 1974).

Some, but not all, post-abscission processes appear to be regulated in a HAE HSL2-dependent manner. Defense-related genes and several genes in the biosynthesis of lignin and suberin have reduced expression levels in *hae hsl2* mutants (Niederhuth et al. 2013). Additionally, the increased cell numbers and cell expansion in constitutively expressed *IDA* plants (Stenvik et al. 2006) and *knat1/bp* mutant plants (Wang et al. 2006; Shi et al. 2011) also suggests regulation in a HAE HSL2-dependent manner, either directly or indirectly. On the other hand, the elevated expression levels of genes involved in callose deposition and senescence and the reduced levels of genes involved in water/fluid transport are both unaffected in *hae hsl2* mutants (Niederhuth et al. 2013). Similarly, genes involved in the biosynthesis and signaling of ethylene and abscisic acid are observed to increase in stamen AZs in an HAE HSL2-independent manner (Niederhuth et al. 2013). Both ethylene and abscisic acid are known regulators of senescence (Tripathi and Tuteja 2007), and abscisic acid is known to affect callose formation and deposition (Flors et al. 2005). How the HAE HSL2-dependent and HAE HSL2-independent processes interact and coordinate to bring about abscission is an important question that has yet to be addressed.

Functional Genomics of Abscission

The combination of classical physiological, genetic, and genomic studies has shown that multiple pathways and processes are coordinated to bring about abscission. Among the pathways and processes known to be involved are hormone signaling from ethylene, auxin (potentially) abscisic acid, and jasmonic acid (Ogawa et al. 2009), as well as the HAE HSL2 pathway, membrane trafficking, and the subsequent responses regulated by these signals. A major task moving forward, therefore, will be elucidation of the network(s) that integrates these different pathways and processes. Resolution of the entire network will require a combination of single-gene analyses and studies using genomic technologies, such as the microarray and RNA-seq studies used in *Arabidopsis* (Cai and Lashbrook 2008; Lashbrook and Cai 2008; Niederhuth et al. 2013). For instance, a microarray study in *Arabidopsis*, that made use of naturally abscising cells marked by green fluorescent protein expressed with the *ADPG2/PGAZAT* promoter, led to the identification of genes previously unassociated with abscission, including *At3g14380*. When T-DNA mutants of *At3g14380* were subsequently examined, they were found to display delayed abscission (Gonzalez-Carranza et al. 2012).

Functional genomic approaches are being applied increasingly to study abscission in species other than *Arabidopsis*.

Microarrays have been used to study gene expression changes of ethylene-induced abscission in citrus (Agustí et al. 2008), benzyl adenine-induced abscission in apple (Botton et al. 2011), shading-induced and NAA-induced abscission in apple (Zhu et al. 2011), and auxin-induced abscission in tomato (Meir et al. 2010). These studies have revealed changes in cell wall modifying enzymes as well as potential roles for hormonal "cross-talk" and factors like nutritional stress in abscission.

Sequencing-based approaches also have been used to analyze abscission. Zhou et al. (2008) sequenced expressed sequence tags to study shade-induced apple abscission (Zhou et al. 2008) and identified genes primarily involved in carbohydrate metabolism. More recently, Gil-Amado and Gomez-Jimenez (2013) used 454 pyrosequencing to sequence the transcriptomes of olive AZs during mature fruit abscission and found significant expression changes to genes involved in sphingolipid turnover. This study provides the first evidence for the potential involvement of sphingolipids in abscission processes (Gil-Amado and Gomez-Jimenez 2013).

Conclusions and Future Directions

Letting go is never easy. For a plant, breaking bonds and discarding a part of itself is a complicated process. Much has been learned of the abscission process in the last two decades, in particular the function of RLKs in the induction of abscission. As the roles of RLKs and their signaling pathways in abscission continue to be elucidated, striking similarities to other RLK signaling pathways are emerging, including peptide ligands (Butenko et al. 2009), downstream MAP kinase cascades (Asai et al. 2002; Meng et al. 2012), membrane trafficking (Robatzek et al. 2006; Russinova et al. 2004), and the involvement of members of the SERK LRR-RLKs as potential co-receptors (Albrecht et al. 2008). Thus, studies of abscission serve as a model for RLK signaling in other processes. The HAE HSL2 signaling pathway is also being explored in other species. Recently, homologs of *HAE* and *IDA* have been discovered in both soybean and tomato and shown to be expressed in AZs (Tucker and Yang 2012).

If letting go is never easy, neither is it easy to understand the reasons why. Moving forward, there are large gaps in our knowledge of abscission that remain to be filled. The formation and development of the AZ and what characterizes these cells at the molecular level is still unclear. To date, only two genes affecting AZ development have been identified. Similarly, although our understanding of the HAE HSL2 signaling pathway is increasing, obvious gaps remain. For example, the actual binding of IDA by HAE and HSL2 has yet to be demonstrated. Also unclear is how the signal from the receptors to the MKKs is transmitted, although presumably this involves a MAP3K and likely other factors. While multiple potential transcription factors have been identified, the actual substrates of the MPKs during abscission are unknown. At the systems level, it is unclear how all the different inputs integrate to bring about abscission. Several possibilities exist. For instance, expression of *IDA* may be regulated by ethylene and auxin (Butenko et al. 2006; Kumpf et al. 2013). Yet, on the other hand, there is a well-known connection between ethylene biosynthesis and signaling and MPK3/6 (Liu and Zhang 2004; Yoo et al. 2008; Hahn and Harter 2009). Likely, a combination of genomic technologies and traditional methodologies carried out in *Arabidopsis* and tomato will be used to fill these gaps in our knowledge and the findings translated into other species of agricultural and economic importance.

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