1   # Advances in abscission signaling

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29 **Abstract**

30 Abscission is a process in plants for shedding unwanted organs such as leaves, flowers, fruits, or

31 floral organs. Shedding of leaves in the fall is the most visually obvious display of abscission in

32 nature. The very shape plants take is forged by the processes of growth and abscission.

33 Mankind manipulates abscission in modern agriculture to do things like prevent pre-harvest

34 fruit drop prior to mechanical harvesting in fruit orchards. Abscission occurs specifically at

35 abscission zones that are laid down as the organ that will one day abscise is developed. A

36 sophisticated signaling network initiates abscission when it is time to shed the unwanted organ.

37 In this article, we review recent advances in understanding the signaling mechanisms that

38 activate abscission. Physiological advances and roles for hormones in abscission are also

39 addressed. Finally, we discuss current avenues for basic abscission research and potentially

40 lucrative future directions for its application to modern agriculture.

41

42 **Keywords**: Abscission signaling, Arabidopsis abscission zones, floral organ abscission, leaf

43 abscission, ligand-receptor pathway, feedback loop

44

Introduction

46 Abscission in plants is the process of shedding unwanted organs. The most recognized

47 abscission event occurs in the northern hemisphere when deciduous plants drop their leaves

48 before winter. However, plants can abscise leaves, branches, flowers, floral organs (petals,

49 sepals, stamen), fruits, and seed pods. In general, plants have a flexible ordered design wherein

50 size, number of organs, and precise shape are not strictly set. If abscission did not occur, plants

51 would look very different. For example, if deciduous trees did not abscise their leaves before

52 winter, they would retain remnants of the previous year's leaves all over themselves. While

53 some of these remnants may break off, the dead leaves that remain would eventually decay

54 and invite disease as well as shield some light from living leaves. In essence, the ordered design

55 we see in plants is shaped by both growth and abscission. It is easy to imagine how

56 reproduction in many plants could be adversely impacted if abscission did not exist. If fruits

57   with seeds were to never fall to the ground, the seeds would not touch the soil without aid of

58   outside forces, like animals or harsh weather.

59   Abscission can be triggered by developmental cues like fruit ripening or fertilization.

60   Flower petals falling off after fertilization is well characterized in Arabidopsis. The environment

61   can also prompt abscission. Photoperiod and cooler temperatures trigger leaf abscission in the

62   fall. By losing leaves in the fall, plants save on energy needed to keep them alive. Also, trees

63   without leaves have less surface area to catch snow and ice, which reduces the risk of branches

64   breaking under excess weight. Plants also cut their transpirational load during drought by

65   abscising leaves. Many plants, such as bean, shed entire flowers when exposed to drought.

66   Without adequate water it would be hard to set seeds, so this adaptation prevents plants from

67   wasting energy starting seed production that they would not be able to finish. Insect feeding

68   also triggers abscission in plants as a protective strategy (Faeth *et al.*, 1981). Leaves can also be

69   shed as a response to bacterial disease. Leaf shedding as a defense response leaves the

70   pathogen feeding on the fallen leaf, giving the plant time to mount a more comprehensive

71   preventative defense response (Faeth *et al.*, 1981). In short, abscission is used by sessile plants

72   to regulate their morphology to suit the environment in which they live.

73   Modern agriculture manipulates the abscission process to its advantage. For example,

74   synthetic auxins and ethylene blockers, which partially block abscission, are sprayed on *Citrus*

75   and apple trees about a month before harvest (Anthony and Coggins Jr., 1999; Yuan and

76   Carbaugh, 2007). This practice prevents fruits from dropping to the ground before mechanical

77   harvesters can collect them. Tomatoes used in the canning industry are bred with the *jointless*

78   mutation, which results in plants with no pedicel abscission zone (Mao *et al.*, 2000). When

79   *jointless* tomatoes are picked, they leave their calyx and stem behind on the plant. This results

80   in less puncture damage to other tomatoes when they are placed together in a harvesting bag

81   (Zahara and Scheuerman, 1988). The future looks bright for further agricultural improvements

82   resulting from altering abscission. For instance, many crop plants are overly sensitive to periods

83   of mild drought common in the agricultural setting. It may be possible to increase yield in beans

84   by preventing flowers from abscising in response to mild drought conditions (Pandey *et al.*,

85   1984).

3

86   Abscission occurs specifically at a specialized region of cells called the abscission zone

87 (AZ). AZs are laid down early in development and often have a band-like appearance. The cells

88 in an AZ are smaller than the surrounding cells and have a densely packed cytoplasm. Once

89 abscission is triggered, AZ cells expand and the middle lamella (the pectin layer that glues two

90 cells together) is dissolved via hydrolytic enzymes, allowing cell separation. After abscission has

91 occurred, a new, protective epidermal layer is laid down over the abscission "scar."

92

93 **The abscission signaling network in Arabidopsis**

94   For years Arabidopsis floral organ abscission has served as the premiere model for

95 understanding abscission at the molecular genetic level. Forward and reverse genetic

96 approaches have revealed a number of components that are necessary for abscission and

97 recently biochemistry has led to some mechanistic insights. Abscission can be divided into 4

98 phases to simplify its explanation. First, abscission zones must develop. Second, abscission

99 signaling is activated. Third, an enzymatic hydrolysis of the abscission zone's middle lamella

100 takes place and AZ cells begin to enlarge. Finally, the abscission scar further differentiates and

101 seals itself (Kim, 2014). The hydrolysis phase has been thoroughly reviewed and will not be

102 discussed in detail here (Niederhuth *et al.*, 2013; Kim, 2014). A model of the physiological

103 phases of abscission is shown in Figure 1.

104   AZ development begins very early in the development of the organ that will later be

105 able to abscise. Some genetics is known about AZ development in Arabidopsis. The

106 transcription factors *BLADE ON PETIOLE 1/2* (*BOP1/2*) are redundantly necessary for its

107 formation (McKim *et al.*, 2008). ARABIDOPSIS THALIANA HOMEOBOX GENE1 (ATH1), a BELL-

108 type transcription factor, is required for stamen AZ placement and development (Gómez-Mena

109 and Sablowski, 2008). The homeodomain transcription factor BREVIPEDICELLUS (BP) prevents

110 floral organ AZs from becoming too big (Wang *et al.*, 2006). The myb transcription factor,

111 *ASYMMETRIC LEAVES1* (*AS1*), establishes the positioning of floral organ AZs (Gubert *et al.*,

112 2014). In tomato, an agamous-like MADS domain transcription factor, *JOINTLESS*, is necessary

113 for formation of the pedicel AZ (Mao *et al.*, 2000). While MADS domain transcription factors

114 regulate abscission activation in Arabidopsis, they have not been shown to regulate AZ

115   development in Arabidopsis (Fernandez *et al.*, 2000; Chen *et al.*, 2011; Patharkar and Walker,

116   2015; Patharkar *et al.*, 2016).

117   There is a broad understanding of the mechanisms that result in the activation of

118   abscission signaling in Arabidopsis. Signaling events in the abscission activation phase initiate

119   the expression of a mixture of cell wall modifying enzymes that dissolve the pectin-rich middle

120   lamella of the AZ. No known Arabidopsis mutants abscise but then fail to further differentiate

121   their AZs after abscission. However, it is likely that signaling components of the abscission

122   activation phase play a role in the final differentiation of the AZ scar. For example, over-

123   expression of *INFLORESCENCE DEFICIENT IN ABSCISSION* (*IDA*), a gene needed for abscission

124   activation, results in over-differentiation of the AZ scar (Stenvik *et al.*, 2006).

125

126   **The abscission receptor complex and perception of processed IDA peptide**

127   Abscission activation is regulated by two receptor-like protein kinases, *HAESA* and

128   *HAESA-like 2* (*HAE/HSL2*), which are redundantly required for Arabidopsis to shed its petals,

129   sepals, and stamen (Jinn *et al.*, 2000; Cho *et al.*, 2008; Stenvik *et al.*, 2008). A peptide released

130   from IDA by subtilisin-like serine proteinase processing is also required for abscission (Butenko

131   *et al.*, 2003; Schardon *et al.*, 2016). Recent work indicates that HAE does not work alone to

132   perceive IDA; rather, HAE works together with SOMATIC EMBRYOGENESIS RECEPTOR-LIKE

133   KINASE 1/2/3/4 (SERK1/2/3/4) (Meng *et al.*, 2016; Santiago *et al.*, 2016). *SERK3* is also known as

134   *BRI1-ASSOCIATED RECEPTOR KINASE 1* (*BAK1*). Importantly, BAK1 is also the co-receptor for

135   both BRASSINOSTEROID INSENSITIVE 1 (BRI1) and FLAGELLIN-SENSITIVE 2 (FLS2) (Chinchilla *et*

136   *al.*, 2007; Nam and Li, 2002). BRI1 is the receptor that perceives brassinosteroid hormones that

137   are responsible for cell expansion and elongation (He *et al.*, 2000). FLS2 is the receptor that

138   perceives a portion of bacterial flagellin and initiates a defense response (Gómez-Gómez and

139   Boller, 2000). IDA stabilizes a protein complex between HAE/HSL2 and SERK 1/2/3/4 in

140   Arabidopsis mesophyll protoplasts and in *Nicotiana benthamiana* epidermal cells (Meng *et al.*,

141   2016). Specifically, the extracellular domains of HAE/HSL2 and SERK 1/2/3 interact with the IDA

142   peptide (Meng *et al.*, 2016; Santiago *et al.*, 2016). Notably, an IDA peptide-HAE-SERK complex

143   has only been shown to exist *in vitro* as well as in Arabidopsis mesophyll protoplast and in

144   *Nicotiana benthamiana* overexpression systems (Meng *et al.*, 2016; Santiago *et al.*, 2016); this

145   complex has yet to be identified in AZs. It will be interesting to see if new intricacies of the

146   ligand-receptor complex can be uncovered in AZs.

147   Recent developments have shed new light on how IDA is processed into a biologically

148   active form and shown the mechanism of IDA's proteolytic processing into a biologically active

149   peptide. A recent study overcame functional redundancy of subtilisin-like proteinases (SBTs) by

150   expressing tissue-specific proteinase inhibitors to show that SBTs are both required for

151   maturation of the IDA peptide and floral organ abscission (Schardon *et al.*, 2016). Furthermore,

152   the study revealed that the mature and highly bioactive IDA peptide is a 14 mer of sequence

153   GVPIPPSAPSKRHN. This mature IDA peptide is at least 10 times more bioactive than the

154   previously proposed PIPP or extended PIPP peptide previously proposed based on sequence

155   conservation (Stenvik *et al.*, 2008; Schardon *et al.*, 2016). The exact contribution of specific SBTs

156   to IDA cleavage and the extent of redundancy are not clear at the moment. SBTs 4.12, 4.13, and

157   5.2 are all proposed to contribute to IDA processing based on their expression patterns and *in*

158   *vitro* activity (Schardon *et al.*, 2016). Interestingly, the *in vitro*, mesophyll protoplast, and

159   *Nicotiana benthamiana* over-expression studies that were used to show that IDA peptide can

160   induce a complex between HAE and SERKs require a version of the peptide where the central

161   proline is modified to hydroxyproline (Meng *et al.*, 2016; Santiago *et al.*, 2016). In contrast,

162   studies involving AZs from Arabidopsis require no such modification for complementation of

163   abscission defects of *ida* mutants or plants blocked in SBT activity (Stenvik *et al.*, 2008;

164   Schardon *et al.*, 2016). This difference between findings in AZs and other non-AZ systems

165   represents an opportunity to further refine our understanding of the abscission signaling

166   mechanism.

167

168   **Amplification of the abscission signal by a MAPK cascade and a positive feedback loop**

169   Downstream of the HAE receptor complex lies a MITOGEN-ACTIVATED PROTEIN KINASE

170   (MAPK) cascade consisting of MKK4/5 and MPK3/6. Knockouts/knockdowns of the MAPK

171   cascade fail to abscise their floral organs. Conversely, expression of constitutively active

172   versions of the MKK4/5 are able to restore abscission in *hae hsl2* double mutants, indicating

173 that the MAPK cascade is epistatic to the HAE receptor complex (Cho *et al.*, 2008). At the

174 moment, it is not clear which MAP triple kinase functions in the abscission pathway, nor is it

175 clear whether there are other intermediates between the HAE receptor complex and the MAPK

176 cascade. A striking discovery revealed that knockdown of MKK4/5 results in less than 20% of

177 normal *HAE* expression as abscission is activated in floral organ abscission zones (Patharkar and

178 Walker, 2015). This result is surprising since *HAE* was thought to be genetically upstream from

179 the MAPK cascade (Cho *et al.*, 2008). Over-expression of the MADS domain transcription factor,

180 *AGAMOUS-LIKE 15* (*AGL15*), blocks abscission but does not alter AZ development, suggesting

181 AGL15 is a negative regulator of abscission (Fernandez *et al.*, 2000). A study in floral receptacles

182 revealed that under native protein levels, AGL15 binds the *HAE* promoter keeping *HAE* from

183 being expressed prior to abscission being activated. Furthermore, once the abscission signaling

184 pathway is activated, the MAPK cascade phosphorylates AGL15 on serine 231 and 257 and de-

185 represses *HAE* expression. Newly synthesized HAE completes a positive feedback loop once it

186 takes its place in the plasma membrane. The positive feedback loop and the MAPK cascade

187 both serve to greatly amplify the starting signal to abscise, which explains how *HAE* expression

188 is increased 27-fold during the process of floral organ abscission. While AGL15 appears to be a

189 major transcription factor regulating abscission, it certainly cannot be the only one. For

190 example, *agl15 agl18* double mutants abscise statistically earlier than wild type, suggesting that

191 AGL15's sister protein, AGL18, plays a partially redundant role with AGL15 (Patharkar and

192 Walker, 2015; Patharkar *et al.*, 2016). Other transcription factors that block abscission when

193 over-expressed have previously been reviewed (Niederhuth *et al.*, 2013). Currently, it is not

194 entirely clear how these other transcription factors fit into the aforementioned positive

195 feedback loop.

196   Proper abscission requires an ADP-ribosylation factor GTPase-activating protein,

197 NEVERSHED (NEV). Mutations in NEV alter the Golgi structure and change the location of the

198 trans Golgi network. *nev* mutants also over accumulate paramural vesicles, which are thought

199 to move HAE and other proteins to the plasma membrane (Liljegren *et al.*, 2009). However, this

200 story is anything but simple. Mutations in three different secondary genes can partially restore

201 vesicle trafficking and restore abscission in *nev* mutants. The first of these three secondary

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202 genes is *EVERSHED* (*EVR*), a receptor-like protein kinase that is also known as *SUPPRESSOR OF*

203 *BIR1 1* (*SOBIR1*) (Leslie *et al.*, 2010). Mutations in *BAK1-INTERACTING RECEPTOR-LIKE KINASE 1*

204 (*BIR1*) have a constitutive pathogen response that can be suppressed by secondary mutations

205 in *EVR/SOBIR1* (Gao *et al.*, 2009). Secondary mutations in *SERK1* can also suppress *nev*

206 phenotypes (Lewis *et al.*, 2010). From a molecular mechanistic standpoint, it is not clear how

207 mutating the co-receptor of HAE could restore abscission in *nev* mutants. The triple mutant

208 *serk1 serk2 bak1* actually has a mild floral organ abscission defect (Meng *et al.*, 2016). Finally,

209 secondary mutations in *CAST AWAY* (*CST*), a receptor-like cytoplasmic kinase, can also suppress

210 phenotypes of *nev* mutants. CST physically interacts with HAE and EVR in Arabidopsis mesophyll

211 protoplasts (Burr *et al.*, 2011). Secondary mutations in *EVR*, *SERK1*, and *CST* all restore

212 abscission in *nev* mutants, but the final AZ scar in these plants is over differentiated. This

213 observation suggests that players involved in the abscission activation phase also function in

214 the final differentiation of the AZ scar. In addition to being shuttled by vesicles, HAE passes

215 through an error checking mechanism in the endoplasmic reticulum as well. The endoplasmic

216 reticulum-associated degradation system (ERAD) ensures HAE is free from defects (Baer *et al.*,

217 2016). When the ERAD system is defective, alleles of *HAE* that generate a partially functional

218 protein can still make it to the plasma membrane and transduce the abscission signal. A model

219 of the abscission activation signaling pathway is shown in Figure 2 and Table1.

220

221 **Physiology, hormones, and the big picture of abscission**

222 At first glance, the molecular mechanisms regulating abscission, that are described

223 above, seem relatively straightforward and logical. However, their depiction is overly simplified

224 since there are several points that are only partially congruent with physiological observations.

225 Therefore, a great many opportunities exist to connect our understanding of molecular

226 mechanisms regulating abscission with the actual physiological changes that occur in AZs during

227 abscission. In our opinion a glaring issue that could be clarified is what exactly does IDA do at

228 the physiological level? Recent literature refers to IDA peptide as a hormone, which it is likely to

229 be (Santiago *et al.*, 2016; Schardon *et al.*, 2016). However, hormones are typically defined as

230 molecules produced in one tissue that exert an effect in another tissue. Currently, no effort has

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231   been made to determine where the IDA peptide acting on a given AZ cell is coming from: the

232   same cell, the immediately adjacent cells, or from more distant cells? Also, why is there a

233   peptide signal at all? Does it help synchronize the abscission process? As drawn in model

234   diagrams, all the molecular components necessary for signaling abscission are produced in each

235   individual cell. *In vitro* experiments show that IDA peptide in agar plates can enter the pedicel

236   of detached flowers and complement *ida* mutants (Stenvik *et al.*, 2008). In short, there could be

237   many future breakthroughs at the interface of molecular signaling and the physiology of

238   complex AZ tissue. An increased understanding of AZ tissue will ultimately push mankind's

239   understanding of cell to cell communication to a new level.

240   A number of more classical plant hormones exert influence over abscission. Ethylene is

241   broadly necessary for abscission in Arabidopsis and crop plants. Arabidopsis mutants defective

242   in ethylene perception, *ethylene response 1* (*etr1*), and ethylene signaling, *ethylene insensitive 2*

243   (*ein2*), have delayed floral organ abscission (Patterson and Bleecker, 2004). Additionally, the

244   MADS domain transcription factor *FOREVER YOUNG FLOWER* (*FYF*) has been proposed to work

245   at the level of ethylene signaling. Over-expression of *FYF* results in delayed abscission (Chen *et*

246   *al.*, 2015). Auxin is generally thought to negatively regulate abscission by making tissue

247   insensitive to ethylene (Sexton and Roberts, 1982). As mentioned above, a cocktail of ethylene

248   blocker (aminoethoxyvinylglycine HCl) and synthetic auxin (2,4-Dichlorophenoxyacetic acid) are

249   used to prevent pre-harvest fruit drop in *Citrus* and apple tree (Anthony and Coggins Jr., 1999;

250   Yuan and Carbaugh, 2007). Jasmonic acid positively regulates floral organ abscission in

251   Arabidopsis. Mutations in the jasmonic acid receptor, *coronatine insensitive 1* (*coi1*), result in

252   delayed floral organ abscission in Arabidopsis (Kim *et al.*, 2013). Hormones other than ethylene,

253   auxin, or jasmonic acid are also likely to influence abscission. Salicylic acid may also regulate

254   abscission. The genes encoding enzymes necessary for salicylic acid synthesis, *ISOCHORISMATE*

255   *SYNTHASE 1/2*, are transcriptionally increased during the process of floral organ abscission (Cai

256   and Lashbrook, 2008). Salicylic acid has a well-established role in regulating senescence, and

257   both floral organs and cauline leaves appear to senesce before they abscise (Guiboileau *et al.*,

258   2010; Patharkar and Walker, 2015, 2016).

259   Many interesting yet relatively unexplained physiological events occur in AZs once

260   abscission is activated. As abscission advances, the cytosol of AZ cells becomes more alkaline.

261   Treatments that slow abscission, like ethylene blockers, prevent this cytosolic alkalization

262   (Sundaresan *et al.*, 2015). Additionally, cytosols of ethylene insensitive mutants in Arabidopsis

263   do not alkalize, while mutants with overly active ethylene signaling are already alkalized before

264   abscission begins. *ida* and *nev* mutants also fail to alkalize the cytosol of their AZ cells. The

265   cytosolic alkalization of AZ cells, associated with abscission, has been shown to occur in

266   Arabidopsis, tomato, and wild rocket (Sundaresan *et al.*, 2015). The reason for the pH change of

267   AZ cell's cytosol is currently a mystery. One hypothesis is that alkaline pH may be optimal for

268   some abscission enzymes.

269   Abscission zone cells also enlarge as abscission occurs. Arabidopsis cauline leaf AZ cells

270   can clearly be seen to begin enlarging slightly prior to abscission. Thus, AZ middle lamella

271   hydrolyses and AZ cell enlargement overlap in timing. In drought triggered-abscission, the final

272   size of AZ cells that have a sealed scar are not noticeably larger than AZ cells at the moment of

273   first cell separation (Patharkar and Walker, 2016). Previous reviews suggest that floral organ AZ

274   cell enlargement only happens after cell separation is complete (Kim, 2014). This notion is likely

275   due to the fact that floral organ AZ cells cannot be visualized nondestructively prior to

276   abscission because sepals and petals cover the AZs. After learning cauline leaf AZ cells were

277   expanding and separating simultaneously, we looked closely at floral organ AZs as cell

278   separation was just beginning. When we removed loosely attached sepals and petals (i.e., when

279   abscission is just beginning but not complete), we observed already enlarged AZ cells. It should

280   be noted that early in the 20th century scientists believed mechanical shearing force from AZ

281   cell enlargement was the primary driving force for abscission (Fitting, 1911; Sexton and Roberts,

282   1982). Currently, no mutants of Arabidopsis abscise but fail to enlarge their AZ cells suggesting

283   that AZ enlargement is necessary for abscission in Arabidopsis.

284

285   **Leaf abscission and abscission in non-model plants**

286   The most detailed explanation of abscission signaling has come from studying

287   Arabidopsis floral organ abscission. The Arabidopsis floral organ system has been used to work

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288   out a number of molecular mechanisms regulating abscission signaling (described above) that

289   would have been more difficult to solve in less genetically tractable systems. Floral organ

290   abscission occurs once fertilization occurs. Since Arabidopsis is self-pollinating, abscission

291   basically occurs in a developmentally timed manner. Recently, it has become clear that cauline

292   leaf abscission triggered by drought requires the same core abscission signaling mechanism as

293   floral organ abscission (Patharkar and Walker, 2016). *IDA*, *HAE/HSL2*, *MKK4/5*, and *NEV* are all

294   required for drought-triggered leaf abscission to occur. This is an interesting finding because

295   cauline leaf abscission is not set on a developmental clock but rather occurs conditionally.

296   Drought to the point of wilting activates HAE expression, and then, once plants are re-watered,

297   leaf abscission occurs (Patharkar and Walker, 2016). HAE expression is also activated in the

298   vestigial pedicel abscission zone in Arabidopsis prior to partial abscission. This observation

299   suggests that fruit abscission may also utilize the same signaling pathway as leaves and floral

300   organs (Patharkar and Walker, 2016). The cauline leaf abscission system in Arabidopsis has two

301   distinct features from the floral organ abscission system that will likely aid researchers in

302   further unraveling the process of abscission. First the cauline leaf AZ can be non-destructively

303   observed from development through abscission because no tissue obscures its view. Second,

304   abscission is not triggered by a developmental stage, rather it is triggered by environmental

305   conditions. This allows separation of abscission events from developmental events. Currently,

306   drought in known to trigger cauline leaf abscission, however, other environmental stimuli may

307   also initiate leaf abscission (Patharkar and Walker, 2016).

308   How conserved is the Arabidopsis abscission signaling module in other species? Recent

309   research indicates that the Arabidopsis abscission module likely extends far past Arabidopsis. A

310   phylogenetic study showed that IDA is conserved in all flowering plants (Stø *et al.*, 2015). *HAE*

311   homologs are up-regulated in Poplar leaf AZs that are abscising due to shading (Jin *et al.*, 2015).

312   *Citrus IDA3* can complement abscission deficiency of Arabidopsis *ida* mutants (Estornell *et al.*,

313   2015). Taken together, these findings are strong evidence that the Arabidopsis abscission

314   signaling module works in other and distantly related species.

315

316   **Future research**

11

317   In our opinion, two areas of basic abscission research stand out as particularly likely to

318   pay big dividends in the future. First, a literature cross reference analysis can provide a number

319   of easy to test leads to extend the existing abscission signaling pathway. Since abscission

320   components overlap with defense and brassinosteroid fields, and drought-triggered abscission

321   has been characterized, mining those fields for connections relevant to abscission could be

322   fruitful. For example, one could look at the defense field and see that BIR1 interacts with BAK1

323   and that mutations in *EVR* can suppress the phenotypes of *bir1* mutants. The power of cross

324   reference analysis will grow as more network hub components are added to the abscission

325   pathway. The ability to work in both floral organ and cauline leaf abscission zones also gives

326   researchers options. Of course, researchers will have to take care in interpreting non-AZ data

327   and applying it to AZs. The AZ is a unique tissue that does not behave like other parts of the

328   plant. For example, AGL15 over-expression represses *HAE* expression in AZs but not in

329   mesophyll protoplasts (Patharkar *et al.*, 2016).

330   A second promising area is research focused on precisely connecting molecular data to

331   the physiology of abscission zones. At the moment, molecular signaling mechanisms have very

332   vague outputs. For the most part, all that can be said for the output of the molecular pathway is

333   that it triggers abscission. However, abscission can be broken down into a number of

334   physiological events, like AZ cell enlargement, AZ cell cytosol alkalization, and middle lamella

335   hydrolysis. How does the molecular pathway affect these different physiological aspects? It is

336   unlikely that all AZ cells behave the same. Using modern physiological methods to break down

337   the AZ into functional groups of cells and events will yield a tangible understanding of what

338   actually happens at the cellular level to allow abscission.

339   Finally, there is also promise to use our basic knowledge of abscission signaling to do

340   applied research that could benefit agriculture. For example, preventing soybeans from

341   shedding flowers in response to mild drought conditions might result in more seed pods come

342   harvest time. *ida* and *hae hsl2* mutants are abscission defective but have no seed yield penalty,

343   making them excellent candidates for manipulation (Patharkar and Walker, 2016). Knocking out

344   their homologs in soybean via CRISPR/Cas9 is doable and would allow for straightforward seed

345   yield trials. Alternatively, yield trials could be conducted where chemicals that delay abscission

346   are sprayed on plants prior to drought treatment. While there is no guarantee that the

347   proposed manipulations to soybean would result in increased grain yield, the mechanism for

348   grain yield improvement is simple and therefore straightforward to assay. Basically, more

349   flowers on the plant could be more seed pods later on. In summary, there are more

350   opportunities in basic and applied abscission research right now, than ever before.

351

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13

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Table 1. Additional abscission signaling components not precisely placed on Figure 2.

| Cellular location	Protein	Reference
1 (plasma membrane)	CST	(Burr *et al.*, 2011)
1 (plasma membrane)	EVR	(Burr *et al.*, 2011)
1 (plasma membrane)	ETR1	(Patterson and Bleecker, 2004)
2 (cytoplasm)	EIN2	(Patterson and Bleecker, 2004)
3 (nucleus)	AGL18	(Adamczyk *et al.*, 2007)
3 (nucleus)	FYF	(Chen *et al.*, 2011)
3 (nucleus)	COI1	(Kim *et al.*, 2013)
3 (nucleus)	BP	(Shi *et al.*, 2011)
3 (nucleus)	KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 2/6	(Shi *et al.*, 2011)
3 (nucleus)	ZINC FINGER PROTEIN 2	(Cai and Lashbrook, 2008)
3 (nucleus)	DNA BINDING WITH ONE FINGER 4.7	(Wei *et al.*, 2010)

Figures
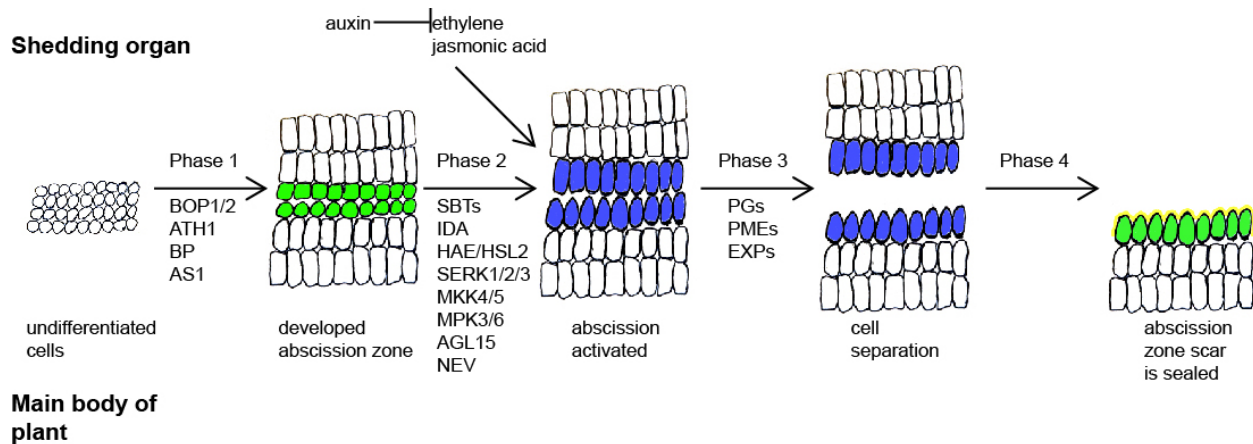
Figure 1. Physiological model of abscission. In phase 1, abscission zones develop. BOP1/2, ATH1, BP, and AS1 are required for abscission zone development. In phase 2, the abscission signaling loop is activated and the indicated proteins are all required. The hormones ethylene and jasmonic acid positively regulate abscission, while auxin negatively regulates abscission by reducing the effect of ethylene. At this step, abscission zone cells begin to enlarge and the pH of their cytosol becomes alkaline. In phase 3, polygalacturonases (PGs), pectin methyltransferases (PMEs), and expansins (EXPs) cause cell separation. These same enzymes, particularly expansins, are likely involved in the cell expansion that occurs before cell separation. Finally, in phase 4, the abscission zone scar is sealed with a protective layer and the pH of the abscission zone cells returns to neutral. Abscission zone cells are depicted in color where green represents neutral cytosolic pH and blue represents alkaline pH. This figure was adapted and updated from (Kim, 2014).
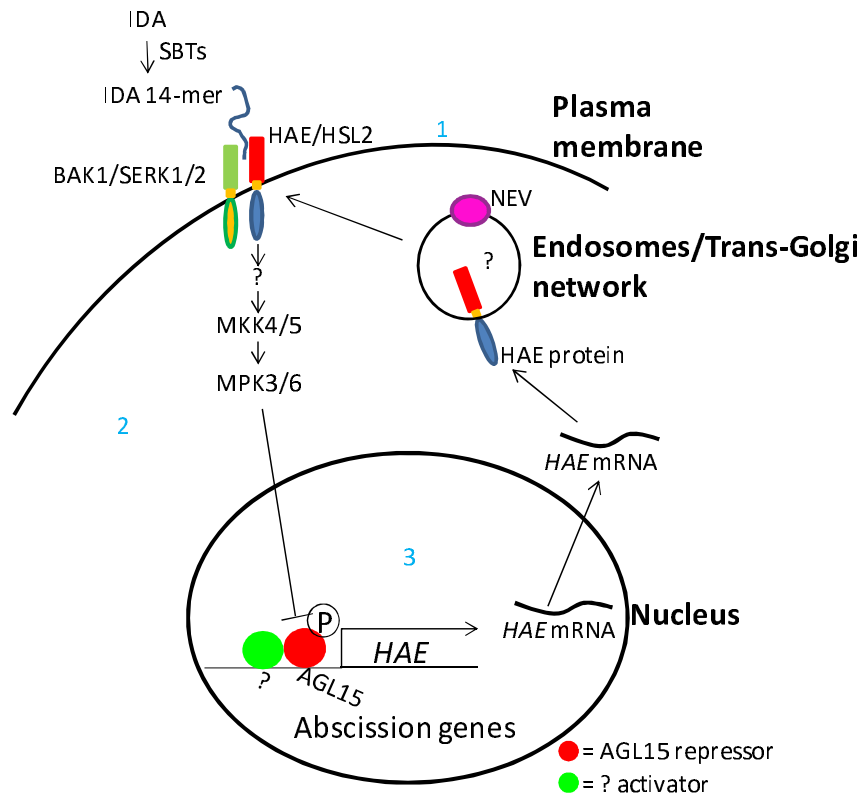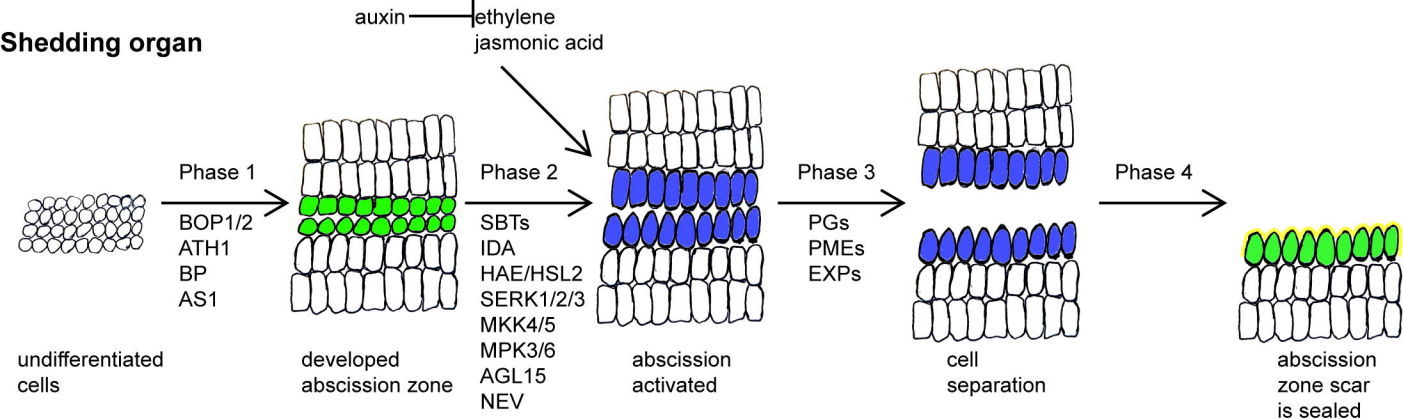
Figure 2. Model of abscission activation signaling pathway. IDA is cleaved by SBTs into a 14-mer peptide that binds HAE and its co-receptor that can be SERK1/2/3 (SERK3 is also known as BAK1). HAE then, through unknown means, activates a MAPK cascade consisting of MKK4/5 and MPK3/6. MPK3/6 then phosphorylate AGL15, which de-represses *HAE* transcription. Newly synthesized HAE is then shuttled back to the plasma membrane in endosome vesicles with the assistance of NEV, completing a positive feedback loop. Blue colored numbers indicate additional components that are located at the plasma membrane (1), cytoplasm (2), and nucleus (3) that cannot be precisely placed on the diagram. The names of these additional proteins are listed in Table 1 and were previously reviewed in detail (Niederhuth *et al.*, 2013).

Shedding organ

auxin —— ethylene
jasmonic acid

Phase 1

BOP1/2
ATH1
BP
AS1

Phase 2

SBTs
IDA
HAE/HSL2
SERK1/2/3
MKK4/5
MPK3/6
AGL15
NEV

Phase 3

PGs
PMEs
EXPs

Phase 4

undifferentiated
cells

developed
abscission zone

abscission
activated

cell
separation

abscission
zone scar
is sealed

Main body of plant