Chapter 22
Signaling Events in Pollen Acceptance or Rejection in the *Arabidopsis* Species

Emily Indriolo, Darya Safavian, and Daphne R. Goring

Abstract The initial events of pollen–pistil interactions are fundamentally important in flowering plants because they influence successful fertilization. These early events include the recognition of pollen grains through signaling events in the pistil that will lead to the acceptance of a compatible pollen grain or the rejection of an incompatible pollen grain. There has been much research into this field in the Brassicaceae, as this family includes many agriculturally important crops such as canola, radish, turnip, and cabbage. However, this review focuses on what is known about the early pollen–pistil interactions in the experimentally tractable *Arabidopsis* genus, including *Arabidopsis thaliana* (a self-compatible species) and *Arabidopsis lyrata* (a self-incompatible species). Compatible pollinations are driven by the ability of the pistil to provide the resources for an acceptable pollen grain to hydrate, germinate, and fertilize the ovule. Self-incompatible species have a receptor–ligand signaling pathway that rejects self-pollen grains, preventing inbreeding and encouraging genetic diversity within the species. There is some overlap between these two pathways, and current research is looking for unknown elements and downstream events following the initial recognition of a pollen grain in *Arabidopsis*.

Keywords Compatible pollen response • E3 ubiquitin ligase • Exocyst • Self-incompatibility • Vesicle secretion

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H. Sawada et al. (eds.), *Sexual Reproduction in Animals and Plants*,
DOI 10.1007/978-4-431-54589-7_22, © The Author(s) 2014

22.1 Introduction

Throughout the course of land plant evolution, various mating strategies have occurred over time, with the most recent innovation being the development of flowers. With flowers, a successful fertilization is typically determined by interactions between the male pollen and the female pistil. Therefore, a great deal of research has focused on pollen–pistil interactions, and one targeted area has been uncovering the molecular and cellular mechanisms of early pollen–pistil interactions in species of the mustard family (Brassicaceae) such as the *Arabidopsis* and *Brassica* genera. In this family, selective cell–cell interaction events occur between the pollen grain and the surface of the pistil. These pollen–pistil interactions trigger an active recognition system to allow for the acceptance of compatible pollen and the subsequent successful fertilization or pollen rejection if the self-incompatibility system is activated. Much of our understanding of these early pollen–pistil interaction events has come from research on the *Brassica* genus (reviewed in Hiscock and Allen 2008; Chapman and Goring 2010; Iwano and Takayama 2012). This chapter largely focuses on reviewing the early stages following compatible and self-incompatible pollinations for the closely related Arabidopsis genus.

22.2 Early Compatible Pollen–Pistil Interactions in *Arabidopsis thaliana*

Pollen first comes in contact with the stigma at the top of the pistil, and the ability of the stigma to discriminate between compatible and other foreign pollen depends on whether the plant possesses wet- or dry-type stigmas. Wet stigmas have abundant surface secretions that indiscriminately capture pollen and allow germination to occur. In contrast, surface secretions are absent in dry stigmas, and as a result there is a much tighter regulation of these early pollen–stigma interaction stages (Dickinson 1995). *Arabidopsis* species possesses a dry stigma that is covered with papillae on the stigmatic surface. Once a compatible pollen grain comes in contact with a stigmatic papilla, the early stages of pollen capture and adhesion, pollen hydration, and germination are closely regulated (Elleman et al. 1992; Preuss et al. 1993; Kandasamy et al. 1994; Zinkl et al. 1999). The ability to conduct genetic screens with relative ease in *A. thaliana* has aided in identifying a number of factors that regulate pollen–stigma interactions (Preuss et al. 1993; Hulskamp et al. 1995; Nishikawa et al. 2005).

22.2.1 Pollen–Stigma Components for Compatible Pollen Acceptance

Following pollination, two components of the pollen grain come in contact with the stigmatic papilla: the exine, which is the sculptured outermost layer of the pollen grain, and the lipid- and protein-rich pollen coat that is present in the pockets of the

exine (Elleman et al. 1992; Kandasamy et al. 1994). The initial binding of compatible *A. thaliana* pollen to the stigmatic papilla was determined to be mediated by the pollen exine, while the pollen coat was not required (Zinkl et al. 1999). Consistent with this, a genetic screen for mutants disrupted in this initial adhesion stage resulted in a number of mutants with exine defects (Nishikawa et al. 2005; Dobritsa et al. 2011). The pollen coat is then involved in further adhesion of the pollen grain to the stigmatic papilla. At this stage, the pollen coat flows out from the pollen grain toward the stigmatic papilla to mix with the lipidic and proteinaceous surface of the stigmatic papilla, creating a more robust connection at the pollen–stigma interface (Elleman et al. 1992; Preuss et al. 1993; Kandasamy et al. 1994; Zinkl et al. 1999). The result is an interface between the pollen grain and the papilla where signaling is hypothesized to occur, and water is transferred from the papilla to the pollen grain for hydration (Elleman and Dickinson 1990; Preuss et al. 1993).

Pollen hydration is necessary for the desiccated pollen grain to become metabolically active and proceed to the next stage of pollen germination and formation of a pollen tube, and components of the pollen–stigma interface are important to this process (Preuss et al. 1993). On the female side, the specific lipid content in the stigmatic cuticle was indirectly implicated in the control of pollen hydration through the study of the *A. thaliana fiddlehead* (*fdh*) mutant. Normally, pollen hydration and pollen tube growth are restricted to mature stigmas and cannot be supported on other tissues (Kandasamy et al. 1994; Ma et al. 2012). However, the *fdh* mutant allowed pollen hydration and pollen tube growth to occur on nonstigmatic surfaces such as the entire shoot epidermis (Lolle and Cheung 1993). Interestingly, *fdh* mutant leaves were found to have increased levels of long-chain lipids, and the *FDH* gene is predicted to encode an enzyme involved in lipid biosynthesis (Lolle et al. 1997; Yephremov et al. 1999; Pruitt et al. 2000).

The importance of pollen coat lipids in the hydration process was determined by the study of impaired pollen hydration in *A. thaliana eceriferum* (*cer*) mutants (Preuss et al. 1993; Hulskamp et al. 1995). The *cer* mutants have defects in the long-chain lipid synthesis, and there was a reduction or loss of pollen coat on the surface of the *cer* mutant pollen grains. As a result, these pollen grains failed to hydrate on the stigma, but this defect could be rescued by high environmental humidity where normal pollen hydration, pollen tube growth, and successful seed set were observed (Preuss et al. 1993; Hulskamp et al. 1995). Thus, work on the *cer* and *fdh* mutants suggests that long-chain lipids are required on both surfaces (i.e., the pollen and the stigma) to support pollen hydration.

Although the molecular mechanism of water transfer from the stigmatic papilla to the pollen grain has yet to be determined, changes to the impermeable pollen coat with the formation of the pollen–stigma interface are proposed to create a passageway for water to flow from the stigma to the pollen grain (Elleman and Dickinson 1986; Elleman et al. 1992; Murphy 2006). This may involve changes in the lipid properties of the pollen–stigma interface through the actions of lipid-binding oleosin-like proteins or lipases that have been identified in the *A. thaliana* pollen coat (Mayfield et al. 2001). For example, the pollen glycine-rich protein 17 (GRP17) contains an oleosin domain that has been implicated in this role (Mayfield and

Preuss 2000). The *A. thaliana grp17* mutant produced pollen grains that were slower in initiation of pollen hydration, although the rate of hydration, once initiated, was similar to the wild type. Compared to wild-type pollen, the *grp17* mutant pollen had a visibly similar pollen coat with a similar lipid composition but was lacking the GRP17 protein (Mayfield and Preuss 2000). Lipids in the pollen–stigma interface may also be enzymatically modified by lipases such as extracellular lipase 4 (EXL4) (Updegraff et al. 2009). A mutation in the EXL4 gene also resulted in changes to pollen hydration. The *exl4* mutant pollen had a pollen coat that was normal in appearance and had a similar lipid profile to wild-type pollen but had reduced esterase activity. Interestingly, the *exl4* mutant pollen initiated hydration at a similar time to wild-type pollen but then displayed a slower rate of hydration (Updegraff et al. 2009). Both the *grp17* and *exl4* mutants displayed mild hydration phenotypes, and it may be that multiple members of the corresponding gene families (Mayfield et al. 2001) need to be knocked out to see a more pronounced hydration defect. Other unknown factors may are also involved in controlling pollen hydration.

Following pollen hydration and germination, the emerging pollen tube penetrates the cell wall of the stigmatic papilla. Changes at the *A. thaliana* stigmatic surface were observed after compatible pollen attachment, including the expansion of the outer layer of the cell wall beneath the grain (Elleman et al. 1992; Kandasamy et al. 1994). In *B. oleracea*, the expansion of the outer layer of the stigmatic cell wall appears to be initiated by factors in the pollen coating as application of isolated pollen coat extracts to the stigma was found to cause cell wall expansion (Elleman and Dickinson 1996). To facilitate the penetration of the developing pollen tube into the stigmatic papillar surface, hydrolytic enzymes from both the pollen and the stigma are thought to cause the breakdown of the waxy cuticle and the underlying cell wall of the stigmatic papilla (Dickinson 1995); these may include enzymes such as serine esterases, cutinases, polygalacturonases, pectin esterases, and expansins (Hiscock and Allen 2008). *A. thaliana* microarray experiments have identified predicted genes for these various enzymes to be enriched in their expression in the pollen (Honys and Twell 2003) or the stigma (Swanson et al. 2005; Tung et al. 2005). A specific example is the *A. thaliana* VANGUARD1 (VGD1) gene, which encodes a pectin methylesterase and is expressed in the pollen grain and pollen tube. VGD1 is required for pollen tube growth through the pistil as the *vgd1* mutant displayed reduced levels of pectin methylesterase activity in the pollen grain and the pollen tube growth proceeded at a much slower rate compared to wild-type pollen (Jiang et al. 2005). After a pollen tube grows through the stigmatic papillar cell wall to the base of the stigma, it enters into the transmitting tract, growing down to an ovule, where fertilization takes place (Lennon et al. 1998; Cheung et al. 2010). A number of factors have also been identified for these later stages (Kessler and Grossniklaus 2011; Takeuchi and Higashiyama 2011).

22.2.2 Signaling Events in the Stigmatic Papilla Regulating Pollen Hydration and Germination

With the stigmatic papilla controlling the very early postpollination stages, starting with pollen adhesion, a specific signaling event is proposed to occur upon contact with compatible pollen. A number of small pollen coat proteins could potentially act as signaling molecules for putative receptors in the stigma papilla (Mayfield et al. 2001; Vanoosthuyse et al. 2001), and several *Brassica* candidates for promoting pollen adhesion have been proposed (Doughty et al. 1998; Luu et al. 1997, 1999; Takayama et al. 2000a). However, there are likely other unknown signaling proteins responsible for activating a cellular response in the stigmatic papilla to promote acceptance of the compatible pollen grain. These secretory vesicles to the specified sites responses include Ca^{2+} spikes, actin polymerization, and microtubule depolymerization, and these events may be linked to polarized exocytosis toward the pollen attachment site, as described next. In *Brassica rapa*, both an actin network and vacuolar network were observed to be established in a direction toward the compatible pollen grain during pollen hydration (Iwano et al. 2007). In *B. napus* and *A. thaliana*, disruption of the microtubule network resulted in increased acceptance of compatible pollen, indicating that microtubule depolymerization is important to this process (Samuel et al. 2011). Ca^{2+} dynamics in the stigmatic papillae were monitored in vivo using transgenic *A. thaliana* plants expressing the yellow cameleon 3.1, a Ca^{2+} indicator that can be used to monitor rapid changes in Ca^{2+} cytoplasmic concentrations (Iwano et al. 2004). Although no increase of $[Ca^{2+}]$ was observed in unpollinated stigmatic papillae, several increases were observed in the stigmatic papillae following pollination. The first increase in $[Ca^{2+}]$ took place in the stigmatic papilla underneath the pollen contact site during the pollen hydration period. A second local increase in $[Ca^{2+}]$ occurred in the stigmatic papilla, again under the pollen attachment site, with pollen germination. Finally, the third and strongest increase in local $[Ca^{2+}]$ occurred with pollen tube penetration of the stigmatic papilla (Iwano et al. 2004). Thus, these Ca^{2+} spikes in the stigmatic papilla underneath the pollen–pistil interface support the premise that the stigmatic papilla responds to the compatible pollen and controls these early postpollination stages.

More recently, Exo70A1 has been identified as a factor required in the stigma for the early responses of the stigmatic papilla to the compatible pollen (Samuel et al. 2009). Exo70A1 is a subunit of the exocyst, an evolutionary conserved protein complex in eukaryotes consisting of eight subunits: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (Hsu et al. 1996; TerBush et al. 1996; Hala et al. 2008). Some of the exocyst subunit genes are present in multiple copies in plant genomes, and Exo70A1 is one member of the Exo70 gene family in *A. thaliana* (Synek et al. 2006; Chong et al. 2010). In yeast and animal systems, the exocyst was determined to act as a tethering complex to dock secretory vesicles to the plasma membrane for polar secretion, and various small GTPases have been found to interact with exocyst

subunits to regulate the assembly, localization, and function of this complex (reviewed in He and Guo 2009; Heider and Munson 2012). Once the vesicles have been tethered at the plasma membrane by the exocyst complex, the SNARE complex catalyzes the fusion of the secretory vesicle to the plasma membrane (Whyte and Munro 2002).

Exo70A1 was identified through work on the self-incompatibility pathway (described in the next section), and this led to the establishment of its role in compatible pollen response in both *B. napus* and *A. thaliana* (Samuel et al. 2009). The *A. thaliana exo70A1* mutant displayed a loss of pollen hydration and pollen tube growth when wild-type pollen was placed on the *exo70A1* mutant stigma (Samuel et al. 2009). Furthermore, this stigmatic defect was rescued by the stigma-specific expression of an RFP:Exo70A1 fusion protein in the *A. thaliana exo70A1* mutant. Confocal microscopy revealed that RFP:Exo70A1 protein was localized to the apical plasma membrane of mature stigmatic papillae (Samuel et al. 2009).

In yeast, Sec3 and Exo70 were found to be located at the plasma membrane before exocyst assembly (Finger et al. 1998; Boyd et al. 2004). Thus, Exo70A1 may play a similar role of being present at the stigmatic papillar plasma membrane before pollination and exocyst assembly. In yeast and mammalian cells, Sec3 and Exo70 were also found to be recruited to the plasma membrane via binding to phosphatidylinositol-4,5-bisphosphate, located at the inner leaflet of the plasma membrane (He et al. 2007; Liu et al. 2007; Zhang et al. 2008). Interestingly, when *A. thaliana* mutants with altered phosphoinositide pools were tested in pollen hydrations assays, wild-type pollen grains were found to have reduced hydration rates on the mutant stigmas, suggesting that specific membrane lipids are also important for these pollen–stigma interactions (Chapman and Goring 2011). Finally, in yeast, the polarized localization of the exocyst has been found to be controlled by the Rho GTPases, Cdc42, Rho1, and Rho3, through interactions with the Sec3 and Exo70 subunits (Robinson et al. 1999; Guo et al. 2001; Zhang et al. 2001; Wu et al. 2010). Exocyst assembly then occurs through an actin-dependent recruitment of the remaining exocyst subunits with the secretory vesicles to the specified sites on the plasma membrane for exocytosis (Boyd et al. 2004; Zhang et al. 2005).

Although RFP:Exo70A1 was localized to the entire stigmatic papillar apical plasma membrane, one would predict that exocyst assembly and vesicle docking would occur in a more localized area, just under the pollen contact site (Samuel et al. 2009). Consistent with this, we have observed, with compatible pollinations in *A. thaliana* and *A. lyrata*, that vesicle-like structures were observed fusing to the papillar plasma membrane under the pollen contact site (Safavian and Goring 2013). This step would require some type of unknown signal, possibly through Exo70A1, to regulate exocyst assembly and secretory vesicle docking under the pollen contact site (Fig. 22.1). The cargo of these secretory vesicles is also unknown, but they presumably contain stigmatic resources for pollen hydration and pollen tube penetration that are released upon fusion with the papillar plasma membrane and with the subsequent discharge of the contents into the apoplastic space. One such candidate cargo could be plasma membrane aquaporins, which could facilitate water transfer (Verdoucq et al. 2008; Postaire et al. 2010) from the stigmatic papilla to the

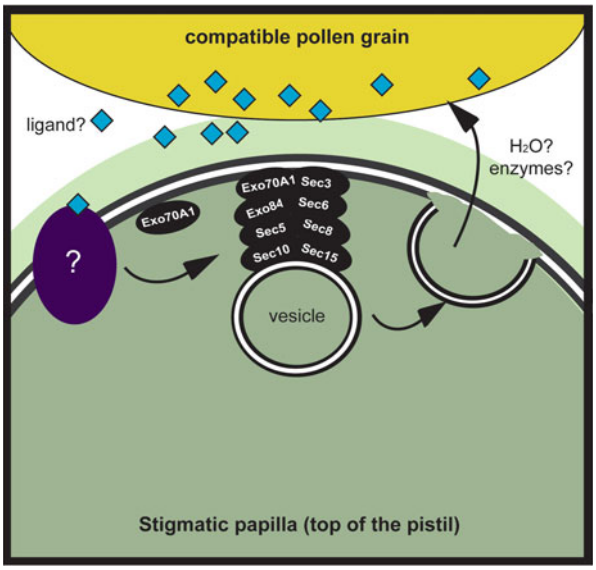

Fig. 22.1 Model of compatible pollen–pistil interactions in *Arabidopsis*. When a compatible pollen grain lands on the stigmatic papilla, an unknown factor or ligand is proposed to be detected by an unknown receptor in the papilla. Polarized vesicle secretion is then targeted to the pollen contact site by the exocyst complex. These vesicles are hypothesized to deliver factors to allow for the transfer of water from the papilla to the pollen grain and to deliver cell wall modification enzymes. The compatible pollen grain is then able to hydrate, and the pollen tube penetrates the stigma to continue onto an ovule or fertilization

pollen grain for hydration. As well, the vesicles may deliver cell wall-modifying enzymes for stigmatic papillar cell wall loosening and pollen tube penetration (Elleman and Dickinson 1996; Samuel et al. 2009).

22.3 Self-Incompatibility in the Genus *Arabidopsis*

Because plants are sessile in nature, flowering plants have evolved various mechanisms to aid in mate selection, and one of those methods is through the development of a self-incompatibility system. In the Brassicaceae, the self-incompatibility system allows the stigma to reject self-pollen and allows for the acceptance of genetically diverse non-self pollen (reviewed in Charlesworth and Vekemans 2005; Iwano and Takayama 2012). Two components control this system: the female pistil determinant, *S* receptor kinase (SRK; Takasaki et al. 2000; Silva et al. 2001), and the male pollen ligand, *S*-locus cysteine-rich/*S*-locus protein 11 (SCR/SP11; Schopfer et al. 1999; Takayama et al. 2000b). The two genes encoding these proteins are highly polymorphic (referred to as *S*-haplotypes), first identified in

Brassica species (Haasen and Goring 2010), and have been characterized in other Brassicaceae species, including *A. lyrata* (Kusaba et al. 2001; Schierup et al. 2001; Mable et al. 2005) and *Capsella grandiflora* (Paetsch et al. 2006; Boggs et al. 2009; Guo et al. 2009). Although much of the research into the self-incompatibility pathway has been performed in the *Brassica* genus, recent discoveries in the *Arabidopsis* genus are described here.

A. thaliana is a self-compatible species that has lost its self-incompatibility system by the pseudogenization of the *SCR*/*SP11* and *SRK* genes (Kusaba et al. 2001; Bechsgaard et al. 2006; Tang et al. 2007; Shimizu et al. 2008; Guo et al. 2011). As *A. thaliana* is easily transformed, a number of studies have reintroduced the *SCR*/*SP11* or *SRK* genes from other Brassicaceae species in an attempt to reintroduce the self-incompatibility trait (Bi et al. 2000; Nasrallah et al. 2002). One approach was to transform *SCR*/*SP11*-*SRK S*-haplotypes from *A. lyrata* and *C. grandiflora* into different *A. thaliana* ecotypes. In some ecotypes such as C24, Cvi-0, Hodja, Kas-2, and Shadara, the expression of these *SCR*/*SP11* and *SRK* genes was able to cause self-pollen rejection while other ecotypes such as Col-0, Mt-0, Nd-0, No, RLD, and Ws-0 remained self-compatible (Nasrallah et al. 2004; Boggs et al. 2009). Although both the *SCR*/*SP11* and *SRK* genes are disrupted in many of the *A. thaliana* ecotypes, Tsuchimatsu et al. (2010) identified some *A. thaliana* ecotypes carrying an intact *SRK* gene. Wei-1 was one of these ecotypes with a functional copy of *SRKa*, but *SCRa* was nonfunctional because of an inversion in the gene coding region (Tsuchimatsu et al. 2010). Transformation of a restored *SCRa* gene into Wei-1 plants resulted in these plants displaying a self-incompatibility phenotype. Interestingly, these plants displayed a change over the course of development, going from being self-incompatible to becoming pseudo-self-compatible; that is, as the flowers became older they were able to accept self-pollen grains that were previously rejected (Tsuchimatsu et al. 2010). As a result of these studies, it was concluded that *SCR*/*SP11* and *SRK* were the only components required to restore the self-incompatibility trait in *A. thaliana* ecotypes. However, the stability of the self-incompatibility trait was variable, depending on the ecotype being used for the transformation studies. This lack of a completely stable self-incompatibility phenotype in *A. thaliana* may be the result of variation in the *A. thaliana* ecotypes studied, or additional factors such as the *ARC1* gene may be required, as discussed in more detail next.

In *Brassica*, the pollen SCR/SP11 ligand, present in the pollen coat, crosses the pollen–papillar interface to bind to the papillar membrane-localized SRK, and SRK becomes phosphorylated (Kachroo et al. 2001; Takayama et al. 2001; Shimosato et al. 2007). SRK exists as a homodimer and is proposed to interact as a transient complex with the *M* locus protein kinase (MLPK) (Murase et al. 2004; Kakita et al. 2007a, b). *B. rapa* MLPK is a receptor-like cytoplasmic kinase that is also localized to the plasma membrane in the stigma (Murase et al. 2004). MLPK exists as two splice variants with different N-terminal ends. MLPKf1 is generally expressed in a broad range of tissues and encodes a protein with an N-terminal myristoylation site, whereas MLPKf2 was found to be stigma specific in expression and has an

N-terminal hydrophobic domain (Kakita et al. 2007a). *A. thaliana APK1b* is the orthologue to *B. rapa* MLPK, and it has a similar pattern of expression with the two different isoforms that encode the same protein variants, one with an N-terminal myristoylation site (*APK1bf1*) and the other with the N-terminal hydrophobic region (*APK1bf2*). Despite the different N-terminal motifs between MLPKf1 and MLPKf2, both the MLPK variants localized to the plasma membrane through their respective N-terminal domains. Both isoforms also interacted with SRK at the plasma membrane, but the interaction was lost if the N-terminal domain was removed (Kakita et al. 2007a, b). Finally, both isoforms could rescue the *mlpk* mutation in *B. rapa*, restoring the self-incompatibility response (Kakita et al. 2007a). A similar role for *A. thaliana* APK1b in the self-incompatibility response has yet to be established. An *apk1b* knockout mutant in the *A. thaliana* Col-0 ecotype did not show any differences from the wild type when the *SCR*/*SP11* and *SRK* transgenes were expressed (Rea et al. 2010; Kitashiba et al. 2011). However, because the transgenic *SCR*/*SP11-SRK A. thaliana* Col-0 plants only display a very weak self-incompatibility response and remain self-compatible (Nasrallah et al. 2002, 2004), it would be difficult to make a definitive conclusion on the role of *APK1b* in *A. thaliana* self-incompatibility.

In the Brassicaceae, the self-incompatibility signaling pathway is rapidly activated in the stigmatic papilla following contact with a self-pollen grain. The end result of this pathway is pollen rejection by preventing compatible pollen responses such as pollen grain hydration and pollen tube penetration into the stigma (described under compatible pollinations; Dickinson 1995). Following SCR/SP11 binding to SRK, the SRK-MLPK complex is proposed to recruit the ARM repeat-containing 1 (ARC1) E3-ubiquitin ligase (Stone et al. 2003; Samuel et al. 2008). *B. napus* ARC1 was the first downstream signaling component identified for SRK and was shown to be required for the rejection of self-pollen (Gu et al. 1998; Stone et al. 1999). Our most recent research into the *ARC1* orthologue in *A. lyrata* has revealed that it is necessary for the self-incompatibility response in *A. lyrata*, similar to *B. napus* (Indriolo et al. 2012). Previously, ARC1 was proposed to be not required in transgenic SCR/SRK *A. thaliana* plants for restoring the self-incompatibility trait because the ARC1 gene was deleted from the *A. thaliana* genome (Kitashiba et al. 2011). Given that the strength of the self-incompatibility trait varied depending which *A. thaliana* ecotype was used to transform with the SCR and SRK genes, we further explored the extent of the *ARC1* gene deletion. An additional 355 ecotypes were surveyed by polymerase chain reaction (PCR), and in all cases, *ARC1* was determined to be deleted (Indriolo et al. 2012), including ecotypes such as Wei-1 that contained a functional SRK gene (Tsuchimatsu et al. 2010). These data demonstrated that the functional *ARC1* gene was likely lost in *A. thaliana* before the loss of a functional *SRK* and *SCR*/*SP11* and perhaps aided in the breakdown of self-incompatibility to self-compatibility in *A. thaliana*. A broader bioinformatics survey of the presence or absence of *ARC1* in several Brassicaceae sequenced genomes gave further support to this idea. This survey included the self-incompatible species of *B. rapa*, *A. lyrata*, and *Capsella grandiflora* and the self-compatible species of

A. thaliana, *Aethionema arabicum*, *Capsella rubella*, *Leavenworthia alabamica*, *Thellungiella halophila*, *Thellungiella parvula*, and *Sysimbrium irio*. It was determined that the *ARC1* gene was frequently deleted in self-compatible species including *A. thaliana*, *A. arabicum*, *L. alabamica*, *T. halophila*, and *T. parvula* whereas it was always found in the genomes of self-incompatible species (Indriolo et al. 2012). This observation was specific to the *ARC1* gene as the most closely related *ARC1* paralogue, *PUB17*, was completely conserved in all the genomes surveyed (Indriolo et al. 2012). Therefore, the presence or absence of *ARC1* appeared to correlate with a switch from self-incompatibility to self-compatibility in the Brassicaceae.

To directly address the requirement of ARC1 for the self-incompatibility trait in *Arabidopsis*, ARC1 was characterized in a natural self-incompatible species, *A. lyrata*. Basic characterization determined that *ARC1* was found to be more highly expressed in the pistil, similar to *B. napus ARC1*, which was shown to be most highly expressed in the stigma (Gu et al. 1998; Indriolo et al. 2012). The expression of *ARC1* was then knocked down in *A. lyrata* by the use of *ARC1* RNAi construct. Similar to previous observations in *B. napus*, transgenic *ARC1* RNAi *A. lyrata* plants exhibited a partial breakdown in the self-incompatibility response, leading to the acceptance of self-pollen and partial seed set (Indriolo et al. 2012). As a result of the aforementioned data regarding self-incompatibility in *Brassica* spp. and *A. lyrata*, one can conclude that the role of *ARC1* is conserved in the self-incompatibility signaling pathway in the core Brassicaceae (Indriolo et al. 2012).

Because the self-incompatibility pathway caused pollen rejection by inhibiting the early postpollination stages (pollen hydration, pollen tube penetration), compatibility factors in the stigmatic papilla would be predicted to be inhibited as part of this response. Following from this, the role of ARC1 in the self-incompatibility pathway was proposed to promote the ubiquitination and degradation of the proposed compatibility factors by the 26S proteasome (Stone et al. 2003). Through an ARC1-interaction screen, Exo70A1 was identified as a candidate compatibility factor targeted by ARC1 (Samuel et al. 2009). As described earlier in the compatible pollen–stigma interactions section, Exo70A1 is a component of the exocyst complex for tethering secretory vesicles at the plasma membrane and is required in the stigmatic papilla for accepting compatible pollen (Samuel et al. 2009). Therefore, the inhibition or removal of Exo70A1 through ubiquitination via ARC1 would block secretory vesicles from fusing to the papillar plasma membrane underneath the pollen contact site, resulting in pollen rejection (Fig. 22.2). This model was tested in *A. lyrata* where both self-incompatible and cross-compatible pollinations can be followed. At 10 min after a cross-compatible pollination, vesicle-like structures were observed fusing to the papillar plasma membrane under the pollen contact site (Safavian and Goring 2013). However, at 10 min after a self-incompatible pollination, there was a complete absence of these vesicle-like structures at the papillar plasma membrane, and autophagic bodies were detected in the vacuole; thus supporting the inhibition of vesicle secretion as part of the self-incompatibility response (Safavian and Goring 2013).

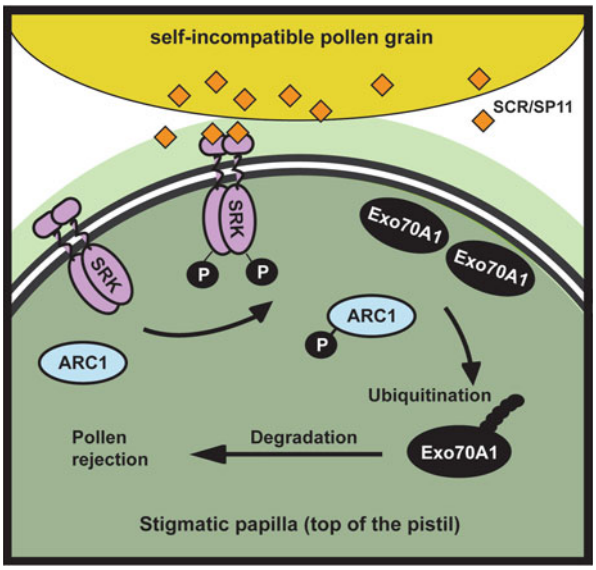

Fig. 22.2 Model of self-incompatible pollen–pistil interactions in *Arabidopsis*. When a self-incompatible pollen grain lands on the stigmatic papilla, the SCR/SP11 pollen ligand binds to the homodimeric receptor, *SRK*, in the stigmatic papilla. SRK then becomes phosphorylated and activated, and is proposed to phosphorylate ARC1. Phosphorylated ARC1 is then proposed to find its target, Exo70A1, at the plasma membrane. ARC1 ubiquinates Exo70A1, and Exo70A1 is targeted to the 26S proteosome where it is then degraded. The removal of Exo70A1 prevents exocyst assembly and the delivery of vesicles to the pollen contact site. As a result, the self-pollen grain is rejected as it is unable to hydrate and the pollen tube cannot penetrate the stigmatic surface

22.4 Conclusions and Future Directions

Much progress has been made recently into the mechanisms underlying pollen–pistil interactions in the *Arabidopsis* genus, but it is quite clear that much still remains unknown. For example, it is surprising that so little is known about what underlies a compatible interaction, such as a putative ligand and receptor to signal a compatible pollen recognition pathway in the stigma. What is clear is that downstream of this pathway, the exocyst complex is required for hydration and pollen tube penetration of compatible pollen in *A. thaliana* and *B. napus* (Samuel et al. 2009). Interestingly, following self-incompatible pollination in *B. napus*, moderate changes in the microtubule network were seen at the apical region of stigmatic papillae where longer microtubule bundles were observed instead of the dense network in unpollinated papillae, although no microtubule shortening or depolymerization was documented. However, a more dramatic localized depolymerization of the microtubule network was observed during compatible pollinations (Samuel et al. 2011). This distinct localized breakdown of the microtubule network was

proposed to be regulated by Exo70A1, triggering successful pollination. Thus, the relationships between the exocyst complex, vesicle secretion, actin polymerization, and microtubule depolymerization is an area for further investigation.

The role of Exo70A1 in compatible pollinations came from its identification in the self-incompatibility signaling pathway in *B. napus* as a target for degradation by ARC1, downstream of the SRK-MLPK complex. Interestingly, the SRK-MLPK-ARC1 module appears to be used in other members in these gene families. For example, in plant innate immunity, the BIK1 receptor-like cytoplasmic kinase is part of a complex that includes the FLS2 receptor kinase, the BAK1 receptor-like kinase, and two ARC1-related proteins, PUB12 and PUB13 (Lu et al. 2011). Following bacterial flagellin recognition by FLS2, BIK1 was shown to enhance the ability of BAK1 to phosphorylate PUB13 (Lu et al. 2011). Similarly, using an in vitro assay, MPLK was found to be much more efficient at phosphorylating *B. napus* ARC1 compared to SRK (Samuel et al. 2008).

Other future research directions that may follow from recent discoveries include further investigating the role of ARC1, the *MLPK* orthologue, APK1b, and Exo70A1 in *Arabidopsis* species. For example, *A. lyrata ARC1* could be transformed into *A. thaliana* expressing the *A. lyrata* SRK and SCR/SP11 genes to determine if a more robust self-incompatibility phenotype is generated. *A. thaliana* Col-0 was previously found to lack a strong self-incompatibility response with only *A. lyrata* SRK and SCR/SP11 being expressed (Nasrallah et al. 2002, 2004), and we have some preliminary data that show a much stronger self-incompatibility response when all three *A. lyrata* genes are expressed in the *A. thaliana* Col-0 ecotype. Another direction is to further examine the role of the Exo70A1 orthologue in *A. lyrata* to investigate if Exo70A1 is targeted for degradation by ARC1 following a self-incompatible pollination. Finally, the ability of some *A. thaliana* ecotypes to mount varying degrees of self-incompatibility responses in the absence of ARC1 support that there are still other signaling components to be discovered in this pathway. With the greater access to bioinformatics and genomics tools in the Brassicaceae, it will be much easier to find candidate genes and perform research in a similar fashion to the *Arabidopsis* species in regard to both compatible and self-incompatible pollinations.

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