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REVIEW

Stigma factors regulating self-compatible pollination

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Abstract Pollination is one of the most important steps during fertilization and sexual reproduction in plants, and numerous cell-cell interaction events occur between the pistil and the pollen grain/tube during this process. The pollen-stigma interaction is a highly selective process which leads to compatible or incompatible pollination. Previous studies in *Solanaceae*, *Papaveraceae*, and *Brassicaceae* provided some important insights into pollen-stigma recognition in self-incompatible systems. In recent years, considerable data have been available regarding pollen-stigma interaction during self-compatible pollination. In this review, we focus on discussing current knowledge on stigma factors that regulate pollen-stigma interaction in self-compatible systems in comparison with self-incompatible systems.

Keywords stigma, signaling, self-compatible, pollination

1 Introduction

The stigma is located at the top of the pistil and is capable of trapping and discriminating pollen grains from relative or unrelated species. The interaction between pollen and the stigma is composed of a number of important events, such as pollen adhesion, pollen hydration and germination as well as pollen tube emergence and growth on the surface of the stigma. Much progress has now been made in understanding of self-compatible pollination involving pollen-stigma interaction (Hiscock and Allen, 2008). It has been shown that pollen exine structure, long-chain lipids and the proteins on the pollen coat play essential roles during pollen-stigma interaction (Zinkl et al., 1999; Zinkl and Preuss, 2000; Fiebig et al., 2000; Mayfield et al., 2001; Suen et al., 2003; Swanson et al., 2004). Recent studies indicate that the stigma is also critical for a successful pollination. The pollen tube grows rapidly in the stigma compared with its growth in a synthetic medium. Ablation of the stigmatic papilla cells by exposure to a cytotoxin in transgenic *Arabidopsis* results in failure of pollen hydration and pollen tube production (Tung et al., 2005). Moreover, pollen tubes grown in media are unable to locate the micropyle of excised ovules unless they grow through an excised upper portion of the pistil or germinate on the stigmas of excised pistils (Higashiyama et al., 1998; Palanivelu and Preuss, 2006). Hence, the pollen-stigma interaction is important not only for pollen adhesion, hydration and germination, but also for pollen tube growth and guidance in the pistil (Fig. 1). In this review, we summarize and discuss the factors and the possible signals on the stigma involved in the pollen-stigma interactions during self-compatible pollination.

2 Exudates on the wet stigma

Stigmas can be classified into two categories, wet and dry, according to whether there is surface secretion. During late development in the wet stigma, fluid secretion including lipids, carbohydrate, proteins and water, is deposited on the stigma surface, which is controlled by STIG1, a cysteine-rich and stigma-specific protein, in tobacco (*Nicotiana tabacum*) and petunia (*Petunia hybrida*) (Verhoeven et al., 2005). Several studies have shown that the exudates on wet stigmas are involved in the interaction with pollen capture, adhesion and hydration. Ablation of the stigmatic secretory zone in tobacco results in an arrest of pollen germination and pollen tube elongation on the stigma (Goldman et al., 1994). Although pollen germination can be improved by subsequent addition of medium containing boron, calcium, and sugar to this ablated stigma, there is a failure of pollen tube penetration into the pistil tissues. The exudate of the petunia stigma is effective in restoring pollen tube penetration into an ablated stigma, whereas that of lily only enables pollen hydration and germination (Sanchez et al., 2004). The exudate of the tobacco stigma contains both saturated and unsaturated fatty acids (Cresti et al., 1986). Application of a fatty-acid composition similar to that of the exudate restores stigma function and pollen tube penetration of stigma-less pistils (Wolters-Arts et al.,

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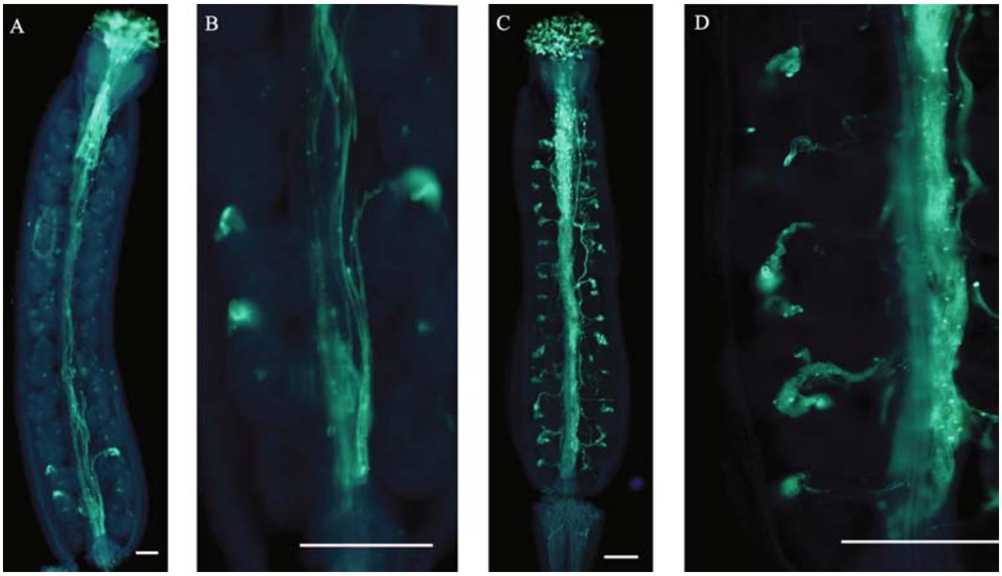

Fig. 1 The pollen tubes of *Raphanus sativus* (A) and *Rorippa dubia* (C) do not enter the micropyle of *Arabidopsis* at 72 and 48 h after pollination, respectively. (B) and (D) show magnified images of the ovary base in (A) and (C), respectively. B: The pollen tubes turn back to the ovules from the bottom of the transmitting tract but cannot enter the micropyle. D: The pollen tubes cannot enter the micropyle. Pollen tubes were stained with aniline blue. Bars, 100 µm.

1998). The collective evidences to date thus demonstrate the importance of lipids in stigmatic exudates for pollen tube growth. These lipids may function by controlling the flow of water to pollen (Wolters-Arts et al., 2002) or by increasing the permeability of the stigma cuticle to water (Pruitt et al., 2000). Additionally, the non-membrane lipids also function in signal transduction (Eyster, 2007). For example, fatty acids can cross cell membranes and communicate directly with the contents of the cell by binding to intracellular receptors as well as G protein-coupled receptors on the plasma membrane (Jump, 2004; Kostenis, 2004). Therefore, it is possible that the lipids in the exudates of the tobacco stigma are involved in signaling pathways that regulate the pollen-stigma interaction. However, lipid signaling transduction is very complex, and the mechanism of lipids in pollination remains to be investigated.

The pollen tube growth in the exudates of wet stigmas is more efficient than that in lipids only (Wolters-Arts et al., 1998), suggesting there are other substances functioning in the pollen-stigma recognition process. Indeed, many proteins have now been identified in these exudates (Kuboyama, 1998; Miller et al., 2000; Kuboyama et al., 2001; Pezzotti et al., 2002; Busot et al., 2008; Wakelin et al., 2009), while little is known about the role of exudate proteins in mediating the interaction between pollen and stigma. A lipid transfer protein, stigma/style Cys-rich adhesin (SCA), from lily (*Lilium longiflorum*) exudates has been shown to be important for pollen tube adhesion (Park and Lord, 2003). Similarly, a lipid transfer protein in the tobacco stigma exudate has been found to function in cell wall-loosening (Nieuwland et al., 2005). However, most of these studies have suggested that exudate proteins improve pollen germination and pollen tube growth in compatible pollination only (de Graaf et al., 2003; Verhoeven et al., 2005). For example, the tomato stigma-specific LeSTIG1 protein can bind the extracellular domains of both pollen-specific receptor kinases LePRK1 and LePRK2, and promote pollen tube growth *in vitro* (Tang et al., 2004).

3 Cuticle of dry stigma

Unlike the wet stigma, the dry stigmas of *Brassica* and *Arabidopsis* are covered with a consecutive cuticle which is a barrier for pathogens and an obstacle to pollen tube penetration (Fig. 2). It has been thought that the cuticle is freely permeable to water and large molecules (Elleman et al., 1988); however, a thickened cutinized stigma surface can prevent compatible pollen from germination (Heslop-Harrison, 2000). The cuticle may also be an obstacle to water transportation during pollen hydration. However, disruption or removal of the pellicle, a membrane-like layer of protein at the surface of the cuticle, leads to the failure of pollen tube entry into the stigma (Hiscock and Allen, 2008).

During compatible pollination, the cuticle can be broken by visiting insects, by an increased turgidity of the stigma (Dafni and Maues, 1998). Esterases may thus play an important role in breaking the cutinized cuticle. It is thought that the pollen coat and the pollen tube are the main sources of esterases in plants. The pollens of *Brassica napus* carry an active cutinase to break the cuticle of the papilla (Hiscock et al., 1994; 2002). In addition, the serine

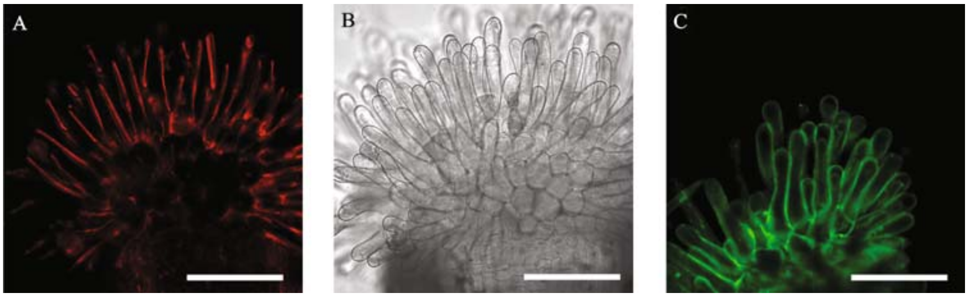

Fig. 2 Lipids and cuticles of the *Arabidopsis* stigma. A: Stigma stained with the fluorescent lipophilic dye Nile red, revealing the localization of lipids on the surface of the papilla cells. B: DIC image of the stigma shown in (A). C: Cuticles of the papilla cells visualized by staining with auramine O. Bars, 100 μm.

esterase inhibitors, diisopropyl phosphorofluoridate or ebelactone B, hinders pollen tube penetration of the stigma surface. This suggests that serine esterases, most probably cutinase(s), are required for pollen tube penetration of the dry cuticularized *Brassica* stigma. The abundant *Arabidopsis* pollen coat proteins, EXL4 and GRP17, function in pollen rapid initiation of hydration on the stigma (Mayfield and Preuss, 2000, Mayfield et al., 2001; Updegraff et al., 2009). EXL4, a member of the GDSL family of esterases/ lipases, is found to have esterase activity, although its substrate and substrate localization are unknown, on the pollen coat or on the stigma surface (Updegraff et al., 2009). In the case of GRP17, its oleosin domain may solubilize lipids, which provides substrates for EXL4 and other pollen coat lipases (Updegraff et al., 2009). Recently, CDEF1, an *Arabidopsis* cutinase, was detected on pollen and tubes, but there is no evidence to support its function in pollination (Takahashi et al., 2010). Whether these esterases function in disrupting the stigma cuticle during pollination remains to be determined. The esterase activity is also detectable on the stigma surface (Hiscock et al., 1994; 2002) and found to increase in the stigma at the receptive stage (Dafni and Maues, 1998). In *Brassica*, the esterase activity is localized at the points of contact between the pollen and stigma (Hiscock et al., 1994), implying that these enzyme activity correlates with the pollen-stigma interaction. Knox et al. (1976) suggested that the esterase interaction from the surface of pollen and the stigma is necessary to digest the cuticle and enable the entry of the pollen tube into the stigma.

During the pathogen response pathways in plants, the cuticle is a source of signals perceived by invading fungi in addition to a physical barrier to microbial invaders. Plants can perceive degradation products of the cuticle which can induce resistance response to fungi (Chassot et al., 2007). In *Brassica*, the molecular weight of pollen cutinase is similar to that of the fungal cutinases, and its activity is inhibited by serine esterase inhibitors, similar to most fungal cutinases (Hiscock et al., 1994). It will be interesting to study whether the cutinase at the contact point between pollen and stigma catalyzes the degradation of the cutinized cuticle of the stigma, and whether the degradation products of the cuticle act as a signal that induces the stigma cell response.

4 Cell wall of the stigma

After cuticle rupture in the dry stigma, the pollen tube grows into the cell wall of the papilla cells. The papilla cells in *Arabidopsis* contain two layer walls, an outer layer which is apparently specific to the stigmatic papillae, and an inner layer comprising the main body of the fibrillar cell wall (Elleman et al., 1988; 1992). The pollen tube grows between these two layers in the dry stigma. In wet-stigma plants with a solid style, the pollen tube grows into the extracellular matrix (ECM) of intercellular spaces in the stigma secretory zone after germination. Thus, cell wall expansion or loosening in the stigma is important for pollen tube growth through the papilla cell wall (Elleman et al., 1992), and a number of enzymes produced either by the pollen, pollen tube or stigma are implicated in this process.

It has been found that the application of an isolated pollen coating from *Brassica oleracea* induces a rapid expansion of the outer layer of the stigmatic wall (Elleman and Dickinson, 1996), indicating that the components of the pollen coat play a role in loosening the stigmatic wall. In maize, the pollen-coat beta-glucanase and xylanase enzymes facilitate the penetration of the stigma wall by the pollen tube via hydrolysis of cellulose and hemicellulose (Suen et al., 2003). The pollen tubes from transgenic maize lines containing little or no xylanase in the pollen coat have been shown not to penetrate the silk as efficiently as wild type tubes (Suen and Huang, 2007). Additionally, many pollen tube-held enzymes were found to be involved in pollen tube growth in the dry stigma, including pectin esterase, pectate lyase and polygalacturonase (Kim et al., 1996; Wu et al., 1996; Bosch et al., 2005). It is thus possible that these enzymes function in the regulation of the stigmatic cell wall. However, the pectin methylesterases may only be essential for normal pollen tube growth and not for the interaction between the pollen tube and the stigma. This is evidenced by the pectin methylesterase-less mutant of *Arabidopsis* which displays pollen tube defects *in vitro* (Bosch et al., 2005; Jiang et al., 2005; Tian et al., 2006). Transcriptional analysis has indicated that the expression of cell wall metabolism proteins (TOBC092C05 for a hydrolase, TOBS004A06 for a pectin acetylesterase, and TOBC003D12 for a pectin methylesterase inhibitor) is increased in the stigmas/styles in tobacco (Quiapim et al., 2009). These proteins are thus implicated in the control of cell wall loosening in the stigmatic secretory zone and transmitting tissue.

The expansins are a family of non-enzymatic proteins that function in cell wall loosening. Some members of this family, belonging to the group-I allergens, have been identified in grass pollen. For example, an expansin in pollen, Zea m1, shows wall-loosening activity in the style, suggesting that group-I allergens play a role in assisting pollen tube growth through the stigma and style by softening the maternal cell walls (Cosgrove et al., 1997; Cosgrove, 2000; Li et al., 2003). In the exudate of the tobacco stigma, a pollen-pistil allergen-like protein (PPAL) was identified (Pezzotti et al., 2002). PPAL is a beta-expansin homolog, but has no cell wall loosening role. In some plant species, hormone functions in the pistil were examined after pollination (Zhang and O'Neill, 1993; Llop-Tous et al., 2000; Mol et al., 2004). Expansins are linked to cell wall changes induced by auxin, gibberellin, cytokinin, ethylene, and brassinosteroids. It is therefore likely that the expansins in the stigma are involved in pollen tube growth regulated by hormones, as shown in *Torenia fournieri* (Wu et al., 2008). In contrast, a lipid transfer protein (LTP) in the tobacco stigma exudate was identified as having a role in cell wall-loosening via its association with hydrophobic wall compounds which causes non-hydrolytic disruption of the cell wall (Nieuwland et al., 2005). Although more than seventy members of the LTP family have been annotated in *Arabidopsis* (Beisson et al., 2003), their roles in pollination have yet to be clarified.

The cell wall of the stigma cells is the site of callose deposition during incompatible pollination (Fig. 3). Sedgley (1979) showed that there is no callose in the receptive female phase on avocado stigmas, but that stigma cell walls accumulate callose during the subsequent male phase of the avocado dichogamous flowers. These results indicate that callose deposition is a developmental regulatory or pollination-induced process. At least three *callose synthase* (*CalS*) genes have been detected in stigma cells of *Arabidopsis* (Dong et al., 2008). Whether these genes are downregulated after compatible pollination or other *CalS* genes are induced during incompatible pollination is unknown.

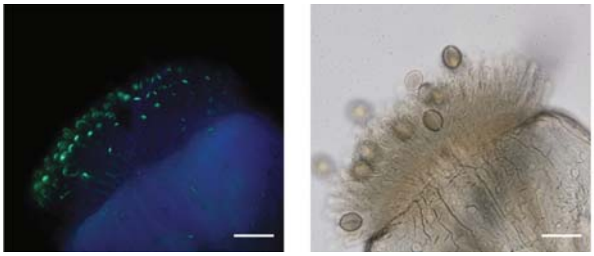

Fig. 3 The pollens of *Raphanus sativus* induce callose deposition onto the *Arabidopsis* stigma at 30 min after pollination. Callose was stained with aniline blue. Bars, 100 μm.

5 Stigma signaling in the pollen-stigma interaction

Ca^{2+} dynamics are regarded as an important regulator of pollen hydration and pollen tube growth. Moreover, Ca^{2+} gradients in pistil induce pollen tube orientation for the ovule (Ge et al., 2009; Shi and Yang, 2009). After pollination the papilla cells in *Arabidopsis* show a remarkable $[Ca^{2+}]_{cyt}$ increase at the site of pollen-grain attachment, when pollen hydration, pollen protrusion and pollen tube penetration of the papilla cell occur. It is implicated that the Ca^{2+} dynamics function in the interaction between pollen and the papilla cells (Iwano et al., 2004). Although many regulators of Ca^{2+} dynamics and effectors of Ca^{2+} signaling have been identified during pollen tube growth regulation, the regulatory mechanism for Ca^{2+} in pollination remains to be elucidated. Lenartowska et al. (2009) reported that calreticulins, proteins involved in cellular Ca^{2+} homeostasis regulation, could be detected in the pollen/pollen tubes and the transmitting tract of pollinated pistil of *Haemanthus*, suggesting a possible role for these proteins in pollen-pistil interaction. Additionally, reactive oxygen species and nitric oxide (NO) signaling were found to be involved in pollen-stigma interaction, and NO exerts its role by modulating Ca^{2+} signaling (McInnis et al., 2006; Hiscock and Allen, 2008; Prado et al., 2008).

The actin cytoskeleton is regarded as a transducer of Ca^{2+} signaling and plays a role in pollen tube growth (Vidali et al., 2001). Recently, actin reorganization in the stigma papilla cells has been shown to be involved in self-pollen "rejection" in *Brassica rapa* (Iwano et al., 2007). Actin in the pollen tube of *Papaver rhoeas* was previously suggested to cope with the mechanical obstacles that arise upon pollen tube growth in the stigma (Gossot and Geitmann, 2007). The actin dynamics in plant cells are regulated by actin binding proteins, and a number of actin-regulating proteins have been suggested to function in the control of pollen tube growth (Gu et al., 2003; Huang et al., 2004; Hussey et al., 2006; Gebert et al., 2008; Yan et al., 2009). Actin depolymerizing factors (ADFs) may also be important regulators of the pollen-stigma interaction in stigma cells, because the expression of several *ADFs* (*ADF3*, *4*, *6*, and *9*) has been detected in *Arabidopsis* stigma cells (Kandasamy et al., 2007).

Previously, it was revealed that the deposition of secretory substances onto the stigma occurs through the release of fluids from internal reservoirs, indicating that vesicular transportation is a fundamental process in stigma cells (Hiscock and Allen, 2008). The endocytosis and exocytosis pathways have been shown to function in both the pollen tube and stigma cell in the self-incompatible system (Ivanov and Gaude, 2009; Lee et al., 2009; Samuel et al., 2009). Ivanov and Gaude (2009) found that the interaction of S-locus receptor kinase (SRK) and S-locus cysteine-rich protein occurs in the plasma membrane and is followed by SRK internalization in endosomes during pollination. Exo70A1 is a putative component of the exocyst complex that regulates polarized secretion. In *Arabidopsis*, Exo70A1 is localized in the cytoplasm in immature stigma cells and can contact the plasma membrane of mature stigmatic papillae. However, Exo70A1 is lost from the plasma membrane after compatible pollination (Samuel et al., 2009). The wild-type pollen cannot hydrate and germinate on the stigmas of transgenic lines that are Exo70A1-deficient. These findings indicate that the Exo70A1-involved exocyst is required for the interaction between the pollen and the stigma (Samuel et al., 2009). Given that the SCA (stigma/stylar cysteine-rich adhesin)/pectin matrix in the lily is transported into the pollen tube, endocytosis is suggested to play a role in the pollen-stigma interaction (Kim et al., 2006). Thus, it is likely that the vesicles are involved in endocytosis and exocytosis, and that the key molecules in vesicles are responsible for the pollen-stigma recognition during self-compatible pollination. In *Brassica* compatible pollination, the vacuolar network orients itself toward the compatible pollen attachment site in the stigma cell, and the vacuolar changes are dependent upon actin bundles (Iwano et al., 2007). Actin is involved in vesicular transportation, endocytosis and exocytosis in the plant cell, and the endocytotic and exocytotic pathways play roles in pollen tube growth (Samaj et al., 2004; Moscatelli and Idilli, 2009; Zárský et al., 2009). It is thus possible that the crosstalk between endocytosis, exocytosis as well as actin is correlated with the pollen-stigma interaction during self-compatible pollination.

Although the cell wall of the stigma is regarded as an obstacle to pollen tube growth, there is evidence to show that the cell wall is important in relaying information from the external stimuli (Humphrey et al., 2008). Incompatible pollination generally induces callose deposition onto the cell walls of the stigma cells, indicating that the cell wall is an important responsive site for the pollen-stigma interactions. Plant cell wall is a highly dynamic, responsive structure which extends to the plasma membrane and underlying cytoskeleton during signaling transduction (Baluska et al., 2003). Many proteins have also been identified in the cell wall as candidate sensors and receptors for plant signaling events, such as wall-associated kinases, lectin receptor kinases, proline-rich extensin receptor kinases, leucine-rich extensin proteins, mechanosensing calcium channel, arabinogalactan proteins and glycosylphosphatidylinositol-anchored proteins (Humphrey et al., 2008). The arabinogalactan proteins and receptor kinases in the pistil have been identified in the self-incompatible response (Zhang et al., 2008; Lee et al., 2009). It may therefore be interesting in future studies to elucidate the functions of the cell wall proteins in modulating pollen-stigma interactions through the regulation of Ca^{2+} dynamics, actin configuration, endocytosis and exocytosis of the plasma membrane (Table 1).

Table 1 Possible stigma factors regulating self-compatible pollination

position	stigma factor	plant species	possible function	reference
exudates of wet stigma	lipids	*Nicotiana tabacum*	regulating the flow of water to pollen from stigma	Wolters-Arts et al., 2002
	lipid transfer protein	*Lilium longiflorum*; *N. tabacum*	pollen tube adhesion on pistil; cell wall-loosening of stigma	Park and Lord, 2003; Nieuwland et al., 2005
	Expansin	*N. tabacum*; *Torenia fournieri*	pollen tube growth in stigma	Pezzotti et al., 2002; Wu et al., 2008
cuticle of dry stigma	esterases	*Brassica napus*	break cuticle of stigma	Hiscock et al., 1994; Hiscock et al., 2002
cell wall of sigma	pectin acetylesterase	*N. tabacum*	cell wall loosening of stigma	Quiapim et al., 2009
	callose	*Persea americana*	incompatible pollen rejection	Sedgley, 1979
intracellular signaling	Ca^{2+}	*Arabidopsis thaliana*; *N. tabacum*	pollen tube orientation for the ovule	Ge et al., 2009; Shi and Yang, 2009
	reactive oxygen species and nitric oxide	*A. thaliana*; *Senecio squalidus*	regulating Ca^{2+} signaling; pollen tube guidance	McInnis et al., 2006; Hiscock and Allen, 2008; Prado et al., 2008
	actin	*B. rapa*	pollen-stigma recognition	Iwano et al., 2007
	endocytosis and exocytosis	*A. thaliana*; *B. rapa*; *L. longiflorum*	pollen-stigma recognition	Kim et al., 2006; Samuel et al., 2009

6 Summary

The pollen-stigma interaction is the first checkpoint for pollen-pistil recognition. A considerable number of studies have attempted to elucidate the molecular mechanisms and signaling pathways underlying the control of this interaction in self-compatible pollination. So far, most of the results converge upon the roles of pollen, particularly in dry stigma species. Since *Arabidopsis* pollen tubes grown through stigma and style tissues show a distinct gene expression profile compared with those grown *in vitro* (Qin et al., 2009), it is thus reasonable to assume that stigmas play active roles in pollen-stigma recognition. The components in the exudates of the wet stigma, the degradation products of the dry stigma cuticle, and the composition of the stigma cell wall may be involved in the early stage of pollen-stigma interaction (Table 1). Identification of these stigma factors using genetic, cytological and biochemical analysis will greatly advance our understanding of self-compatible recognition.

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