Love is a battlefield: programmed cell death during fertilization

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Received 11 November 2013; Revised 20 December 2013; Accepted 14 January 2014

Abstract

Plant development and growth is sustained by the constant generation of tremendous amounts of cells, which become integrated into various types of tissues and organs. What is all too often overlooked is that this thriving life also requires the targeted degeneration of selected cells, which undergo cell death according to genetically encoded programmes or environmental stimuli. The side-by-side existence of generation and demise is particularly evident in the haploid phase of the flowering plants cycle. Here, the lifespan of terminally differentiated accessory cells contrasts with that of germ cells, which by definition live on to form the next generation. In fact, with research in recent years it is becoming increasingly clear that the gametophytes of flowering plants constitute an attractive and powerful system for investigating the molecular mechanisms underlying selective cell death.

Key words: Antipodal cells, fertilization, gametes, gametophyte, pollen tube, programmed cell death, synergids.

Introduction

Unlike animals, flowering plants generate their germ cells in specialized haploid structures, termed gametophytes. The female gametophyte (FG) generates the egg cell, which upon fusion to a sperm cell develops into the embryo. As a characteristic feature of angiosperms, the egg cell adjoining central cell is also fertilized, giving rise to the embryo-nourishing tissue, termed the endosperm. The two immotile sperm cells required for fertilization are transported by the vegetative cell of the male gametophyte, or pollen. After pollen germination, the vegetative cell transforms into a long tip-growing pollen tube, which targets the ovules and enters the female gametophyte through the micropyle. Short-range pollen tube attraction is mediated by two egg cell adjoining synergids, which represent one of two accessory cell types formed in the FG. The second accessory cell type are antipodals, which form at the opposite pole of the FG adjacent to the central cell. While gametic cells by definition live on to form the next generation, the accessory cells of the male and female gametophytes of Arabidopsis thaliana degenerate at the end of the haploid life phase (Sundaresan and Alandete-Saez, 2010; Drews and Koltunow, 2011; Sprunck and Groß-Hardt, 2011). Programmed cell death (PCD) has many faces, and for comprehensive reviews we refer the reader to Bozhkov and Lam (2011), van Doorn (2011), Olvera-Carrillo et al. (2012), and Reape and McCabe (2013). In this review, we will focus on the causal events and molecular mechanisms underlying the demise of pollen tubes, synergids, and antipodal cells after they have fulfilled their functions.

Abbreviations: AAC2, ATP/ADP carrier 2; ACA, autoinhibiting Ca2+ ATPase; ACC, 1-aminocyclopropane-1-carboxylic acid; amc, abstinence by mutual consent; CSLD, cellulose synthetase-like gene D; fer, feronia; FG, female gametophyte; GCD1, GAMETE CELL DEFECTIVE 1; HGA, homogalacturonan; HR, hypersensitive response; ire, lorelei; MLO, mildew-resistant locus O; n-synergid, non-receptive synergid; PCD, programmed cell death; PME, pectin methyltransferase; PMEI, pectin methyltransferase inhibitor; PRC2, polycomb group repressive complex 2; RAC/ROP, RHO GTPases; ROS, reactive oxygen species; r-synergid, receptive synergid; sir, sirene; SYCO, SYCO ARATH.

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Antipodal cell death

In the model plant Arabidopsis, the three antipodal cells degenerate at around the time of fertilization. In contrast, in several grasses, such as maize and wheat, antipodal cells proliferate, forming up to several hundred cells, which persist during endosperm development, where they appear to serve a nutritive function (Diboll and Larsson, 1966; Raghavan, 1997; An and You, 2004; Holloway and Friedman, 2008).

There is reasonable evidence to assume that the PCD competence of antipodal cells requires both antipodal inherent factors and signalling cues provided by the female gametes.

Hiding from death: reprogramming of antipodal cells correlates with an increased antipodal lifespan

Several mutants have been described in which antipodal reprogramming is associated with a failure to undergo PCD. In mutants defective in the pre-mRNA splicing factors LACHESIS, CLOTHO, and ATROPOS, antipodals enlarge towards the centre, disintegrate their membranes, and exhibit central cell characteristic nuclear fusion. The morphological abnormalities correlate with the molecular reprogramming of antipodals into central cells and an extended antipodal lifespan (Groß-Hardt et al., 2007; Moll et al., 2008). Similarly, antipodal PCD is repressed upon the overexpression of auxin biosynthesis genes, which has been shown to activate egg cell identity in antipodals (Pagnussat et al., 2009). Given that, in all instances, cell identity rather than position is altered, these results argue against chalaza-derived positional cues as the sole source of antipodal PCD. Instead, they suggest that antipodal differentiation is associated with the acquisition of a PCD competence not present in other FG cells.

Live and let die: antipodal cell death requires signalling cues from female gametes

Defects in the cysteinyl-tRNA synthetase SYCO ARATH (SYCO) cause nuclear fusion defects in the central cell, and extend the antipodal lifespan. In the FG, SYCO is expressed and required exclusively in the central cell, indicating that antipodal PCD is under the regulatory control of the central cell. The protein is targeted to mitochondria, and ultrastructural analysis indicates that SYCO is necessary for mitochondrial integrity in the central cell (Kägi et al., 2010). It is well established that mitochondria play a critical role in PCD, in both plants and animals (Green and Reed, 1998; Reape and McCabe, 2008). They integrate various cell death stimuli, and one key death-promoting readout is the release of cytochrome c. Depending on the organism and cellular context, mitochondrial dysfunction has been shown to correlate both positively and negatively with cell and organismal ageing (Lee et al., 2003; Copeland et al., 2009). It has long been assumed that mitochondria affect the cellular lifespan in a cell-autonomous manner (Diamond and McCabe, 2011).

However, the antipodal lifespan in the FG has been shown to be causally linked to mitochondrial integrity in the central cell (Fig. 1). This is evidenced by targeted disruption of the electron transport chain in wild-type central cells: A dominant version of the ATP/ADP translocator AAC2 (aac2A199D) results in membrane uncoupling, and central cell-specific expression of aac2A199D phenocopies all aspects of syco-1 mutants, including an extended lifespan of antipodal cells (Kägi et al., 2010).

Mitochondrial integrity is required not only for central cell-antipodal communication but also for cross-talk between the egg cell and central cell, which appear to synchronize their development through monitoring their respective metabolic status. Wu et al. (2012) have shown that the ubiquitously expressed mitochondrial protein GAMETE CELL DEFECTIVE 1 (GCD1) is required for proper maturation of female gametes and PCD of antipodal cells. Intriguingly, expression of GCD1 in either of the female gametes enhances maturation in the other respective gamete. Conversely, targeted disruption of mitochondrial function by aac2A199D in the central cell prevents egg cell maturation, while egg cell-specific expression of aac2A199D interferes with nucleus fusion in the central cell and antipodal PCD (Wu et al., 2012). To date, it is unclear whether the extended lifespan of the antipodals is an indirect result of delayed central cell maturation, or whether antipodal PCD requires a second, central cell-independent, egg cell signal (Fig. 1).

Considering that the cell death mechanisms are conserved to some extent between animals and plants (Lam, 2004), it is not surprising that non-cell-autonomous mitochondrial-dependent mechanisms have also been identified in animals. As a matter of fact, Durieux et al. (2011) report that artificial electron transport chain impairment in neurons of Caenorhabditis elegans is communicated to the intestine where the mitochondria-specific unfolded protein response is activated, resulting in an extended lifespan. Although speculative at this stage, the non-cell-autonomous effects of mitochondria on other cells could also provide a mechanistic framework for the trophic theory of cell survival, which holds that cell death in neurons or cultured oligodendrocytes is modulated by trophic factors from neighbouring cells.

Fig. 1. Schematic representation of the female gametophyte containing two synergids (grey outline), one egg cell (red outline), one central cell (orange outline), and three antipodal cells (green outline). The lifespan of antipodal cells is controlled by mitochondria-dependent signalling from the egg and the central cell, revealing gamete maturation and vitality as important parameters for antipodal PCD. Whether the egg cell effect is direct or mediated by the central cell (dotted black lines) is currently unclear. For further details, see text.

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(Bergmann et al., 2002). Durieux et al. (2011) have coined the term ’mitokine’ for the mobile mitochondria-dependent longevity cue. Recent research has suggested that in animals, the criteria for such a mitokine are met by the fibroblast growth factor Fgf21. This starvation hormone is generated and released in response to mitochondrial stress (Kim et al., 2012) and its overexpression extends the lifespan in mice (Zhang et al., 2012). Plant mitokines have not yet been described; however, obvious suspects for mitochondria-generated factors include free calcium, ATP, and reactive oxygen species (ROS). In this respect, it is interesting that a recent analysis of ROS reporters has identified the central cell as a main source of ROS in the FG (Martin et al., 2013).

Synergid cell death

Synergids play a prominent role during fertilization as they represent the primary interface between male and female gametophytes (Punwani and Drews, 2008). They not only ensure short-range pollen tube attraction but, in addition, control pollen tube growth arrest and mediate sperm cell release. These processes have been comprehensively reviewed elsewhere (Chevalier et al., 2011; Kessler and Grossniklaus, 2011; Hamamura et al., 2012; Palanivelu and Tsukamoto, 2012; Dresselhaus and Franklin-Tong, 2013).

Digging one’s own grave: synergids attract pollen tubes and mediate sperm cell release

By faithfully ensuring pollen tube reception, synergids promote their cell death as the process of fertilization triggers sequential degeneration of both synergids. While we are still far from fully understanding the processes that govern the demise of these cells, work in recent years has provided substantial insights into some of the molecular mechanisms that regulate synergid PCD.

In many plant species, the two synergids appear to differ neither morphologically nor molecularly. Still, their mode and timing of PCD is distinct, depending on whether they are the one contacted by the pollen tube or not. Accordingly, we will in this review refer to the synergids as receptive and non-receptive synergids, (synergid and nr synergid, respectively). In compliance with the lack of visible differences between both synergids, in many species the choice of the receptive synergid seems to be random (Christensen et al., 1997; Huang and Russell, 1992) and elegant live-cell imaging studies in A. thaliana and Torenia fournieri have suggested that synergid PCD is only triggered upon physical contact between the synergid and pollen tube (Higashiyama et al., 2000; Sandaklie-Nikolova et al., 2007).

In contrast, in tobacco, synergid fate is accomplished prior to pollen tube arrival. This is evidenced by ultrastructural modifications and the accumulation of membrane-bound calcium in the prospective synergid prior to pollen tube arrival (Huang and Russell, 1992; Tian and Russell, 1997). Similarly, in Triticum aestivum, synergid degeneration precedes pollen tube entry and the pollen tube selectively penetrates the synergid containing an already degenerated nucleus (An and You, 2004). The initial selection of the synergid might in these cases follow positional cues, as it was shown for apple that it is the most micropylar cell which adopts synergid fate (Maria et al., 2007).

The degeneration process of the synergid has been described in detail in several organisms and involves various PCD hallmarks, including cell shrinking, disintegration of the nucleus and major organelles, and chromatin condensation, which, in apple, is associated with an increase in DNA methylation (Huang and Russell, 1992; Russell, 1992; Murgia et al., 1993; Christensen et al., 1997; Tian and Russell, 1997; Huck et al., 2003; An and You, 2004; Maria et al., 2007; Sandaklie-Nikolova et al., 2007).

The molecular basis of synergid PCD is not understood; however, these cells exhibit an extended lifespan in verdandi (vdd) mutants, which are characterized by misspecified synergids that occasionally express an antipodal marker. This result suggests that synergid inherent factors are required to realize cell death at the micropylar pole of the FG (Matias-Hernandez et al., 2010). While the nature of the PCD signal is as yet unknown, analysis of the gfa2 mutant has suggested a role for mitochondria in the regulation of synergid cell death. GFA2 encodes a J-domain-containing protein similar to the yeast Mdj1p and functions as a chaperone in the mitochondrial matrix. While gfa2 ovules faithfully attract pollen tubes, both synergids fail to degenerate (Christensen et al., 2002) (Fig. 2). Synergid degeneration is also affected upon down-regulation of ZmES4. This defensin-like protein is produced in synergids and causes the opening of potassium channels in the pollen membrane, a prerequisite for pollen tube burst (see below) (Amien et al., 2010). Notably, male-derived factors also contribute to synergid PCD, as evidenced by an extended synergid lifespan in plants defective for three pollen-expressed MYB genes (see below) (Leydon et al., 2013).

The degeneration of the synergid requires gamete fusion

The degeneration of the synergid is delayed with respect to that of the synergid. It has long remained unclear what triggers PCD of the synergid; however, making use of male gametophytic mutants, Kasahara et al. (2012) and Beale et al. (2012) have uncovered an important role for gamete fusion during this process (Fig. 2). Pollination of wild-type stigmas with pollen heterozygous for either duo1, duo3-1, or hap2/gcs1 revealed that the delivery of mutant sperm was associated with the survival of the synergid and the attraction of additional pollen tubes. It is anticipated that the effectiveness of a given pollen tube block is negatively correlated with (i) the time it takes to be established and (ii) the amount of pollen grains applied to the stigma. The latter parameter varies between experiments and can possibly explain why Kasahara et al. observed a maximum of two pollen tubes while Beale et al. detected up to four pollen tubes (Beale et al., 2012; Kasahara et al., 2012, 2013).

A requirement for gamete fusion for the establishment of a pollen tube block was also observed in plants defective for AtEC1.1–AtEC1.5. These genes encode small cysteine-rich
Regulation of \(n_s\)synergid PCD

The gamete fusion dependency of \(n_s\)synergid PCD implies that a mechanism must exist to communicate the fertilization status of the egg and central cell to synergids. Recent work has implicated the gaseous hormone ethylene as an important player in this process. Ethylene signal transduction converges on EIN3 and EIL1, two transcription factors which are both necessary and sufficient to induce an ethylene response (Chao et al., 1997). In ein3 eil1 double mutants and ein3 single mutants, PCD of the \(n_s\)synergid is unaffected; however, the \(n_s\)synergid lifespan is extended and the mutants attract supernumerary pollen tubes. Notably, double fertilization is not affected, indicating that ethylene signalling is necessary to couple fertilization and \(n_s\)synergid degeneration. In fact, the process of fertilization results in the accumulation of the ethylene response reporter EIN3. Moreover, constitutive proteins, which accumulate in storage vesicles in the egg cell prior to fertilization. After sperm cell arrival, EC1-containing vesicles are exocytosed, thereby triggering the relocalization of HAP2/GCS1 from the pollen tube endomembrane system to the plasma membrane (Fig. 2). This sperm activation is required for gamete fusion, and \(ec1.1\)–5 pentuple mutants attract supernumerary pollen tubes (Sprunk et al., 2012).

To dissect the importance of egg and central cell fertilization in this process, Maruyama et al. (2013) made use of the male gametophytic mutants \(cdka:1\) and \(kokopelli\), that induce single fertilization events (Aw et al., 2010; Ron et al., 2010). Surprisingly, the fusion of a sperm to either the egg or the central cell did not result in \(n_s\)synergid degeneration, but pollen tube attraction is only terminated if both female gametes are fertilized (Fig. 2). This dual control system implies that pollen tube attraction continues in cases of single fertilization. In fact, a second pollen tube, when delivering wild-type sperm, is able to rescue the single fertilization defect (Maruyama et al., 2013), indicating that the \(n_s\)synergid is part of a fertilization recovery mechanism that ensures maximum reproductive success in cases of compromised pollen fitness. In these cases, the female gametes can become fertilized by sperm of different paternal origin (Maruyama et al., 2013), a process referred to as heterofertilization.

In the future, heterofertilization might serve as an attractive tool to dissect the relative contribution of genetic factors to either embryo or endosperm development.

The fertilization-dependent degeneration of the \(n_s\)synergid can, in retrospect, explain the extended \(n_s\)synergid lifespan that has been observed in gametophytic mutants exhibiting a sperm cell reception defect, including lorelei (\(lre\)), fenrial siren (\(fer/sir\)), and abstinence by mutual consent (\(amc\)) (Huck et al., 2003; Rotman et al., 2003; Escobar-Restrepo et al., 2007; Boisson-Dernier et al., 2008; Capron et al., 2008; Tsukamoto et al., 2010). A common denominator of \(lre\), \(fer/sir\), and \(amc\) mutants is a failure of the pollen tube to rupture and to arrest growth and, consequently, fertilization is prevented. \(LRE\) encodes a putative glucosylphosphatidylinositol (GPI)-anchored protein and \(FER/SIR\) a receptor-like serine/threonine kinase, which both localize to the plasma membrane of synergids (Escobar-Restrepo et al., 2007; Capron et al., 2008; Tsukamoto et al., 2010). On the male side, pollen tube growth arrest and rupture require the redundant action of the three closely related transcription factors \(MYB97\), \(MYB101\), and \(MYB120\) (Leydon et al., 2013). In the respective triple mutants, the failure of sperm cell reception is frequently accompanied by an extended lifespan of both synergids, indicating that the degeneration of the \(n_s\)synergid relies on paternal cues as well (Fig. 2).

### Coupling fertilization and \(n_s\)synergid degeneration

The gamete fusion dependency of \(n_s\)Synergid PCD implies that a mechanism must exist to communicate the fertilization status of the egg and central cell to synergids. Recent work has implicated the gaseous hormone ethylene as an important player in this process. Ethylene signal transduction converges on EIN3 and EIL1, two transcription factors which are both necessary and sufficient to induce an ethylene response (Chao et al., 1997). In ein3 eil1 double mutants and ein3 single mutants, PCD of the \(n_s\)synergid is unaffected; however, the \(n_s\)synergid lifespan is extended and the mutants attract supernumerary pollen tubes. Notably, double fertilization is not affected, indicating that ethylene signalling is necessary to couple fertilization and \(n_s\)synergid degeneration. In fact, the process of fertilization results in the accumulation of the ethylene response reporter EIN3. Moreover, constitutive...
ethylene response in *ctrl* mutants and external application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) through central cell microinjection are sufficient to induce the degeneration of both synergids independently of fertilization (Völz *et al.*, 2013). These results stress the important role of ethylene for the degeneration of the *nsyn* synergid and suggest that the initial *syn* synergid degeneration is governed by an ethylene-independent mechanism. Whether ethylene signalling is involved in communicating egg cell fertilization, central cell fertilization, or both is currently unclear (Fig. 2).

The situation is less ambiguous with mutants defective in components of the polycomb group repressive complex 2 (PRC2). Making use of live imaging techniques, Maryama *et al.* (2013) could show that mutants defective in the central cell-expressed *MEA, FIS2, or FIE* genes attract supernumerary pollen tubes, despite successful gamete fusion. Other mutants which were affected in chromatin modifications exhibited a functional pollen tube block. These results suggest that PRC2-mediated gene silencing is involved in either realizing or sensing the molecular switch from a non-fertilized to a fertilized gamete profile or regulates the communication of the fertilization status to synergids (Maryama *et al.*, 2013) (Fig. 2).

**Synergid PCD: a domesticated form of the hypersensitive response?**

There is accumulating evidence for a substantial overlap in the molecular control of pollen tube reception and fungal invasion (Dresselhaus and Marton, 2009). The hypothesis that both processes have evolved from a common symbiosis-regulating module was first put forward by Kessler *et al.* (2010) who observed that the receptor-like kinase FER is not only involved in pollen tube reception but, in addition, mediates the compatible interaction between plant epidermal cells and tip-growing powdery mildew hyphae. Moreover, in synergids, FER is required for pollen tube-triggered relocation of NORTIA, a mildew-resistant locus O (MLO) protein, which, similarly to FER, mediates pollen tube reception and fungal invasion (Kessler *et al.*, 2010). A common plant response to pathogen infection is the hypersensitive response (HR), which is characterized by a ROS-dependent oxidative burst (Nanda *et al.*, 2010). In this respect it is interesting that FER can activate RHO GTPases (RAC/ROP), which act upstream of NADPH-dependent ROS generation (Duan *et al.*, 2010), and the expression of *FER* positively correlates with ROS levels in root hairs. High ROS levels have also been observed in the *syn* synergid after pollen tube reception (Martin *et al.*, 2013), and it is thus conceivable that FER-dependent ROS signalling is also involved in sperm cell discharge. At the functional level, the ROS link is supported by the finding that defects in the *AMC* gene result in synergid PCD defects. *AMC* encodes a peroxin essential for protein import into the ROS-detoxifying peroxisomes. As the name suggests, the defect only becomes evident when both sides, male and female, are mutant (Boisson-Dernier *et al.*, 2008), suggesting that the redox balance is controlled by both sexes.

The finding that pollen tube arrival triggers an oscillation of cytoplasmatic Ca\(^{2+}\) in synergids (Iwano *et al.*, 2012) provides a further parallel to the plant immune response, where the elevation of cytoplasmic Ca\(^{2+}\) constitutes an early alarm reaction (Ma *et al.*, 2012). Finally, and along this line, it is noticeable that ethylene has previously been shown to be implicated in the regulation of HR-associated PCD (Trobacher, 2009). In the light of these results, it thus appears conceivable that synergid PCD represents a ‘domesticated’ form of HR-associated cell death.

**PCD of the vegetative cell**

Fertilization-associated PCD of accessory cells is not restricted to the FG but involves the male gametophyte as well. After being recognized at the micropylar end of the synergids, the pollen tube continues to grow until it reaches a site beyond the filiform apparatus, where it ruptures (Leshem *et al.*, 2013). This process releases the two sperm cells into the synergid (Sandaklie-Nikolova *et al.*, 2007) and terminates the life of the vegetative cell. The discharge of the pollen tube content appears to be an explosive, rapid mechanism (Higashiyama *et al.*, 2000) and relies on signalling cues generated by the *syn* synergid (see above). It is currently unclear whether the synergid degeneration process contributes to sperm release; however, synergid PCD can be excluded as the sole trigger of pollen tube rupture, as this process is not disturbed in *feronia* and *nortia* mutants (Huck *et al.*, 2003; Kessler *et al.*, 2010). Thus, at least three distinct processes are activated during pollen tube reception, one causing synergid degeneration, a second mediating pollen tube growth arrest, and a third which results in pollen tube burst. On the molecular level, cation dynamics appear to play a critical role for the PCD of the vegetative cell, which, in maize, are in part regulated by the defensin-like cysteine-rich protein ZmES4 (Amien *et al.*, 2010). ZmES4 accumulates in vesicles in the secretory zone of mature synergid cells before pollen tube arrival and is released during the fertilization process. While external ZmES4 application causes a species-specific explosive burst at the pollen tube tip, RNA interference (RNAi) approaches result in a failure of pollen tube burst, and synergid degeneration, and sperm cell release. ZmES4 is structurally similar to invertebrate toxins and its application causes the opening of the inward rectifying shaker potassium channel KZM1. Depolarization of the pollen plasma membrane together with K\(^{+}\) influx causes water uptake, and the resulting osmotic pressure might provide the energy for pollen tube rupture (Amien *et al.*, 2010).

Besides the potassium channel, a Ca\(^{2+}\) pump also plays a role in pollen tube burst regulation. Ca\(^{2+}\) dynamics are mediated by the controlled influx and efflux of Ca\(^{2+}\) ions. The influx is a passive process mediated by Ca\(^{2+}\) channels, and glutamate receptor-like channels have recently been shown to facilitate Ca\(^{2+}\) influx across the plasma membrane of pollen tubes (Michard *et al.*, 2011). In contrast, Ca\(^{2+}\) efflux is actively accomplished by antiporters or pumps (Bose *et al.*, 2011). The *Arabidopsis* genome encodes 10 autoinhibiting Ca\(^{2+}\) ATPase (ACA) pumps, which become activated by Ca\(^{2+}\)
or calmodulin. The ACA9 high affinity Ca$^{2+}$ pump is primarily expressed in the pollen and localizes to the plasma membrane. Mutants defective in ACA9 exhibit a defect in sperm cell discharge (Schiøtt et al., 2004). Synergid PCD is not impaired in these mutants, suggesting that synergid degeneration occurs independently of ACA9-mediated pollen tube rupture.

Premature pollen tube rupture is prevented by ANXUR1 and ANXUR2. These receptor-like kinases are the closest homologues to FERONIA and are preferentially expressed in pollen where they localize to the tip of the pollen tube. anxur1 anxur2 double mutant pollen tubes grow through the stigma, but already burst and discharge their cytoplasmic content on their way to the transmitting tract (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). The suggested model for ANXUR-dependent signalling proposes that ANXUR might be constitutively active in inhibiting pollen tube burst and maintaining tip growth until the pollen tube arrives at the FG. After pollen tube reception, a ligand secreted from the synergids deactivates ANXUR, resulting in tube burst. This ligand is proposed to operate downstream of FERONIA (Bosch and Hepler, 2009; Boisson-Dernier et al., 2009; Miyazaki et al., 2009).

PCD and cell wall modifications

Ultimately, the processes mediating pollen tube rupture have to target cell turgor and/or wall stiffness. In fact, it has been shown that the esterification status of pectin homogalacturonan (HGA) plays a critical role in pollen tube stabilization (Bosch and Hepler, 2005; Jiang et al., 2005; Woriedh et al., 2013). HGA is a homopolymer composed of (1,4)-α-L-galacturonic acid, which is synthesized in the Golgi. Following esterification and secretion into the apoplast, pectin methyl esterases (PMEs) catalyse the demethylesterification, resulting in the exposure of negatively charged carboxyl groups which can be cross-linked by Ca$^{2+}$ and thereby enhance cell wall stiffness (Bosch and Hepler, 2005). A mutation in the pollen-expressed PME VANGUARD1 (VGD1) reduces PME activity and vgd1 pollen tubes grow more slowly and burst prematurely in vitro (Jiang et al., 2005). It has recently been shown that a PME inhibitor (PMEI), which binds and blocks the active site of the demethylsterification enzymes (Bosch and Hepler, 2005), is expressed in the male and female gametophyte, as well as in the transmitting tract of maize. ZmPMEI1 accumulates at the pollen tube tip as well as at bending sites, and the application of recombinant ZmPMEI1 to an in vitro growing pollen tube causes pollen tube burst at a subapical region (Woriedh et al., 2013).

Cell wall-modifying enzymes also play a critical role for synergid integrity: CSLDs are cellulose synthase-like genes, and CSLD2 and CSLD3 are thought to catalyse the synthesis of β-1,4-glucan polysaccharides, which assemble into cellulose-like microfibrils. It has recently been shown that mutants defective in CSLD2 and CSLD3 exhibit premature synergid disintegration (Yoo et al., 2012). Until now, it is, however, unclear whether pollen tube arrival is associated with the modulation of CSLD activity profiles.

Conclusion and outlook

The degeneration of the pollen tube, of synergids, and of antipodal cells accompanies and characterizes the completion of the haploid life phase. While sharing a common fate, these haploid cells differ remarkably with respect to their cell death trigger and timing of PCD. Also the molecular mechanisms underlying accessory cell death appear to differ substantially. This is evidenced by the fact that most mutants are affected in the cell death of only a single cell type. Pollen tube reception mutants represent an exception to this rule; however, in these cases, the synergid PCD defect appears to be a secondary consequence of a fertilization failure.

The example of synergids has unravelled an unprecedented regulatory potential for synergid lifespan extension. At this point, it is unclear whether the degeneration of the other two cell types can be modulated for adaptive purposes as well. In addition, it will be interesting to determine the biological significance of Ca$^{2+}$ and ROS accumulation in the FG and to dissect the regulatory network underlying the coordination of the successive PCD events.

Major advances in our understanding of male–female gametophyte interaction have only been possible because of advanced microscopy approaches, and the establishment of novel in vitro systems and ever more sophisticated live-cell imaging techniques (Kurihara et al., 2013) promises to accelerate further the rapid movement of an exciting field.

Acknowledgements

We thank members of the RG-H laboratory for helpful comments on the manuscript. Work in the RG-H laboratory is supported by the Universities of Tübingen and Bremen, and grants from the Deutsche Forschungsgemeinschaft (DFG), the Volkswagenstiftung, and the state of Baden-Württemberg.

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