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Killing me softly - programmed cell death in plant reproduction from sporogenesis to fertilization

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Abbreviations
PCD, programmed cell death; MMC, Megaspore Mother Cell; TF, transcription factor; NAC, NAM ATAF1/2 CUC2; ROS, reactive oxygen species; SI, self-incompatibility; GPI, glycosylphosphatidylinositol; GPI-AP, GPI-anchored protein.

Abstract
Regulated or programmed cell death (RCD or PCD) is a fundamental biological principle integral to a considerable variety of functions in multicellular organisms. In plants, different PCD processes are part of biotic and abiotic stress responses, but also occur as an essential aspect of unperturbed plant development. PCD is particularly abundant during plant reproduction, eliminating unwanted or no longer needed cells, tissues or organs in a precisely controlled manner. Failure in reproductive PCD can have detrimental consequences for plant reproduction. Here we shed a light on the latest research into PCD mechanisms in plant reproduction from sex determination over sporogenesis to pollination and fertilization.

Introduction

Regulated or programmed cell death (RCD or PCD) denotes actively controlled processes that cause the death, and in some cases the corpse removal, of cells. As genetically programmed operations, RCD processes are generally considered to have evolved to serve specific functions and provide tangible selective advantages. In animals and plants, RCD events occur in a plethora of subroutines. Generally, one can differentiate between stress-induced RCD and physiological forms of PCD occurring in the absence of exogenous environmental perturbation [1]. In plants, these contextual categories have been recognized as environmental PCD (triggered by external biotic or abiotic agents) and developmental PCD (occurring as an integral part of development; hereafter referred to as PCD) [2]. Over the last two decades, a growing number of PCD routines have been described in plants, and many are essential for plant growth and reproduction [3–6].

In this review, we discuss recent findings regarding PCD processes occurring during plant reproduction from meiosis to fertilization. There are numerous post-fertilization PCD processes during seed development and germination; these have been excellently reviewed elsewhere [7,8]. Here, we focus on the regulation of PCD in sex determination, micro- and megasporogenesis, and nucellus degeneration. Further, we will cover PCD processes occurring during pollination, self-incompatibility responses, floral senescence, and double fertilization. An overview of genes regulating these processes is given in table 1.

PCD during sex determination

Sex determination in plants is a complex process that holds great agronomical potential for fruit and seed production [9]. Unisexual flowers (Figure 1) can develop by different means, many of which involve PCD processes at various stages of flower development. In maize (*Zea mays*), PCD suppresses carpel development in all flowers of the male inflorescence (tassel), as well as in half of the flowers in the female inflorescence (ear) [10]. Several classical “tasselseed” mutants exhibit defects in carpel suppression, leading to conspicuous seed formation in the tassel [11,12]. Recently, RAMOSA 3 (RA3) was shown to act with GRASSY TILLERS 1 (GT1) to regulate carpel suppression, revealing a surprising pleiotropy where genes involved in vegetative development were recruited for floral sculpting [13••]. Interestingly, RA3 and GT1 appear to regulate genes implicated in PCD, including homologs of the Arabidopsis (*Arabidopsis thaliana*) NAC transcription factors (TFs) KIRA1 and ANAC087 [13–15].

In several fruit crops, PCD processes have been implicated in unisexual flower development. In persimmon (*Diospyros kaki*), PCD causes the developmental arrest of carpel primordia in male flowers [16], whereas PCD underlies stamen abortion in female flowers of the tung tree (*Vernicia fordii*) [17]. In *Opuntia robusta*, premature tapetum degeneration causes male sterility of female flowers, while

ovules abort through PCD in male flowers [18]. In the dioecious kiwifruit (*Actinidia spec.*), failure to produce pollen in flowers of female individuals is caused by delayed tapetum PCD, while flowers on male plants develop non-functional rudimentary carpels [19]. The type-C cytokinin response regulator Shy Girl (SyGI), which is located on the Y-chromosome of the kiwifruit genome, acts as a dominant suppressor of carpel development [20•]. SyGI may act by reducing cytokinin-controlled meristematic activity, thereby conveying sex determination without PCD [21], showcasing that processes other than PCD may be employed in sex determination.

PCD during microsporogenesis

The tapetum is the innermost cell layer of the anther, surrounding and supporting pollen development (Figure 1). Pollen maturation and wall formation relies on the precise timing of PCD in tapetal cells [22], which occurs at late microsporogenesis stages [23].

Multiple regulators of tapetal PCD have been identified, several of which are conserved between Arabidopsis and rice (*Oryza sativa*) (reviewed in [2]). In both species, gibberellic acid (GA) affects tapetum differentiation through GAMYB TFs, as well as DYSFUNCTIONAL TAPETUM 1 (DYT1) in Arabidopsis and its ortholog UNDEVELOPED TAPETUM 1 (OsUDT1) in rice. In Arabidopsis, DYT1 positively regulates MALE STERILITY 1 (MS1) and ABORTED MICROSPORES (AMS), which in turn drives expression of the reactive oxygen species (ROS)-producing enzyme RESPIRATORY BURST OXIDASE HOMOLOG E (RBOHE). In rice, TAPETUM DEGENERATION RETARDATION (OsTDR, an AMS homologue) and PERSISTENT TAPETAL CELL 1 (OsPTC1, an MS1 homologue) are positively regulated by both OsGAMYB and OsUDT1. Both OsTDR and OsPTC1 positively regulate the lipid transfer protein OsC6 and the cysteine protease OsCP1, which are thought to promote tapetal PCD. OsUDT1 acts upstream of bHLH142 to promote tapetal PCD via OsTDR and ETERNAL TAPETUM 1 (OsEAT1) [2]. Further downstream, OsEAT1 promotes the expression of two aspartic proteases (OsAP25 and OsAP37), which induce cell death when ectopically expressed [24].

More recently, mutation of an AT-hook nuclear localized (AHL) family protein, PERSISTENT TAPETAL CELL 2 (OsPTC2), has been shown to phenocopy *ptc1*. OsPTC1 and OsPTC2 may function in the same tapetal PCD pathway by targeting shared downstream genes, such as *OsEAT1*, *OsCP1*, and *OsAP25* [25,26•]. However, *ptc2* differs from *ptc1* in its abnormal pollen wall patterning, which is reminiscent of an Arabidopsis pollen wall defective mutant, *transposable element silencing via AT-hook* (*tek*) [27], underscoring the relevance of AHL family proteins in pollen wall patterning processes in both monocots and dicots. Several new factors promoting rice tapetal PCD have been recently described, such as ENHANCED DOWNY MILDEW 2-LIKE (OsEDM2L) and OsMYB103, whose mutants both show delayed tapetal cell death [28,29]. Interactions among OsEDM2L, bHLH142, and OsTDR activate

OsEAT1 expression, thereby promoting tapetal PCD [28]. Other recently published mutants, for instance *glucose 6-phosphate/phosphate translocator* (*osgpt1*) [30], and *defective in callose, exine and tapetum 1* (*dcet1*) [31] show delayed tapetal PCD and abnormal pollen wall patterning. However, the underlying mechanisms and pathways remain to be elucidated.

Male fertility can be affected by both delayed and premature tapetal PCD. Downstream of the DELLA protein REPRESSOR OF GA1-3 (RGA), the *rga target 1* (*osrgat1)* mutant showed premature tapetal degeneration, while OsRGAT1 overexpression delayed PCD [32•]. Additionally, the rice *osaldh2b* mutant, affecting a mitochondrial aldehyde dehydrogenase, exhibited premature tapetal PCD and an upregulated expression of *OsTDR* [33]. Likewise, the *earlier degraded tapetum 1* (*edt1)* mutant exhibits premature tapetal cell death along with a decrease in ATP concentration and fatty acid content in anthers [34]. Surprisingly, known tapetal PCD-promoting genes (*OsTDR*, *OsEAT1*, *OsAP25*, and *OsCP1*) are downregulated in *edt1*, suggesting this premature cell death might be independent of the canonical PCD network [34].

In addition to the established PCD network, autophagy has been implicated in tapetal differentiation and PCD. In normal conditions, autophagy-deficient mutants in rice display sporophytic male sterility [35], while fertility is not affected in core autophagy mutants in Arabidopsis [36]. However, autophagy is activated by high temperature stress in Arabidopsis, and autophagy-deficient mutants displayed impaired male fertility characterized by incomplete degeneration of tapetum cells [37•]. In the light of climate change, these finding might have important implications for crop reproduction.

PCD during megasporogenesis

Megasporogenesis starts with the specification of the Megaspore Mother Cell (MMC). After MMC meiosis produces four haploid megaspores, one functional megaspore persists, while the remaining megaspores degenerate (Figure 1) [38]. Little is known about the molecular mechanisms underpinning monospory and functional megaspore selection; although PCD has been proposed to cause megaspore degeneration in *Medicago sativa*, further studies are required to confirm this in other species [38,39]. An Arabidopsis septuple mutant of the cyclin-dependent kinase inhibitor genes ICK1-7/KRP1-7 (interactor/inhibitor of cyclin-dependent kinase/Kip-related proteins) developed ovules with multiple MMCs, functional megaspores and functional female gametophytes [40]. An ICK4/KRP4-YFP (yellow fluorescence protein) fusion protein was shown to be expressed highly in degenerating megaspores, but was absent in the functional megaspore, leading the authors to propose ICK4/KRP4 (and potentially other redundant ICKs/KRPs) as a positive regulator of megaspore degeneration [40]. However, a simultaneous study on a triple ick/*krp4-6-7* mutant concluded that KRPs function in a network ensuring the switch from mitosis to meiosis in MMCs; this suggests that the surplus functional megaspores in

the *krp* septuple mutant are the result of delayed mitotic exit of the MMC before meiosis, rather than a failure to degrade non-functional megaspores after meiosis [41].

Recently, small RNAs have been implicated in the degeneration of non-functional megaspores, as *miR822* mutants exhibit extranumerary surviving meiotic products with functional megaspore identity that can mitotically divide, but fail to form mature female gametophytes [bioRxiv 2021, doi:10.1101/2021.10.18.464879]. MiR822a interacts with ARGONAUTE9 to negatively regulate Cysteine/Histidine-Rich C1 domain proteins and restrict meiotically derived cell survival to a single megaspore. The authors postulated that miR822a functions as an intercellular signal for PCD, thereby mediating a pathway to eliminate female haploid products after meiosis [bioRxiv 2021, doi:10.1101/2021.10.18.464879]. These exciting data might bring us a step closer to understanding how cell death mechanisms ensure monosporic development in plants.

PCD during nucellus development

The nucellus is a short-lived tissue present in the developing ovule and in the early seed supporting gametophyte and embryo/endosperm development. The nucellus degenerates in a species-specific, spatiotemporal pattern before, during, or after fertilization (Figure 1). In Arabidopsis, a part of the nucellus degenerates prior to fertilization, while the other part persists until after fertilization [42]. Nucellar degeneration before fertilization starts in the cells adjacent to the developing female gametophyte, and control of auxin fluxes has been implicated in this process. A mutant in the auxin efflux carrier PIN1 shows inhibited nucellar degeneration, while in a *pin3/4/7* triple mutant, auxin-enriched nucellar cells degenerate precociously with altered cell death morphology, suggesting that nucellar auxin accumulation might promote PCD [43]. The nucellar cells that persist until after fertilization can be further divided into one domain that degenerates during early seed development, and another domain that persists longer [42]. Prior to fertilization, polycomb-group proteins repress endosperm development and nucellar degeneration, while MADS box TF AGAMOUS-LIKE 62 (AGL62) relieves the Polycomb-mediated repression to permit post-fertilization nucellar degeneration. Downstream of AGL62, TRANSPARENT TESTA 16 (TT16) and the MADS box TF GORDITA promote nucellar degeneration [42]. Why one part of the nucellus persists while another degenerates is not clear yet. Possibly, the nucellus acts as an early sugar sink, and provides the reserves it transiently accumulates to the growing endosperm during degeneration [44].

In many cereals, nucellar degeneration occurs only after fertilization and in a centripetal fashion (reviewed in [45]). Some cereals develop a unique tissue dedicated to nutrient transport called the nucellar projection. Multiple PCD-associated factors have been identified in the nucellar projection of barley (*Hordeum vulgare*), such as the toxin-like protein JEKYLL [46] and numerous proteases, including

various VACUOLAR PROCESSING ENZYMEs (VPEs). Suppression of VPE2 paralogs in barley reduced PCD in the nucellar projection, compromising sucrose transport into the endosperm and grain filling [47]. In contrast to domesticated cereals, nucellar projection PCD in Brachypodium *(Brachypodium distachyon*) occurs slower, suggesting that domestication selected for a rapid nucellar projection cell death to allow more extensive endosperm expansion. Interestingly, Brachypodium lacks direct VPE2 orthologs, which might explain slower nucellar projection breakdown rates in this wild relative of barley and wheat [48].

PCD in self-incompatible pollen-pistil interactions

In many angiosperm taxa, self-incompatibility (SI) mechanisms have evolved to recognize and reject self-pollen. SI prevents self-fertilization and inbreeding, and thus promotes genetic diversity. Currently, three major SI mechanisms have been intensively investigated at the molecular level: Brassicaceae SI, *S*-RNase-based SI in several taxa (including Solanaceae, Rosaceae, and Plantaginaceae), and Papaveraceae SI [49].

In *S*-RNase-based SI, self-*S*-RNase exerts RNase toxicity and triggers a signaling cascade in the self-pollen, leading to tip-localized ROS disruption, actin cytoskeleton depolymerization, vacuole collapse, cytoplasmic pH decrease, caspase-like protease activation and nuclear DNA degradation, culminating in self-pollen tube PCD [50,51]. In poppy (*Papaver rhoeas*), SI is controlled by the female *S*-determinant PrsS (*P. rhoeas* stigma *S*), a small secreted stigma protein [52], and the male S-determinant PrpS (*P. rhoeas* pollen *S*), a pollen membrane protein [53]. Cognate PrpS-PrsS interaction induces a calcium-triggered signaling network causing cytosolic pH drop, increase of ROS and nitric oxide, cytoskeleton depolymerization and actin foci formation, mitogen-activated protein kinase and DEVDase-like activation, and finally growth arrest coupled with self-pollen PCD [54]. Recently, poppy SI-induced ROS accumulation was found to result in the rapid, extensive, predominantly irreversible oxidation of pollen proteins. Importantly, the oxidative modification was observed in cytoskeleton proteins and energy production-associated enzymes, suggesting that ROS contributes to SI-PCD by reducing cellular activities [55].

PCD processes are involved in self-pollen rejection in both *S*-RNase-based SI and poppy SI (Figure 1); although these two types of SI-PCD arose independently during evolution, they share common changes of cellular physiology during PCD. Whether this is a coincidence or of evolutionary significance remains an intriguing question.

Notably, co-expression of cognate poppy *PrpS-PrsS* in the self-compatible model plant Arabidopsis resulted in self-incompatible Arabidopsis “SI” lines, demonstrating that heterologous *PrpS-PrsS* can function in Arabidopsis, and providing new opportunities to investigate poppy SI-PCD in a model plant

[56]. Moreover, ectopic *PrpS-PrsS* expression in Arabidopsis roots induces root growth arrest and an SI-like PCD response [57•]. Using genetically encoded reporters and sensors in Arabidopsis "SI" pollen, the dynamic cellular changes of cytosolic calcium concentration, pH level, cytoskeleton integrity, clathrin-mediated endocytosis, and vacuolar morphology occurring in SI-induced PCD were studied in unprecedented detail [58]. Recently, the glycosylphosphatidylinositol (GPI) inositol deacylase HIGHLANDER1/AtPGAP1 was identified as a critical regulator of the poppy SI response in a forward genetics screen for SI-PCD suppressors in Arabidopsis "SI" lines [59••]. *AtPGAP1* mutation results in a "three-footed" configuration of GPI-anchored proteins (GPI-APs), which does not affect the targeting of GPI-APs to the plasma membrane [59,60], but affects their cleavage and release from membranes *in vivo* [59••]. These results suggest that functional PrsS-PrpS interaction requires one or several cleavable GPI-APs, the identification of which provides interesting directions for future research.

PCD regulating plant fertility by floral organ senescence

Flower receptivity is temporally restricted to a tightly controlled, species-specific window, which can last mere hours in some species, and span months in others. If pollination does not occur within this window, floral organs start to senesce and irrevocably lose their reproductive potential (Figure 1) [61]. Investigating the senescing non-pollinated stigma tissue in Arabidopsis, cellular and transcriptional hallmarks of PCD processes were discovered in degenerating stigmatic papilla cells [14••]. Via transcriptional profiling, the NAC TFs ORESARA1 (ORE1) and KIRA1 (KIR1) were found to promote senescence-induced papilla PCD. Simultaneous KIR1 and ORE1 loss of function resulted in a two- to four-fold extension of stigma life span. However, flower fertility was only moderately extended, suggesting that stigma viability is only one of the factors determining receptivity in Arabidopsis [14••]. Maize stigmata, or silk strands, have long been known to undergo a senescence process characterized by tissue collapse in the silk base at the junction to the ovary [62]. Profiling senescence-induced transcriptome changes in the silk base, various differentially expressed TFs were identified, including several orthologs of KIR1, dubbed KIR1-LIKEs (KILs) [63]. Among them, KIL1/NAC36 is sufficient to induce cell death when inducibly expressed in maize, tobacco, and Arabidopsis, suggesting that PCD regulatory networks are functionally conserved between dicots and monocots. While overexpressing KIL1 in senescent silks yielded early onset of silk degeneration and a reduced fertile window, KIL1 mutation delayed silk senescence and extended fertility—demonstrating this gene's central role in modulating silk senescence and fertility in maize [63].

Cell death in stigmata also occurs after pollination, appearing to interact with SI mechanisms in Brassica species. In *Brassica rapa*, compatible pollination accelerates stigma senescence and papilla cell death in an ethylene-dependent manner, and ethylene treatments were sufficient to induce stigma

senescence independent of pollination [64]. Interestingly, ethylene- or age-induced stigma senescence correlated with a breakdown of SI, suggesting that SI mechanisms are lost or suppressed in ageing flowers, as well as young flowers in ageing plants [64,65].

PCD during double fertilization

On the stigma, compatible pollen grains germinate a tip-growing pollen tube (PT) to transport the immotile sperm cells to the female gametophytes. Guided by sporophytic and female gametophytic cues, the pollen tubes grow through the stigma, style, and ovary to reach the ovules harboring the female gametophytes to accomplish double fertilization [66]. In Arabidopsis, the gametophytic synergid cells attract a pollen tube to the micropyle by secreting cysteine-rich peptides, AtLUREs and XIUQIUs [67,68]. Pollen tube reception is precisely and tightly controlled by a series of signaling events between the pollen tube and the receptive synergid to coordinate pollen tube growth arrest, disintegration of the receptive synergid, pollen tube burst to release the sperm cells, and finally the demise of the remaining persistent synergid [69]. Hence, the pollen tube cell and both synergid cells perish during the fertilization process (Figure 1), but if PCD pathways known from canonical ~~d~~PCD systems (e.g. xylem, tapetum, or root cap [70] [83]) operate in this context remains unclear.

When the pollen tube enters the micropyle, receptive synergid-expressed receptors, including the receptor-like kinase FERONIA (FER) and the GPI-AP LORELEI (LRL), sense pollen tube arrival and halt its growth [71-73]. During this process, FER mediates ROS accumulation in a Ca^{2+}-dependent manner in the synergid cells to induce pollen tube rupture [74]. Recently, the synergid-expressed MILDEW RESISTANCE LOCUS *O* (MLO) protein NORTIA was found to bypass FER signaling, acting as a secondary booster to promote pollen tube burst [75•].

The receptive synergid degenerates concomitantly with pollen tube rupture, allowing the released sperm cells to access the egg and central cells [76,77]. Death of the receptive synergid is regulated by the interaction of two REPRODUCTIVE MERISTEM (REM) TFs, VALKYRIE (VAL)/VERDANDI (VDD) and MADS-box complex SEEDSTICK-SEPALLATA3 [78]. Vacuolar acidification mediated by AP1G and V-ATPases was observed during receptive synergid degeneration [79]. While the receptive synergid degenerates, the persistent synergid remains alive until successful fertilization. Upon double fertilization, the persistent synergid is removed to cease the attraction of additional pollen tubes. Interestingly, this removal is not achieved by PCD, but by a cell fusion event followed by specific degradation of the persistent synergid nucleus in the central cell cytoplasm [80]. Ethylene has been implicated in persistent synergid removal based on the observation that the persistent synergid maintains its integrity longer in ethylene-insensitive mutant *ein3 eil1* [81]. However, an ethylene-deficient ACC oxidase (ACO) quintuple mutant shows no PCD-related phenotype in synergid cells

[82••], suggesting that EIN3 and EIL1 might regulate synergid cell degeneration in an ethylene-independent way. Interestingly, the receptor-like kinases FER, ANJEA, and HERCULES RECEPTOR KINASE 1 that control pollen tube rupture through interaction with the ovular peptide ligand RAPID ALKALINIZATION FACTOR 34 (RALF34) [83], also prevent the arrival of multiple pollen tubes at the ovule [84•]. These kinases interact with the pollen tube-expressed RALF6, 7, 16, 36, and 37 at the ovary septum, establishing a pollen tube block that prevents the micropylar attraction of excess pollen tubes. With pollen tube rupture, RALF signaling at the septum stops and allows the surviving persistent synergid to attract a second pollen tube as a backup should fertilization by the first pollen tube fail [84•].

Recently, MITOGEN-ACTIVATED PROTEIN KINASE 4 (MPK4) was implicated in preventing premature synergid cell death via the resistance protein SUMM2 [85••]. The *mpk4* mutants show premature synergid cell death accompanied by ROS accumulation, which can be reverted by ROS scavengers and SUMM2 mutation. Without SUMM2, synergid cells fail to die upon pollen tube arrival, with the pollen tube surviving and continuing to grow [85••]. These results suggest parallels between immunity-related and synergid cell death during plant reproduction.

Concluding remarks

A multitude of cellular disintegration events is involved in successful reproductive development in plants. Several mechanisms are in place to kill cells in a targeted fashion, and a key question is in how far there are core PCD executers at work in reproductive PCD, and if they are shared with PCD events occurring during vegetative development [70]. Systematic comparative research will be necessary to shed a light on this still little understood but fascinating aspect of plant biology.

Author contributions

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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- ●● This paper reveals that MPK4 and SUMM2 are involved in the regulation of synergid PCD by affecting ROS homeostasis. Importantly, MPK4 and SUMM2 are known as cell death pathway genes acting in plant immunity, suggesting a parallel regulation between the PCD of immunity and plant reproduction.

Figures

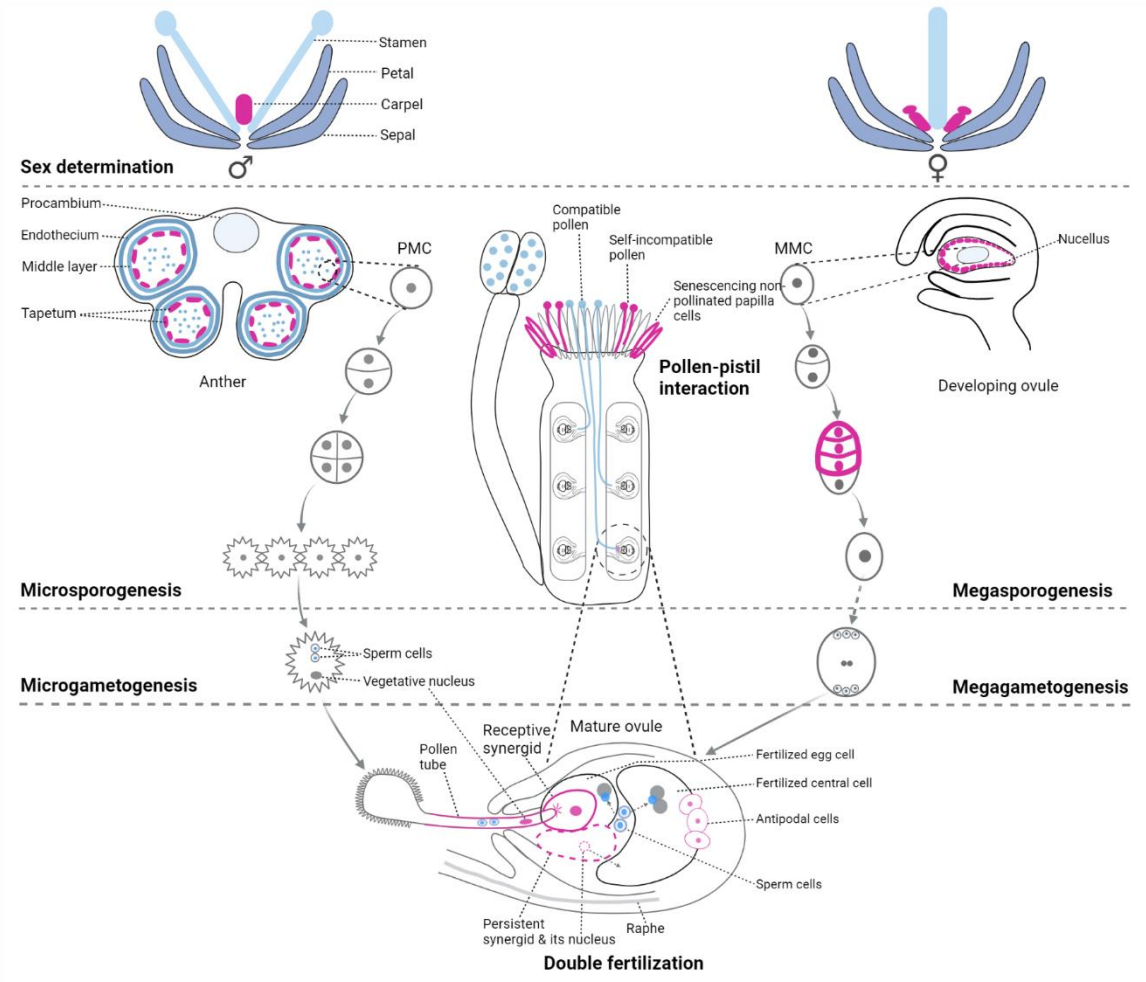

Figure 1: Tissues and cells undergoing developmentally controlled PCD (in magenta) from sporogenesis to fertilization. In unisexual flowers, PCD can causes the abortion or developmental arrest of carpel primordia in male flowers, and stamen abortion in female flowers. During sporogenesis, PCD occurs in supporting sporophytic tissues including the anther tapetum and the nucellus in the developing ovules. After meiosis, non-functional megaspores degenerate. During pollination, some self-incompatibility mechanisms operate by arresting and killing self-pollen on the stigma. Lastingly unpollinated stigmata undergo senescence-triggered PCD, terminating flower fertility. After timely and compatible pollination, the pollen tube grows towards the ovule attracted by the female gametophytic synergids. Upon pollen tube arrival, both the pollen tube cell and the receptive synergid disintegrate, allowing the sperm cells to contact the egg cell and the central cell. The persistent synergid is eliminated after successful fertilization by fusing to the central cell. The antipodal cells in various species do not appear to have specific functions and degenerate before or after fertilization (not covered in this review). Abbreviations are: PMC, pollen mother cell; MMC, megaspore mother cell. The Figure was created with BioRender.com.

Table 1 Overview of recently identified players regulating reproductive PCD processes

PCD occurs during	Gene locus	Gene product	Species	Reference
Sex Determination	*GRMZM2G014729*	RAMOSA3	Maize	[13]
	At4g28530; At5g39610	KIRA1; ORESARA1	Arabidopsis	[14]
	At5g18270; At3g04060	ANAC087; ANAC046	Arabidopsis	[15]
	Achn384741	SyGI	Kiwifruit	[20]
Microsporogenesis	*LOC_Os02g57520*	OsPTC2	Rice	[26]
	LOC_ Os08g39250	OsEDM2L	Rice	[28]
	LOC_Os04g39470	OsMYB103	Rice	[29]
	LOC_Os08g08840	OsGPT1	Rice	[30]
	LOC_Os08g02330	DCET1	Rice	[31]
	At1g19530	RGAT1	Arabidopsis	[32]
	LOC_Os06g15990	OsALDH2b	Rice	[33]
	Os11g0696200	EDT1	Rice	[34]
	At3g19190; At5g17290; At5g45900; At3g07525	ATG2; ATG5; ATG7; ATG10	Arabidopsis	[37]
Megasporogenesis	*At2g23430; At3g50630; At3g24810; At3g19150; At1g49620; At5g48820; At2g32710; At3g12280*	ICK1; ICK2; ICK3; ICK4/KRP6; ICK5/KRP7; ICK6; ICK7/KRP4; RBR1	Arabidopsis	[40]; [41]
	At5g03552	MIR822	Arabidopsis	[bioRxiv]*
Nucellus development	*At5g60440; At5g23260; At1g31140; At4G24960;*	AGL62; TT16; GOA; HVA22d	Arabidopsis	[42]; [44]
	At1g73590; At1g70940; At2g01420; At1g23080	PIN1; PIN3; PIN4; PIN7	Arabidopsis	[43]
	At3g28007; At4g39210	SWEET4; APL3	Arabidopsis	[44]
	HORVU2Hr1G091880.1; HORVU2Hr1G092090; HORVU2Hr1G092080.15	VPE2a; VPE2b; VPE2d	Barley	[47]
	LOC103932244	DGK4	Pear	[51]

Self-incompatible pollen-pistil interaction	*At3g27325*	AtPGAP1	Arabidopsis	[59]; [60]
Floral organ senescence	*Zm00001eb077580*	KIL1	Maize	[63]
	At5g03730	CTR1	Chinese cabbage	[64]
Double fertilization	*At4g08869; At4g08875*	AtLURE1.7; AtLURE1.8	Arabidopsis	[68]
	At2g17430	NTA/AtMLO7	Arabidopsis	[75]
	At5g60140; At5g18000	VALKYRIE; VERDANDI	Arabidopsis	[78]
	At1g60070; At1g23900;	AP1G1; AP1G2;	Arabidopsis	[79]
	At3g20770; At2g27050	EIN3; EIL1	Arabidopsis	[82]
	At4g39110; At2g21480; At1g28270; At2g33775	BUPS1; BUPS2; RALF4; RALF19	Arabidopsis	[83]
	At1g60625; At1g60815; At2g32835; At2g32785; At2g32788	RALF6; RALF7; RALF16; RALF36; RALF37	Arabidopsis	[84]
	At4g01370; At1g12280	MPK4; SUMM2	Arabidopsis	[85]

* Tovar-Aguilar et al., 2021 [bioRxiv 2021, doi:10.1101/2021.10.18.464879]