PERSISTENT TAPETAL CELL1 Encodes a PHD-Finger Protein That Is Required for Tapetal Cell Death and Pollen Development in Rice\textsuperscript{1[C]}\textsuperscript{[W]}\textsuperscript{[OA]}

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In higher plants, timely degradation of tapetal cells, the innermost sporophytic cells of the anther wall layer, is a prerequisite for the development of viable pollen grains. However, relatively little is known about the mechanism underlying programmed tapetal cell development and degradation. Here, we report a key regulator in monocot rice (Oryza sativa), PERSISTANT TAPETAL CELL1 (PTC1), which controls programmed tapetal development and functional pollen formation. The evolutionary significance of PTC1 was revealed by partial genetic complementation of the homologous mutation MALE STERILITY1 (MS1) in the dicot Arabidopsis (Arabidopsis thaliana). PTC1 encodes a PHD-finger (for plant homeodomain) protein, which is expressed specifically in tapetal cells and microspores during anther development in stages 8 and 9, when the wild-type tapetal cells initiate a typical apoptosis-like cell death. Even though ptc1 mutants show phenotypic similarity to ms1 in a lack of tapetal DNA fragmentation, delayed tapetal degeneration, as well as abnormal pollen wall formation and aborted microspore development, the ptc1 mutant displays a previously unreported phenotype of uncontrolled tapetal proliferation and subsequent commencement of necrosis-like tapetal death. Microarray analysis indicated that 2,417 tapetum- and microspore-expressed genes, which are principally associated with tapetal development, degeneration, and pollen wall formation, had changed expression in ptc1 anthers. Moreover, the regulatory role of PTC1 in anther development was revealed by comparison with MS1 and other rice anther developmental regulators. These findings suggest a diversified and conserved switch of PTC1/MS1 in regulating programmed male reproductive development in both dicots and monocots, which provides new insights in plant anther development.

Programmed cell death (PCD) has been defined as a sequence of genetically regulated events that lead to the elimination of specific cells, tissues, or whole organs (Lockshin and Zakeri, 2004). In multicellular organisms, PCD is essential for defense responses, to limit the spread of pathogens, and for development processes (Lam, 2004). Studies in animals have grouped PCD into three categories: apoptosis, necrosis, and autophagic cell death (Lockshin and Zakeri, 2004; Bras et al., 2005). Apoptosis is known to be promoted by the activation of a family of Cys proteases (caspases; Wolf and Green, 1999), leading to cell shrinkage, nuclear condensation and fragmentation, and “apoptotic body” formation (Adrain and Martin, 2001). Necrosis is associated with uncontrolled cell death, frequently due to overwhelming stress, when the cell is unable to enter the normal apoptotic pathway, and is characterized by morphological swelling rather than cell shrinkage (Bras et al., 2005). Autophagic cell death is characterized by the formation of autophagic vacuoles and dilation of the mitochondria and endoplasmic reticulum as well as enlargement of the Golgi (Bras et al., 2005).

PCD plays a fundamental role in developmental processes in plants, including leaf senescence, the removal of aleurone cells, root cap cells, and xyleogenesis (Pennell and Lamb, 1997; Kuriyama and Fukuda, 2002; Rogers, 2005). PCD is also of special importance for plant reproduction, including embryonic de-
opment, tapetal degradation, anther dehiscence, pollen germination and pollen tube growth, pistil development, and pollen-pistil interactions (Wu and Cheung, 2000). Similar to the hallmarks of animal apoptosis, DNA fragmentation and mitochondrial changes are found in plant PCD (Danon et al., 2000), although it is rare to observe apoptotic bodies in plants (McCabe and Pennell, 1996). There are also no close sequence homologs of classical caspases in the plant genome, although caspase-like activities have been linked to plant PCD (Woltering, 2004; Reape et al., 2008). Currently, the signaling pathway and molecular mechanisms underlying plant PCD are largely unknown.

Male reproductive development in higher plants is a complex biological process involving the correlated differentiation of anther tissues and the generation of haploid microspores/pollen (Liu and Qu, 2008). The developed anther consists of the meiotic cells (also called microsporocytes) at the center, surrounded by the anther wall with four somatic layers: the epidermis, the endothecium, the middle layer, and the tapetum, from the surface to the interior (Goldberg et al., 1993). The tapetum, the innermost cell layer of the anther wall, plays a crucial role in regulating programmed anther development, microspore/pollen formation, and pollen wall formation (Li et al., 2006; Parish and Li, 2010). Tapetal cell development and differentiation are critical for the early events in male reproduction, including meiosis; however, during late pollen development, tapetal degeneration, triggered by an apoptosis-like process, is also vital for viable pollen formation (Papini et al., 1999; Varnier et al., 2005; Li et al., 2006; Aya et al., 2009). Currently, although several genes encoding putative transcription factors have been reported to be associated with tapetal function and degeneration, such as Arabidopsis (Arabidopsis thaliana) MYB33/MYB65 (Millar and Gubler, 2005), DYSFUNCTIONAL TAPETUM1 (DYT1; Zhang et al., 2006), ABORTED MICROSPORE (AMS; Sorensen et al., 2003; Xu et al., 2010), and MALE STERILITY1 (MS1; Wilson et al., 2001; Ito and Shinozaki, 2002) and rice (Oryza sativa) GAMYB (Kaneko et al., 2004; Aya et al., 2009; Liu et al., 2010), UNDEVELOPED TAPETUM1 (UDT1; Jung et al., 2005), TAPETUM DEGENERATION RETARDATION (TDR; Li et al., 2006), and MADS3 (Hu et al., 2011), their detailed functional roles in regulating tapetal PCD during anther development are unclear. We have shown that the Arabidopsis ms1 mutant displays altered tapetal development, with a lack of normal PCD and abnormal tapetal degeneration associated with large autophagic vacuoles and mitochondrial swelling (Vizcay-Barrena and Wilson, 2006). Previously, we also reported that TDR, the ortholog of the Arabidopsis AMS gene, plays a key role in tapetal PCD in rice; trd shows delayed tapetal degeneration and nuclear DNA fragmentation as well as aborption of microspores after release from the tetrad. Moreover, TDR is able to directly bind the promoter of a Cys protease gene, OsCPI1, in vivo and in vitro (Li et al., 2006). Recently, GAMYB has been shown to be regulated by GA, and the gamyb tapetal cells appear swollen and have defects in PCD (Aya et al., 2009; Liu et al., 2010).

Tapetal PCD is a physiological process in which timing is critical for pollen maturation and male fertility. To address the mechanism underlying tapetal PCD in the model crop rice, we have identified a key regulator, PERSISTENT TAPETAL CELL1 (PTC1), which controls programmed tapetal development and degradation during rice anther development. Loss of function of PTC1 results in uncontrolled tapetal cell proliferation and swelling, delayed DNA fragmentation, abnormal Ubisch bodies/orbicules, and pollen wall development, causing complete male sterility. PTC1 encodes a putative PHD-finger (for plant homeodomain) protein that is transiently expressed in tapetal cells and microspores. Gene expression analysis revealed that PTC1 affects expression associated with tapetal function and pollen exine formation. Furthermore, the conserved and crucial roles of PTC1 and its Arabidopsis ortholog MS1 were compared by genetic complementation and regulatory analysis. This work provides new insight into a molecular switch of programmed male reproductive development in both dicots and monocots.

RESULTS
Isolation of the ptc1 Mutant

From our rice mutant library (Chu et al., 2005; Chen et al., 2006; Wang et al., 2006), the male-sterile mutant ptc1 was identified. ptc1 displayed complete male sterility and normal female development when tested by reciprocal cross analysis. All of the F1 progeny were fertile, with an approximate 3:1 ratio for phenotypic segregation in F2 plants (fertility:sterility = 189:55; χ² = 0.65), indicating that this mutation is due to a single recessive locus. Compared with wild-type plants, ptc1 exhibited normal vegetative development (Fig. 1A). During the reproductive stage, ptc1 plants developed normal panicles and floral organs (Fig. 1, B–D), but ptc1 anthers were smaller, white, and lacked viable pollen grains (Fig. 1, D and E).

Defects of Anther Wall and Pollen Development in ptc1

Pollen development in the monocot rice can be characterized into similar stages to those observed in the dicot Arabidopsis (Zhang and Wilson, 2009). To further characterize the defects in ptc1, anther morphology was observed during the various developmental stages. Histological analysis indicated that early events in anther and pollen development progressed normally until after meiosis and microspore release. The epidermis, endothecium, middle layer, tapetum, and pollen mother cells formed normally in ptc1 anthers at stage 6 before meiosis (Fig. 2, A and E). During meiosis (stage 8a), both the wild-type and ptc1
pollen mother cells became associated with the tapetal layer and the tapetum appeared vacuolated (Fig. 2, B and F); subsequently (during stage 8b), tetrads with four haploid microspores formed and the middle cell layer became very thin and degenerated in both wild-type and ptc1 cells (Fig. 2, C and G).

From stage 9 (free microspore released from the tetrad) to early stage 10 (vacuolated microspore), the wild-type tapetum became condensed, less vacuolated, and stained strongly (Fig. 2, D and I). In contrast, ptc1 tapetal cells were more vacuolated and less stained (Fig. 2, H and M). From late stage 10 to stage 11 (mitosis I), the wild-type tapetum appeared to undergo degeneration, and the cytoplasmic constituents remained densely stained and clearly discernible (Fig. 2, J and K); however, the ptc1 tapetum became abnormally enlarged, and cytosolic constituents seemed to be secreted into the anther locule (Fig. 2, N and O). Meanwhile, the wild-type microspores maintained a defined shape (Fig. 2, J and K), while the ptc1 microspores degenerated (Fig. 2, N–P; Supplemental Fig. S1). At stage 13, when the mature pollen grains were formed, the wild-type tapetal layer and middle layer were completely degraded, and round, densely stained pollen grains were observed (Fig. 2L). However, at this stage in the ptc1 anther, only cell debris of both tapetal cells and pollen grains remained (Fig. 2P).

Transmission electron microscopy was used to gain greater insight into the ptc1 tapetal and microspore abnormalities. From stage 9 onward, the wild-type tapetal cytoplasm became condensed, with swollen and less defined organelles, which showed a loss of membrane integrity and a diffuse cellular organization, indicative of apoptotic degeneration (Fig. 3, A and B). Cytoplasmic condensation was reduced in the ptc1 tapetum, with membrane and organellar integrity maintained (Fig. 3C). The ptc1 tapetum had a high density of organelles, particularly mitochondria and endoplasmic reticulum, which appeared morphologically intact, giving the appearance of a highly active tissue without any signs of tapetal breakdown and apoptosis (Fig. 3, D, G, and H). Strikingly, extensive tapetal proliferation was also observed in ptc1, resulting in tapetal extrusions into the anther locule (Fig. 3, E–H). These extruded regions appeared to have a continuous outer membrane with the surrounding tapetal cells and contained an extremely high density of organelles, lipid bodies, and electron-dense materials (Fig. 3, G and H), implying that unregulated proliferation of the tapetum cellular contents was occurring. Ubisch bodies/orbicules containing electron-opaque materials were evident on the inner tapetal surface, facing the anther locule in both the wild type and ptc1 (Figs. 3, B and C, and 4, A and E). Orbicules (i.e. Ubisch bodies) are sporopollenin particles seen in secretory tapeta, which are thought to be involved in transporting materials from the tapetum to microspores (Huysmans et al., 1998). Orbicule development was seen in the mutant but was decreased in size compared with the wild type, and by late stage 10 it showed a reduction of both the electron-opaque central region and the surrounding electron-dense sporopollenin (Fig. 4, A and E). Interestingly, orbicules were present on the membrane flanking the tapetal extrusions but were not seen on the extruded regions, suggesting that these extrusions may have been pushed out as a consequence of proliferation from specific regions of the tapetal cells (Fig. 3, F and G, arrow). The two layers of the pollen exine, nexine and sexine (Li and Zhang, 2010), were evident in both the wild type and ptc1, although the mutant exine was much reduced and the microspore plasma membrane appeared undulating (Fig. 4, B and F). Secreted pollen wall material was evident in the wild-type locule, and the orbicules were surrounded by densely stained materials, presumed to be sporopollenin precursors (Fig. 4C). The ptc1 tapetum appeared to have less accumulation of pollen wall precursor materials (Fig. 4, A and E).

Consistent with this, the wild-type microspore developed a well-organized pollen wall, with extensive banding of electron-transparent material (Fig. 4, C and D), whereas ptc1 developed a thin and abnormal pollen exine, with fewer electron-transparent channels across the pollen wall (Fig. 4, G and H). The ptc1
The mutant appeared to have a reduced bacular structure, with no spine structures as well as an abnormal nexine, which was uneven on the internal microspore surface (Fig. 4, G and H). This was supported by scanning electron microscopy (SEM), which indicated that the surface of wild-type pollen grains exhibited a regular pattern (Fig. 4, I and K) with spine distribution on the exine (Fig. 4, C and D), whereas *ptc1* pollen showed a lack of the elaborate patterning, resulting in a smooth surface (Fig. 4L). Also, *ptc1* pollen was about half the size of wild-type pollen at stage 11 (Fig. 4, I and J). These observations indicate that *ptc1* is unable to undergo normal tapetal PCD and pollen wall formation; instead, it undergoes tapetal proliferation and extrusion into the locule, followed by a subsequent rapid necrosis-like cell death during late anther development, leading to male sterility. Therefore, we named this mutant *ptc1*.

**ptc1 Tapetal Cells Have Less DNA Fragmentation**

Internucleosomal DNA cleavage, visualized as ladders, is considered a hallmark of apoptosis in animals (Danon et al., 2000), whereas necrotic cell death is associated with random DNA degradation resulting in a DNA smear (Schumer et al., 1992). Attempts to observe laddering in DNA isolated from stages 9 and 10 anthers of wild-type and *ptc1* plants failed due to technical difficulties, presumably since tapetal DNA was a minor component of the isolation. Therefore, DNA fragmentation was analyzed in situ using the Terminal Transferase dUTP Nick End Labeling (TUNEL) assay. Tapetal DNA fragmentation was not detected at stage 7 in both the wild type and *ptc1* (Fig. 5, A and C). However, at early stage 9, when the microspore was released from the tetrad, DNA fragmentation signal could be seen in the wild type (Fig. 5B), which is consistent with our previous data (Li et al., 2006), indicating the initiation of tapetal PCD. No TUNEL signal was detected in the *ptc1* mutant (Fig. 5, C and D), implying that regulated DNA fragmentation does not occur in the *ptc1* tapetal cells.

**Map-Based Cloning of PTC1**

To identify PTC1, a map-based cloning approach was adopted. *PTC1* was mapped between two markers, Lh903-5 and Lh903-8, on chromosome 9 (Fig. 6A; Sup-
plemental Table S1); this 63.6-kb region on bacterial artificial chromosome clone AP005308 contained seven putative genes (http://www.ncbi.nlm.nih.gov/). Six of these genes encoded hypothetical proteins, but one gene, Os09g0449000 from the National Center for Biotechnology Information (or Os09g027620 from The Institute for Genomic Research [TIGR]), encoded a PHD motif transcription factor. Sequencing this gene in ptc1 revealed a single nucleotide T insertion in the putative second exon, causing a frame-shift mutation and altered reading frame after the 162nd amino acid, resulting in a lack of the PHD motif (amino acids 620–670; Fig. 6, B and C). To further verify that the male-sterile phenotype resulted from this mutation, calli induced from flowers of homozygous ptc1 plants were transformed with Agrobacterium tumefaciens EHA105 containing a 5.255-kb genomic DNA fragment consisting of 3,043 bp upstream and the entire 2,212-bp genomic region of Os09g0449000. This fragment was able to rescue the male-sterile defect of ptc1 in 35 plants (Fig. 6, D and E), demonstrating that the mutation in Os09g0449000 is responsible for the sterility in ptc1.

By comparing the cDNA sequence revealed by our 5’ end and 3’ end RACE-PCRs and the genomic sequence, the structure of PTC1 was shown to comprise a 2,043 bp coding region with three exons and two introns (Fig. 6B). We also identified a 46-bp untranslated upstream region (UTR) and a 146-bp 3’ UTR (Supplemental Fig. S2); a nuclear localization signal (RRRKR) and a conserved PHD domain were predicted at the N and C termini, respectively (Fig. 6C).

PTC1 Is Preferentially Expressed in the Tapetum and Microspores from Stage 8 to Stage 9 during Anther Development

The main morphological defect of ptc1 is male sterility, with no obvious vegetative phenotype suggesting anther-associated expression. According to the RiceGE (Gene Expression Atlas Data Sources), PTC1 expression is highest in the 10- to 15-cm inflorescence (stages 7–10), gradually decreasing in the 15- to 20-cm inflorescence (stages 10–12; http://signal.salk.edu/cgi-bin/RiceGE). Recent expression profile data from laser microdissection coupled with microarray analysis also suggest that PTC1 is expressed in both the tapetum and microspores at stage 8 (Hobo et al., 2008). Consistent with this, our reverse transcription (RT)-PCR and quantitative RT-PCR (RT-qPCR) results indicated that PTC1 was preferentially expressed in the anther from stage 8 to stage 9 (Fig. 7, A and B). Strikingly, ptc1 displayed an increased level of the mutant PTC1 transcript (Fig. 7, A and B). This suggests either auto down-regulation of the PTC1 transcript or that the mutant transcript may be more stable and accumulates more than the wild type.

In transgenic plants expressing the PTC1pro::GUS fusion (driven by the 3,043-bp PTC1 upstream region), GUS staining was specifically detected in the anther...
from stages 8 to 9 and at a low level in stage 10 (Fig. 7, C and D). No GUS expression was seen in the palea, lemma, pistil (Fig. 7, C and D) or other organs. Maximal GUS staining, albeit at a very low level, was seen in the tapetal tissues, but GUS was also apparent within the microspores (Fig. 7E), indicating that PTC1 plays a specific function in tapetal cells during anther development.

**Transcriptome Analysis of Wild-Type and ptc1 Anthers**

Transcript profiling of *ptc1* was performed on anthers at two developmental stages: (1) stage 8, during meiosis and tetrad formation, and (2) stage 9, during young microspore development, using Agilent 4*44K* rice whole genome DNA microarrays. An empirical Bayes method was used to analyze the data (Smyth, 2004), a false discovery rate (FDR) cutoff of 0.5% was used for the initial filtering of candidate genes, and a secondary selection of greater than log2 fold difference was then applied. Putative functions of genes were obtained from TIGR (http://tigrblast.tigr.org/euk-blast). Furthermore, the microarray expression data were verified by RT-qPCR using 10 differentially expressed genes; the correlation (94%) of fold change between the RT-qPCR data was closely comparable to that obtained from microarray analyses (Supplemental Fig. S3).

Microarray analysis revealed 2,943 genes with a 2-fold or greater expression change over the two developmental stages in *ptc1* anthers compared with the wild type (570 down-regulated and 278 up-regulated at stage 8; 1,252 down-regulated and 843 up-regulated at stage 9; Supplemental Data Set S1). Since PTC1 expression is specific to the tapetum and microspores, the *ptc1* data were compared with the laser microdissection-

**Figure 4.** Transmission electron microscopy and SEM of the wild type and *ptc1*. A to H, Orbicule and pollen exine structure of wild-type (A–D) and *ptc1* (E–H) plants from stage 9 to stage 11. A and E show orbicule development at stage 10 in the wild type (A) and the *ptc1* mutant (E). I and J, The outer surface structure of wild-type (I) and *ptc1* (J) pollen at stage 10 by SEM analysis. K and L, Magnification of wild-type (K) and *ptc1* (L) pollen exine. Ba, Bacular; Ne, nexine; Or, orbicule; T, tapetal layer; Te, tectum. Bars = 1 μm (G, K, and L), 10 μm (I and J), and 500 nm (A–D, E, F, and H).

**Figure 5.** DNA fragmentation detection in wild-type and *ptc1* anthers. TUNEL signals of stage 7 and early stage 9 are shown in wild-type (A and B) and *ptc1* (C and D) anthers. There is no detectable DNA fragmentation signal at stage 7 in the wild type (A) and *ptc1* (C). Wild-type tapetal cells exhibit obvious DNA fragmentation signals at early stage 9 (B). No DNA fragmentation signal is seen in *ptc1* anthers (D) at early stage 9. Bars = 50 μm.
coupled microarray data of wild-type rice microspores/pollen and the tapetum (Hobo et al., 2008). We identified 1,569 down-regulated genes and 848 up-regulated genes (2,417 in total) with a change in expression of 2-fold or more in the tapetum and/or microspores at stages 8 and 9 between the wild type and ptc1 (Supplemental Data Set S1). Some genes previously identified as critical for postmeiotic anther development were down-regulated in the ptc1 microarray data (Table I), including a Cys protease (OsCP1; Lee et al., 2004), a fatty acyl-CoA reductase homologous to Arabidopsis MS2 (Aarts et al., 1997), lipid transfer proteins (Osc4, OsC6, YY1, and Os09g0525500; Tsuchiya et al., 1992; Hihara et al., 1996), a stilbene synthase (YY2; Hihara et al., 1996), BURP domain-containing proteins (RA8 and OsRAFTIN; Jeon et al., 1999), a P450 family member (CYP704B2; Li et al., 2010), a ribosome-inactivating protein (RA39; Jeon et al., 1999), and the putative homolog of AtMYB103 (Higginson et al., 2003; Zhang et al., 2007). Consistent with the RT-qPCR analysis, PTC1 showed up-regulation in the ptc1 mutant (2.97-fold at stage 8, 5.49-fold at stage 9), whereas genes involved in early tapetal and pollen regulation, such as CYP703A3 (Aya et al., 2009), TDR (Li et al., 2006), UDT1 (Jung et al., 2005), and GAMYB (Kaneko et al., 2004; Liu et al., 2010; Table I), showed no significant change.
To understand the regulatory role of PTC1, we grouped the 2,417 genes into four COG (for Cluster of Orthologous Groups of proteins) categories: I, information storage and processing (144 genes); II, cellular processes and signaling (363 genes); III, metabolism (551 genes); and IV, poorly characterized genes (1,359 genes; Fig. 8; Supplemental Table S2).

Postmeiotic tapetal PCD is thought to play an important role in pollen development through providing materials for pollen wall formation (Li et al., 2006). Endopeptidases have been implicated as executors of PCD and are grouped into Ser, Cys, aspartic, metallo- peptidases, and Thr endopeptidases (Rawlings and Barrett, 1999). Two genes encoding putative aspartic peptidases, and Thr endopeptidases (Rawlings and Barrett, 1999) were down-regulated in ptc1 (Table I). However, 15 mid- to late-stage transcription factors, including homologs of ATMYB99 and other MYB family members, bHLH domain protein, ACTIVATOR PROTEIN2 domain-containing protein, and zinc-finger proteins, showed changed regulation in ptc1 (Supplemental Table S3).

Functional Conservation of PTC1 in Plants

To gain additional insights into the phylogenetic relationship between PTC1 and its close homologs, BLASTP searches were conducted with the full-length amino acid sequence of PTC1. An unrooted tree of PTC1 and its 25 homologs from moss to flowering plants was constructed (Fig. 9; Supplemental Data Set S2). PTC1 was located in the same clade with Arabidopsis MS1 (Wilson et al., 2001; Ito and Shinozaki, 2002; Arizumi et al., 2005). In this clade, PTC1 homologs were encoded by a single subfamily member in rice, Sorghum bicolor, Arabidopsis, grape (Vitis vinifera), poplar (Populus trichocarpa), Ricinus communis, and Physcomitrella patens (Fig. 8). No putative PTC1 close homolog was identified in green algae. In addition, another homolog of PTC1, Arabidopsis MALE MEIOCYTE DEATH1/DUET, previously reported as involved in male gametophyte meiosis (Yang et al., 2003), was located in a separate clade. Subsequently, we used the full-length PTC1 protein to search for closest relatives in EST databases. Among thoseESTs with E values higher than 2.00E-12, we found that most of the PTC1 homologous ESTs were from reproductive organs in monocot species (Supplemental Table S4). Together, these observations imply an evolutionarily conserved PTC1-related pathway in reproductive development ranging from moss to higher plants.

The phylogenetic analysis indicates that PTC1 and Arabidopsis MS1 are orthologs with 43.76% overall identity (Fig. 6C), suggesting that they may have similar functions. To confirm the evolutionary relatedness of these two genes, we conducted functional complementation of the ms1 mutant with PTC1. Arabidopsis ms1 shows aberrant tapetal PCD after microspore release, abnormal sporopollenin deposition leading to pollen breakdown (Vizcay-Barrena and Wilson, 2006). A binary plasmid carrying the Arabidopsis MS1 promoter (2.9 kb) and PTC1 coding region fused with the synthetic GFP was introduced into

seen in ptc1 for cell wall biogenesis-related genes, encoding pectinesterases and polygalacturonases (Supplemental Table S3).

In agreement with the observed pollen exine defect, 175 genes putatively related to lipid transport and metabolism and secondary metabolite biosynthesis, transport, and catabolism had expression changes in ptc1 (Supplemental Table S3).

Transcription factors such as MYB and basic helix-loop-helix (bHLH) families have been shown to play key roles in modulating anther development; many of these involved in the early stages in pollen development were unchanged in ptc1 (Table I). However, 15 mid- to late-stage transcription factors, including homologs of ATMYB99 and other MYB family members, bHLH domain protein, ACTIVATOR PROTEIN2 domain-containing protein, and zinc-finger proteins, showed changed regulation in ptc1 (Supplemental Table S3).
**Table 1. Expression in the ptc1 mutant of genes previously reported as altered in expression by TDR, GAMYB, and UDT1 and involved in anther development**

The number 1 indicates no changed expression, and “Down” indicates reduced expression in the mutant. Other numbers indicate the fold change in ptc1.

| Gene Locus | Gene Name in Rice | Arabidopsis Ortholog | Gene Annotation | Fold Change (Log[Mutant/Wild Type]) |
|------------|-------------------|----------------------|----------------|------------------------------------|
| Os03g0167600 | OsMS2 | MS2 | Fatty acyl-CoA reductase | -3.0 -5.4 Down Down Down |
| Os04g0470600 | OsMYB103 | AtMYB103 | MYB family transcription factor | 1 2.7 1 1 Down |
| Os07g0556800 | RA39 | | Ribosome-inactivating protein | -5.7 -4.2 1 Down 1 |
| Os08g0131100 | CYP703A3 | CYP703A2 | Cytochrome P450 | 1 1 1 Down 1 |
| Os08g0496800 | OsRAFTIN | | BURP domain-containing protein | 1 -2.2 Down Down 1 |
| Os08g0546300 | OsC4 | | LTPL44 lipid transfer protein precursor | -2.5 -11.5 Down Down Down |
| Os09g0449000 | PTC1 | MS1 | PHD-finger domain-containing protein | 1.6 2.5 1 Down 1 |
| Os09g0525500 | YY1 | | LTPL45 lipid transfer protein precursor | 1 -5.5 Down 1 1 |
| Os10g0484800 | YY2 | | Stilbene synthase | 1 -1.3 Down Down 1 |
| Os07g0549600 | UDT1 | DYT1 | bHLH domain transcription factor | 1 1 1 1 |
| Os02g0120500 | TDR | AMS | bHLH domain transcription factor | 1 1 Down 1 1 |
| Os04g0670500 | OsCP1 | | Cys protease | -1.4 -4.9 Down 1 Down |
| Os09g0480900 | RA8 | | BURP domain-containing protein | 1 -1.4 Down 1 1 |
| Os11g0582500 | OsC6 | | LTPL68 -LTP family protein precursor | 1 -2.46 Down Down 1 |
| Os03g0168600 | CYP704B2 | CYP704B2 | Cytochrome P450 | 1 -1.2 Down Down 1 |
| Os01g0812000 | GAMYB | | MYB33/65 | 1 1 1 Down 1 |

**Figure 8.** Functional classification by COG analysis of genes that were up-regulated and down-regulated at least 2-fold in ptc1.

MS1/ms1 heterozygous plants. Three lines were identified that carried the PTC1 gene in the homozygous ms1/ms1 background (Supplemental Fig. S4); all showed rescue of fertility, although silique length and seed set were slightly reduced compared with the wild type (Fig. 10, A–C). SEM analysis also confirmed that the characteristic ridged pollen exine pattern was restored by PTC1 (Fig. 10D) as compared with the immature ms1 pollen, which appeared very small with irregular exine patterning (Fig. 10E). These results indicate conservation of PTC1/MS1 function in regulating anther and pollen development of dicots and monocots, possibly from moss to higher plants.

To further explore the conserved regulatory role, comparison of microarray data between ptc1 and ms1 was conducted (Yang et al., 2007). BLASTP analysis using the 260 genes changing in ms1 identified 130 genes and their close homologs changing in ptc1, representing 50% of the genes changing in ms1; 30% (79 of 260) of these genes had similar changed expression in the two mutants (Supplemental Data Set S3). Nine of these down-regulated genes were related to lipid and secondary metabolism for pollen exine development, including APG proteins (anther-specific Pro-rich-like protein), lipid transfer protein, β-ketoacyl-CoA synthase, calcosin-related proteins, pollen-specific protein, and trans-
caffeoyl-CoA 3-O-methyltransferase. In addition, two genes encoding chalcone synthases and ATP-binding cassette transporter were up-regulated (Supplemental Data Set S3) and eight genes putatively required for pectin modification and degradation were down-regulated (Supplemental Data Set S3).

Comparison of the PTC1 Regulatory Role with Those of GAMYB, UDT1, and TDR in Anther Development

To aid characterization of the regulatory role of PTC1, the expression profiles of the gamyb-2, udt1, and tdr mutants were compared (Jung et al., 2005; Zhang et al., 2008; Aya et al., 2009). We observed that 40.3% (93 of 231) of the genes changing in tdr, 10.5% (128 of 1,225) in udt1, and 24.2% (210 of 870) in gamyb-2 had expression changes in ptc1 (Supplemental Data Set S3).

However, transcription factors involved in regulating early tapetal differentiation or sporopollenin synthesis, including GAMYB (Kaneko et al., 2004; Aya et al., 2009; Liu et al., 2010), UDT1 (Jung et al., 2005), TDR (Li et al., 2006), and CYP703A3 (Aya et al., 2009), showed no obvious change (Table I), suggesting that they act upstream of PTC1 (Fig. 11). Conversely, PTC1 was shown to have reduced expression in gamyb-2 mutants (Aya et al., 2009) but no obvious expression change in udt1 and tdr (Jung et al., 2005; Zhang et al., 2008).

Twelve genes showed expression changes (11 down-regulated and one up-regulated) in all four mutants (gamyb-2, udt1, tdr, and ptc1; Supplemental Data Set S3); seven are putatively related to lipid metabolism and transport, including 3-oxoacyl reductase, β-ketoacyl-CoA synthase, fatty acyl-CoA reductase (OsMS2), GDSL-like lipase, and LTP family proteins (LTLPL44, OsC6, LTLPL2), indicating the importance of lipid metabolism for tapetal and pollen development. Three genes are putatively involved in facilitating the transport of water and small neutral molecules (aquaporin protein) and cell wall/membrane/envelope biogenesis (pectinesterase and subtilisin homolog OsSub12), respectively. One gene putatively encodes a plant thionin family protein precursor, THION18, and another one has unknown function (Os08g0216900). This suggests that these genes are essential for tapetum and

Figure 9. Phylogenetic analysis of PTC1 and its related proteins. An unrooted maximum likelihood tree was created by MEGA 3.1 using the PTC1-related sequences from rice (Os), Physcomitrella patens (Pp), Zea mays (Zm), Arabidopsis (At), Vitis vinifera (Vv), Populus trichocarpa (Pt), Ricinus communis (Rc), and Sorghum bicolor (Sh). The EEH58473 protein of Micromonas pusilla and CAL54119 of Ostreococcus tauri were used as an outgroup. Bootstrap values are shown as percentages. Black dots indicate PTC1 subfamily members. [See online article for color version of this figure.]

Figure 10. Analysis of the conserved role of PTC1 and MS1. A, Wild-type Arabidopsis siliques. B, Transgenic lines of MS1pro:PTC1 cDNA in the Arabidopsis MS1−/− mutant, displaying rescue of ms1 fertility, although the siliques were slightly shorter than in the wild type. C, Arabidopsis MS1−/−. Bars = 5 mm for A to C. D, The observation of pollen surfaces of transgenic lines containing PTC1+/ms1− by SEM. Bar = 10 μm. E, The pollen of Arabidopsis ms1 mutant (MS1−/−). Bar = 500 nm.
possibly by GAMYB suggesting that regulation may involve multiple membrane proteins and in vitro (Li et al., 2006; Aya et al., 2009; Fig. 11), is regulated by both TDR and GAMYB in vivo.

DISCUSSION

Indirectly regulated by genes acting upstream of pollen exine development and are probably directly or indirectly regulated by genes acting upstream of PTC1, possibly by GAMYB, UDT1, and TDR. Interestingly, OsC6 is regulated by both TDR and GAMYB in vivo and in vitro (Li et al., 2006; Aya et al., 2009; Fig. 11), suggesting that regulation may involve multiple members of the transcriptional regulatory network.

PTC1 Determines a Conserved and Diversified Switch of Apoptosis and Necrosis in Tapetal Cells

Timely initiation of tapetal PCD is essential for the regulated release of wall materials, including carbohydrates, lipidic molecules, and other nutrients, from the tapetum to the developing microspore. At stage 8, the wild-type rice anther initiates an apoptosis-like cell death in the tapetum, displaying typical apoptotic hallmarks including cell shrinkage and fragmentation of chromatin DNA (Papini et al., 1999; Li et al., 2006). However, chromatin condensation, as indicated by TUNEL analysis, does not occur in the ptc1 tapetum (Fig. 5, C and D). Instead, the mutant tapetum appears highly metabolically active, with increased numbers of mitochondria and endoplasmic reticulum and the apparent proliferation of cellular constituents (Fig. 3, F–H). Normal tangential tapetal cell wall breakdown does not appear to occur in ptc1; therefore, instead of materials being secreted out into the locule, they are retained within the mutant tapetum. Tapetal PCD fails to occur in the pcl1 tapetum, which appears to go through overproliferation, resulting in the extrusion of cytoplasmic contents, which are constrained by the persistent plasma membrane, into the locule (Fig. 3). This phenotype is distinct from other tapetal mutants that have been described, such as Arabidopsis amts1 (Xu et al., 2010) and rice tdr (Li et al., 2006) mutants, which exhibit tapetal defects involving swelling of the tapetum as a consequence of vacuole increase rather than cytoplasmic proliferation. Subsequent to this proliferation, very rapid degeneration of the tapetum and microspores occurs in ptc1 via necrosis-like breakdown. Therefore, in ptc1, there seems to have been a departure from the standard PCD pathway to one of uncontrolled proliferation, altered organelle organization, and subsequent necrotic death. In ms1, the Arabidopsis ortholog, although a lack of DNA fragmentation and tapetal PCD is also seen, and the cytoplasm of the tapetum cells and microspores becomes granular and abnormally vacuolated (Vizzay-Barrena and Wilson, 2006), the changes in tapetal and organelle proliferation are not observed. This may reflect the differences in tapetal development in monocots and dicots. Rice and Arabidopsis both have secretory tapeta where the cells remain intact until their disintegration (Huysmans et al., 1998; Furness and Rudall, 2001). However, the tapeta of rice and other cereals exhibit characteristic orbicules/Ubisch bodies, which are thought to export tapetum-produced sporopollenin precursors across the hydrophilic cell wall to the locule. Orbicules have not been observed in the Brassicaceae, including Arabidopsis, which contain unique secretory tapeta with specialized organelles such as tapetosomes. Whether the observed tapetal proliferation in ptc1 compared with Atms1 is a consequence of these differences remains to be determined.
In animals, necrosis is thought to be an uncontrolled and passive form of cell death caused by severe nonphysiological and/or physical/chemical factors (Galluzzi et al., 2007). However, recent evidence suggests that necrosis can be genetically regulated in a similar manner to apoptosis (Vande Velde et al., 2000; Zong and Thompson, 2006; Hitomi et al., 2008). To date, the molecular control of necrosis in both plants and animals remains largely unknown. In plants, a group of proteins such as Nep1 (for necrosis and ethylene-inducing peptide)-like proteins found in many plant pathogens have the ability to trigger a necrotic response with the leakage of cell metabolites (Bae et al., 2006). In addition, cell lines in suspension culture under higher temperature conditions displayed necrotic responses (McCabe and Leaver, 2000; Burbridge et al., 2007; Reape et al., 2008). As far as we know, there are no reports of nuclear gene mutations displaying proliferation and subsequent severe necrosis-like cell death in plants. Therefore, our work provides a system to investigate necrosis-like cell death in plants and to investigate the role of membrane breakdown and tapetal proliferation in triggering the regulation of PCD in the anther.

Cys proteases are thought to be involved in the process of plant cell death (Solomon et al., 1999), although currently no caspase homologs have been identified in plants, but emerging evidence indicates that Cys/Ser proteases may function as caspase-like executors (Beers et al., 2000; Woltering, 2004). In agreement with the role of PTC1 in regulating tapetal PCD, cell death-associated genes encoding Cys proteases show altered expression in ptc1. OsCP1, encoding a Cys protease, is regulated by TDR at the transcriptional level and is expressed in the tapetum (Li et al., 2006), and reduction of OsCP1 expression causes reduced viability of pollen grains (Lee et al., 2004). OsCP1 expression is also down-regulated in ptc1 (Table I). Another gene (Os03g0689300) encoding a plasma membrane ATPase was down-regulated and is thought to be responsible for maintaining ATP levels that can regulate a switch between apoptosis and necrosis (Kroemer et al., 1998; Proskuryakov et al., 2003; Supplemental Data Set S3).

The data indicate that PTC1 plays a critical role in the regulation of tapetal PCD, although it is currently unclear how this occurs. PTC1 may act, alongside other transcription factors, for example TDR, by directly regulating executor proteases and other PCD initiators. Alternatively and possibly more likely, PTC1 may act in an indirect way by regulating overall tapetal function and pollen wall biosynthesis. If abnormal, this then causes prolonged activity of the tapetum and the avoidance of PCD induction, which then causes the induction of death by necrosis.

**PTC1 Controls Pollen Wall Formation**

*PTC1* encodes a PHD-finger protein that is specifically expressed in tapetal cells and microspores. The PHD domain comprises about 50 to 80 amino acids, contains a conserved Cys2-His-Cys2 motif, and is found mainly in proteins involved in eukaryotic transcription regulation (Aasland et al., 1995). Consistent with the defective pollen wall in *ptc1*, gene expression profiling analysis indicates that *PTC1* regulates genes related to lipid synthesis, metabolism, and transport for anther development and pollen wall formation.

Lipidic pollen exine is made of sporopollenin, an extremely resilient material derived from the polymerization of fatty acid metabolites and phenolic acid (Piffanelli et al., 1998; Morant et al., 2007). Even though the pollen grains of rice and Arabidopsis have similar pollen exine layers, rice has a distinct pollen wall ontology compared with Arabidopsis. The outer surface of Arabidopsis pollen grains has elegant reticulate cavities with abundant pollen coat (tryphine) deposited inside the pollen exine. Whereas rice pollen have a smooth and particulate exine pattern that has more interlayer space between the nexine (foot layer) and sexine compared with Arabidopsis (Li et al., 2010; Li and Zhang, 2010). This is likely to be a consequence of different pollination strategies; Arabidopsis belongs to insect-pollinated (entomophilous) plants, in contrast to rice, which is wind pollinated (anemophilous; Ariizumi and Toriyama, 2011).

The expression of genes involved in flavonoid and lipid biosynthesis, including fatty acyl-CoA reductase (homologous to MS2), chalcone synthase, and P450 proteins, is down-regulated in *ptc1* (Table I; Supplemental Data Set S1). In Arabidopsis, *MS2* encodes a fatty acyl reductase that is thought to convert fatty acids into fatty alcohols for sporopollenin production and exine formation (Aarts et al., 1997). We recently reported that *CYP704B2* is expressed in the tapetum and microspores and catalyzes the production of ω-hydroxylated fatty acids with 16- and 18-carbon chains, which are essential for the formation of both anther cuticle and pollen exine during rice male reproductives development (Li et al., 2010).

Tapetal cells are able to actively synthesize lipidic precursors that are transported in rice by orbicules onto the microspore surface. *RAFTIN* has been shown to be present in pro-orbicules, to accumulate in orbicules, and then to be targeted to the microspore exine (Wang et al., 2003). In *ptc1*, orbicules were smaller in size with less sporopollenin material surrounding the reduced electron-transparent central region (Fig. 4, A and E) and had reduced expression of *RAFTIN* (Table I). This leads to abnormal assembly of the mutant pollen wall, with a reduced amount of channels within the wall and a smooth surface (Fig. 4, F–H, K, and L). In addition, reduced expression of some LTP family members (i.e. *OsC6*) was observed in the *ptc1* mutant (Table I), implying associated alterations in lipid movement with the mutant tapetum. *OsC6* encodes a small fatty acid-binding protein that is required for postmeiotic anther development in rice; silencing of *OsC6* causes defective development of orbicules and pollen exine (Zhang et al., 2010).
PTC1 Plays a Conserved Role in Plant Anther Development

PTC1 shares homology with a number of proteins containing the PHD-finger motif in animals, yeast, and higher plants (Halbach et al., 2000). Phylogenetic analysis indicates that Arabidopsis MS1, poplar PtMS1, and rice PTC1 form a separate group within the entire family (Ito et al., 2007), suggesting that PTC1 and its close homologs may have a conserved role in plant reproductive development. Consistent with this hypothesis, PTC1 driven by the MS1 promoter is able to rescue pollen wall development and pollen fertility of the homozygous ms1 mutant, suggesting a conserved role regulating programmed anther development in monocot and dicot species (Fig. 10).

Moreover, PTC1 and MS1 show similar tapetal expression patterns from late tetrad to microspore release (Yang et al., 2007). Also, neither ms1 (Ito et al., 2007) nor ptc1 could be rescued using the equivalent wild-type gene driven by the cauliflower mosaic virus 35S promoter, suggesting that the precise control of MS1/PTC1 expression is critical for pollen development. MS1 expression in the ms1 mutant shows increased expression (Wilson et al., 2001; Yang et al., 2007), suggesting that MS1 is able to negatively regulate its own expression. Similarly, we also observed an elevated and prolonged expression level of PTC1 in the ptc1 mutant. This implies that a conserved mechanism regulates the transcription of MS1 and PTC1 in both rice and Arabidopsis, with functional MS1/PTC1 down-regulating their own expression in the wild-type plants. Furthermore, genes involved in RNA processing and modification are down-regulated in both ptc1 (OsPAB184) and ms1 (PAB3; Supplemental Data Set S2). OsPAB184 encodes a poly(A)-binding protein that is the homolog of Arabidopsis PAB3. PAB3 has been shown to be specifically expressed in the tapetal cell and pollen at the young microspore stage (Belostotsky, 2003) and can promote mRNA degradation (Chekanova and Belostotsky, 2003). Therefore, we speculate that down-regulation of both OsPAB184 and PAB3 may result in the transcript accumulation of PTC1/MS1 in the mutants.

Microarray analysis also revealed that PTC1 and MS1 may have partly conserved and common regulatory pathways for pollen wall development and tapetal cell death. For example, a gene (Os02g0672500) with reduced expression in both ptc1 and ms1, encoding a mitochondrial import inner membrane translocase, is predicted to mediate the protein import pathway in mitochondria (Hong et al., 2006).

Regulatory Network of Tapetal PCD

Several transcription factors have been shown to regulate tapetum development and degeneration by analyzing the tapetum-defective mutants, including rice gamyb, ud11, and tdr (Kaneko et al., 2004; Jung et al., 2005; Li et al., 2006; Liu et al., 2010). However, ptc1 has distinct morphological changes from these mutants. UDT1 and TDR are bHLH transcription factors, and GAMYB encodes a MYB domain transcription factor. Tapetal cells in gamyb, tdr, and ud11 display delayed degeneration and abnormal enlargement, occupying the locule space and causing microspore abortion and defective pollen exine (Kaneko et al., 2004; Jung et al., 2005; Li et al., 2006; Zhang et al., 2008; Liu et al., 2010). Furthermore, no expression alteration of PTC1 was observed in tdr and ud11 (Jung et al., 2005; Zhang et al., 2008), while significant reduction of PTC1 was seen in gamyb-2 (Aya et al., 2009), suggesting that PTC1 may act downstream of GAMYB in rice anther development and, consistent with this, that PTC1 is expressed after GAMYB (Fig. 11).

In summary, we have identified a PHD-finger protein, PTC1, as a conserved key regulator for tapetal PCD in plant pollen development. The mutation of PTC1 caused tapetal proliferation, lack of PCD-based tapetal degeneration, and subsequent necrosis-like cell death in tapetal cells, resulting in abnormal pollen wall development, aborted pollen grains, and male sterility.

MATERIALS AND METHODS

Mutant Materials and Growth Conditions

All rice (Oryza sativa) plants were grown in the paddy field of Shanghai Jiao Tong University. The F2 mapping population was generated from a cross between the ptc1 mutant (japonica) and Guan Lu Ai (indica; Chu et al., 2005; Liu et al., 2005; Chen et al., 2006; Wang et al., 2006). In the F2 population, male-sterile plants were selected for genetic mapping.

Characterization of the Mutant Phenotype

Plants and flowers were photographed with a Nikon E995 digital camera. ptc1 tetrads and microspores were stained with 0.2% 4,6-diamino-2-phenylindole dihydrochloride n-hydrate to observe microspore development. Observation of anther development by semithin sections and transmission electron microscopy were performed as described by Li et al. (2006). SEM was performed as described by Zhang et al. (2008).

Isolation of PTC1 Full-Length cDNA

To obtain the full-length cDNA of PTC1, RACE-PCR was performed with the GeneRacer kit (Invitrogen) as described before (Yuan et al., 2009). The PTC1 cDNA was amplified using 2 μl of RACE product from rice anther RNAs (stage 9) with Phusion hot-start high-fidelity polymerase (New England Biolabs). PTC1-specific primers PTC1 Race 5R and PTC1 Race 3R were used for 5‘ RACE, and primers PTC1 Race 3R1 and PTC1 Race 3R2 were used for 3‘ RACE. The cDNA of PTC1 was amplified using primers PTC1 FL-cDNA F and PTC1 FL-cDNA R.

Cloning of PTC1 and Complementation

A 5,255-bp wild-type genomic fragment of PTC1 with a 3,043-bp 5‘ upstream region and a 2,212-bp genomic region (including introns) was amplified using the primer pair PTC1 com F and PTC1 com R. Then, the fragment was cloned into the entry vector pENTR/D-TOPO and destination vector pGWB7 to produce plasmid pGWB7/PTC1. The pGWB7/PTC1 plasmid was transferred into Agrobacterium tumefaciens EHA105 and introduced into the ptc1 mutant plants by A. tumefaciens-mediated transformation (Zeng et al., 2007). Pollen grains of transgenic lines were assayed by I2-KI staining. Transgenic lines were identified by PCR amplification using primers B7-com
screen F and PGWB7 vector primer B7-com screen R. The segregation of fertility plants and sterile plants was approximately 3:1 for line 1 (23 fertility:7 sterility) and line 2 (16 fertility:6 sterility) of the T1 generation.

Arabidopsis (Arabidopsis thaliana) MS1 gene promoter (2,981 bp) was amplified by primers ATMS1 Pro F and ATMS1 Pro R, and the PTC1 cDNA was amplified by primers PTC1 FL-F DNA F and PTC1 FL-CDNA R. The two fragments were ligated together by KpnI and EcoRI restriction enzymes in the pBluescript SK+ vector (the plasmid was designated pMOG1). Then, the GFP fragment including the MS1 terminator (GFPt, including the GFP fragment) was amplified by primers GFP F and GFP R, and the GFP fragment, digested with BamHI and SacII, was ligated to pMOG1 by BglII and SacII restriction enzymes (the plasmid was designated pMOG2). Then, the whole sequence, including the MS1 promoter, PTC1 cDNA, GFP, and MS1 terminator, was digested with KpnI and XbaI and inserted into the pBIn19 vector (the plasmid was designated pMOG3). Plasmid pMOG3 was then transferred into Agrobacterium tumefaciens (lods or B), which is the log-odds that the gene is differentially expressed. The annotations of genes were obtained from TIGR (http://rice.plantbiology.msu.edu/).

Sequence data from this article for the cDNA and genomic DNA of PTC1 can be found in the GenBank/EMBL data libraries under accession numbers NM_010169854, GU597363, and AC053038.

**Supplemental Data**

The following materials are available in the online version of this article.

**Supplemental Figure S1.** 4,6-Diamino-2-phenylindole dihydrochloride n-hydrate staining of microspores of wild-type and ptc1 plants from stage 8 to stage 11.

**Supplemental Figure S2.** Nucleotide and amino acid sequences of PTC1.

**Supplemental Figure S3.** RT-PCR analyses of gene expression levels in ptc1.

**Supplemental Figure S4.** RT-PCR analysis of AIM51 and PTC1 expression in the transgenic lines containing MS1silentPTC1 cDNA in Arabidopsis.

**Supplemental Table S1.** Markers used for mapping the PTC1 gene.

**Supplemental Table S2.** Functional classification by COG analysis of genes that were up-regulated or down-regulated in ptc1 as identified by Bayes analysis (FDR < 0.5%) and showing a greater than log, fold change in expression.

**Supplemental Table S3.** List of genes that were up-regulated or down-regulated at least 2-fold in the ptc1 tapetum and microspores.

**Supplemental Table S4.** ESTs of PTC1 homologs in monocot species.

**Supplemental Table S5.** Primer sequences used in this study.

**Supplemental Data Set S1.** Genes changed in the data of ptc1 microarray.

**Supplemental Data Set S2.** Text file of the protein alignment among rice PTC1 and other members on the phylogenetic tree.

**Supplemental Data Set S3.** Comparison of the ptc1 microarray data with those of gamyb, ud11, and tdr.

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