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Transient silencing mediated by *in vitro* synthesized double-stranded RNA indicates that PsCdc14 is required for sporangial development in a soybean root rot pathogen

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In many eukaryotic organisms, Cdc14 phosphatase regulates multiple biological events during anaphase and is essential for mitosis. It has been shown that Cdc14 is required for sporulation in the potato blight pathogen *Phytophthora infestans*; however, the role that the Cdc14 homolog (PsCdc14) plays in the soil-borne soybean root rot pathogen *P. sojae* remains ambiguous. *PsCdc14* is highly expressed in sporulation, zoospore, and cyst life stages, but not in vegetative mycelia and infection stages, suggesting that it contributes to asexual reproduction and thus the spread of the disease. Double-stranded RNA (dsRNA) mediates gene silencing, a post-transcriptional and highly conserved process in eukaryotes, involving specific gene silencing through degradation of target mRNA. We combined *in vitro* dsRNA synthesis and a polyethylene glycol-mediated transformation system to construct a dsRNA-mediated transient gene silencing system; and then performed a functional analysis of PsCdc14 in *P. sojae*. *PsCdc14* mRNA was dramatically reduced in transformants after protoplasts were exposed in *in vitro* synthesized *PsCdc14* dsRNA, resulting in low sporangial production and abnormal development in *P. sojae* silencing lines. Furthermore, dsRNA-mediated transient gene silencing could enable elucidation of *P. sojae* rapid gene function, facilitating our understanding of the development and pathogenicity mechanisms of this oomycete fungus.

***Phytophthora sojae*, PsCdc14, sporangium, transient gene silencing**

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Phytophthora sojae, one of more than 80 species of *Phytophthora* that causes notorious diseases on a wide range of agriculturally and ornamentally important plants, is the casual agent of stem and root rot in soybean. *P. sojae* is responsible for annual yield losses estimated to be USD 1–2 billion worldwide [1,2]. This oomycete fungus is a hemibiotroph and is homothallic, propagating through clonal zoospores and sexual oospores**,** producing nondehiscent sporangia when soils are saturated [3]. The development and spread of zoospores and subsequent infection of soybean roots is favored by saturated soil conditions [4]. Oospores are readily produced in susceptible soybean roots and can survive in crop residues and soil for many years [5].

An important feature of *P. sojae* is the formation of the sporangia during asexual development, the central part of the disease cycle of this pathogen. Sporangia develop on the termini of specialized aerial hyphae if humidity and oxygen levels are high. Although in some species such as *P. infestans*, sporangia are released freely from aerial hyphae and serve as agents of dispersal, the sporangia of *P. sojae* are not readily released from the hyphae. The asexual sporangia of *P. sojae* have a remarkable ability to germinate in

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two different ways. At higher temperatures, direct germination occurs, and hyphae emerge through the sporangial wall. Plant colonization can then occur through openings in a host such as stomata, lenticels, or wounds. Indirect germination, also known as zoosporogenesis, is predominant at cooler temperatures [3,6]. Relatively little is known about the molecular biology of zoospore formation or germination, although a few participant genes have been studied in *Phytophthora* [7,8].

Coordinating nuclear division with growth and the cell cycle is central to the development of eukaryotes. The transmission of genetic information from one generation to the next requires the accurate replication of DNA during S-phase, and the faithful partitioning of chromosomes during mitosis requires the coordination of several cellular events. *CDC14* is one of the key regulators. *CDC14* encodes a protein phosphatase that is essential for mitotic exit and meiotic progression [9–11]. Cdc14p dephosphorylates key mitotic targets, leading to the coordinated inactivation of mitotic cyclins, proper spindle disassembly, and completion of cytokinesis [12–14]. During the meiotic cell cycle, Cdc14p has been proposed to coordinate spindle disassembly and the two consecutive chromosome segregation events [15,16]. During most of the mitotic cell cycle, Cdc14p is kept inactive and sequestered in the nucleolus. Released from the nucleolus, Cdc14p can dephosphorylate S-phase and M-phase mitotic cyclin substrates to coordinate the metaphase to anaphase transition [17–19]. The return of Cdc14p to the nucleolus signals the completion of mitosis [20,21]. Multiple regulatory pathways have been proposed to regulate Cdc14p phosphatase activity during meiosis as well [22,23]. Cdc14p belongs to a subfamily of dual-specificity protein phosphatases that can dephosphorylate phosphotyrosine and phosphoserine/phosphothreonine residues [24]. Cdc14p is conserved in fungi, worms, and mammals [25,26]. In *P. infestans*, *piCdc14* complemented a mutation in its *S. cerevisiae* ortholog, suggesting that it could be a mitotic regulator; however, the gene exhibits a pattern of expression strikingly different from that observed in yeast and other studied species. *piCdc14* is not transcribed during normal hyphal growth but is expressed only during the formation of sporangia, and appears required for sporulation. These results can be placed in the context of the adaptation of Cdc14 functions to different modes of growth during eukaryotic evolution [7].

Exposure of eukaryotic cells to double-stranded RNA (dsRNA) led to post-transcriptional degradation of homologous mRNA sequences [27]. Known as RNA interference (RNAi), this process was initially described in nematodes and subsequently demonstrated in fungi, plants, insects, and animals [27,28]. The dsRNA molecules that trigger RNAi can be delivered into cells by microinjection, transfection, or transformation. In *P. infestans*, transient silencing mediated by *in vitro*-synthesized dsRNA has been reported [29–32]. Target genes were silenced from 12 to 17 days after the *P. infestans* protoplasts were exposed to dsRNA, and the gene silencing triggered by dsRNA was sequence-specific [32]. These reports show that dsRNA-mediated transient gene silencing can be used to generate detectable phenotypes and should provide a valuable tool for functional genomics in *Phytophthora*.

Our analysis of the transcription of *PsCdc14* in *P. sojae* indicated that it is highly upregulated during sporulation, zoospore, and cyst stages. We combined *in vitro* dsRNA synthesis and a polyethylene glycol (PEG)-mediated transformation system to construct a dsRNA-mediated transient gene silencing system, and then performed a functional analysis of PsCdc14 in *P. sojae*.

1 Materials and methods

1.1 Growth of *Phytophthora sojae* life cycle stages and plant inoculation

P. sojae strain P6497 and all transgenic *P. sojae* isolates used were routinely grown on 10% V8 media at 25°C in the dark, as described by Erwin [2]. To obtain axenically prepared mycelium, hyphal tip plugs of P6497 were used to inoculate 30 mL of sterile clarified 10% V8 broth in 90 mm petri dishes. Stationary mycelial cultures were incubated at 25°C in the dark for 3 d. Sporulating hyphae were prepared by repeatedly washing 2-day-old hyphae incubated in 10% V8 broth with sterile distilled water, and incubating the washed hyphae in the dark at 25°C for 4–8 h until sporangia had developed on most hyphae. Zoospores were filtered using Miracloth (Calbiochem) and collected by centrifugation at 2000×*g* for 2 min. Cysts were obtained by vortexing a zoospore suspension for 30 s and then centrifuging at 2000×*g* for 2 min. Cysts were germinated in clarified 5% V8 broth for 2 h. The inoculation of soybean leaves with *P. sojae* mycelia, used for qRT-PCR analysis, was performed as described by Chen [33]. Mycelia were harvested from infected leaves at 1.5 hours post infection (hpi), and at 3 hpi, and then infected leaves were harvested at 6, 12, 24, and 48 hpi. All collected samples were immediately frozen in liquid nitrogen and stored at −70°C prior to RNA extraction.

1.2 RNA extraction

Total RNA from various stages of the *P. sojae* life cycle was extracted from frozen samples ground in liquid nitrogen using a NucleoSpin RNA II RNA extraction kit (Macherey-Nagel), following the manufacturer's protocol. The integrity of the RNA was tested by agarose gel electrophoresis. Prior to cDNA synthesis, all RNA samples were treated with DNase I (Takara, D2270), following the manufacturer's protocol. First strand cDNA was synthesized from 1 to 5 μg of total RNA by oligo (dT) priming using a M-MLV reverse transcriptase kit (Invitrogen), following the manufacturer's

protocol.

1.3 qRT-PCR analysis of gene expression

For gene expression analysis, SYBR green qRT-PCR assays were performed. Primer pairs (Table 1) were designed to anneal specifically to each of the genes for qRT-PCR analysis. The *actA* gene from *P. sojae* was used as a constitutively expressed endogenous control, and the expression of each gene was determined relative to *actA* using the $\Delta\Delta C_t$ method [30]. Expression of *PsCdc14* at different life cycle stages was compared with the level of its expression in a calibrated sample of cDNA from a mycelium. Although there was a high expression of *PsCdc14* in sporulating mycelia, zoospores, and cysts, the basal levels of *PsCdc14* mRNA in mycelia could be detected using the sensitivity of real time PCR. The expression of *PsCdc14* in the mycelial cDNA sample was assigned the value of 1.0. For expression of each gene in potentially silenced lines, the calibrated sample was the expression value from the wild type strain P6497 (WT) for each gene; this expression number was assigned the value of 1.0. qRT-PCR assays for gene expression were carried out using three biological replicates.

1.4 Transformation of *P. sojae*

We transformed *P. sojae* using a polyethylene glycol (PEG)-mediated protoplast transformation strategy [34] with the following modifications: Two-day-old *P. sojae* mycelial mats, cultured in pea broth medium, were rinsed and washed in 0.8 mol L^{-1} mannitol, then placed in enzyme solution (0.4 mol L^{-1} mannitol, 20 mmol L^{-1} KCl, 20 mmol L^{-1} MES (2-(N-Morpholino)ethanesulfonic acid hydrate), pH 5.7, 10 mmol L^{-1} $CaCl_2$, 7.5 mg mL^{-1} lysing enzyme (from *Trichoderma harzianum*; Sigma L1412), and 3 mg mL^{-1} cellulase (from *Trichoderma reesei*; Sigma C8546), and incubated for 40 min at 25°C with 100 r min^{-1} shaking. The protoplasts were harvested by centrifugation at 1500 r min^{-1} for 3 min and resuspended in W5 solution (5 mmol L^{-1} KCl, 125 mmol L^{-1} $CaCl_2$, 154 mmol L^{-1} NaCl, and 31 mg mL^{-1} glucose) at a concentration of 1×10^6 protoplasts mL^{-1}. After 30 min, the protoplasts were centrifuged at 1500 r min^{-1} for 4 min and resuspended in an equal volume of MMg solution (0.4 mol L^{-1} mannitol, 15 mmol L^{-1} MgCl2, and 4 mmol L^{-1} MES, pH 5.7) to allow protoplasts to swell. The protoplasts were then diluted to 5000–10000 in each 1 mL MMg solution, about 100 μg dsRNA was added and incubated for 10 min on ice. Three 580 mL aliquots of freshly made polyethylene glycol solution (40% (v/v) polyethylene glycol 4000, 0.3 mol L^{-1} mannitol, and 0.15 mol L^{-1} $CaCl_2$) were slowly pipetted into the protoplast suspension and gently mixed. After 20 min incubation on ice, 10 mL pea broth containing 0.5 mol L^{-1} mannitol was added, and the protoplasts were incubated overnight to regenerate. The regenerated protoplasts were suspended in liquid pea agar (40°C) containing 0.5 mol L^{-1} mannitol, and then plated. Visible colonies could be observed after 24 h incubation at 25°C. A total of 20 single colony transformants were selected and propagated on V8 agar for further testing.

2 Results

2.1 Identification and characterization of *PsCdc14* in *P. sojae*

Using the previously reported amino acid sequence of *P. infestans* Cdc14 [7] as a query, a tBLASTN search program was used to identify a Cdc14 homology from the *P. sojae* genome database (http://genome.jgi-psf.org/Physo1 1/Physo1_1.home.html; March 2009). The gene (protein ID: 108222) was named *PsCdc14*, and the predicted ORF was 1281 bp, encoding 426 amino acids. The sequence of *PsCdc14* transcript was confirmed by sequencing the RT-PCR product, using cDNA from sporulating mycelial mRNA as a PCR template. *PsCdc14* and *piCdc14* shared 81% and 86% identity at DNA and amino acids level, respectively. The PsCds14 amino acid sequence was analyzed through a conserved domain search (CD-search) at http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi (May 2009) [35]. The region between amino acids 219 and 322 was predicted to be a dual specificity phosphatase catalytic domain, showing about 70% similarity with the Cdc14 motif from *S. cerevisiae* and other species. The region between the 275 and 285 amino acid sites was a 100% match with the tyrosine phosphatase active site (VHCKAGLGRTG) (Figure1). As CDC14 is conserved in eukaryotes, phylogenetic analysis of Ykt6 proteins was performed. This revealed that *Phytophthora* CDC14 is closer to the CDC14 in plants than it is to that in fungi (Figure 2).

2.2 Expression analysis of *PsCdc14* at different life stages of *P. sojae*

Real time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to characterize expression profiles of *PsCdc14*. RNA samples were isolated from cultured mycelia, sporulating mycelia, zoospores, cysts, germinating cysts, and susceptible soybean tissues infected with *P. sojae* strain P6497, at 1.5, 3, 6, 12, 24, and 48 hpi. *PsCdc14* was expressed at relatively low levels at mycelium and infection stages; however, its expression dramatically increased up to 180-fold in sporulating mycelia, 250-fold in zoospores, and over 1100-fold in cysts (Figure 3).

2.3 Transient silencing of *PsCdc14* expression

To ensure the specificity of dsRNA-mediated gene silencing, the synthesized dsRNA should not share more than 20 bp

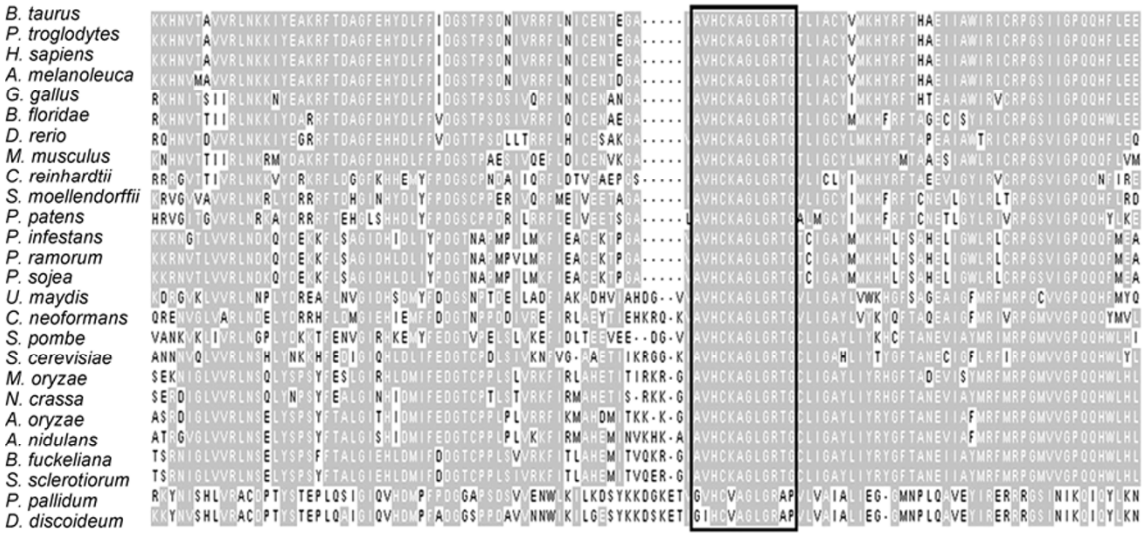

Figure 1 ClustalW alignment of conserved acid amino sequences (segments) of Cdc14 dual specificity phosphatase catalytic domain, from *Bos taurus* (XP_002686214), *Pan troglodytes* (XP_001135275), *Homo sapiens* (ABF74568), *Ailuropoda melanoleuca* (XP_002920815), *Gallus gallus* (NP_001171207), *Branchiostoma floridae* (XP_002602511), *Danio rerio* (CAP09233), *Mus musculus* (NP_001116461), *Chlamydomonas reinhardtii* (XP_001699757), *Selaginella moellendorffii* (XP_002969311), *Physcomitrella patens* (XP_001773592), *Phytophthora infestans* (AAP38170), *Phytophthora ramorum* (71621), *Phytophthora sojae* (108222), *Ustilago maydis* (XP_762334), *Cryptococcus neoformans* (XP_566871), *Schizosaccharomyces pombe* (NP_594716), *Saccharomyces cerevisiae* (YFR028C), *Magnaporthe oryzae* (XP_362192), *Neurospora crassa* (XP_963738), *Aspergillus oryzae* (XP_001819929), *Aspergillus nidulans* (XP_662661), *Botryotinia fuckeliana* (XP_001548549), *Sclerotinia sclerotiorum* (XP_001586094), *Polysphondylium \pallidum* (330787), *Dictyostelium discoideum* (DDB0238561). The box indicates the tyrosine phosphatase active site.

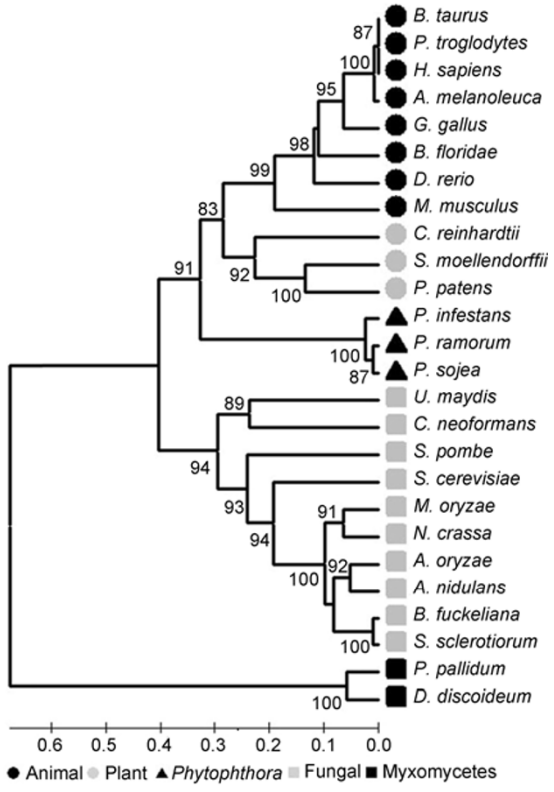

Figure 2 Phylogenetic analysis of Cdc14. Dendrogram of Cdc14 sequences derived from *Phytophthora* and several other eukaryotes. Alignments were made using ClustalW, and dendrograms produced using Mega4.

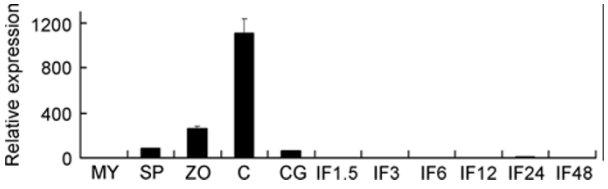

Figure 3 *PsCdc14* expression at different developmental and infection stages, as monitored by qRT-PCR. MY, Mycelium; SP, sporulating mycelium; ZO, mixed zoospores and cysts; C, cysts; CG, germinating cysts; IF1.5, IF3, IF6, IF12, IF24 and IF48, infected susceptible soybean cultivar samples at 1.5, 3, 6, 12, 24, and 48 hours post-inoculation, respectively. Expression was equilibrated based on the expression level in mycelium, set at a value of 1. The qRT-PCR experiment was repeated twice with independent samples.

identity with any other sequences in the genome of *P. sojae*. According to the genome alignment output from the Joint Genome Institute (JGI) *P. sojae* assembly V 1.0 database, the dsRNA was designed for the specific N terminal portion of *PsCdc14* (Figure 4). Transient transformation of *P. sojae* protoplasts was mediated using polyethylene glycol/calcium chloride (PEG) methods [36,37]. *P. sojae* isolate P6497 was used as the wild type recipient strain and the transformant from the regenerated protoplast without dsRNA was used as the control. In addition to the high expression of *PsCdc14* in sporulating mycelia, zoospores, and cysts, the basal levels of *PsCdc14* mRNA in mycelia can be detected using qRT-PCR. Subsequently, qRT-PCR was performed with the extracted RNA from new mycelia to determine whether the

Table 1 Primers used

Primer name	Primer sequence	Application	Application size (bp)
Cdc14T7F	GTAATACGACTCACTATAGGGTCTCGAGGTGGCGATCGAGTT	dsRNA synthesis	191
Cdc14R	GCTTCTTGTTGAGTGTCTCGG		
Cdc14F	TCTCGAGGTGGCGATCGAGTT	dsRNA synthesis	191
Cdc14T7R	GTAATACGACTCACTATAGGGGCTTCTTGTTGAGTGTCTCGG		
Cdc14qRT-F	CATCCGCAGCATTATGGTCC	Real-time PCR	134
Cdc14qRT-R	AGCGCATGTACTTCTGGTCG		
ActA qRT-F	ACTGCACCTTCCAGACCATC	Real-time PCR	165
ActA qRT-F	CCACCACCTTGATCTTCATG		

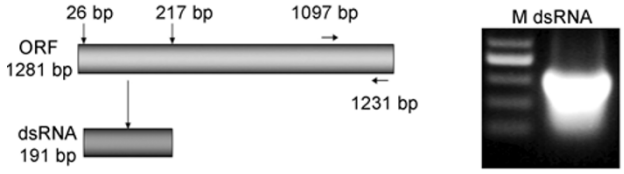

Figure 4 The synthesis of *PsCdc14* dsRNA and qRT-PCR primers. A, A 191 bp fragment from 26 to 217 bp was selected as the dsRNA synthesis region, and the primers located at 1097 and 1231 bp were used for mRNA abundance detection using qRT-PCR. B, *In vitro* synthesized dsRNA products were examined on ethidium bromide-stained gels.

dsRNA-treated lines were silenced for *PsCdc14* expression. Relative expression values were assessed 9 days after exposure to *PsCdc14* dsRNA or the control. Results from two independent experiments are presented in Figure 5. The *PsCdc14*-silenced lines showed a reduction in *PsCdc14* mRNA abundance, with mRNA levels ranging from 10% to 30% of the controls. Successful silencing of *PsCdc14* was therefore achieved using RNA silencing.

2.4 Phenotypic characterization of *PsCdc14*-silenced lines

Colony morphology and growth rates of the *PsCdc14*-silenced lines were similar to those of the wild-type recipient strain P6497 and the control transformant. However, at 10 days after exposure to dsRNA, all the *PsCdc14*-silenced mutants failed to sporulate. In some lines, no sporangia were observed (T2, T3). Some lines produced immature sporangia that were much smaller than the normal sporangia of the wild type and the control transformant (T1). Some lines formed very few normal sporangia (T4) (Figure 6). These results indicate that the *PsCdc14* that was partially silenced by dsRNA-mediated transient transformation was defective in sporulation. *PsCdc14* is thus required for *P. sojae* sporulation.

3 Discussion

Completion of the cell cycle requires the temporal and spatial coordination of chromosome segregation with mitotic spindle disassembly and cytokinesis. In *Saccharomyces cerevisiae*, the protein phosphatase Cdc14 is a key regulator of late mitotic events [38]. In *P. infestans*, *piCdc14* is essential for effective sporulation [7]. *PsCdc14*, a *Cdc14* homolog with a single copy in *P. sojae*, shares a similar transcriptional pattern to *piCdc14* in *P. infestans*. This suggests that *PsCdc14* and *piCdc14* might play the same role in development and sporulation in each species. Although other predicted *P. sojae* proteins have the conserved tyrosine phosphatase active site that is present in the C-terminal region of Cdc14, the 191 bp synthesized dsRNA was designed at the N-terminal region to ensure target specificity. We found that the *PsCdc14*-silenced transformants were impaired in sporangium formation. Few, if any, sporangia were produced by the gene-silenced transformants. Sporangia that did develop were reduced in size compared with the control strains. These results are consistent with previously described *P. infestans Cdc14*-silencing, indicating that the gene plays a conserved role in *Phytophthora* sporangial generation, despite differences in sporangial biology be-

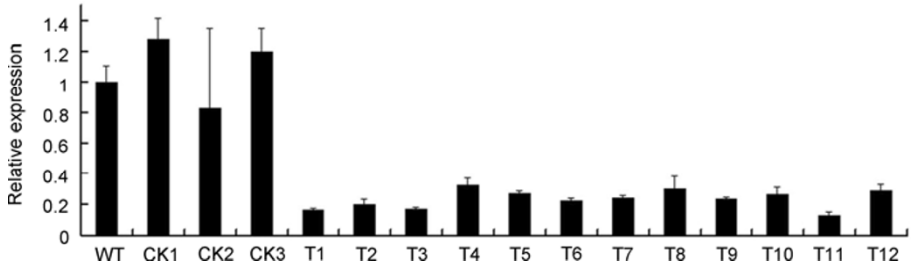

Figure 5 The *PsCdc14* relative expression levels in individual RNAi lines were monitored using qRT-PCR. RNA samples were taken from silencing lines 9 days after introduction of *PsCdc14* dsRNA into protoplasts. T1–T12 represent the regenerated lines treated by dsRNA. WT is the wild type P6497 strain and CK1-3 is the transformant regenerated without dsRNA introduction.

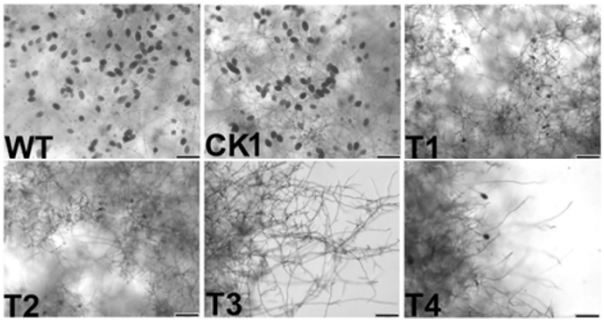

Figure 6 Sporangial formation (bottom) in wild type *P. sojae* (WT), control transformant (CK1), and *PsCdc14*-silenced mutants (T1–T4). The *PsCdc14*-silenced lines (T1–T4) showed a significant reduction in the presence or absence of sporangial generation. Scale bars, 100 μm.

tween the two species.

In other eukaryotes, Cdc14s are constitutively transcribed [11]. However, like *piCdc14* in *P. infestans*, *PsCdc14* was expressed at a relatively low level in mycelium and infection stages, but its expression dramatically increased in sporulating mycelia, zoospores, and cysts. We propose that the function of *PsCdc14* may be to synchronize nuclear behavior during sporulation and maintain dormancy in spores until germination. The low level of expression of *PsCdc14* in vegetative hyphae might reflect the evolution in some eukaryotes of networks for regulating nuclei that are absent in *P. sojae*. *Phytophthora* may have lost the normal pattern of Cdc14 expression during its evolution and have adapted to life without a well-regulated cell cycle.

Our dsRNA-mediated gene silencing experiments supported the essential role of *PsCdc14* in *P. sojae* sporulation. Assigning a precise role to *PsCdc14* is complicated by studies of other species that indicate the protein may have several functions. The specific substrates of Cdc14 that are critical for the decline in Cdk1 activity that accompanies mitosis have been well established. Similarly to Cdk1, Cdc14 has a preference for phospho-Ser/Thr-Pro motifs [39], suggesting that Cdc14 may directly reverse some Cdk1-dependent phosphorylation events. Cdc14 has been shown to dephosphorylate multiple substrates, including Sli15, Ask1, Fin1 and Ase1, which are important for stabilization and extension of the anaphase spindle [40–43]. Cdc14 is also important for the segregation of repetitive ribosomal DNA and telomeric regions of chromosomes during anaphase [44–46], although the relevant target(s) for these processes have not been fully elucidated. In addition, Cdc14 has a role in nuclear positioning and proper segregation of replicated DNA in both the daughter and mother cells [47].

A variety of transformation protocols delivering sense, antisense, and hairpin DNA into *Phytophthora* cells can result in the silencing of endogenous gene targets in *P. infestans*, *P. sojae*, and other *Phytophthora* species [48]. However, the stable transformation of *Phytophthora* is not only difficult and time-consuming but also may suffer many problems such as a low ratio of gene integration, unstable transformants, lengthy transformation regeneration times, unpredictable copy number, and chromosome insertion effects [48]. Nonetheless, researchers continue to optimize transformation protocols for *Phytophthora* species, especially *P. infestans*, to simplify and improve the efficiency of transformation [32,34]. A stable transformation method for *P. sojae* is available and has been employed successfully in the functional characterization of G-protein subunit component *PsGPA1*, avirulent gene *Avr1a*, transcription factor *PsCRZ1*, and SNARE protein PsYKT6 [36,37,49,50]. However, the different transformation protocols do not work equally well on different strains of this organism, and adapting improvements described for *P. infestans* or other species are not always successful for *P. sojae*. Current *P. sojae* transformation protocols are thus useful, but need to be improved and expanded to meet the needs of researchers. New and potentially high-throughput tools such as dsRNA-mediated RNAi need to be developed for *P. sojae*. Gene silencing by *in vitro* synthesized dsRNA (150–300 bp in size), directly delivered into protoplasts with Lipofectin, was first reported in *P. infestans* [32]. A marker gene, GFP, and two *P. infestans* genes, *inf1* and *cdc14*, were transiently silenced. A reduction of target gene mRNA and a detectable phenotype was observed 12–17 days after introduction of the appropriate dsRNA. Based on previous reports and our own experience, we combined the dsRNA method with a PEG mediated *P. sojae* transformation method, generating a convenient and cost-effective RNAi transient silencing protocol. Whether PEG or Lipofectin is more efficient in mediating dsRNA delivery for *Phytophthora* is not known since we did not directly compare the two compounds. Nonetheless, from our work on *PsCdc14*, we have demonstrated that PEG-mediated protoplast exposure to dsRNA triggers gene silencing with resulting phenotypes. This transient gene- silencing protocol is a simple but powerful tool that will assist functional genomics in *P. sojae*. An advantage of the transient silencing of *P. sojae* compared with stable transformation is that there is no need to construct silencing vectors. The simple requirement is the production of dsRNA from a PCR product with T7 RNA polymerase binding sites at each end. Thus, our results substantiated and expanded upon claims that dsRNA-directed RNAi offers a rapid and convenient method to silence target genes in oomycete species.

In summary, we have created the first transient silencing protocol for *P. sojae*, and successfully silenced *PsCdc14*. We have shown that silencing *PsCdc14* results in phenotypic changes in transformants, which affect sporulation, and follow the predicted function of this gene. Our described methods are important for *Phytophthora* researchers and will enable more feasible studies of gene function.

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