MINIREVIEWS

Molecular Genetics of Pathogenic Oomycetes

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Parasitic and pathogenic lifestyles have evolved repeatedly in eukaryotes (93). Several parasitic eukaryotes represent deep phylogenetic lineages, suggesting that they feature unique molecular processes for infecting their hosts. One such group is formed by the oomycetes. Traditionally, due to their filamentous growth habit, oomycetes have been classified in the kingdom Fungi. However, modern molecular and biochemical analyses suggest that oomycetes have little taxonomic affinity with filamentous fungi but are more closely related to brown algae (heterokonts) in the Stramenopiles, one of several major eukaryotic kingdoms (2, 56, 93). The most notable and best-studied oomycete species is *Phytophthora infestans*, the Irish famine pathogen. *P. infestans* causes late blight, a devastating and reemerging disease of potatoes and tomatoes (4, 17, 18, 82, 90–92). Other oomycetes include destructive plant and animal pathogens, as well as saprophytes that are beneficial to the environment (56).

Despite their peculiar phylogenetic affinities and economic importance, oomycetes were chronically understudied at the molecular level. However, in recent years, increased awareness of the evolutionary history of oomycetes as unique eukaryotic microbes resulted in the emergence of a group of researchers specializing in oomycete genetics. The evolution of this community has been driven by the biological properties of oomycetes, which require alternative methodologies. Technical developments, such as routine DNA transformation, use of reporter genes, and genetic manipulation using gene silencing have facilitated the discovery and functional analyses of several interesting genes. With recent efforts in genomics and functional genomics and the resulting resources, genetic research on oomycetes has entered an exciting phase. This review provides an overview of oomycete biology, with a particular emphasis on molecular genetics. It describes the molecular and genomic resources available for these organisms and discusses the current status of, and future perspectives in, basic studies of pathogenic oomycetes. Unfortunately, too little is known about the molecular genetics of the pathogenicity of oomycetes other than *Phytophthora* to warrant a comprehensive review. Therefore, the section on infection mechanisms focuses on *Phytophthora*. For additional information on various aspects of oomycete biology, the reader is referred to a number of recent reviews (4, 38, 39, 43, 82, 91, 92, 99, 100).

OOMYCETE BIOLOGY

Diversity and pathology. Oomycetes form a diverse group of fungus-like eukaryotic microorganisms, also known as water molds, that include both saprophytes and pathogens of plants, insects, crustaceans, fish, vertebrate animals, and various microorganisms (Table 1) (56). The multitude of saprophytic oomycetes inhabit primarily aquatic and moist soil habitats. They have a positive effect on nutrient cycling through their key role in the decomposition and recycling of organic matter (56). Plant-pathogenic oomycetes cause devastating diseases in numerous crop, ornamental, and native plants. They include more than 60 species of the genus *Phytophthora* that are arguably the most devastating pathogens of dicotyledonous plants (1, 13, 14). *Phytophthora* spp. cause enormous economic damage to important crop species such as potatoes, tomatoes, peppers, soybeans, and alfalfa, as well as environmental damage in natural ecosystems. Virtually every dicot plant is affected by one or more species of *Phytophthora*, and several monocot species are infected as well. The most notable pathogenic oomycete is *P. infestans*, the Irish potato famine pathogen. This species causes late blight, a ravaging disease of potatoes and tomatoes (4, 17, 18). Introduction of this pathogen to Europe in the mid-nineteenth century resulted in the potato blight famine and the death and displacement of millions of people. Today, *P. infestans* remains a devastating pathogen, causing losses as high as $5 billion in potato production worldwide (12). The appearance of highly aggressive and fungicide-insensitive strains in North America and Europe in the 1990s resulted in a new wave of severe and destructive potato and tomato late-blight epidemics (17–19, 90–92).

Other economically important *Phytophthora* diseases include root rot of soybean, caused by *Phytophthora sojae*; black pod of cocoa, a recurring threat to worldwide chocolate production, caused by *Phytophthora palmivora* and *Phytophthora megakarya*; dieback and related root rot diseases in crops and native plant communities, caused by *Phytophthora cinnamomi*; and sudden oak death, caused by the recently discovered *Phytophthora ramorum*. This disease is decimating oak trees along the Pacific coast of the United States and might be expanding to other hosts, such as redwoods, and to other regions in North America (49, 83). At least one species, *Phytophthora brassicae* (previously known as *Phytophthora porri*), infects the model plant *Arabidopsis thaliana*, and this pathosystem is thought to be easier to dissect at the genetic level than agronomically important *Phytophthora* diseases (84).

Important oomycete plant pathogens also occur outside the

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TABLE 1. Major genera of pathogenic oomycetes

Genus	Host(s)	Description
Albugo	Plants	Obligate pathogens of plants
Aphanomyces	Animals and plants	Mostly pathogens of aquatic animals, but includes pathogens of plants
Bremia	Plants	Obligate pathogens of plants
Lagenidium	Animals	Pathogenic species in this genus infect aquatic animals
Peronospora	Plants	Obligate pathogens of plants
Phytophthora	Plants	Most extensively studied genus, with more than 60 species that cause some of the most severe diseases of dicot plants
Plasmopara	Plants	Obligate pathogens of plants
Pythium	Plants, animals, and microbes	Mostly plant pathogens, but includes mycoparasites and at least one species that infects humans
Saprolegnia	Animals	Pathogenic species in this genus infect aquatic animals, mainly fish

genus *Phytophthora* (1). These include the obligate biotrophs *Plasmopara viticola* (the agent of downy mildew of grapevine) and *Albugo*, *Bremia*, and *Peronospora* species, which cause white rust and downy mildew on several crops. Other important pathogens include more than a hundred species of the genus *Pythium*, which are abundantly present in water and soil habitats and cause a diversity of plant diseases, mainly in root tissue. *Pythium* infections are usually limited to the meristematic tips, epidermis, cortex of roots, and fruits (110), but occasionally, severe *Pythium* infections occur when the pathogen moves deeper into plant tissue and reaches the vascular system. Some *Pythium* species, such as *Pythium oligandrum*, are essentially beneficial and reduce infections caused by more-severe pathogenic microbes (58). This can occur directly, through antagonistic effects or mycoparasitism, or indirectly, by induction of defense responses in plants (74).

Animal-pathogenic oomycetes, such as species in the genus *Saprolegnia*, can cause severe losses in aquaculture and fisheries (7). At least one species, *Pythium insidiosum*, is known to infect various mammals, including humans, horses, and dogs (62, 63, 94). *P. insidiosum* colonizes cutaneous and subcutaneous tissues and can invade blood vessels and bones, resulting in fatal lesions (81). One genus, *Aphanomyces*, includes both plant- and animal-pathogenic species (1, 7). A facultatively parasitic oomycete, *Lagenidium giganteum*, infects the larval stage of many mosquito species, and spore formulations of this organism have been used for biocontrol of mosquitoes (117).

Unique biological features. The oomycetes have a number of remarkable biological features that distinguish them from several other eukaryotic microorganisms. For example, the cell walls of oomycetes are mainly composed of β-1,3-glucan polymers and cellulose; unlike fungal cell walls, they contain little chitin (13, 14). Nonetheless, chitin synthase genes are widely distributed among oomycete species (52, 66, 114), and the chitin synthase inhibitor polyoxin D caused significant reduction in growth in *Saprolegnia* (8), indicating that chitin is a minor but important component of the oomycete cell wall. Other singular features include the energy storage carbohydrate mycolaminarin, a β-1,3-glucan that is also found in kelps and diatoms; diploidy at the vegetative stage; and complex life, infection, and sexual cycles (13, 14, 56). Within the oomycetes, *Phytophthora* spp. are both sterol and thiamine auxotrophs and typically require exogenous sources of β-hydroxy sterols for sporulation and thiamine for growth (14).

Genome size. The genome size of oomycetes varies widely, ranging from 18 to 250 Mb, based on estimates made using image analysis of nuclear Feulgen stainings, reassociation kinetics, and contour-clamped homogeneous electric field (CHEF) gel electrophoresis (54, 57, 64, 97, 112). For example, whereas the *P. sojae* genome was estimated at 62 to 98 Mb (54, 112), the *P. infestans* genome size was calculated to be approximately 237 Mb (97). *Pythium* species may have the smallest genomes of oomycetes, with estimates ranging from 18.8 to 41.5 Mb based on electrophoretic karyotyping (57). The genome size of *Saprolegnia monoica* was estimated at 51 Mb based on CHEF gel electrophoresis (64).

The haploid chromosome counts of many oomycete species are not exactly known due to the presence of several small chromosomes that are difficult to resolve under light microscopy. Microscopic analyses revealed that *P. infestans* has 8 to 10 chromosomes (88), whereas *P. sojae* has 10 to 13 chromosomes (89). Using CHEF gel electrophoresis, Tooley and Carras (95) resolved at least 7 to 13 bands for *Phytophthora megasperma*, 6 for *Phytophthora cactorum*, 4 to 5 for *Phytophthora boehmeriae*, and 3 to 4 for *Phytophthora capsici*. The chromosomes of several other species, including *P. infestans*, failed to separate. In other studies using CHEF gel electrophoresis, 7 to 20 bands could be resolved for 18 *Pythium* species (57), and 8 bands were separated for *S. monoica* (64).

Genome organization. Not much is known about the organization of oomycete genomes outside the genus *Phytophthora*. *Phytophthora* genomes are characterized by an abundance of repetitive sequences. In *P. sojae*, five families of tandemly repeated sequences were identified following genomic subtraction of chromosomal DNA from different isolates of the pathogen (55). These sequences varied in copy number between isolates and were all localized on a single chromosome of *P. sojae*. In *P. infestans*, the majority of the repeated DNA families (covering at least 50% of the genome) have been identified following screening of genomic libraries (36). These repeated elements were grouped into 33 distinct families that were either tandemly repeated or dispersed throughout the *P. infestans* genome (36). Copy numbers varied from 70 to 8,400 per haploid genome. A number of these repeated sequences occurred in other, distantly related *Phytophthora* species. However, some elements were specific to *P. infestans* and the closely related *Phytophthora mirabilis*. Two such sequences were used to develop specific primers for sensitive and reliable detection as well as quantification of *P. infestans* in plant samples (37). A family of repeated sequences, PCIS, was also reported for *Phytophthora cryptogea* (71). These elements varied from 100 to 1,200 bp and were not detected by hybridiza-

tion in 20 other *Phytophthora* species, except for a few copies in *P. cinnamomi*.

Only limited genome sequencing has been published so far for oomycete species, and our understanding of gene organization and structure remains sketchy. Several functional genes are known to be located in high-density gene islands separated by clusters of repetitive sequences. For example, seven genes, including three genes encoding necrosis-inducing proteins (PsojNIP family), were tightly clustered in a 10,835-bp region of the *P. sojae* genome (79). Open reading frames were separated only by an average of 497 bp (range, 102 to 714 bp). A similar high gene density was also described for the *ipiO* and *ipiB* clusters of *P. infestans* (76).

Genome instability. Many oomycetes, including *P. infestans* and several other *Phytophthora* species, are known to exhibit tremendous phenotypic variation, both in the field and in culture, even during asexual reproduction (9, 10, 16, 22, 34). The genetic basis of this phenomenon is not clear, but it could be due to genome instability, perhaps caused by transposable elements, gene conversion, mitotic recombination, and/or dispensable chromosomes (10, 22, 34, 57). Sequences similar to transposable elements are abundant in *Phytophthora* genomes. For example, sequences with similarity to the *copia* and *Gypsy*/*Ty* classes of retrotransposons have been described (34, 96). *Gypsy*-like sequences were detected in 29 species of *Phytophthora* and varied in abundance from 10 to 10,000 copies per genome (34). Sequences with similarity to DNA transposable elements of the *mariner* class were identified from expressed sequence tag (EST) databases of *P. infestans* and *P. sojae* and were subsequently found to be abundant in *Phytophthora* genomes (42, 78; W. Morgan and S. Kamoun, unpublished data).

Phytophthora may have also evolved specific mechanisms to generate rapid genetic variation. Recently, mitotic gene conversion was observed to occur in hybrid strains of *P. sojae* at remarkably high frequencies, as high as 3×10^2 conversions per locus per nucleus per generation (10). The conversion tracts were shorter than 1 kb, with no evidence for crossing over or mitotic recombination (10). Mitotic gene conversion resulted in the reassortment of loci that show close linkage during meiotic recombination, suggesting that in nature this mechanism could rapidly generate unique genetic types (10). Other mechanisms for genetic variation, such as mitotic recombination and parasexuality, have been proposed by several authors (reviewed by Goodwin [22]) and may also contribute to phenotypic instability.

Gene structure. Promoters from non-oomycete species do not function in *Phytophthora*, suggesting the presence of a unique transcriptional machinery (29, 30). Canonical eukaryotic elements, such as the TATA box, were not always detected in oomycete promoters, as occasionally reported for fungal promoters. The transcriptional start sites of several genes have been determined and are typically only 50 to 100 bp upstream of the start codon (76). This suggests that oomycetes bear highly compact transcripts, with some of the shortest untranslated regions noted for eukaryotes (73). A conserved 16-bp sequence motif, GCTCATTYBNNNWTTY, surrounds the transcriptional start site of many oomycete genes (61, 76) (Fig. 1). This motif has facilitated the prediction of transcribed regions from genomic sequences. Sequences resembling AT-rich
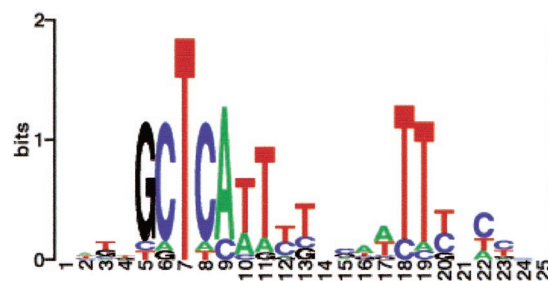

FIG. 1. Sequence conservation at the oomycete transcriptional start site. Sequences surrounding known and predicted transcriptional start sites (61, 76) were identified from 15 oomycete gene sequences deposited in GenBank (release 131.0) and aligned manually. Note that the exact position of the transcriptional start site varies depending on the gene examined but often corresponds to position 8 (C) or 9 (A). Consensus sequences were calculated at http://www.bio.cam.ac.uk /seqlogo.

polyadenylation signals are commonly found downstream of oomycete open reading frames.

A majority of oomycete genes examined to date do not contain introns. Analysis of *Phytophthora* sequences in GenBank release 131.0 (September 2002) revealed 21 intron-containing genes out of 63 genes examined (33%). These 21 genes contained a total of 32 introns, or an average of 1.5 introns per gene. Introns averaged 79 bp (range, 26 to 172 bp) and contained conserved sequences at the 5′ and 3′ exon-intron junctions (5′-GTRNGT. . .YAG-3′) (Fig. 2). A motif, CTAAC, believed to be important for lariat formation during splicing, has been reported in several intron sequences (48, 75). *P. infestans* and *P. sojae* transcribed sequences are characterized by a GC content of approximately 58% (3, 78). This feature has facilitated the identification of *Phytophthora* ESTs from cDNA libraries constructed from infected plant tissue, since transcribed sequences of plants average a GC content of only 42% (3, 28, 78). Conservation around the ATG translation start codon follows the eukaryotic consensus ACCATGA (Fig. 3). Codon usage in *Phytophthora* is typically biased toward GC-rich codons (101) (http://www.oardc.ohio-state.edu/phytophthora /codon.htm).

MOLECULAR AND GENOMIC RESOURCES

DNA transformation. Novel protocols and vectors for transformation of oomycetes had to be developed, since vectors suitable for transformation of filamentous fungi did not work in *Phytophthora*, probably due to different sequence requirements for the transcriptional machinery (29, 30). A number of promoters, such as *ham34* and *hsp70*, from the downy-mildew oomycete *Bremia lactucae*, showed strong activity in transient transformation assays of *P. infestans* protoplasts and were subsequently incorporated into transformation vectors (29). The standard transformation protocol is based on liposome-polyethylene glycol (PEG)-mediated transformation of protoplasts, followed by regeneration and antibiotic selection on agar medium (31, 35). High frequency rates of cotransformation (up to 50%) were observed, especially if the two plasmids were linearized with restriction enzymes with compatible ends (33). This finding turned out to be quite useful, as the gene of
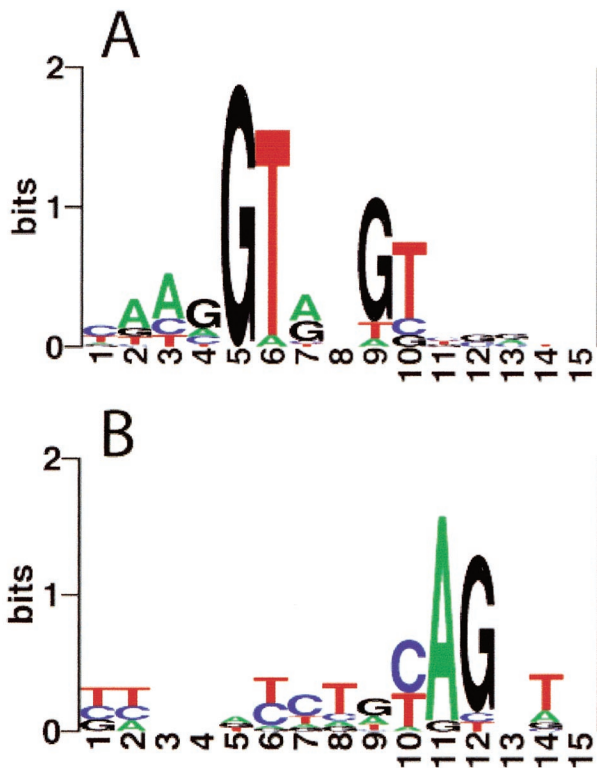
FIG. 2. Sequence conservation in *Phytophthora* introns. Consensus sequences for 5′ exon-intron junctions (A) and 3′ intron-exon junctions (B) were calculated based on 19 *Phytophthora* introns (GenBank release 113.0) at http://www.bio.cam.ac.uk/seqlogo. The intron sequences start at position 5 (A) and end at position 12 (B).

interest can be rapidly cloned into convenient expression cassettes and cotransformed with the selection plasmid. So far, homologous recombination has not been detected, and the introduced DNA is integrated into the genome through heterologous recombination. High rates of tandemly integrated plasmids are often observed with the PEG-liposome method. Overall, the rates of transformation remain limited for large-scale applications; however, a single transformation experiment with *P. infestans* can lead to as many as 200 independent transformants. Several species, such as *P. infestans*, *P. sojae*, *P. palmivora*, *Phytophthora parasitica*, *Pythium aphanidermatum*, and *S. monoica*, have been transformed using the PEG-liposome protoplast protocol (5, 20, 31, 35, 65, 108; J. J. Weiland, Abstr. 21st Fungal Genet. Conf., abstr. 284, 2001).

Alternative transformation protocols, based on microprojectile bombardment (C. Cvitanich and H. Judelson, Abstr. 21st Fungal Genet. Conf., abstr. 343, 2001), *Agrobacterium tumefaciens* (I. Vijn and F. Govers, Abstr. 10th Int. Congr. Mol. Plant-Microbe Interact., abstr. 283, 2001), or electroporation of zoospores (B. Tyler, personal communication), have been reported recently and should offer enhanced opportunities for genetic analyses of *Phytophthora*.

Reporter genes. Several reporter genes, including those encoding β-glucuronidase (GUS) and the green fluorescent protein (GFP), have been used successfully in *Phytophthora* (5, 32, 108). *Phytophthora* transformants expressing the GUS reporter gene have been used to monitor disease progression in planta,

to evaluate disease resistance, to study promoter expression, and to visualize morphological structures during development (32, 45, 106). For example, a transgenic *P. infestans* strain containing a transcriptional fusion between the promoter of the plant-induced *ipiO* gene and GUS proved useful in determining spatial patterns of expression of the *ipiO* promoter during infection of potatoes (106).

Gene silencing. Classical gene disruption approaches have not been successful with *Phytophthora*, since homologous recombination occurs at very low levels. Therefore, attempts at targeted gene knockout have centered on the gene-silencing strategies that have proven successful in plants. Gene silencing has now been applied to several *Phytophthora* species to generate strains deficient in particular gene products (20, 44, 107; M. Latijnhouwers, A. Laxalt, T. Munnik, and F. Govers, Abstr. 21st Fungal Genet. Conf., abstr. 9, 2001). Following transformation of *P. infestans* with sense, antisense, and promoterless constructs of the endogenous single-locus *inf1* gene, silencing of *inf1* was observed in as many as 20% of the transformants (107). Silencing was accompanied by the complete absence of *inf1* mRNA and INF1 protein and proved stable over repeated vegetative cultures of the pathogen both in vitro and in products (44, 107). The mechanism of gene silencing is largely unknown; however, nuclear run-on assays have indicated that silencing is regulated at the transcriptional level (107). No hypermethylation was observed in either transgenic or endogenous sequences of *inf1* (107), but alterations in heterochromatin formation in regions surrounding silenced genes have been noted recently (P. van West, personal communication). Overall, it is likely that the gene silencing observed in *P. infestans* occurs via mechanisms similar to the RNAi phenomenon reported for numerous eukaryotes. Several groups are currently testing whether constructs that generate double-stranded RNA could be used to increase the frequency and efficiency of gene silencing in *Phytophthora*.

Due to the stability and total efficacy of the silencing phenomenon, functional analyses can be performed with the silenced strains (20, 44). There are a number of advantages in using gene silencing for functional analyses. For example, promoterless cDNA clones can be used without modifications to generate silenced strains (107), suggesting that pools of cDNA clones could be assayed rapidly and simultaneously. Also, since
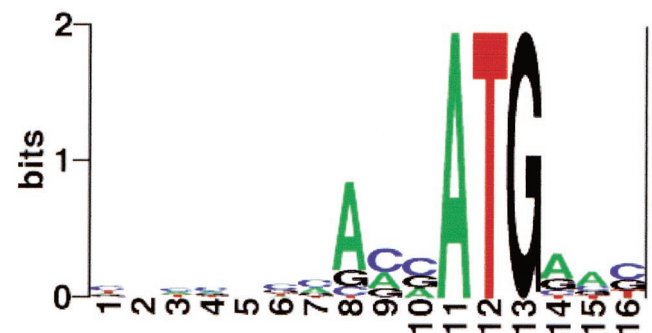

FIG. 3. Sequence conservation around translational start codons of *Phytophthora*. Consensus sequences surrounding the ATG start codon (−10 to +3) were calculated based on 50 gene sequences (GenBank release 113.0) at http://www.bio.cam.ac.uk/seqlogo.
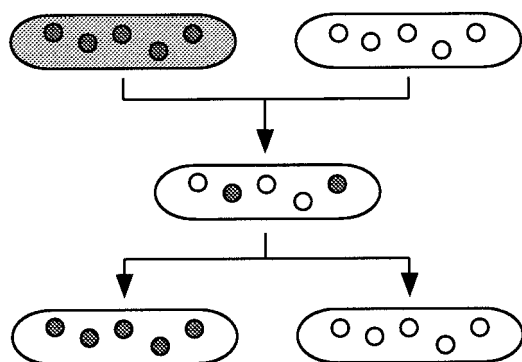
FIG. 4. Schematic illustration of internuclear transfer of gene silencing in *Phytophthora* (107). Protoplast fusion of an antisense transgenically silenced strain (unshaded nuclei, unshaded cytoplasm) and a wild-type strain (shaded nuclei, shaded cytoplasm) resulted in heterokaryotic strains (mixed unshaded and shaded nuclei) that exhibit gene silencing (unshaded cytoplasm). Nuclear separation using single zoospore cultures derived from the heterokaryotic strains resulted in silenced strains (unshaded cytoplasm) independently of the presence of the transgene.

gene silencing is known to affect sequences with similarities higher than 80% (DNA:DNA), this technique offers the opportunity to simultaneously silence several members of a closely conserved gene family.

Transfer of gene silencing. An interesting phenomenon is the so-called internuclear transfer of gene silencing (Fig. 4). Efficient silencing of the *inf1* gene was manifested in heterokaryotic mycelia, obtained after protoplast fusion of a transgenically silenced strain and a nonsilenced strain (107). This observation suggests the involvement of a *trans*-nuclear silencing factor and rules out DNA-DNA interactions as the basis for gene silencing. Furthermore, homokaryotic wild-type strains, obtained following nuclear separation of silenced heterokaryotic strains, displayed stable gene silencing, indicating that the presence of nuclear transgenic sequences was not essential to ensure and maintain silencing of the endogenous *inf1* gene. Apparently an internuclear process, perhaps based on a *trans*-acting silencing factor, is responsible for spreading of gene silencing in heterokaryotic *P. infestans* strains (107). The easy transfer of gene silencing from one genetic background to another by passage through a heterokaryon should prove a very useful technology for constructing strains in which multiple genes are silenced. In addition, engineering silencing in a limited number of nuclei of a hypha might be sufficient to ultimately silence the entire mycelium.

Genetic maps. Several resources have been developed that facilitate analyses of the *Phytophthora* genome, including genetic maps for *P. infestans* and *P. sojae* (103, 115). A detailed genetic map of *P. infestans* based on amplified fragment length polymorphism (AFLP) markers has been published (103). The data were generated from 73 F_1 progeny from a cross between two homokaryotic and diploid isolates of *P. infestans*. A total of 183 AFLP markers, 7 restriction fragment length polymorphism (RFLP) markers, and the mating type locus were mapped into 10 major and 7 minor linkage groups covering a total of 827 centimorgans (cM). A linkage map of *P. sojae* was constructed using 106 F_2 individuals from two crosses (115). The map was based on 22 RFLP markers, 228 randomly amplified polymorphic DNA (RAPD) markers, and 7 avirulence genes, and it covered 10 major and 12 minor linkage groups for a total of 830.5 cM. Recently, May et al. (59) updated the *P. sojae* genetic map by analyzing 106 F_2 individuals from two additional crosses. The resulting integrated map consisted of 35 RFLP, 236 RAPD, and 105 AFLP markers and 10 avirulence genes (59).

Several avirulence genes, involved in triggering genotype-specific resistance, have been mapped in the *P. infestans* and *P. sojae* maps, and their cloning is under progress (53, 59, 100, 104, 105, 116). Highly representative bacterial artificial chromosome (BAC) libraries are also available and have facilitated the physical mapping of *Phytophthora* genomes (80, 116).

Genomic resources. Structural genomic projects are well under way for *P. infestans*, *P. sojae*, and *P. brassicae* (4, 40, 42, 50, 78, 99, 113) (Table 2). The resources generated through these projects consist mainly of tens of thousands of ESTs from a variety of developmental and infection stages. Genome sequencing of *Phytophthora* remains limited but is expected to accelerate significantly in the coming years, since funding for shotgun sequencing of *P. sojae* has been secured (B. Tyler, personal communication).

With the availability of large EST data sets, the community investigating *Phytophthora* has embarked on functional genomic analyses (4, 40, 79). For example, computational tools and algorithms have been developed to mine sequence data sets for genes predicted to function in virulence and host-specificity or to form targets for chemical control (40). This includes an algorithm, PexFinder, that identifies genes encoding extracellular proteins from ESTs (T. Torto, A. Styer, and S. Kamoun, submitted for publication; http://www.oardc.ohio-state.edu /phytophthora/pexfinder). Rapid assays for testing *Phytophthora* genes in planta for virulence function are well established and are being applied to validate candidate genes (40, 41, 79). A database, the *Phytophthora* Functional Genomics Database (PFGD), that combines functional assays and expression data with transcript analysis and annotation has recently been funded by the National Science Foundation Plant Genome Program and will be available online at http://www .pfgd.org (M. E. Waugh, personal communication).

TABLE 2. Current resources for oomycete genomics

Species	Host(s)	Reference(s)	URL
Phytophthora infestans	Potato and tomato	Syngenta *Phytophthora* Consortium (50), *Phytophthora* Genome Consortium (113)	http://www.ncgr.org/programs https://xgi.ncgr.org/pgc
Phytophthora sojae	Soybean	*Phytophthora* Genome Consortium (113)	https://xgi.ncgr.org/pgc
Phytophthora brassicae[a]	*Arabidopsis thaliana*	Syngenta *Phytophthora* Consortium (50)	http://www.ncgr.org/programs

[a] This species was previously known as *Phytophthora porri*.

MECHANISMS OF INFECTION BY *PHYTOPHTHORA*

Several key cellular events, such as adhesion to the host surface, penetration, and colonization of host tissue, take place during plant infection by oomycetes. Oomycete species can also manipulate biochemical and physiological processes in their host plants through a diverse array of virulence or avirulence molecules, known as effectors. In susceptible plants, these effectors promote infection by suppressing defense responses, enhancing susceptibility, or inducing disease symptoms. Alternatively, in resistant plants, effectors are recognized by the products of plant resistance genes, resulting in host cell death and effective defense responses known as the hypersensitive response (HR).

Adhesion, penetration, and colonization of host tissue. In *Phytophthora*, infection generally starts when motile zoospores released from sporangia reach a leaf or root surface, encyst, and germinate (26, 43). Adhesion of cysts to plant surfaces occurs rapidly following zoospore encystment (26, 27). The cysts germinate, and the germ tubes swell to form appressoria or appressorium-like structures that facilitate adhesion and penetration of plant surfaces. In root-infecting species, penetration can occur in between cells without the aid of an appressorium (26).

Little is known about the developmental processes that lead to appressorium formation. Hardham (26) proposed that appressorium formation may result from the difficulty that germlings experience in attempting to penetrate plant surfaces. Some members of a small gene family, *Car*, encode extracellular mucin-like proteins and are up-regulated in germinating cysts and appressoria shortly before penetration of plant tissue; these could function in adhesion (23). Recently, CBEL, a cellulose binding protein of *P. parasitica*, was shown to be essential in adhesion to cellulosic substrates (20). *P. parasitica* strains in which the *CBEL* gene was silenced were impaired in their ability to attach to cellophane membranes but remained able to infect tobacco plants (20). CBEL may also play a role in signaling, since the purified protein induces defense responses in tobacco plants (111).

Penetration and colonization of host tissue involves the secretion of a range of degradative enzymes that break down physical barriers to infection (60). Several ESTs with significant similarity to degradative enzymes such as cutinases, proteases, endo- and exoglucanases, and chitinases have been identified (42, 61, 78; T. Torto and S. Kamoun, unpublished data). A few *Phytophthora* genes encoding degradative enzymes have been characterized in detail, including genes encoding phospholipases (67), a β-glucosidase/β-xylosidase (6), exo-1,3-β-glucanases (61), an endo-1,3-β-glucanase (61), and endopolygalacturonases (endo-PGs) (24, 98). The endo-PG family is remarkable in many respects. In *P. cinnamomi*, endo-PGs form a major family with at least 19 members (24). Birth-and-death evolution, reticulate evolution, and diversifying selection have been detected in *P. cinnamomi* endo-PGs and may have contributed to the evolution of this structurally diverse and complex family (24). Phylogenetic analyses have indicated that *Phytophthora* endo-PGs are more similar to fungal endo-PGs than to their plant and bacterial counterparts (24, 98). Similar phylogenetic affinity to fungal sequences was also observed for exo-1,3-β-glucanases and an endo-1,3-β-glucanase

from *P. infestans* (61). The phylogenies of these enzymes are unexpected and contrast with phylogenies obtained using ribosomal sequences or compiled protein sequences from mitochondrial and housekeeping chromosomal genes (2, 51, 72, 102). These exceptional phylogenies could reflect convergent evolution through which phylogenetically distinct enzymes evolved to share significant similarity, perhaps by targeting similar substrates. On the other hand, horizontal gene transfer events may have taken place in which plant pathogenic oomycetes acquired endo-PG and glucanase genes from fungi.

Suppression of host defense responses. *Phytophthora* effectors that suppress host defense responses have been described in several pathosystems (25, 87, 118). Suppression of host defenses can occur through the production of inhibitory proteins that target host enzymes. Recently, genes encoding secreted proteins that inhibit soybean endo-β-1,3-glucanase have been cloned from *P. sojae* (85). These proteins, termed glucanase inhibitor proteins (GIPs), show significant structural similarity to the trypsin class of serine proteases but bear mutated catalytic residues and are proteolytically nonfunctional as a consequence (85). GIPs are thought to function as counterdefensive molecules that inhibit the degradation of β-1,3- and β-1,6-glucans in the pathogen cell wall and/or the release of defense-eliciting oligosaccharides by host endo-β-1,3-glucanases (85). Another class of secreted inhibitory proteins, containing one to three domains of Kazal-type serine protease inhibitors, was identified by data mining of *Phytophthora* ESTs. One of these proteins, EPI1 from *P. infestans*, was found to inhibit and interact with tomato proteases, suggesting a novel type of defense-counterdefense cross talk between plants and *Phytophthora* (M. Tian and S. Kamoun, unpublished data).

Induction of defense responses and disease-like symptoms. Several *Phytophthora* effector molecules are known to induce a variety of cellular defense responses in plants (15, 20, 46, 69, 70, 79, 86, 111). Some of these effectors induce defense responses in both susceptible and resistant plants and are referred to as general elicitors. Based on similarity to self and non-self recognition models of the animal innate immune system, these general elicitors have been likened to pathogen-associated molecular patterns (PAMPs), which are surface-derived molecules that induce the expression of defense response genes and the production of antimicrobial compounds in host cells (21, 68). However, in many cases the contribution of general elicitors to the infection process remains ambiguous due to the lack of genetic evidence. For example, orthologous proteins from *P. parasitica* (NPP1) and *P. sojae* (PsojNIP) have recently been reported to induce necrosis on all tested dicotyledonous plants, including host plants (15, 79). The NPP1 and PsojNIP proteins fulfill many criteria for functioning as PAMPs, including the occurrence of functional orthologues in the oomycete *Pythium aphanidermatum*, as well as in many species of pathogenic filamentous fungi and bacteria, but the absence of similar sequences in plant genomes (15, 79, 109). Nevertheless, in *P. sojae*, the *PsojNIP* gene was expressed late in the infection of soybeans and thus may function as a toxin by facilitating colonization of host tissue during the necrotrophic phase of growth (79).

Other effectors induce defense responses specifically in resistant plants and are known as specific elicitors. For example, *P. infestans*, as well as other *Phytophthora* species, produces

10-kDa extracellular proteins, known as elicitins, which induce the HR and other biochemical changes associated with defense responses in tobacco species but not in potatoes or tomatoes (46, 77). Tobacco is resistant to most *Phytophthora* species, and recognition of elicitins is thought to be one component of this resistance. Direct evidence for this model was obtained using *P. infestans* strains engineered by gene silencing to be deficient in the elicitin INF1. These strains induced disease lesions on the wild tobacco species *Nicotiana benthamiana*, suggesting that the elicitin INF1 conditions avirulence for this plant species (46). Some strains of *P. parasitica* are known to naturally infect tobacco and cause black shank disease. These strains evade the defense surveillance system of tobacco either by not producing elicitins or by exhibiting down-regulation of elicitin genes in-planta (11, 47).

OUTLOOK

Oomycetes evolved the ability to infect plants and animals independently of other eukaryotic microbes, and they have probably developed unique mechanisms of pathogenicity. With the availability of a respectable molecular toolbox and a multitude of gene sequences, significant progress has been made in understanding the molecular basis of infection by oomycetes. However, most of our knowledge remains limited to economically important species in the *Phytophthora* genus, and little is known about infection by other plant- or animal-pathogenic oomycetes. Future research should exploit emerging information about *Phytophthora* genetics to ask pertinent questions about oomycete pathology and evolution. Are there common mechanisms of infection among filamentous microbes? How did the effector gene arsenal emerge and evolve? To what extent do these effectors vary between plant- and animal-pathogenic oomycetes? How did coevolution with host species shape the structure of effector genes? The increased availability of genome sequences will offer unique opportunities to address these questions and perform genomic comparisons among pathogenic oomycetes and between oomycetes and other eukaryotic microbes. Such studies will improve our overall understanding of the evolution of parasitic and pathogenic lifestyles in eukaryotes.

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