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CURRENT PERSPECTIVE ESSAY
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Insights from Sequencing Fungal and Oomycete Genomes: What Can We Learn about Plant Disease and the Evolution of Pathogenicity?

Fungi and oomycetes are the causal agents of many of the world's most serious plant diseases and are unique among the microbial pathogens in being able to breach the intact surfaces of host plants, rapidly establishing infections that can have disastrous consequences for large-scale agricultural production. The ability to cause plant disease is not a common trait among the many saprotrophic and mutualistic fungal species, but it is a very widespread one, occurring throughout the fungal kingdom (James et al., 2006). Recently, there have been a number of studies published describing the genome sequences of a diverse set of fungi and oomycetes (Table 1), including one published in this issue of *The Plant Cell* (Hane et al., 2007), and this provides an opportunity to review what we have learned so far from sequencing the genomes of pathogenic and free-living fungi and also to look forward to the mass of genome sequence information that is likely to be generated in the next few years. The deployment of low-cost, high-throughput DNA sequencing technologies and large-scale functional genomics to eukaryotic plant pathogens will provide new insight into their biology and into the evolution of pathogenicity.

THE RAPID PACE OF FUNGAL GENOME SEQUENCING

There are currently genome sequences from 12 plant pathogenic fungi and two oomycetes that are publicly available, out of a total of >40 fungal genomes that have now been sequenced (Table 1). A significantly higher number of projects is in progress, and the dramatic fall in the price of DNA sequencing, coupled with the new high-throughput sequencing technologies, strongly suggest that there will soon be many more sequences, both of new species and of isolates of the same species, supplying information regarding gene inventory conservation, pathogenic adaptation, evolutionary history, and allelic variability of virulence-associated genes. Here, we have brought together some observations based on analysis of the fungal and oomycete genomes that have been reported so far. We set out to explore the evolution of phytopathogenicity in microbial eukaryotes and the features present within predicted gene inventories that are common to plant pathogenic species and that may be fundamental to their biology.

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EVOLUTIONARY GENOMICS OF THE EUKARYOTIC PHYTOPATHOGENS

With increased genome sampling from a range of fungal species, it is now becoming possible to resolve a fungal phylogeny using multiple concatenated gene alignments (Fitzpatrick et al., 2006; James et al., 2006). Strikingly, this approach consistently has shown that nutritional modes of life and cellular characteristics are a mosaic across the fungal phylogeny. For example, fungi that form spores with flagella (chytrids) are not monophyletic and do not form a single unified branch, suggesting that loss of the cilium/flagellum occurred at least four times throughout the kingdom fungi (James et al., 2006). Furthermore, the yeast and filamentous mode of growth and the ability to parasitize plant tissue are both interdispersed traits across the fungal kingdom. Comparison of 42 fungal taxa focusing mainly on ascomycetes (Fitzpatrick et al., 2006) and extended to represent the diversity of the eukaryotes suggests that plant pathogenic mechanisms rose separately on at least five occasions within the fungi and a total of seven times across the eukaryotes, as shown schematically in Figure 1. Of course, increased genome and taxon sampling may alter the model presented, but clearly the rise of phytopathogenesis has involved multiple phenotype acquisitions and a complex evolutionary history, suggesting that even within relatively closely related ascomycete groups, pathogenic and saprotrophic organisms are likely to have many key differences. Recent work by Wapinski et al. (2007) defined the appearance, duplication, and loss of genes across the ascomycetes and also demonstrated radical differences across the fungi, including numerous acquisitions and losses occurring among the ascomycete phylogeny. The Pezizomycota, which include many phytopathogenic fungal species, was only represented by four genomes in the study, two of which were phytopathogens, yet this analysis suggested that *Magnaporthe grisea* has gained 3240 novel genes and has undergone 193 unique duplications and 1860 unique gene losses. The other phytopathogen sampled, *Fusarium graminearum*, had a similar strikingly novel gene complement compared with its sister ascomycetes, including 2513 novel genes, 305 unique duplications, and 711 unique losses (Wapinski et al., 2007). Comparative genome analysis of the *Stagonospora* genome (Hane et al., 2007), when considered with the genomic comparisons discussed above, and the differential complements of genes across the ascomycete phylogeny, suggest that

Table 1. Publicly Available Phytopathogenic Fungal and Oomycete Genome Sequences

Pathogen Species[a]	URL
Ascomycota	
Leotiomycetes	
Botrytis cinerea (Grape/other host grey rot)	http://www.broad.mit.edu/annotation/genome/botrytis_cinerea/Home.html
Sclerotinia sclerotiorum (Multihost rot diseases)	http://www.broad.mit.edu/annotation/genome/sclerotinia_sclerotiorum/Home.html
Sordariomycetes	
F. graminearum (Wheat/barley head blight)	http://www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html
Fusarium oxysporum (Multi-host wilt disease)	http://www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html
Fusarium verticillioides (Maize seed rot)	http://www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html
Magnaporthe grisea (Rice blast disease)	http://www.broad.mit.edu/annotation/genome/magnaporthe_grisea/Home.html
Nectria haematococca (Pea wilt)	http://genome.jgi-psf.org/Necha2/Necha2.home.html
Dothideomycetes	
Mycosphaerella fijiensis (Banana black leaf streak)	http://genome.jgi-psf.org/Mycfi1/Mycfi1.home.html
Mycosphaerella graminicola (Wheat leaf blotch)	http://genome.jgi-psf.org/Mycgr1/Mycgr1.home.html
Stagonospora nodorum (Wheat glume blotch)	http://www.broad.mit.edu/annotation/genome/stagonospora_nodorum/Home.html http://wwwacnfp.murdoch.edu.au/Mission.htm
Heterokontophyta	
Oomycetes	
P. ramorum (Sudden oak death)	http://genome.jgi-psf.org/Phyra1_1/Phyra1_1.home.html
P. sojae (Soybean stem/root rot)	http://genome.jgi-psf.org/Physo1_1/Physo1_1.home.html
Basidiomycota	
Pucciniomycetes	
Puccinia graminis (Cereal rusts)	http://www.broad.mit.edu/annotation/genome/puccinia_graminis
Ustilaginomycetes	
U. maydis (Corn smut disease)	http://www.broad.mit.edu/annotation/genome/ustilago_maydis/Home.html

[a] Species are grouped by phylum and class. The most common or most widely recognized disease associated with each species is listed in parenthesis below the species name.

the rise of ascomycete phytopathogenesis has involved radically different evolutionary genomic changes. Increased genome sampling is therefore required to fully understand the nature and evolutionary background of phytopathogenic genomic innovations.

Phytopathogenesis is not a lifestyle limited to the fungi, however, as there are two other major groups of eukaryotic phytopathogenic microbes branching in very different places in the eukaryotic phylogeny (Figure 1). The oomycetes (such as *Phytophthora* sp and *Hyaloperonospora*) group along with the brown algae and the diatoms within the heterokonts (stramenopiles), while the plasmodiophorids (e.g., *Plasmodiophora brassicae*, which cause a range of plant diseases, including clubroot of brassicas) group together with the heterotrophic cercozoan protozoa within the Rhizaria (Cavalier-Smith and Chao, 2003; Simpson and Roger, 2004). It has recently been demonstrated that lateral gene transfers have occurred between the fungi and the oomycetes (Richards et al., 2006). Based upon the predicted function of these lateral gene transfers, it seems likely that they were important for the acquisition of osmotrophy and perhaps pathogenesis. For example, one strong candidate for gene transfer from the fungal lineage to the oomycetes encodes a putative protocatechuate, 3,4-dioxygenase β-subunit (*PcaH*), a key functional component of the β-ketoadipate pathway, which would provide a means to use aromatic compounds from the environment. Such acquisitions may have been important for the origin of the oomycete plant pathogens that descended from a phototrophic ancestor bearing a plastid acquired from the endosymbiosis of a red algae (Tyler et al., 2006). The study by Richards et al. (2006) demonstrates that distantly related plant pathogens may have striking genetic similarities within an unrelated genomic background. Horizontal gene transfer, however, continues to impact the host range and virulence of pathogens. Ability to synthesize the ToxA host-specific toxin, for example, was transferred laterally from *Stagonospora nodorum* to the wheat tan spot fungus *Pyrenophora tritici-repentis* at some point in the recent past, prior to 1941, allowing tan spot to emerge as a significant disease on wheat cultivars containing the *Tsn1* gene (Friesen et al., 2006).

SYNTENY

The availability of an increasing number of fungal genome sequences has allowed comparisons of gene order or synteny between different species. Generally the large degree of evolutionary distance between the species where genomes have been sequenced has meant that no significant synteny, or gene order conservation, has been detected between species of

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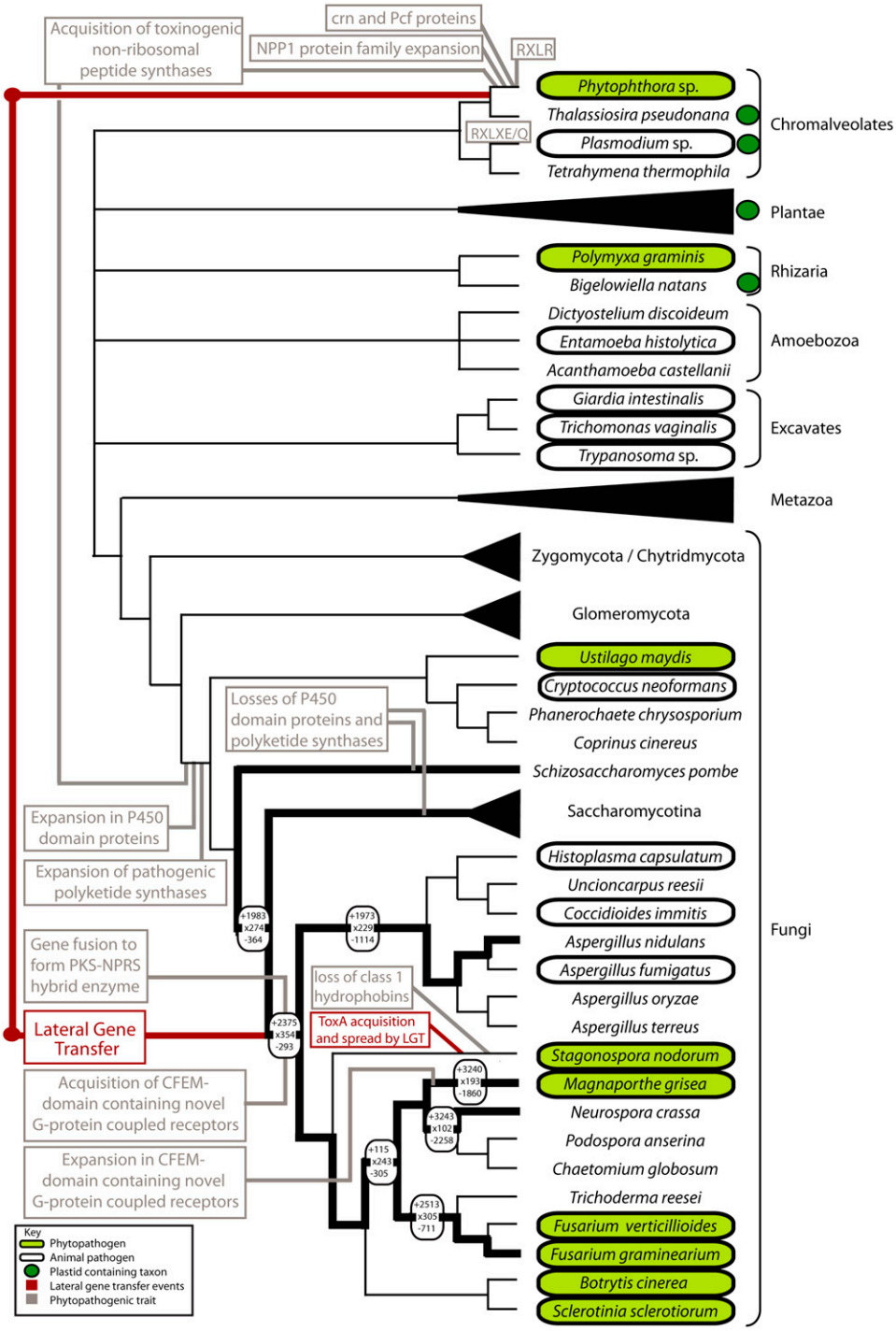

Figure 1. Schematic Representation of a Cladogram of the Fungi Showing a Selection of Pathogenicity-Associated Traits.

The cladogram shown is reinterpreted from Fitzpatrick et al. (2006) with additional eukaryotic branches attached to represent the diversity of the eukaryotes where representative genome projects are available or under analysis. Branches with thick black lines are analyses from the Wapinski et al. (2007) study of gene gains, losses, and duplications in the kingdom Fungi. Values of gain, loss, and duplication from this study are illustrated in boxes on

a different genus (Dean et al., 2005; Hane et al., 2007). Comparisons between three species of *Aspergillus* have shown that a majority of each genome could be mapped to conserved syntenic blocks in at least one other genome, but there have been extensive rearrangements (Galagan et al., 2005). There are also large regions lacking synteny, particularly subtelomeric regions, which in the aspergilli are enriched for secondary metabolite biosynthetic genes, thought to play a role in niche adaptation and opportunistic pathogenicity. It can be hypothesized that rapid rearrangement of such subtelomeric regions might facilitate species-specific evolution of these genes. There are some examples of small-scale synteny between fungal species. For example, the quinate cluster consists of seven coregulated genes that control the catabolism of quinate (Giles et al., 1991). This cluster is widespread amongst filamentous ascomcyetes. Although the clustering of these genes is conserved amongst filamentous ascomycetes, there is no conservation of gene order or orientation (Dean et al., 2005; Hane et al., 2007).

It is clear that with increased genomic data we will be able to pinpoint true lineage-specific pathogenic innovations and broader pathogenic mechanisms conserved among many species, to track the rise and spread of key pathogenic factors (such as effector proteins), and to identify both specific and general targets for disease intervention. Some examples of characteristics of the gene inventories of phytopathogenic fungi and oomycetes are described in the following sections.

CELL SURFACE RECEPTORS

To successfully infect a host plant, plant pathogenic fungi and oomycetes must make appropriate responses to a variety of environmental cues, including the chemical and physical characteristics of the host plant surface. This is achieved via cell surface receptors that respond to external stimuli and relay that information into the cell. The genomes of animals contain large numbers of G-protein–coupled receptors (GPCRs), which are characterized by having seven transmembrane helices. They bind exogenous ligands and relay signals into the cell by activating intracellular signaling pathways via conformational changes in G-proteins (Hamm, 1998). The genomes of the model ascomycete yeast species *Saccharomyces cerevisiae* and *Schizosaccharomyce pombe* contain only three and four GPCRs, respectively. Analysis of the genomes of filamentous phytopathogenic ascomycete fungi, however, reveals a significant number of additional putative GPCRs, as many as 61 in the rice blast fungus *M. grisea* and 84 in the head blight pathogen *F. graminearum* (Dean et al., 2005; Cuomo et al., 2007). Furthermore, a novel class of putative GPCRs has only been found so far in the genomes of filamentous ascomycetes (Kulkarni et al., 2005). This group of putative receptors includes Pth11, a plasma membrane protein that activates appressorium differentiation in response to inductive surface cues in *M. grisea* (DeZwaan et al., 1999). The Pth11 protein contains an extracellular epidermal growth factor (EGF)–like domain at the N terminus followed by seven transmembrane domains. There are 12 putative GPCRs that contain the extracellular EGF-like domain (known as the CFEM domain) in the *M. grisea* genome. Comparative genome analysis reveals that there has been an expansion in CFEM domain–containing receptor-encoding genes in the genome of *M. grisea* when compared with the nonpathogenic ascomycete fungi *Neurospora crassa* and *Aspergillus nidulans*, as shown in Figure 1. Analysis of the genome of the Dothideomycete wheat pathogen *S. nodurum* reveals only three Pth11-like proteins in contrast with other ascomycete pathogens. Two of these putative GPCR genes were studied further and did not have a virulence-associated phenotype when corresponding deletion mutant strains were created (Hane et al., 2007), suggesting diversification and redundancy in GPCR function among the fungi. Interestingly, the genome of *M. grisea* also contains six genes that code for receptors similar to a cAMP receptor from the amoebozoan *Dictyostelium discoideum* (Dean et al., 2005), suggesting the capacity to respond to an exogenous cAMP signal. Three similar genes are found in the saprotrophic fungus *N. crassa*, indicating that this may be a conserved character among sordariomycetes (Galagan et al., 2003). Interestingly, the genomes of the oomycete phytopathogens *Phytophthora sojae* and *Phytophthora ramorum*, which contain 24 putative GPCR genes, also contain four homologs of the *Dictyostelium* cAMP receptor in addition to 12 putative GPCRs, each with a C-terminal intracellular phosphatidylinositol-4-phosphate 5-kinase domain similar to RpkA from *Dictyostelium*, which might allow GPCR-mediated signal transduction through phosphoinositide second-messenger synthesis rather than via heterotrimeric G-proteins (Tyler et al., 2006).

In *M. grisea*, Pth11 is thought to act upstream of a cAMP-dependent signaling pathway that is required for infection-related development (DeZwaan et al., 1999). The cAMP response pathway operates across the fungal kingdom, including within *S. cerevisiae*. However, in *S. cerevisiae*, protein kinase A acts downstream of the glucose-sensing Gpr1 GPCR (Rolland et al., 2001) and the genome of this yeast contains no Pth11 homologs.

Figure 1. (continued).

the relevant branches (+, gain; x, duplication; −, loss). Boxes show pathogenicity-associated traits identified empirically or by genome analysis and their expansion and distribution among fungal and oomycete species. Some traits have been acquired, for example, such as presence of polyketide synthases, early in evolution of these microbes, subsequently expanded in pathogenic and some saprotrophic organisms but lost from groups such as the hemiascomycete yeasts. The key shows the diagrammatic conventions.

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Thus, pathogenic fungi, such as *M. grisea*, have acquired the ability to respond to a variety of environmental cues using novel classes of receptors that activate conserved intracellular signaling pathways, giving new triggers to old weapon systems.

SECRETED PROTEINS

Fungi and oomycetes, although phylogenetically very distantly related, are both osmotrophic microorganisms. This means that they live by secreting into the external environment enzymes that degrade polymers encountered there, such as cellulose, lipid, protein, and lignin, and transport the resulting simple sugars, amino acids, and fatty acids into the growing cell for use. Therefore, to a large degree the ecological niche that a fungus or oomycete occupies is defined by the products that it secretes, be they small molecules such as toxins, proteinaceous effectors to perturb host signaling or metabolism, or the array of hydrolytic enzymes that digest extracellular macromolecules into smaller subunits that the fungus can use. The secretome is defined as the set of soluble secreted proteins encoded by a genome. The secretome for a fungal species is usually predicted by analyzing the set of translated gene sequences using bioinformatics tools, such as SignalP 3.0 (Bendtsen et al., 2004), which detects the presence of a signal peptide in the N terminus of a protein, or WoLF PSORT (Horton et al., 2007), which uses a variety of known signal motifs as models to predict the subcellular localization of a protein. However, these tools rely on correct prediction of gene structure (especially the 5′-end) and have generally been trained on secreted proteins from the model yeast *S. cerevisiae*, such that the resulting predicted secretome may not be very accurate in the absence of direct experimental proof of secretion. Nevertheless, secretome sets based on genome sequence have been predicted for a number of fungal species, although the different methodologies applied for each species means that comparisons must be treated with caution.

It is clear that a diverse array of proteolytic and carbohydrate-degrading enzymes is secreted by numerous fungi, reflecting the variety of nutritional sources they can use. The various ecological niches occupied by different fungal species are reflected in the gene families present in the secretome. For example, the white-rot basidiomycete fungus *Phanerochaete chrysosporium* secretes enzymes involved in lignin degradation, such as lignin and manganese-dependent peroxidases, copper radical oxidases, extracellular FAD oxidases, and multicopper oxidases (Martinez et al., 2004). The secretome of *Aspergillus oryzae*, a fungus used in the production of traditional Japanese fermentation foods such as soy sauce, contains large numbers of α-amylases, pectinolytic enzymes, and proteases (Machida et al., 2005). These enzymes are essential for the ability of the fungus to ferment rice and soybean products. Many species of plant-pathogenic fungi secrete a battery of glycoside hydrolases, polysaccharide lyases, and esterases, which the fungi use to degrade the host-plant cell wall for nutrition and in some cases to aid entry into plant tissue. Many saprophytic fungi gain nutrition from decaying plant material and therefore also secrete cell wall degrading enzymes. The total numbers of plant cell wall–degrading enzymes secreted by a group of sequenced fungal species are summarized in Table 2. From this relatively limited sample and crude comparison, there seems no obvious correlation between the ability to infect plants and the number of cell wall–degrading enzymes present within the genome. Saprophytic species, such as *A. nidulans*, produce more glycosyl hydrolases and polysaccharide lyases than any of the phytopathogenic species. Notably, the biotrophic phytopathogen *Ustilago maydis* produces considerably fewer cell wall–degrading enzymes than either *M. grisea* or *F. graminearum*. This is consistent with the necessity of minimizing plant cell wall damage (and the release of cell wall fragments that may trigger plant defense responses) for the biotrophic lifestyle of *U. maydis*. Although *M. grisea* has a clear and extended biotrophic stage of development (Kankanala et al., 2007), it is likely that the formation of necrotic lesions and conidiogenesis is essentially a necrotrophic stage of development that require the action of cell wall hydrolases.

EFFECTORS

Infection of the host plant by biotrophic and hemibiotrophic fungi and oomycetes also requires the secretion of protein effectors that suppress plant defenses and alter cellular metabolism to suit the needs of the invading pathogen. The delivery of such proteins to the plant cell is not trivial and requires traversal of the plant plasma membrane. Bacterial phytopathogens deliver effector proteins into host cells via a specialized type III secretion system (Alfano and Collmer, 2004; Grant et al., 2006). A large group of potential effectors have been found in the genomes of the oomycete pathogens *P. sojae* and *P. ramorum* (~350 in each genome) (Tyler et al., 2006). These proteins share a putative host cell targeting motif with effector proteins encoded by four known avirulence genes cloned from

Table 2. Number of Plant Cell Wall Degrading Enzymes Secreted by Fungal Species (Kämper et al., 2006)

Species	Glycosyl Hydrolases	Polysaccharide Lyases	Carbohydrate Esterases
A. nidulans	128	19	8
Coprinus cinereus	42	3	4
Cryptococcus neoformans	21	1	2
F. graminearum	80	16	17
M. grisea	121	4	13
N. crassa	71	3	9
P. chrysosporium	50	0	1
S. cerevisiae	12	0	1
S. pombe	5	0	0
U. maydis	30	1	2

P. sojae, *Phytophthora infestans*, and the oomycete obligate biotroph *Hyaloperonospora parasitica* that are recognized in the host cytoplasm by intracellular resistance gene products (and therefore must be delivered into plant cells). The protein sequences of these effectors all share an RXLR EER motif at their N terminus. This is similar to the RXLXE/Q host cell targeting signal that is required for the translocation of proteins from the malaria parasite *Plasmodium falciparum* into host red blood cells (Figure 1). The RXLR EER motif appears to function as a signal that mediates trafficking of effector proteins into host cells (Birch et al., 2006; Tyler et al., 2006; Whisson et al., 2007), perhaps acting through a translocation protein in the plant plasma membrane or by stimulating endocytosis in a host plant cell at the haustorial interface (Talbot, 2007). With the availability of numerous genomes, it is now possible to study the evolutionary history of host cell targeting mechanisms by comparing the phylogeny of host effector proteins with the presence and absence of N-terminal motifs shown to mediate trafficking of effector proteins. This approach will define whether functional motifs, such as RXLR-EER motifs, are ancient and conserved eukaryotic protein properties and present in other heterokonts, alveolates, and Rhizaria or instead are the product of recent convergent evolution.

So far, RXLR EER motifs have not been reported in proteins secreted by fungal phytopathogens, and identifying a host cell targeting sequence in fungal effectors is a major goal for future analysis of fungal phytopathogen genomes. Nevertheless, groups of potential secreted effectors, without identifiable host cell targeting motifs, have been identified in the secretome of the rice blast fungus *M. grisea* (Dean et al., 2005). These include 21 proteins containing a novel variant of a chitin binding motif. Chitin binding proteins, such as Avr4 from *Cladosporium fulvum*, have been shown to protect the fungal cell wall from plant chitinases (van den Burg et al., 2006). Interestingly, this particular chitin binding domain-encoding gene only seems to have evolutionarily related genes present in animals; there seems to be no Avr4 homolog in any sequenced fungal genome so far sampled. There is also a family of novel proteins that may interact with rice GTP binding proteins and four proteins that are similar to the necrosis-inducing peptide (Npp1) of *P. infestans* (Fellbrich et al., 2002). However, homologs of this peptide are also found in the secretomes of saprophytes, such as *A. nidulans* (Tyler et al., 2006), but not in the basidiomycete biotroph *U. maydis*. The two sequenced oomycete genomes both contain a large family of Npp1-like proteins (40 members in *P. ramorum* and 29 in *P. sojae*). These oomycetes also secrete large numbers of protein toxins from other families, including Crn (crinkling and necrosis-inducing) and PcF proteins. By contrast, the genomes of *Phytophthora* species contain smaller numbers of cytochrome P450s and nonribosomal peptide synthases (NRPSs) than filamentous fungi and no polyketide synthases (PKSs; Tyler et al., 2006). This suggests that oomyctes may synthesize fewer toxic small molecules than fungi and instead rely more on protein toxins for necrotrophic growth.

The diversity of proteins found in the secretome is striking, and 70% of proteins secreted by *U. maydis*, for instance, cannot be assigned a function. Of these unknown proteins, two-thirds have no known homologs even among other fungi (Kämper et al., 2006). It is also notable that 18.6% of secreted *U. maydis* proteins are arranged in 12 gene clusters, and most of these are highly expressed in a coordinated manner within infected plant tissue. Deletion of five of these clusters resulted in changes in virulence, suggesting that many of these proteins are involved in pathogenicity (Kämper et al., 2006). Sequence comparisons between two *Phytophthora* species suggest that secreted proteins are evolving more rapidly than the proteome overall, because 17 and 11% of *P. sojae* and *P. ramorum* secreted proteins, respectively, are unique compared with 9 and 4% of proteins in the total proteome of the two species. The rapid diversification of the secretomes is also evident from the large number of proteins (77%) that occur in multigene families (Tyler et al., 2006) and the diversifying selection evident among the secreted RXLR effectors of oomycetes (Win et al., 2007).

A large number of secreted proteins from the glume blotch pathogen *S. nodorum* do not have homologs in any other sequenced fungi, providing more evidence for lineage-specific novel secretome diversity (Hane et al., 2007). This pattern is consistent with the large differences in gene loss, duplication, and acquisition across the fungi (Wapinski et al., 2007). As sequencing technology become more accessible, it is more likely that different strains of the same species (perhaps with different host ranges) will be sequenced. This is important because it will allow the identification of variations between strains, pinpointing genes that show a history of rapid variation, potentially indicative of host–pathogen coevolution and/or diversifying selection under this selective pressure (Win et al., 2007). Such genes are therefore candidates for further research because they are likely to encode proteins involved in interactions with the host plant. When coupled with large-scale gene functional analysis studies that are now possible in fungal pathogens (Jeon et al., 2007), this will allow rapid functional definition of effector-encoding genes.

SECONDARY METABOLIC PATHWAYS

Toxins are important virulence factors for a number of fungal diseases. Nonspecific toxins, such as the trichothecenes produced by *Fusarium* species, damage cells of a number of plant species, whereas host-specific toxins, such as the toxins produced by different pathotypes of *Alternaria alternata*, determine the host range of the fungus (Sweeney and Dobson, 1999). Some fungal toxins are small peptides (for example, the Npp1-like proteins referred to in the previous section), but many are secondary metabolites that are low molecular weight molecules with a variety of structures.

Necrotrophic pathogens use toxins to elicit plant cell death and derive nutrition from dead plant tissue (Howlett, 2006).

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Toxin biosynthetic pathways have been elucidated in a many fungal species, for example, the synthesis of sirodesmin in *Leptosphaeria maculans*, the blackleg pathogen of canola (Gardiner et al., 2004). A number of key enzyme families are involved in toxin biosynthesis (as well as other secondary metabolic pathways), including PKSs, NRPSs, and cytochrome P450 monooxygenases. Ascertaining the number of genes encoding enzymes from these families is useful in predicting the capacity of an organism to produce secondary metabolites (including toxins). Table 3 shows the number of genes encoding members of these families in the genomes of selected fungi and oomycetes. It is notable that there are relatively low numbers of genes encoding these enzymes in the genomes of the oomycete phytopathogens compared with the true fungi. As mentioned in the previous section, the *Phytophthora* species secrete a large variety of small toxic proteins and may well produce a smaller number of secondary metabolite toxins than phytopathogenic fungi. The biotroph *U. maydis* also has very few PKS- and cytochrome-P450–encoding genes in its genome. Although some saprophytic fungi have lower numbers of PKS- and NRPS-encoding genes in their genomes than the phytopathogens, the genome of the saprophyte *A. nidulans* has more PKS and NRPS-encoding genes than the pathogen *M. grisea*. The genome of *A. oryzae* contains 30 PKS-encoding genes (Machida et al., 2005). Phylogenomic analysis of PKS-encoding genes has shown a wide divergence between different fungal genomes, even in the same genus, reflecting perhaps the variety of different secondary metabolites that these species produce (Kroken et al., 2003). However, comparisons of the number of PKS and NRPS families between fungi reveals far more than the capacity of a fungus to produce toxins. This is because members of these enzyme families are involved in the synthesis of many other secondary metabolites with potential roles in fungal development or perhaps as effectors within plant cells, altering cell morphology and perturbing cell signaling. For example, fungal metabolites are able to perturb calcium signaling (cyclosporin; Horsburgh et al., 1980), sterol synthesis (lovastatin; Alberts, 1990), microtubule polymerization (taxol; Strobel et al., 1996), actin assembly and disassembly (cytochalasin [Athlin et al., 1988] and phalloidin [Cooper, 1987]), Golgi function (brefeldin A; Misumi et al., 1986), and a host of other cellular processes. Of course, some fungi additionally produce homologs of plant growth regulators, such as gibberellins, which were first discovered in *Gibberella fujikuroi* (Gordon, 1959), demonstrating the capacity of fungal metabolites to fulfill effector-type roles in host plants.

PKSs are also involved in the synthesis of a number of pigments, including melanin, and NRPSs are necessary for the synthesis of iron-chelating siderophores. The genome of the rice blast pathogen *M. grisea* also encodes a number of PKS-NRPS hybrid enzymes. One of these, Ace1, is involved in the synthesis of a secondary metabolite that is recognized by rice cultivars carrying the *Pi33* resistance gene and therefore acts, genetically, as an avirulence gene. *ACE1* is only expressed in appressoria during fungal penetration of rice leaves, consistent with an effector function for the secondary metabolic product during plant infection (Bohnert et al., 2004). The PKS-NRPS hybrid genes are composed of several unique protein domains. This gene and gene architecture are found in a number of filamentous ascomycetes but appear to be absent from the hemiascomycete yeasts (e.g., *Saccharomyces*) and the basidiomycetes (e.g., *Ustilago*), suggesting that the PKS-NRPS hybrid enzymes were the product of a unique gene fusion that occurred in a primitive branch of the filamentous ascomycete evolutionary tree (Figure 1).

Table 3. Number of PKS-, NRPS-, and Cytochrome P450–Encoding Genes in the Genomes of Selected Fungi and Oomycetes (Data on NRPS Not Available for *U. maydis*)

Species	PKS	NRPS	PKS-NRPS	Cytochrome P450
A. nidulans	27	13	1	102
M. grisea	23	6	8	115
N. crassa	7	3	0	39
P. chrysosporium	9	7	0	148
P. sojae	0	4	0	30
P. ramorum	0	4	0	24
S. nodorum	19	8	1	103
U. maydis	3	0	0	19

CONCLUSIONS

Pathogenicity is a complex phenotype in multicellular eukaryotic pathogens, such as fungi and oomycetes, because it encompasses the ability to develop infection structures, such as appressoria and haustoria, invade living plant tissue, and form propagules for further spread of the disease, in addition to the perturbation of host cell signaling and metabolism that characterizes bacterial pathogenesis (Grant et al., 2006). Analysis of fungal genome sequences to date has revealed a large capacity to respond to external environmental cues and secrete a diverse set of proteins. Identifying the subset of secreted proteins and metabolites that fulfill effector functions in plant cells is likely to be the principal area of focus for many plant pathologists in the forthcoming years, aided in the oomycetes by definition of a host cell targeting sequence. Using an evolutionary framework to compare the genomes of plant pathogenic eukaryotes provides a powerful means of identifying conserved pathogenic mechanisms from lineage-specific pathogenic innovations and adaptations and can also reveal where lateral gene transfer has played a role in new virulence-associated functions (Figure 1). Sequencing closely related isolates from the same genus and species of a particular phytopathogen promises to reveal an even greater level of functional diversity in virulence-associated traits and will also facilitate a much greater appreciation of the

evolution of eukaryotic microbial pathogenicity. It is an exciting prospect.

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