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Review

Fungal model systems and the elucidation of pathogenicity determinants

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ABSTRACT

Fungi have the capacity to cause devastating diseases of both plants and animals, causing significant harvest losses that threaten food security and human mycoses with high mortality rates. As a consequence, there is a critical need to promote development of new antifungal drugs, which requires a comprehensive molecular knowledge of fungal pathogenesis. In this review, we critically evaluate current knowledge of seven fungal organisms used as major research models for fungal pathogenesis. These include pathogens of both animals and plants; *Ashbya gossypii*, *Aspergillus fumigatus*, *Candida albicans*, *Fusarium oxysporum*, *Magnaporthe oryzae*, *Ustilago maydis* and *Zymoseptoria tritici*. We present key insights into the virulence mechanisms deployed by each species and a comparative overview of key insights obtained from genomic analysis. We then consider current trends and future challenges associated with the study of fungal pathogenicity.

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1. Introduction

A small fraction of the estimated 5 million fungal species are responsible for devastating diseases affecting agriculture and human health. Emerging infectious diseases (EIDs) caused by fungi are increasingly recognized as major threats to food security and animal health (Brown et al., 2012; Fischer et al., 2008). Dispersal and emergence of fungal diseases are furthermore promoted by human activity, primarily through global trade which lacks sufficient biosecurity measures, and may be exacerbated by the impact of climate change (Brasier, 2008; Fisher et al., 2012; Gange et al., 2007; Harvell et al., 1999; Ratnieks and Carreck, 2010; Verweij et al., 2009). Fungal pathogens are characterized by a remarkable genetic flexibility that facilitates rapid evolution and adaptation to the host or environment (Calo et al., 2013). These features, when combined with Darwinian selection, support the emergence of new

lineages with increased virulence or altered host range (Croll and McDonald, 2012). This highly adaptive behavior is promoted by an immense fungal genome plasticity (Calo et al., 2013). Besides sexual reproduction, parasexualilty (Pontecorvo, 1956), aneuploidy (Selmecki et al., 2006), transposons, telomere instability (Starnes et al., 2012) or horizontal gene transfer (Ma et al., 2010; Richards et al., 2011) amongst others enable adaptive evolution by promoting mitotic recombination, independent chromosomal assortment, gain or loss of chromosomes and translocations. Moreover, fungi exhibit morphogenetic plasticity which enables them to colonize and invade tissue as hyphae, which primarily extend at their tips (Fischer et al., 2008), while often forming differentiated spores, infection structures, fruiting bodies and unicellular, yeast cells, that can aid rapid dispersal (Sudbery et al., 2004).

In this review, we cover an array of fungal species, encompassing diverse taxonomic groups, that cover several hundred million years of evolution (Fig. 1). We include two major fungal pathogens of humans, *Aspergillus fumigatus* and *Candida albicans*, a facultative pathogen of both animals and plants, *Fusarium oxysporum*, and plant pathogenic species *Magnaporthe oryzae*, *Mycospherella graminicola, Ashbya gossypii* and *Ustilago maydis* which display infection-associated dimorphism and cellular differentiation. Collectively, this provides an overview of diverse mechanisms of pathogenesis and some unifying themes, while also encompassing some of the best-studied and understood fungal pathosystems.

The purpose of the review is to highlight both specific differences and unifying features of some of the best-studied fungal pathogens and to highlight the significant challenges that remain in developing a deep understanding of fungal pathogenesis.

2. *Aspergillus fumigatus*

Aspergillus fumigatus is a filamentous fungus that can be isolated from compost soil, where it proliferates in organic debris and plays an essential role in carbon and nitrogen recycling. *Fumigatus* is the most important air-borne fungal pathogen and can grow at temperatures up to 55 °C, while its spores survive temperatures of up to 70 °C (Brakhage and Langfelder, 2002). The fungus propagates asexually with release of thousands of spores per conidial head into the atmosphere. Due to their small size (2–3 μm diameter), the spores can easily reach lung alveoli (Latge, 2001).

A first description of pulmonary aspergillosis was published in 1842 by physician John H. Bennett (Supplementary Fig. 1A), who noted the presence of a fungus in the lungs of a post mortem patient with pneumothorax. Almost 50 years elapsed, however, before *A. fumigatus* was recognized as the primary cause of the infection (Barnes, 2004). Today, *A. fumigatus* conidia infect millions of susceptible individuals, causing allergies associated with asthma, allergic sinusitis and bronchoalveolitis (Denning et al., 2013). In cavities in the lungs of tuberculosis patients, *A. fumigatus* spores germinate and develop into a fungus ball, or non-invasive aspergilloma (Riscili and Wood, 2009). In addition to these forms of aspergillosis, which are not life-threatening, patients with altered immune status such as leukemia patients or transplant patients are at risk to develop invasive aspergillosis (IA), with an estimated number of more than 200,000 cases per year (Brown et al., 2012; Garcia-Vidal et al., 2008). Mortality rates for IA reach around 50% of the cases when patients are treated and increase to more than 90% when the diagnosis is missed or delayed (Brown et al., 2012).

2.1. Overview of the *A. fumigatus* genome

A first draft of the *A. fumigatus* clinical isolate Af293 genome was published in 2005 (Nierman et al., 2005). Eight chromosomes were identified containing about 10,000 genes (Table 2). Three years later, the genome sequence of a second *A. fumigatus* isolate, A1163, was released (Fedorova et al., 2008). Comparative analysis showed conservation of 98% of the sequence between the two

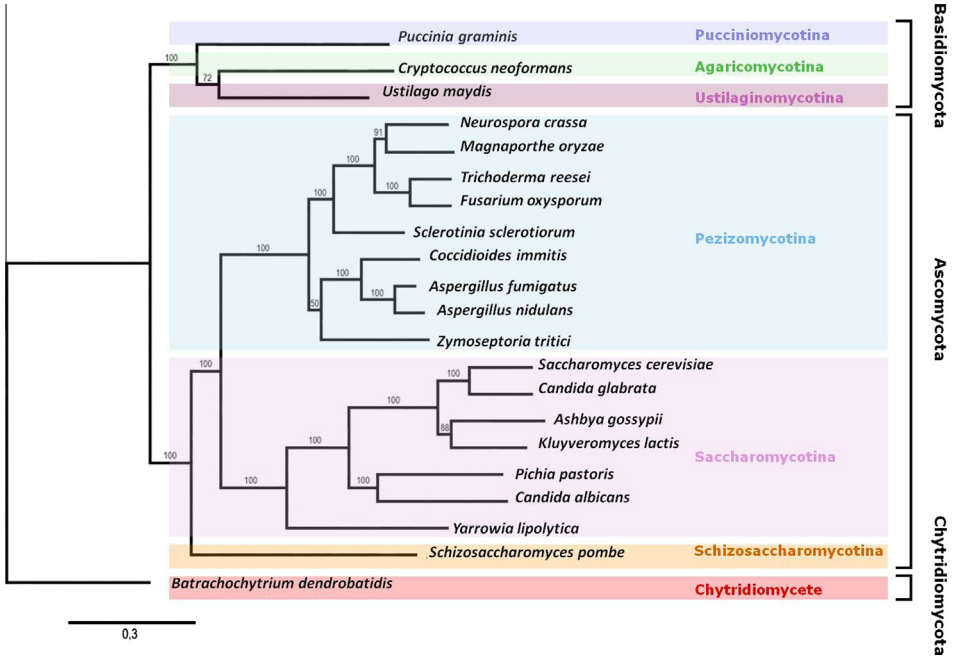

Fig. 1. Phylogenetic tree. Fungal species phylogeny generated from a concatenated alignment of 49 conserved fungal genes using maximum likelihood. The tree covers 21 taxa and 9033 amino acid positions. Bootstrap values for each node are reported as percentages. To generate the tree, protein sequences of the 49 selected conserved single copy genes from the 21 fungal species were aligned using Clustal W (Larkin et al., 2007) and the obtained conserved sequence blocks were sampled with G-blocks (Talavera and Castresana, 2007). All the aligned sequences were then concatenated to one file using Galaxy (Goecks et al., 2010). Finally, PhyML (Guindon et al., 2010) was used to generate the phylogenetic tree with 100 bootstraps for branch support and LG as the amino acid substitution model as identified by ModelGenerator (Keane et al., 2006) (Keane et al., 2006). The tree was visualized using TreeDyn (Chevenet et al., 2006).

Table 1
Overview of biological features.

Species	Human fungal pathogens		Plant fungal pathogens				
	Aspergillus fumigatus	*Candida albicans*	*Magnaporthe oryzae*	*Fusarium oxysporum*	*Zymoseptoria tritici*	*Ustilago maydis*	*Ashbya gossypii*
Taxonomy	Phylum: Ascomycota Class: Eurotiomycetes Order: Eurotiales Family: Trichocomaceae Genus: *Aspergillus* Species: *A. fumigatus*	Phylum: Ascomycota Subphylum: Saccharomycotina Class: Saccharomycetes Order: Saccharomycetales Family: Saccharomycetaceae Genus: *Candida* Species: *C. albicans*	Phylum: Ascomycota Class: Sordariomycetes Order: Magnaporthales Family: Magnaporthaceae Genus: *Magnaporthe* Species: *M. oryzae*	Phylum: Ascomycota Class: Sordariomyctes Order: Hypocreales Family: Nectriaceae Genus: *Fusarium* Species: *F. oxysporum*	Phylum: Ascomycota Class: Dothideomycetes Order: Capnodiales Family: Mycosphaerellaceae Genus: *Zymoseptoria* Species: *Z. tritici*	Phylum: Basidiomycota Class: Ustilaginomycetes Order: Ustilaginales Family: Ustilaginaceae Genus: *Ustilago* Species: *U. maydis*	Phylum: Ascomycota Subphylum: Saccharomycotina Class: Saccharomycetes Order: Saccharomycetales Family: Saccharomycetaceae Genus: *Eremothecium* Species: *A. gossypii*
Predominant cell-type	Multinucleate; septated filaments	Budding yeast, pseudohyphae and true hypha (in which elongated yeast cells remain attached after cytokinesis)	Filamentous mycelium	Filamentous mycelium, Microconidia	Dimorphic fungus: yeast-like cells/filamentous mycelium	Unicellular budding yeast	multinucleate; septated filaments
Sexual cycle	Yes, but in nature predominantly asexual	Parasexual cycle (mating of diploid cells followed by mitosis and chromosome loss instead of meiosis	Yes, but in nature predominantly asexual	Not identified	Yes, occurs during epidemics	Yes, occurs only inside the plant	not identified
Mating-type system	Bipolar heterothallism *MAT1-1*, *MAT1-2*	MATa and MATα	Bipolar heterothallism *MAT1-1*, *MAT1-2*	MAT1 gene identified and expressed in *F. oxysporum*. A mixed distribution of MAT1-1 and MAT1-2 alleles in *Fusarium* species complex	Bipolar heterothallism *MAT1-1*, *MAT1-2*	Tetrapolar: *a* locus (2 alleles) *b* locus (~ 20 alleles)	bipolar; MATa/α
Spores	Uninucleate conidia and binucleate ascospores	Chlamydospore	Conidia (asexual spore) and ascospores (sexual spore)	Microconidia, macroconidia, clamydospores	Ascospores and pycnidiospores	Diploid spores, teliospores	Ascospores
Pathogenicity	Animals (can cause asthma, aspergilloma, invasive aspergillosis)	Candidiasis (skin infections) and Candidemia (presence of *Candida albicans* in blood) in immuno-compromised individuals	Rice and some monocotylous plants (e. g. barley, wheat)	Fusarium wilt on plant crops Emerging cause of fusariosis in humans	Bread and durum wheat (septoria tritici leaf blotch)	Corn and teosinte plants	Cotton/citrus fruits
Other features	Toxin production	Homothallic and heterothallic mating and haploid mating also possible, but rare		Fusarium species complex are pathogenic especially to agriculture plants		Highly resistant to UV radiation	Riboflavin overproducer

Table 2
Overview of fungal genome data.

Species	Human fungal pathogens		Plant fungal pathogens				
	Aspergillus fumigatus	*Candida albicans*	*Magnaporthe oryzae*	*Fusarium oxysporum*	*Zymoseptoria tritici*	*Ustilago maydis*	*Ashbya gossypii*
Genome size (Mb)	29.420	14.88	41.03	61.36	39.7	20.50	8.76
Chromosomes	8	8	7	15	21	23	7
GC content (%)	50.0	33.3	51.6	48.4	51.7	57.0	51.8
Number of Genes	9783	6354	12827	17708	10900	6788	4726
Non-coding RNAs (tRNAs)	179	132	325	308	Unknown	104	192–293 (216)
Introns	Average 1.8 per gene	415 introns in the entire genome	Average 1.8 per gene	Unknown	Average 1.5 per gene	3093 introns in the entire genome, average 0.46 per gene	226 introns in the entire genome
Avg. gene size/intergenic region	1.64 kb/1.22 kb	1.47 kb/858 bp	2 kb/1.4 kb	1.3 kb	⩾1.15 kb	1.74 kb/973 bp	~1.9 kb/~340 bp
Transposons	8 (Predicted DDE1 transposon-related ORF & putative transposase, induced by exposure to human airway epithelial cells)	3 (Zorro3-R ZORRO1, Zorro2-1; member of L1 clade of transposons and encodes a potential DNA-binding zinc-finger protein)	9.7% of genome comprises repeated sequences longer than 200 base pairs (bp) and with greater than 65% similarity	28% of the genome identified as repetitive sequence	20%	1.1% DNA with similarity to transposons	no -
	Predicted LINE (long interspersed nuclear elements), LINE-like reverse transcriptase			Retroelements (copia-like and gypsy-like, LINEs (long interspersed nuclear elements) and SINEs (short interspersednuclear elements). DNA transposons (Tc1-mariner, hAT-like, Mutator-like, and MITEs)		(hobS, tigR retroelements)	
	Predicted gypsy transposon- related ORF Predicted mariner Ant1 transposon-related ORF						
S. cerevisiae homology	60%	64%	36.9%	Unknown	22.5%	33% (20% identity cut-off)	95%
Mitochondrial DNA (kb)	~32	41	34.95	34.48	44	56.8	~23.5
References	Nierman et al. (2005)	Braun et al. (2005)	http://www.broadinstitute.org	http://www.broadinstitute.org	Orton et al. (2011)	Kamper et al., 2006	Dietrich et al. (2004)
	Fedorova et al. (2008)	Mitrovich et al. (2007)	Dean et al. (2005)	Rep and Kistler (2010)	Goodwin et al. (2011)		

isolates, with the majority of the remaining 2% being strain-specific genes clustered in blocks from 10 to 400 kb in size, including a large number of pseudogenes and repeat elements (Fedorova et al., 2008). Comparison of the sequenced *A. fumigatus* genomes with those of closely related species such as *Neosartorya fischeri* and *Aspergillus clavatus* showed the presence of lineage-specific genes often localized to the sub-telomeric regions. These genomic islands were suggested to represent a gene repository that could play a role in adaptation to different environments, such as compost or a living host (Fedorova et al., 2008). Transcriptome studies based on RNA-Seq and proteome studies are routinely carried out with *A. fumigatus* (Horn et al., 2012; Kroll et al., 2014). The updated features of the *A. fumigatus* genome are available at the *Aspergillus* Genome Database website (Table 3).

2.2. Assessing virulence of A. fumigatus

To understand the infection process of *A. fumigatus* and identify fungal factors involved in pathogenesis, appropriate infection models are required. Mouse infection models have been crucial for the study of pathogenicity, for the characterization of host-pathogen interactions and for development of therapeutic approaches (Clemons and Stevens, 2005). According to the immunosuppression regimen, mouse infection models are divided into neutropenic or non-neutropenic models (Liebmann et al., 2004a). Because it is believed that infection starts by inhalation of conidia, in mouse infection models, conidia are administered by intranasal, intra-tracheal inoculation or inhalation. Mouse models however, have disadvantages, including an open question about their reliability as models for human diseases (Seok et al., 2013), as well as increasing public concern regarding animal welfare and the clinical value of animal research, which is resulting in increasing regulatory demands. This, together with the requirement for costly facilities and personnel, is driving the development of alternative infection models for fungal pathogens. The larvae of the greater wax moth *Galleria mellonella*, for example, provide important advantages. They are readily available from suppliers at convenient cost, they are large enough (around 2.5 cm in length) to be easily inoculated (Mylonakis, 2008) and can be maintained at 37 °C, which means that infections can be studied at the temperature at which they occur in humans (Mylonakis, 2008). However, results obtained with such models are of limited value because there are significant differences in the immune responses of invertebrates and mammals. Embryonated chicken eggs have also been used as an alternative infection model for analysis of fungal pathogenicity in *A. fumigatus,* with promising results (Jacobsen et al., 2010).

2.3. Current research interests

2.3.1. Virulence determinants

It is debated whether *A. fumigatus* has true virulence factors or simply physiological characteristics, such as thermo-tolerance, that enable the fungus to grow in an immuno-compromised human host. Several virulence determinants of *A. fumigatus* have, however, been characterized. These determinants include the siderophore-mediated iron uptake system (Schrettl et al., 2004), and the *pksP* gene, which is involved in the biosynthesis of dihydroxynaphthalene (DHN) melanin, which forms the gray-green spore pigment (Heinekamp et al., 2012; Horn et al., 2012; Langfelder et al., 1998; Thywissen et al., 2011; Volling et al., 2011). DHN melanin inhibits both apoptosis and acidification of conidia-containing phagolysosomes of macrophages (Thywissen et al., 2011; Volling et al., 2011). Mechanistically, it was reported that DHN melanin inhibited assembly of v-ATPase on the phagosomal membrane (Heinekamp et al., 2012). *A. fumigatus* also possesses immune-evasion mechanisms which reduce recognition of both immune effector cells and the complement system (Aimanianda et al., 2009; Behnsen et al., 2008, 2010; Horn et al., 2012). It can be expected that virulence is a multifactorial process and thus more virulence-associated traits will be discovered.

2.3.2. Signaling involved in virulence

Several genes of the cAMP signal transduction pathway are required for pathogenicity. These include genes encoding adenylate cyclase (AcyA), protein kinase A (PKA) and a stimulating G_{α} protein-encoding gene designated *gprA* (Liebmann et al., 2004b; Zhao et al., 2006). Recently, the cAMP signaling pathway has also been related to regulation of the DHN melanin production (Abad et al., 2010; Grosse et al., 2008).

The calcineurin/calmodulin signaling pathway is required for formation of cell shape and pathogenicity in *A. fumigatus*. Deletion of the gene encoding the catalytic subunit of calcineurin phosphatase (*calA*) resulted in a branching defect and limited growth, as well as attenuation of virulence (da Silva Ferreira et al., 2007; Juvvadi et al., 2013). A target of calcineurin is the zinc finger transcription factor CrzA, which is implicated in germination, polarization, cell wall structure, asexual development and virulence (Cramer et al., 2008). Of the four MAPKs found in *A. fumigatus*, it was shown that MpkA regulates the cell wall integrity pathway and SakA the hyperosmotic glycerol pathway (Jain et al., 2011; May et al., 2005; Rispail et al., 2009).

Two GPCRs were shown to be required for virulence in *A. fumigatus*: GprC and GprD (Gehrke et al., 2010). Little is known, however, about the downstream multi-subunit G-proteins. *A. fumigatus* encodes only three Gα subunits, while other *Aspergilli* harbor up to four Gα proteins. For *gpaB*, most likely involved in cAMP signal transduction, its involvement in pathogenicity was demonstrated (Liebmann et al., 2004b; Rispail et al., 2009).

The seven transmembrane domain (7TMD) protein PalH was recognized as a putative pH sensor and also shown to be required for virulence on mice (Grice et al., 2013). Three histidine kinase receptors TcsA/Fos-1, TcsB and TcsC have been characterized. Deletion of *tcsA* led to a strain with reduced virulence in a systemic murine model while the only phenotype detectable for Δ*tcsB* was reduced sensitivity to SDS (Grice et al., 2013). TcsC has been shown to play a role in the high osmotic pressure response, and growth in the presence of nitrate as nitrogen source (McCormick et al., 2012). Finally, a number of a cell wall stress sensors have been described, including Wsc1, Wsc2, Wsc3 and MidA. Wsc1 is involved in resistance against echinocandins. MidA appears to be essential for thermotolerance at elevated temperatures and to counteract the effects of cell wall-disrupting compounds such as congo red and calcofluor white. Wsc1, Wsc3 and MidA show also some overlapping roles in radial growth and conidiation. The role of Wsc2 remains to be elucidated (Dichtl et al., 2012). Another conserved signal cascade is the CPC system, which links environmental stresses to amino acid homeostasis. Deletion of the transcriptional activator CpcA led to reduced virulence, while deletion of the sensor kinase CpcC resulted in increased sensitivity towards amino acid starvation (Abad et al., 2010).

2.3.3. Host perception and response

In healthy individuals, inhaled conidia of *A. fumigatus* are rapidly attacked by the immune system of the host. Physical barriers of the lung and the innate immunity are of major importance for defense. Epithelial cells of the upper respiratory tract contribute to elimination of microbes through secretion of mucus and cilia-mediated active transport (Brakhage, 2005). In alveoli, there are epithelial cells, called pneumocytes, responsible for secretion of a pulmonary surfactant with antimicrobial activity (Balloy and Chignard, 2009). A decisive role for elimination of pathogens is played by macrophages and neutrophils. Alveolar macrophages

Table 3
Overview of molecular tools.

Species	Human fungal pathogens		Plant fungal pathogens				
	Aspergillus fumigatus	*Candida albicans*	*Magnaporthe oryzae*	*Fusarium oxysporum*	*Zymoseptoria tritici*	*Ustilago maydis*	*Ashbya gossypii*
Transformation	Protoplasts & electroporation	Lithium acetate, spheroplast fusion & electroporation	PEG/$CaCl_2$ & ATMT (Agrobacterium-mediated transformation)	Protoplasts & ATMT	ATMT	Protoplasts	Electroporation & protoplasts
Minimal homology for gene deletion	>500 bp	50 bp	500 bp	>1000 bp	500 bp	500 bp	45 bp
Episomal elements	no	No episomal plasmids but integrative plasmids include CIp10 (artificial integration at RPS10 locus), pDUP/PDIS (shuttle vector for integration at NEUT5 l)	no	self-replicative ARS (active replicating system) plasmid pFNit-Lam-Tlam, linear	no	UmARS plasmids	ScARS plasmids
Promoters [c] constitutive [r] regulatable	[r] Pyomelanin promoter, *PTet* system, Xylose-regulated promoter	[c] *PADH1,* [r] *PACT1,* [r] *PTEF3,* [c] *PEF1-α2,* [r] *PGAL1,* [r] *PPCK1,* [r] *PSAP2,* [r] *PMRP1,* [r] *PHEX1,* [r] *PMET3,* [r] *PMAL2,* [r] *PTET,* [r] *PSAT1*	[r] *PICL1* [r] *PNiA1* [c] *PMPG1*	[r] Thiamine repressed *P*sti35 promoter [c] *PgpdA*	[c] *Pacu-3* [c] *Pgpd*	[r] *PTet* system [r] *Pnar1*, [r] *Pcrg1*; [c] *Potef*	*PTEF1*, *PHIS3*; [r] *PMET3* [r]*PTHI13*
Commonly used selection markers	Hygromycin B, Pyrithiamine, Phleomycin	*URA3, LEU2, HIS1, ARG4, MH3,* Nourseothricin, FLP and Cre-Lox technology	Hygromycin B, Sulfonylurea, Glufosinate, Geneticin G418, Nourseothricin	Hygromycin B, Phleomycin	Hygromycin B, Bialaphos, Geneticin G418, Carboxin	Hygromycin B, Phleomycin, Carboxin, Nourseothricin, Geneticin G418, FLP technology	Hygromycin B, Ag*LEU2*, *GEN3*/*KanMX*, Nourseothricin
Reporter genes	*lacZ*, GFP	*ADH1, ACT1, TEF3, EF1-α2, GAL1, PCK1, SAP2, MRP1, HEX1, MET3, MAL2, TET, SAT1*	GFP, GUS, HcRed	GFP, ChFP	GFP	GFP, GUS	*lacZ*, GFP
Fluorescent protein labels	GFP, mCherry	mCherry, GFP, YFP, CFP, RFP, Venus	GFP, RFP, mCherry, YFP	GFP; ChFP	GFP	GFP, RFP, mCherry, CFP, YFP	Codon optimized
Cytochemical dyes	DAPI, Calcofluor White, Mito tracker	Calcoflour White, Alcian Blue	Calcofluor White, DAPI, FM4-64, WGA	Calcofluor White, DAPI, FITC	DAPI, Calcofluor White, WGA alexa, Mito tracker	DAPI, FM4-64, Filipin	DAPI, FM4-64, Calcofluor White, Mito tracker, Filipin
Arrays	Affimetrix, Febit, Roche, TIGR (RNA-seq mostly replacing the array platforms)	Custom made DNA microarray, Affymetrix	Custom made DNA microarray, Affymetrix	Custom made DNA microarray, Affymetrix	Custom made DNA microarray, Affymetrix	Custom made DNA microarray, Affymetrix representing 6297 genes	Does not apply
Pathogenicity models	Animal models: mouse and embryonated chicken eggs	Reconstituted epithelial models: chick chorio-allantoic model Non-mammalian models: *C. elegans, D. melanogaster, G. mellonella*, *B. mori*, Zebrafish Mammalian models: murine intravenous model, murine gastrointestinal colonization and dissemination model	Rice, barley	Plant models:Tomato plants, tomato fruits, apple fruits; Mammalian model: immunodepressed mice;. Invertebrate model: *G. mellonella*	Wheat	Corn plants	Does not apply

Strains	CEA10 CEA17 akuB	SC5314, NGY152, CAI4, RM1000, WP17, SN87, SN95, SN152	Guy11, P1-2, 70-15, PH14, TH3, FR13, BR88	*F. oxysporum* f. sp. *lycopersici* wild type 4287 (FGSC 9935)	IPO323	FB1 (a1b1), FB2 (a2b2), FB6a (a2b1), FB6b (a1b2), SG200 (a1mfa2 bE1/bW2), FBD11 (a1a2 b1b2), AB31 (a2 P_{crg1}:bE1 P_{crg1}:bW2) AB33 (a2 P_{nar1}:bE1 P_{nar1}:bW2)	ATCC10895 CBS102347 and derivatives
Bioinformatic/ genome databases	Aspergillus Genome Database	Candida Genome Database	Magnaporthe comparative Database	Fusarium Comparative Database	*Mycosphaerella graminicola* (anamorph: *Septoria tritici*)	Ustilago maydis *Database*	*Ashbya* Genome Database
	http://www.aspgd.org/	http://www.candidagenome.org/	http://www.broadinstitute.org/annotation/genome/magnaporthe_comparative/MultiHome.html	http://www.broadinstitute.org/annotation/genome/fusarium_group/MultiHome.html	http://genome.jgi.doe.gov/Mycgr3/Mycgr3.home.html	http://mips.helmholtz-muenchen.de/genre/proj/ustilago/	http://agd.vital-it.ch/index.html
						http://www.broadinstitute.org/annotation/genome/ustilago_maydis.2/Home.html	

act very fast and within 30 h can kill 90% of inhaled conidia *via* phagocytosis (Balloy and Chignard, 2009). Neutrophils form the highest number of intravascular phagocytes. During infection, neutrophils are recruited to alveoli and phagocytose conidia and germlings. The direct killing mechanisms are, however, still unclear. Neutrophils also produce neutrophil extracellular traps (NETs) in response to *A. fumigatus*, which consist of chromatin covered with granular proteins that display some antimicrobial activity (Bruns et al., 2010).

Host protein receptors are involved in triggering the immune response against *A. fumigatus*, including secreted complement factors or those exposed on the surface of phagocytes, such as dectin-1 (Steele et al., 2005). As long as inhaled *A. fumigatus* conidia are covered with a proteinaceous layer formed by hydrophobins, the fungus is masked and immunologically inert (Aimanianda et al., 2009). Once conidia start to germinate, the rodlet layer is not present on the cell surface, leading to exposure of surface components, recognized by immune cells. C-type lectin and Toll-like receptors on host immune cells have, for instance, been proposed to bind to fungal ligands and are involved in activation of the immune response through production of cytokines and chemokines, prostaglandins and reactive oxygen intermediates (Brakhage et al., 2010).

2.3.4. Development of anti-infective strategies

The current guideline-recommended therapy against invasive aspergillosis is the triazole antibiotic voriconazole, which is superior to the toxic polyene amphotericin B deoxycholate (Herbrecht et al., 2002; Steinbach, 2013). While a 2002 clinical trial found an improved response with voriconazole (52.8%) versus amphotericin B (31.6%), the field is still in need of improved antifungals, with entirely novel targets. As recently reported (Steinbach, 2013), a combination therapy of antifungals may be promising, akin to other medical disciplines whereby agents with different mechanisms are employed to provide a synergistic response. In addition, the future holds promise for novel developments such as the generation of immunotherapies based on T cells or dendritic cells. These therapies are based on *in vitro* proliferation of these cell types upon stimulation by fungus-specific antigens, leading to cell-based vaccination (Lehrnbecher et al., 2013).

2.3.5. Contribution of fungal secondary metabolism to virulence

Based on the genome sequence of *A. fumigatus*, it is estimated that the fungus encodes at least 39 secondary metabolism gene clusters, which lead to biosynthesis of various low molecular weight compounds. These compounds are often synthesized by non-ribosomal peptide synthetases (NRPSs) or polyketide synthases (PKSs) (Brakhage, 2013; Inglis et al., 2013). Some of the compounds produced by these secondary metabolism gene clusters have been identified, such as siderophores, DHN melanin and gliotoxin and their involvement in virulence shown (Scharf et al., 2014; Schrettl et al., 2004). It is likely, however, that other compounds of *A. fumigatus* will contribute to virulence.

2.4. Conclusions

In the last 20 years, considerable progress has been made in development of advanced genetic tools for *A. fumigatus*. While more than 400 mutant strains have been generated (Horn et al., 2012), only a few virulence determinants have been characterized. At present, systems biology approaches are being explored as an potential means to describe the interaction between human host and pathogen. However, the huge amount of data generated by RNA-seq and proteome analyses of both pathogen and host cells, requires development of sophisticated bioinformatic tools. Complete genome-wise gene knock-out libraries would facilitate

screening efforts such as analysis of the mode of action of antifungal compounds, but are not yet available for *A. fumigatus*. In parallel, huge efforts are being made to gain insight into virulence mechanisms by analyzing the interaction of *A. fumigatus* with different cellular systems such as neutrophils, macrophages, epithelial cells and dendritic cells with increasing complexity, such as combining different cellular systems.

To bridge basic research on the pathobiology of *A. fumigatus* with clinical requirements, a so-called Translational Systems Biology approach has been started which aims to integrate different "omics" data levels and image-based data of host-pathogen interactions into network and spatio-temporal computational models. The main goals are to identify and validate new drugs and new drug targets, increase the efficiency of pathogen identification during an infection and identify effective therapies (Horn et al., 2012).

3. *Candida* species

Candida albicans is normally a commensal of the human microflora, but is also a classic opportunistic pathogen causing mucosal thrush, blood stream and systemic infections, termed candidaemia and invasive candidiasis, respectively. *Candida* infections are normally associated with individuals who are immunocompromised or traumatized due to major surgery, transplantation and invasive medical treatments that disrupt the normal protective bacterial microflora. Five species account for around 95% of all Candida infections, namely *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis* (Pfaller et al., 2009). Infections are associated with high mortality rates which can vary between 20% and 60%, depending on the specific risk factors and patient group (Calderone and Clancy, 2012).

C. albicans belongs to the *Saccharomycetaceae*, which includes yeasts, mycelial and pleiomorphic organisms (Table 1). Many of this family can undergo complete sexual cycles but *C. albicans* has not been demonstrated to undergo meiosis leading to ascospore formation, although most of the *S. cerevisiae* homologs for meiosis-associated genes are present in its genome.

An important feature of biology of *C. albicans* is its ability to exist in different morphological forms (Supplementary Fig. 2B) under different environmental conditions. The major vegetative forms of *C. albicans* are: (i) budding yeast cells, (ii) elongated co-joined yeast cells that undergo synchronous cell division called pseudohyphae and (iii) un-constricted, branching, true hyphae that grow exclusively by apical extension (Sudbery, 2011). Each of these growth forms is associated with changes in cell cycle regulation, expression of morphology-specific gene sets and differences in host immune response. Therefore, morphogenesis of *C. albicans* impacts directly on pathogenesis (Gow and Hube, 2012). Most monomorphic mutants are attenuated in virulence (Sudbery et al., 2004). *C. albicans* also forms asexual chlamydospores which are seldom, if ever, observed in human tissues.

Another important cell type in *C. albicans* is the mating competent opaque cell. The white-opaque phenotypic switch was first described in the 1980s (Slutsky et al., 1985, 1987) and later shown to be critically important in determining mating competence of opposite mating types (Hull and Johnson, 1999; Hull et al., 2000; Magee and Magee, 2000). Several transcription factors including Wor1, Czf1, Wor2, and Efg1 play important roles in controlling white-opaque switching (Miller and Johnson, 2002; Zordan et al., 2006). Diploid strains will mate if partners are in the opaque form and homozygous (***aa*** or $\alpha\alpha$) for the MLT (mating-type-like) locus (Miller and Johnson, 2002), and mating partners form elongated conjugation tubes called shmoos that undergo chemotropism and fusion (Lockhart et al., 2003). After fusion, the tetraploid nucleus undergoes concerted chromosome loss to regenerate the diploid state (Supplementary Fig. 2B). Homothallic (same sex) mating of diploids has also been reported in strains lacking the Bar1 protease which inactivates α-pheromone, enabling autocrine signaling (Alby et al., 2009).

Recently, a screen for aneuploid isolates discovered strains that were fully haploid for all eight chromosomes but retained either a *MLT***a** or a *MLT*α locus on chromosome 5. These strains were often unstable and showed reduced overall fitness but could either mate or autodiploidise to regenerate diploid variants (Hickman et al., 2013). The haploid strains of *C. albicans* have a full morphogenetic repertoire and have significant potential in development of new genetic strategies including forward genetic screens for analysis of *C. albicans* biology.

3.1. Comparative genomics in Candida species

Of the pathogenic *Candida* species, *C. albicans*, *C. tropicalis* and *C. parapsilosis* are diploid species belonging to the so-called "CTG clade", characterized by a somatic CTG codon reassignment in their genomes. The CTG codon encodes serine and not leucine and therefore results in the fungus mistranslating heterologous recombinant proteins (Santos and Tuite, 1995). This discovery paved the way for generation of codon-corrected reporter genes and genetic markers required for molecular genetic analyses. *C. glabrata* and *C. krusei* are more distant relatives of the CTG clade species. They are haploid and more closely related to *S. cerevisiae* than to *C. albicans*. Members of the CTG clade have not undergone an ancient whole genome duplication, which significantly shaped the genomes of the *S. cerevisiae* group of fungi.

C. albicans is typical of most pathogenic Candida species in having a genome size of around 6000 predicted genes (Table 2) (Butler et al., 2009). The nearest phylogenetic relative to *C. albicans* is *C. dubliniensis*, yet this species is considerably less virulent than *C. albicans* and many more distantly related *Candida* species (Jackson et al., 2009). Expansion of certain virulence-associated gene families often occurs in the sub-telomeric regions of *Candida* species – such as with the EPA gene family of adhesins of *C. glabrata* (Kaur et al., 2007). The genetic diversity of such cell wall proteins within a species is often further increased by expansion of trinucleotide repeat regions within rapidly evolving genes. Also, a number of gene families are clearly enriched in pathogenic species of *Candida* including those encoding classes of cell wall proteins, iron assimilation properties and major facilitator and other transporters (Butler et al., 2009). Genetic diversity within and between species is further increased by recombination events that have taken place between Major Repeat Sequences (MRS) and previous retrotransposons. Other chromosome changes such as the formation of partially aneuploid strains and strains with isochromosomes have been shown to affect antifungal drug sensitivities (Selmecki et al., 2006).

3.2. The toolbox for C. albicans

C. albicans diploidy and codon usage present significant technical difficulties in performing functional genetics (see above). Consequently, bespoke nutritional markers used in gene disruption protocols and reporter genes had to be developed for use in this organism (Table 3). Genes from other organisms naturally devoid of CTG codons or which have been codon-corrected, or fully codon-optimized genes, have been used to enable heterologous expression (Fonzi and Irwin, 1993; Gerami-Nejad et al., 2009).

The progenitor method of gene disruption methodologies in *C. albicans* has been based on the so-called "Ura-blaster" method (Fonzi and Irwin, 1993). The use of this system is associated with some well recognized problems, mainly related to the issue that null mutants are heterozygous for *URA3* and the Ura3 gene product

can become limiting *in vivo* affecting virulence (Brand et al., 2004). In order to circumvent this, *URA3* can be integrated into a high expression locus and alternative genetically marked strains have been generated based on complementation of *leu2*, *his1* and *arg4* which do not have compromised virulence. Other *C. albicans* strains have been generated that use non-nutritional-based selectable markers such as nourseothricin and flipper (FLP) or Cre-lox recombinase to rapidly excise selectable markers (Table 3). In addition, several systems have been developed that use regulated promoters to control expression of a single functional allele (Samaranayake and Hanes, 2011).

A wide range of *in vitro, ex vivo* and *in vivo* assays have been developed to assess the virulence of wild type and mutant strains of *Candida* species (Table 3). *In vitro* assays include exposure to primary cell cultures or cell lines of epithelial immune cells and the measurement of cell damage by the release of chromium-51 or

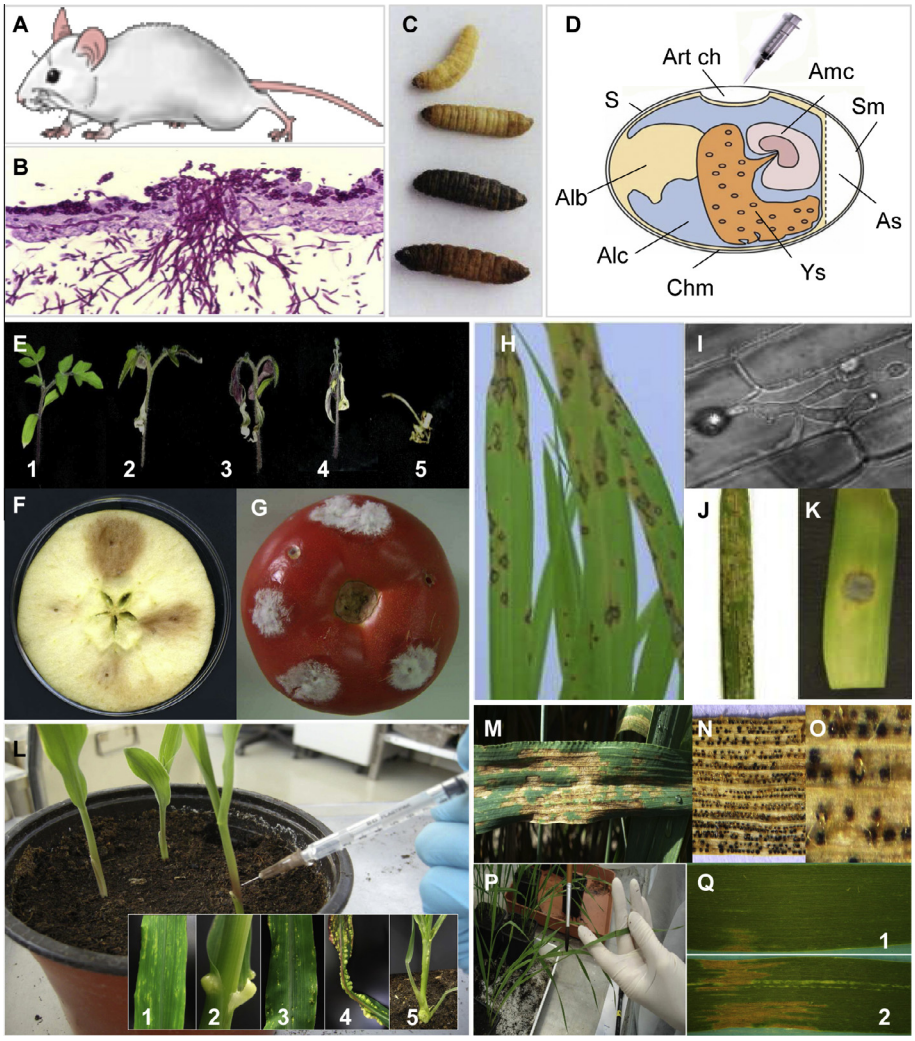

Fig. 2. Pathogenicity assays. **(A)** Laboratory rodents used as models for fungal research of *A. fumigatus*, *C. albicans* and *F. oxysporum*. Immunosuppression of the host can be achieved via cortisone or corticosteroid treatment to mimic a leukopenia. Ways of infection include injection in the tail vein to generate a disseminated infection, inhalation directly from spore suspension and respiration in an aerosol chamber to generate pulmonary infection. **(B)** *C. albicans* invading the chicken embryo chorioallantoic membrane (CAM) (adapted from Gow et al., 2003). **(C)** Larvae of the greater wax moth (*Galleria mellonella*) used to investigate virulence of *A. fumigatus, C. albicans and F. oxysporum.* Tipically, the infection is performed via micro-injection in the posterior pseudopod. Progression of the fungal infection is associated with melanization of the larvae. **(D)** Embryonated eggs used as infection models in *A. fumigatus and C. albicans* research. The egg must be perforated at the blunt end and on the side, where an artificial air chamber is then generated applying a negative pressure from the blunt end hole. After perforation of the shell membrane, the inoculum is injected into the artificial air chamber onto the chorioallantoic chamber using a sterile syringe. The holes can then be sealed with paraffin. Art ch, artificial air chamber S, shell; Amc, amniotic cavity; Sm, shell membrane; As, air sac; Ys, yolk sac; Chm chorioallantoic membrane; Alc, allantoic cavity; Alb, albumin. **(E–G)** *F. oxysporum* assays. (E) Tomato plant root assay. Two week old tomato seedlings (cultivar Money Maker) are inoculated with *F. oxysporum* strains by immersing the roots in a microconidial suspension for 30 min, planted in vermiculite and incubated in a growth chamber at 28 °C. Evaluation is performed using a disease index for *Fusarium* vascular wilt going from 1 = healthy plant to 5 = dead plant. (F-G) Invasive growth assay on living fruit tissue. Apple fruits (F) or tomato fruits (G) are inoculated with *F. oxysporum* strains and incubated in a humid chamber at 28 °C for 3 days. **(H–K)** *M. oryzae* assays. (H) Typical oval-shaped lesions on rice leaves, cultivar (cv) Co-39, generated after spray inoculation of the pathogen (strain Guy11) (kindly donated by Dr. Michael J. Kershaw). (I) Invasive hyphae on rice sheath at 29 h post-inoculation obtained from a rice leaf sheath assay. (J) Spray inoculation of barley, 6 days post-inoculation (kindly donated by Dr. Michael J. Kershaw). (K) Drop inoculation assay on barley, 6 days post-inoculation. **(L)** *U. maydis* strains are injected into 7-day-old maize seedlings. Disease symptoms are scored according to different categories based on tumor size. 1. Chlorosis. 2. Small swelling at ligula or stem. 3. Small tumors on the leaves. 4. Large tumors on leaves. 5. Heavy tumors on the base of the stem and/or dead plant. **(M–Q)** *Z. tritici.* Symptoms on wheat leaves. (M) Natural infection on the third leaf in field tests (cv. CYMMIT). (N) Pycnidia produced by the strain IPO94269 on wheat leaves (cv. Obelisk), 21 days post inoculation (dpi). (O) Close up of (N) showing pycnidia and cirrhi. (P) Inoculation of *Z. tritici* onto the second leaf using a paintbrush in greenhouse. (Q) Symptoms produced by different isolates of *Z. tritici* on flag leaves at 35 dpi: isolates INRA08-FS0002 (1) and isolate IPO323 (2) on cv. Apache (E) (Suffert et al., 2013). Images kindly donated by Dr. Frederic Suffert and Dr. Thierry C. Marcel.

lactate dehydrogenase from the human cells or the measurement production of pro-inflammatory and anti-inflammatory cytokines by using ELISA or Cytometric Bead Arrays (CBA) as indicators of immune activation. Interactions between *Candida* and phagocytes can also be visualized and quantified by live imaging video microscopy and fluorescence-activated cell sorting (FACS) analysis.

Various *in vivo* models including invertebrate models, mini-hosts and mouse models have been developed for *Candida* research (Table 3 and Fig. 2). Invertebrate models include *Drosophila melanogaster*, *Caenorhabditis elegans*, the larval stages of the moth *Galleria mellonella* and the silk worm, *Bombyx mori*. These models have often been shown to be able to recapitulate the rank virulence order of strains as assessed in mice.

The vertebrate mini-host model Zebrafish, *Danio rerio,* which has both innate and adaptive immune systems has also been used to measure *Candida* virulence, host response to infectious agents, the efficacy of antifungal drugs and the cell biology of phagocyte–pathogen interactions, however the main vertebrate infection model is still heavily focussed on murine studies. Normally, yeast cells are intravenously injected via tail vein and mouse mortality is analyzed usually over two to four weeks. Fungal organ burdens are often assessed in the kidney, which is one of the main target organs in the mouse and in other tissues and these assays can be complemented by measurements of *in vivo* cytokine levels. A chick embryo chorioallantoic membrane (CAM) assay has also been used as a pathogenicity model and invasion assay for *C. albicans* (Fig. 2B).

A *Candida* gut infection model has also been described which allows disease progression across the gastrointestinal mucosa and subsequent systemic dissemination of the fungus to be investigated (MacCallum, 2012).

3.3. Current research interests

3.3.1. Genome functional analysis

The recent discovery of *C. albicans* haploid strains is likely to lead to emergence of new libraries of mutants and forward genetic screens that will facilitate studies of developmental processes and the search for antifungal drugs and drug target identification protocols.

3.3.2. Altered host responses

A second major field of endeavor has been related to taking advantage of emerging data from genome sequencing of the human genome to identify polymorphisms that correlate with altered host resistance. Such methodologies have already contributed significantly to our understanding of the recognition and signaling pathways that articulate host responses (Lionakis and Netea, 2013; Plantinga et al., 2012). These studies have radically altered our understanding of disease conditions such as chronic mucocutaneous candidiasis, orophayngeal candidiasis, persistent candidaemia and vulvo-vaginal infections by identifying specific immune defects in relevant pathways. It is likely that this field will remain highly fertile in the next years.

3.3.3. Yeast-to-hypha transition

A third area that has remained a major research focus over many years is the regulation of the yeast-to-hypha transition (Sudbery, 2011). The main pathways and cell biological processes that regulate morphogenesis have been the subject of intense interest and activity, with recent work defining how stress adaptation induces filamentous growth and how the elements of the cytoskeleton, vesicle secretion pathway and cell cycle machinery articulate the modification of cell shape. Hyphal development of *C. albicans* has become the most worked on system of filamentous growth in fungi and this is likely to retain its position as a pre-eminent model system for fungal developmental biology.

3.3.4. Importance of the cell wall in immune recognition

Yeast–hyphal switching is but one of the ways in which *Candida* species can alter their surface shape and surface properties. A major field of research for this organism has therefore focused on understanding how the specific surface chemistry of the cell wall activates, represses or modulates immune recognition and immune function (Gow et al., 2011). Surface components are also being investigated in the context of development of vaccine and new generation diagnostics, therefore a focus on cell wall chemistry and host-fungus interactions will continue to have importance in both basic and translational research.

3.4. Conclusions

Candida research has moved forward quickly in the molecular era with the constant enrichment of the field with new methodologies. This has propelled research particularly in *C. albicans*, which has emerged as one of the most studied microbial pathogens. Future work is seeing the translation of these technologies to the so called "non-*albicans*" species to address relevant clinical and biological questions. Some of the biggest clinical questions are still to be answered and the need for vaccines, sensitive diagnostics and new drugs to improve clinical outcome remain strong research drivers for the future.

4. *Fusarium oxysporum*

The first description of *Fusarium* was made by Link in 1809 (Supplementary Fig. 1C). In 1940, Snyder and Hansen grouped all the species of the genus in 9 taxa and reclassified the infrageneric group called *elegans* into a single *F. oxysporum* species, designating different *formae speciales* (ff. spp.) based on their pathogenicity on different plant species (Snyder and Hansen, 1940). To date, more than 150 ff. spp. have been reported (Michielse and Rep, 2009). Interestingly, molecular phylogenetic studies have revealed substantial genetic diversity among isolates, supporting the current view that *F. oxysporum* represents a species complex. In 1998, a pioneering study established that field isolates of the f. sp. *cubense* have polyphyletic origins, suggesting that the capacity to infect a given plant host has arisen several times during evolution (O'Donnell et al., 1998).

Fusarium oxysporum causes vascular wilt disease. Spores present in the soil germinate in response to signals from the plant host and differentiate infection hyphae, which adhere to the plant roots and penetrate them directly without the need for specialized infection structures (Supplementary Fig. 2C). Root penetration appears to occur predominantly through natural openings at the intercellular junctions of cortical cells or through wounds (Perez-Nadales and Di Pietro, 2011). Once inside the root, hyphae grow inter- and intracellularly to invade the cortex and cross the endodermis, until they reach the xylem vessels. The fungus then uses the xylem as a conduit to colonize the host. Disease symptoms include wilting, chlorosis, necrosis, premature leaf loss, browning of the vascular system and stunting, which eventually will lead to plant death (Michielse and Rep, 2009). Small oval-shaped microconida, falcate macroconidia and thick-walled chlamydospores are formed on the dead plant tissue and in soil. *F. oxysporum* can survive in soil for extended time periods, either as chlamydospores or by growing saprophytically on organic compounds until a new cycle of infection starts (Agrios, 2005)

Although a sexual stage has not yet been described, the genome contains apparently functional mating type idiomorphs (MAT1-1 and MAT-1-2) similar to those found in sexual Fusarium species. The *mat1-1-1* gene encodes a protein carrying an α-box motif, whereas *mat1-2-1* encodes a transcription factor with a high-mobility-group (HMG) DNA binding domain. *F. oxysporum* MAT genes are expressed and all the predicted introns are correctly processed (Arie et al., 2000). These observations indicate that, similar to other fungal pathogens previously thought to lack sex, the presence of a cryptic sexual cycle may remain to be discovered in *F. oxysporum*.

The genome sequence of the tomato pathogenic isolate *F. oxysporum* f. sp. *lycopersici* was published in 2010 (Ma et al., 2010). Since then, eleven additional *F. oxysporum* strains have been sequenced by the Broad Institute. The availability of the complete genome sequences (accesible at the Fusarium Comparative Database, Table 3) as well as of molecular tools and well-established pathogenicity assays has allowed to address the genetic bases and evolutionary origins of pathogenicity and host range in *F. oxysporum*.

4.1. Overview of the F. oxysporum genome

The genome sequencing, assembly and annotation of *Fusarium oxysporum* f. sp. *lycopersici* was performed by the Broad Institute as part of the Fusarium Comparative Sequencing Project. A striking feature is that 28% of the *F. oxysporum* genome corresponds to repetitive sequences, including many retroelements and short interspersed elements (SINEs) as well as class II transposable elements (TEs) (Table 2). Comparison of the *F. oxysporum* genome with those of *F. graminearum* and *F. verticillioides* led to the discovery of four supernumerary chromosomes that are enriched for TEs and for genes putatively related to host–pathogen interactions (Ma et al., 2010). These so-called lineage-specific (LS) regions contain more than 95% of all DNA transposons. Only 20% of the predicted genes in the LS regions could be functionally classified on the basis of homology to known proteins. Many encode predicted secreted effectors, virulence factors, transcription factors and proteins involved in signal transduction but less housekeeping proteins. Recent data suggest that LS regions of *F. oxysporum* strains with different host specificities may differ considerably in sequence.

4.2. Assessing virulence of F. oxysporum

A variety of virulence assays have been developed in *F. oxysporum* to study invasive growth and pathogenicity (Table 3). The tomato root infection assay is performed by immersing the roots of two week-old seedlings in a microconidial suspension in distilled water, followed by planting in vermiculite and maintenance in a growth chamber at 28 °C (Di Pietro and Roncero, 1998). The severity of disease symptoms has been traditionally measured using a disease index ranging from 1 (healthy plant) to 5 (dead plant) (Fig. 2E). Alternative methods involve measurement of vascular browning, determination of the plant weight above the cotyledons and using a disease index on a scale from 0 (healthy plant) to 4 (very small wilted or dead plant) (Michielse et al., 2009). More recently, disease severity has been plotted as a percentage of plant survival against time by recording mortality each day for 30–45 days. This method allows statistical analysis of survival rates by the Kaplan–Meier method and comparison among groups using the log-rank test (Lopez-Berges et al., 2012, 2013). *In planta* quantification of fungal biomass is performed by extracting total genomic DNA from infected tomato roots and/or stems at different times post-infection, followed by quantitative real-time PCR analysis (Pareja-Jaime et al., 2010) using the *Fusarium*-specific *six1* gene (Rep et al., 2004) and normalization to the tomato *gadph* gene. Quantitative assessment of root penetration by *F. oxysporum* germ tubes during the initial 12–24 h after inoculation was performed using scanning electron microscopy (Perez-Nadales and Di Pietro, 2011). The ability of fungal germlings to attach to roots is determined by incubating host roots with fungal spores for 24–48 h in potato dextrose broth diluted 1:50 with water (Di Pietro et al., 2001; Prados Rosales and Di Pietro, 2008).

Rapid invasive growth assays on tomato or apple fruit tissue (Fig. 2E and F) are performed by injecting a microconidial suspension into tomato fruits or apple slices and evaluating invasive growth and maceration of the surrounding fruit tissue (Di Pietro et al., 2001). The *in vitro* cellophane penetration assay has been shown to correlate significantly with *in vivo* pathogenicity on tomato plants. For this assay, the fungus is allowed to grow on a cellophane membrane placed on a solid agar medium plate. The cellophane with the fungal colony is removed 2–4 days after inoculation and the ability of the fungus to penetrate the membrane to reach the underlying medium is evaluated (Prados Rosales and Di Pietro, 2008).

A mouse infection model was established for the tomato pathogenic *F. oxysporum* f. sp. *lycopersici* strain, making this the first fungal isolate to serve as a dual model for the study of fungal pathogenicity in plants and mammals (Ortoneda et al., 2004). Immunosuppressed mice are inoculated by injecting microconidia into a lateral vein of the tail. Mortality of the animals is recorded each day for 15 days and survival rates are estimated as described above for the plant root infection assay. Fungal tissue burden in kidneys and lungs at 7 days post-infection was also evaluated using standard plating methods (Lopez-Berges et al., 2012, 2013; Ortoneda et al., 2004) and histopathological analysis (Schäfer et al., 2014). The greater wax moth *Galleria mellonella* has been used as a non-vertebrate infection model to reduce the need for using mammals for *in vivo* testing (Fig. 2C). *F. oxysporum* was able to proliferate inside the haemocoel of *G. mellonella* larvae and to kill and colonize the insects. Importantly, most genes required for full virulence on immune-suppressed mice also played a significant role in *G. mellonella* infection (Navarro-Velasco et al., 2011).

4.3. Current research interests

4.3.1. Role of MAPK cascades in virulence

The *F. oxysporum* MAPK Fmk1, an orthologue of the yeast Fus3/Kss1 MAPKs, was found to be essential for virulence on tomato plants (Di Pietro et al., 2001). Infection-related processes such as invasive growth, vegetative hyphal fusion and root adhesion (Di Pietro et al., 2001; Prados Rosales and Di Pietro, 2008) absolutely require Fmk1 and are negatively controlled by the nitrogen source ammonium (Lopez-Berges et al., 2010). Because this MAPK is widely conserved among fungi and determines pathogenicity in all plant pathogens studied so far (Rispail et al., 2009), a major effort has been directed towards elucidating the upstream and downstream components of this signaling cascade. The homeodomain transcription factor Ste12 was shown to function downstream of Fmk1 and to be required for invasive growth, the most critical of the Fmk1-regulated functions for plant infection (Rispail and Di Pietro, 2009). The mucin-like transmembrane protein Msb2 was recently characterized as an upstream component of the cascade (Perez-Nadales and Di Pietro, 2011). In addition to the Fmk1 pathway, orthologues of the *S. cerevisiae* high osmolarity Hog1 and cell integrity Mpk1 MAPK signaling cascades have also been identified in *F. oxysporum* and are currently under investigation. The Rho-type GTPase Rho1, which functions upstream of Mpk1, was found to be essential for morphogenesis and pathogenicity (Martinez-Rocha

et al., 2008). Future studies will address how signaling through different MAPK cascades is orchestrated to control infectious growth in *F. oxysporum*.

4.3.2. Lineage specific (LS) chromosomes

Sequence characteristics of the genes present on the LS genome regions indicate a distinct evolutionary origin from the core genome, suggesting that they could have been acquired through horizontal transfer from another *Fusarium* species. This idea was experimentally supported by the finding that co-incubation of two strains of *F. oxysporum* can result in transfer of small LS chromosomes from a tomato pathogenic to a non-pathogenic strain, converting the latter in a pathogen. This led to the hypothesis that horizontal chromosome transfer in *F. oxysporum* can generate new pathogenic lineages (Ma et al., 2010). The genetic and cellular mechanisms underlying these processes are currently the subject of intensive study.

4.3.3. Secreted effectors and gene-for-gene system

Host specificity between different races of *F. oxysporum* f. sp. *lycopersici* and tomato cultivars is determined by a set of pathogen genes encoding small secreted cysteine-rich effector proteins termed SIX (Secreted In Xylem) (Rep et al., 2004). One of these proteins, Avr1, *triggers a* resistance response in tomato plants carrying the matching resistance *(R)* gene, *I-1*. Intriguingly, Avr1 also functions as a virulence effector by suppressing disease resistance conferred by two other *R* genes, *I-2* and *I-3* (Houterman et al., 2008). Most *six* genes are located on the same LS chromosome (chromosome 14), also called the pathogenicity chromosome and are associated with chromosomal sub-regions enriched for DNA transposons (Ma et al., 2010). Expression of most *six* genes is specifically induced *in planta* and requires the transcription factor Sge1, which is located on a core chromosome (Michielse and Rep, 2009).

4.3.4. F. oxysporum as a model for fungal trans-kingdom pathogenicity

F. oxysporum f. sp. *lycopersici* isolate FGSC 9935 was the first fungal strain reported to cause disease both on plant (tomato) and mammalian hosts (immunodepressed mice) (Ortoneda et al., 2004). Since then, a number of fungal genes have been identified that are either required for pathogenicity on tomato but not on mice, such as those encoding the Fmk1 MAPK or the small G protein Rho1 (Di Pietro et al., 2001; Martinez-Rocha et al., 2008), or for virulence on mice but not on plants, such as the pH response factor PacC (Caracuel et al., 2003) or the secreted Pathogenesis Related 1 (PR-1)–like protein Fpr1 (Prados-Rosales et al., 2012). Recently HapX, a transcription factor that governs iron homeostasis, was characterized as the first virulence determinant required for both plant and animal infection in the same fungal strain (Lopez-Berges et al., 2012). Similarly, the velvet protein complex, a conserved regulator of fungal development and secondary metabolism, contributes to infection of plants and mammals, in part by promoting the biosynthesis of the depsipeptide mycotoxin beauvericin (Lopez-Berges et al., 2013). Most of the evidence obtained so far suggests that fungal pathogenicity on plants and animals may have fundamentally distinct evolutionary origins.

4.4. Conclusions

The establishment of *F. oxysporum* as a plant and animal infection model, the use of molecular genetic approaches in this species and the genomic characterization of different *f.f. spp.* has advanced our understanding on several key aspects related to fungal pathogenicity as well as our knowledge of the evolutionary origins and key mechanisms underlying the parasitic lifestyle of fungi. In the future, *F. oxysporum* is likely to continue providing valuable new insights into the molecular bases of both host specificity and pathogenicity on evolutionary distant hosts.

5. *Ashbya gossypii*

The filamentous ascomycete *Ashbya gossypii* belongs to the genus *Eremothecium* and causes stigmatomycosis described by Ashby and Nowell in 1926 (Supplementary Fig. 1D). Stigmatomycosis or 'yeast spot disease' was also linked to other *Eremothecium* species, e.g. *Eremothecium coryli* (syn. *Nematospora coryli*) and is a fungal disease that occurs in a number of crops, especially cotton and pistachio but also soybean, pecan or citrus fruits. These fungi are wound parasites and are transmitted by hemipteran insects (Dietrich et al., 2013). *A. gossypii* was also identified as a natural overproducer of riboflavin/vitamin B_2. In the 1970s, chemical synthesis of riboflavin was replaced by biotechnological means using strains of *A. gossypii, Candida famata or Bacillus subtilis* (Stahmann et al., 2000). *Ashbya* belongs to the *Saccharomycete* family of yeasts. This close relationship to yeast like fungi, the small genome size (9 Mb) and its molecular genetic tractability has turned *Ashbya* into an interesting model system for fungal growth, development and genome evolution (Dietrich et al., 2004; Steiner et al., 1995; Wendland and Walther, 2005).

Needle-shaped *Ashbya* spores, often connected by whip-like filaments, contain a single haploid nucleus each. Spore germination generates a ball-shaped germ cell (Wendland and Walther, 2005) (Supplementary Fig. 2D). Polarity establishment requiring the Cdc42-GTPase module results in germ tube emergence and the establishment of polarized hyphal growth. (Wendland and Philippsen, 2001). Once polar growth is established, filamentous growth is maintained (Supplemental Fig. 2D). *Ashbya* filaments are septate and multi-nucleate. Nuclear divisions in a common cytoplasm are asynchronous based on localized G1-cyclin transcript accumulation (Gladfelter et al., 2006). In juvenile *Ashbya* mycelia, additional axes of polarity are generated by lateral branches. *Ashbya* mycelia mature upon compartmentalization of germlings by septation. Hyphal maturation, which occurs approximately 20 h after the isotropic-to-polar switch that formed the first germ tube, results in a dramatic increase in growth speed (10-fold) to about 200 μm/h (Kohli et al., 2008). In mature mycelia lateral branching is largely repressed and instead new axes of polarity are established by apical, dichotomous tip-branching events. This generates the characteristic Y-shaped hyphae of matured mycelia (Wendland and Walther, 2005).

At the end of the growth phase riboflavin overproduction is initiated and a developmental programme results in the formation of sporangia from septate hyphal segments. Sporangia will usually harbor two bundles of 4 needle shaped uninucleate spores. The genome of *A. gossypii* strain ATCC 10895 contains four copies of a *MAT**a*** mating type locus that harbors conserved **a**1 and **a**2 transcriptional regulators (Dietrich et al., 2013; Wendland and Walther, 2005). Three of the mating-type loci are close to the telomeric ends on chromosomes 4, 5, and 6 respectively, which is reminiscent of the position of the silent mating-type cassettes HMR and HML on chromosome III in *S. cerevisiae*. The putative active mating-type locus on chromosome 6 shows conserved synteny to active *MAT*-loci in Ascomycetes (Wendland and Walther, 2005). Generally, in sexually reproducing Ascomycetes mating partners of two opposite mating-types (in *S. cerevisiae* **a** or α) are attracted by a pheromone signal relay that leads to cell fusion, karyogamy, and formation of diploid nuclei that can undergo meiosis and spore formation. Although the pheromone response pathway is

conserved in *Ashbya*, deletion of key elements of this pathway, e.g. the pheromone receptor genes *STE2* and *STE3* or the downstream transcription factor *STE12* does not abolish sporulation (Wendland et al., 2011).

5.1. Comparative genomics in Eremothecium

Several genomes within the genus *Eremothecium* have been entirely sequenced and annotated. The first was that of *A. gossypii*, while the genome of *E. cymbalariae* represents that of the type strain of the genus that was isolated by Borzi in 1888 (Dietrich et al., 2004; Wendland and Walther, 2011). Recently, an *A. gossypii* isolate from Florida was shown to bear 99% sequence identity with the known *A. gossypii* genome. In addition, *Ashbya aceri* represents a new species, which shows 90% identity with *A. gossypii* and its genome organization differs from *A. gossypii* only by eight translocation events (Dietrich et al., 2013).

A comparison of the *A. gossypii* ATCC10895 and *E. cymbalariae* genomes revealed the basis for genome evolution in the *Eremothecium* genus. The *E. cymbalariae* genome contains 9.7 Mb (excluding rDNA-repeats) on eight chromosomes while that of *A. gossypii* is only 8.8 Mb distributed on only seven chromsomes (Table 2). This genome reduction is largely due to shorter intergenic regions as the number of genes is almost identical. In *Ashbya,* chromosome number was reduced by breakage of one chromosome at the centromere and fusion of the chromosome arms to telomeres of other chromosomes (Gordon et al., 2011).

E. cymbalariae hosts one (potentially non-functional) TY3-Gypsy transposon whereas in *A. gossypii* no transposable element or LTR-remnants were found. Furthermore, the *Ashbya* genome shows a very high GC content of ~52 % compared to 40 % in *E. cymbalariae* and in other *Saccharomyces* complex species. Such a high GC content may be the result of evolutionary recombination events that drive GC-content (Wendland et al., 2011).

5.2. Current research interests

5.2.1. Riboflavin production

Riboflavin/vitamin B_2 is essential for basic cellular metabolism since it is a precursor for many flavin-based coenzymes including flavin mononucleotide and flavin adenine dinucleotide. While many microorganisms, plants and fungi can synthesize riboflavin *de novo* from various carbon sources, humans lack this ability and must obtain this vitamin from their diet.

Today, *A. gossypii* is one of the main organisms used for biotechnological riboflavin production. Riboflavin production in *Ashbya* is highly increased at the end of the growth phase during sporulation. Recently, *Ashbya gossypii* was isolated from mouth parts of heteropteran insects feeding on milkweed and oleander, which produce toxic alkaloids. This led to the interesting hypothesis that riboflavin produced by *A. gossypii* helps these insects to feed on these plants (Dietrich et al., 2013). Previously, it was shown that riboflavin provides some UV-protection for the spores (Stahmann et al., 2001). On the molecular level, recent studies found that Yap1, a transcription factor that regulates responses to oxidative stress, also provides a link to riboflavin production (Walther and Wendland, 2012). Engineering of strains to increase riboflavin production included e.g. increasing the pool of riboflavin precursors GTP and glycine (Kato and Park, 2012). The molecular details linking riboflavin production and sporulation are currently being investigated.

5.2.2. Hyphal growth

Based on comparative biology studies between *S. cerevisiae* and *Ashbya*, it was found that multiple Rho-protein modules are involved in processes of cell polarity establishment, cell wall integrity and maintenance of polarized hyphal growth (Wendland and Philippsen, 2001). Further studies characterized polarisome components Spa2 and Bni1 and the Ras-like GTPase Bud1 and their roles in maintaining hyphal cell polarity (Bauer et al., 2004; Schmitz et al., 2006). Recently, the transmembrane protein Axl2 was found to be involved in polarity establishment, polarity maintenance and stress response (Anker and Gladfelter, 2011). Hyphal maturation in *Ashbya* results in increased growth speed and suppresses lateral branching in favor of dichotomous branching at the hyphal tips. Tip splitting was shown to be dependent on the activity of the p21-activated kinase Cla4 and its downstream effector Pxl1, which may coordinate polarity factors and vesicle processing during tip splitting and fast hyphal elongation (Wendland and Walther, 2005). Comparative analyses between *Eremothecium* species and *S. cerevisiae* will elucidate gene expression differences, transcriptional circuitry, the analysis of protein complexes and polarized transport. This will shed light on how *Bauplan* differences can be generated from apparently the same set of genes.

5.2.3. Septation

The actin cytoskeleton plays a major role in polarized hyphal growth. Actin is found in three structures: as linear actin filaments emanating from the polarisome at hyphal tips, as contractile actin rings at sites of septation and as actin patches at sites of endocytosis. Generating linear actin filaments at sites of polarized growth is a function of the polarity establishment machinery and involves Cdc42, the formin Bni1, and the polarisome (Schmitz et al., 2006; Wendland and Walther, 2005).

Localization of septal sites is directed via cortical cues, so called "landmarks", which usually are membrane-associated proteins that direct proteins or protein complexes to specific sites in the cell. In *A. gossypii*, the yeast Bud3 landmark homolog was shown to be involved in septum formation. Bud3 localizes to septal sites and *bud3* mutants are partially defective in actin ring formation due to the mislocalization of Cyk1 (ScIqg1), which is essential for actin ring formation (Wendland and Walther, 2005). Cyk2/Hof1 is also required for actin ring formation and its SH3-domain is required for actin ring integrity (Kaufmann and Philippsen, 2009). The coiled-coil domains of the Cdc11 and Cdc12 septins are required for septin ring formation and phosphorylation of septins plays an essential role for septin dynamics (Meseroll et al., 2013). The novel process of septin assembly, termed 'annealing' was found to depend on membranes which allows cells to use this intrinsic property of septins at specific sites at the cell cortex (Bridges et al., 2014).

5.2.4. Nuclear division and movement

Cellular compartments within *Ashbya* hyphae are generated via septation. These compartments are multi-nucleate and will be transformed into sporangia at a later developmental stage. In contrast to *S. cerevisiae,* nuclei in *A. gossypii* divide asynchronously (Gladfelter et al., 2006). The lengths of the nuclear division cycles of individual nuclei can range from 45 min up to 200 min and asynchrony seems to be intrinsic since it returns rapidly after artificial synchronization. Recently, specific localization of a G1 cyclin transcript by the Whi3 RNA-binding protein has been shown to contribute to this asynchrony (Gladfelter et al., 2006).

In *S. cerevisiae*, mitotic divisions require two regulatory networks, the Cdc fourteen early anaphase release (FEAR) and the mitotic exit network (MEN), both of which drive exit from mitosis by activating the Cdc14 phosphatase. While the *A. gossypii* FEAR network seems to be conserved in regulating the M/G1 transition, the *A. gossypii* MEN pathway was shown to have diverged significantly from its anticipated role in exit from mitosis and appears to play a crucial role in septum formation (Finlayson et al., 2011).

During hyphal growth, nuclei need to migrate long distances toward the growing tip using cytoplasmic microtubules. In addition to this long-range migration, nuclei of *A. gossypii* show also extensive oscillation, rotation and occasional bypassing of one another. Similar to *S. cerevisiae*, spindle pole bodies (SPBs) are the only microtubule organizing centers in *A. gossypii.* The laminar SPB ultra structure is similar between *S. cerevisiae* and *A. gossypii*, although there are differences at the cytoplasmic phase and additional types of cMTs (long tangential cMTs) were identified. Accordingly, significant differences regarding the control of cMT dynamics and the role of MT-dependent motor proteins were identified between *S. cerevisiae* and *A. gossypii* (Grava and Philippsen, 2010).

5.2.5. Genome evolution

Comparative genomics are being employed to analyze evolutionary trajectories in *A. gossypii* and *E. cymbalariae*. Broad changes can be seen in terms of the size of clusters of synteny between both species or to the yeast ancestor. The *E. cymbalariae* genome bears closer resemblance to the yeast ancestor in terms of number of chromosomes, arrangement of mating-type loci, conservation of telomeric loci and GC content (Wendland and Walther, 2011). Establishing other genome sequences within this genus will certainly provide more detailed insight into *Eremothecium* genome evolution. This could lead, for example, to the compilation of the genome of an *Eremothecium* ancestor at the base of the *Eremothecium* lineage that could be compared with other ancestral genomes, e.g. that of the yeast ancestor just prior to the whole genome duplication.

5.3. Conclusions

Ashbya has a key role in the biotechnological production of riboflavin. Molecular studies will further elucidate the co-regulation of riboflavin production and sporulation. Comparative biology with *A. gossypii* and *S. cerevisiae* will help to understand the regulatory mechanisms governing the *Bauplan* differences of yeast and hyphal cells. This may also result in understanding the molecular mechanisms of 'yeast spot disease'.

6. *Magnaporthe oryzae*

The plant-pathogenic fungus *Magnaporthe oryzae* is a filamentous ascomycete that causes blast disease on rice (*Oryza sativa*), the principal disease of cultivated rice worldwide, with yield losses varying between 10% and 30% (Talbot, 2003). The pathogen affects several other grasses such as wheat, finger millet and barley (Talbot, 2003) (Table 1). *Magnaporthe oryzae* B.C. Couch was first isolated from rice (*Oryza sativa*) by F. Cavara in 1892 as *Pyricularia oryzae* (Couch and Kohn, 2002; Rossman et al., 1990). Rice blast disease has, however, been known since 7000 BC, when domestication of *O. sativa* started in the Yangtze Valley in China (Couch et al., 2005) (Supplementary Fig. 1E). *M. oryzae* was described as rice fever disease in China in 1637 and only later did it become more widely known as the rice blast fungus (Ou, 1985). The actual name "blast" refers to the fast expansion of the disease within the rice fields.

The life cycle of *M. oryzae* consists of a hemi-biotrophic infection cycle and predominantly asexual mode of reproduction (Supplementary Fig. 2E). The sexual stage exists but is rarely found in nature in rice-infecting populations (Notteghem and Silué, 1992; Talbot et al., 1993b). Analyses of *M. oryzae* populations showed that in most areas only one of the mating types, MAT1-1 or MAT1-2, predominates or when both are present, isolates lack fertility (Notteghem and Silué, 1992). However, it is possible to initiate sexual reproduction by pairing fertile strains of opposite mating types (Notteghem and Silué, 1992; Valent et al., 1991). This leads to development of fruiting bodies (perithecia) which enclose asci filled with octads of ascospores (Valent et al., 1991).

During its asexual cycle *M. oryzae* produces three-cell conidia which are spread by dew drop splash (Talbot, 2003). The infection cycle starts when a conidium lands on a rice leaf (Hamer et al., 1988). A polarized germ tube emerges from the tip and elongates, becoming flattened against the surface, a phase known as hooking. At this stage, physical cues such as hydrophobicity, surface hardness and plant signals such as cutin monomers, trigger the formation of specialized infection structures called appressoria (Talbot et al., 1993a). The appressorium is a single dome-shaped melanised cell, which is able to break the cuticle by translating high internal turgor into mechanical force (Dixon et al., 1999). It contains a pore at its base, from which a penetration peg emerges to break through the cuticle of the rice leaf, initiating invasive growth. Invasive hyphae are surrounded by the plant plasma membrane and ramify through plant tissue for 3–4 days until lesion formation occurs (Kankanala et al., 2007). After 72 h, almost 10% of biomass of a rice leaf can be *M. oryzae* hyphae in heavy infections (Talbot et al., 1993b). After 4–5 days, disease lesions are visible on the plant surface from which conidiophores develop to produce conidia that spread to new plants (Talbot et al., 1993a).

In the last 20 years, *M. oryzae* has emerged as a model organism in phytopathology, especially as a system for the study of the plant-pathogen interaction. The availability of methods for *in vitro* cultivation and stimulation of appressorium development, together with the availability of the genome sequence and tools for classical and molecular genetics makes this fungus an excellent experimental model (Wilson and Talbot, 2009).

6.1. Overview of the M. oryzae genome

In 2002, the Fungal Genome Initiative (FGI) released a draft genome sequence of *M. oryzae* strain 70–15. The final genome sequence, obtained by whole-genome shotgun sequencing, was published in 2005 (Dean et al., 2005). The total size of the *M. oryzae* genome is 41.7 Mb and is organized in 7 chromosomes (Table 2). The actual total number of genes is predicted to be between 12,827 and 16,000 with a density of at least one gene every 4 Kb. Repetitive DNA is prevalent in the genome with 15 families of transposable elements present. There are 4734 *M. oryzae* genes in common with *S. cerevisiae*.

6.2. Assessing virulence of M. oryzae

Magnaporthe oryzae has emerged as a major model organism for plant microbe interactions. The fungus can be grown *in vitro* and infection structures can be generated on artificial surfaces such as glass or Teflon® (Polytetrafluoroethylene) (Table 3). This characteristic and the availability of standard pathogenicity assays in both rice and barley make *M. oryzae* an excellent model to study plant microbe interactions. Among pathogenicity assays, the most widely used method is spray-inoculation of 3–4 leaf staged rice seedlings. Plants are stored for 1 day at high relative humidity (RH > 95%) and then transferred to growth chambers for 5–10 days, until symptoms are detectable (Fig. 2H) (Valent et al., 1991). Virulence can be quantified according to different criteria such as lesion number per leaf, average lesion size and lesion sporulation rate (spores/cm^2). The rice leaf sheath assay readily allows observation of invasive hyphae inside cells (Kankanala et al., 2007) (Fig. 2I). Spray and drop-inoculation of barley are also used as pathogenicity assay (Fig. 2J and K). Sterilized onion epidermis and detached rice leaves using a spot inoculation method can also be used to monitor penetration and invasion (Fig. 2K).

6.3. Current research interests

6.3.1. Mechanisms of fungal infection

The appressorium is a specialized infection structure which allows *M. oryzae* to invade its host. Generation of turgor inside the appressorial dome results in production of a penetration peg. The penetration peg is formed following cytoskeletal repolarization mediated by septin GTPases (Dagdas et al., 2012). Septins form a toroidal ring structure at the base of the appressorial pore, which leads to the reorientation of the F-actin cytoskeleton and initiation of membrane curvature and peg development (Dagdas et al., 2012). Regulation of cytoskeleton rearrangement is controlled through the regulated synthesis of reactive oxygen species by NADPH oxidases (Ryder et al., 2013). Discovery of this toroidal structure at the base of the appressorium has opened new research questions regarding the remodeling of septins which lead to aggregation of F-actin and how these processes are coordinated with turgor generation. The characterization of these intracellular mechanisms will be critical for understanding successful appressorium formation and initial plant infection caused by *M. oryzae* in the rice plants.

6.3.2. Plant pathogen interactions in the rice blast fungus M. oryzae

During plant tissue invasion *M. oryzae* secretes a battery of effector proteins to counteract plant defense responses and manipulate the host and facilitate fungal growth. Effectors suppress PAMP (Pathogen-Associated Molecular Patterns) triggered immunity responses of the plant to allow a successful invasion and disease. *M. oryzae* secretes both apoplastic and cytoplasmic effectors during infection. Cytoplasmic effectors accumulate in the biotrophic interfacial complex (BIC). A well-known example is *M. oryzae* Avr-Pita effector that confers resistance to rice cultivars expressing the R gene Pita. Avr-Pita is translocated and accumulated at the BIC, however, very little is known about its virulence function except that it appears to interact with its cognate resistance protein Pi-ta when present (Jia et al., 2000). It has recently been reported that cytoplasmic effectors may be secreted via an unconventional secretion system involving the exocyst complex (Giraldo et al., 2013). Apoplastic effectors, on the other hand, are secreted through the conventional tip secretion pathway (Giraldo et al., 2013). Interesting ongoing studies aim to determine the translocation motif for effectors going through the BIC and what distinguishes these from effectors accumulating in the apoplast.

6.3.3. Signaling

Several signaling cascades are implicated in plant infection by *M. oryzae* including MAP kinase, cAMP-signaling and the Ca^{2+}/calcineurin pathways. All of these pathways mediate the intracellular response to environmental signals perceived from the plant and the prevailing environmental conditions.

M. oryzae possesses three MAP kinase pathways including the MAPKs Osm1, Mps1 and Pmk1 (Dixon et al., 1999; Xu and Hamer, 1996; Xu et al., 1998). Null mutants of the Osm1 MAP kinase show defects in sporulation and increased sensitivity to osmotic stress but remain fully pathogenic, indicating that appressorium turgor responses are unaffected by loss of hyperosmotic stress adaptation (Dixon et al., 1999). The Mps1 MAP kinase is involved in cell wall integrity and *M. oryzae* null *mps1* mutants are non-pathogenic because they are not able to form the penetration peg responsible for cuticle penetration (Xu and Hamer, 1996; Xu et al., 1998). Null mutants of Pmk1 MAP kinase are unable to produce appressoria or invade the host (Xu and Hamer, 1996).

The cAMP signaling pathway is also involved in pathogenicity and required for formation of the appressoria (Adachi and Hamer, 1998). Cyclic AMP (cAMP) produced by the adenylate cyclase binds to the regulatory subunit of the the cAMP-dependent protein kinase A (*RPKA*), releasing the catalytic subunit PKA (*CPKA*). Active CPKA activates by phosphorylation several target proteins. Mutants of the adenylate cyclase gene MAC1 are not able to form appressoria, a defect which can be restored by addition of exogenous cAMP (Adachi and Hamer, 1998; Wilson and Talbot, 2009). Constitutive activation of e CPKA by a mutation in *RPKA1* leads to restoration of the appressorium development defect of *Δmac1* mutants (Choi and Dean, 1997). Δ*cpka* mutants are able to form appressoria but they are non-functional and fail to penetrate (Wilson and Talbot, 2009). These results suggest that PKA mediated activation of the cAMP pathway is essential to form functional appressoria.

The Ca^{2+}/calcineurin pathway also plays an important role in the growth and pathogenicity of *M. oryzae*. Environmental stress increases intracellular Ca^{2+} level and leads to the binding of Ca^{2+} to calmodulin. The Ca^{2+}/calmodulin complex activates calcineurin, a highly conserved protein phosphatase heterodimer, which dephoshorylates the transcription factor Crz1 to induce the expression of stress responsive genes. Deletion of the *CRZ1* gene results in lower sporulation and highly reduced pathogenicity (Choi et al., 2009).

Studies have shown that there is an interaction between Pmk1 MAPK pathway and cAMP signaling (Li et al., 2012) and it is likely that there is cross talk between Mps1 MAPK and Ca^{2+}/calcineurin pathways, similar to that found in yeast (Li et al., 2012). One current line of research is to establish the nature of these interactions and to elucidate which processes they affect.

6.4. Conclusions

Although the first biological studies on *Magnaporthe oryzae* were performed more than 100 years ago, it is only within the last 20 years that it has attained model organism status. With highly efficient cells specialized in penetration (appressoria) and infection (biotrophic infectious hyphae) and the possibility to study these structures *in vitro* and *in vivo*, *M. oryzae* is a valuable model to study plant-pathogen interactions. Several genetic tools, including transformation by homologous recombination, availability of reporter genes for live cell imaging, together with a fully sequenced genome and extensive expression data and mutant collections has led to new insight into the process of plant infection.

Research on *M. oryzae* has focused primarily on the molecular biology of plant-pathogen interaction, identification of virulence determinants by mutation, fungal infection mechanisms and signaling pathways required for pathogenicity. Applying this knowledge to disease control will be critical in future. The current spread of wheat blast disease in South America, including regions in Brazil, Bolivia and Paraguay has opened up new research involving comparative genomics and epidemiology of populations, affecting rice, wild grasses and wheat. It will be critical to develop new and more durable control strategies for this devastating disease.

7. *Ustilago maydis*

Corn smut disease caused by *U. maydis* has interested biologists and farmers alike for more than 250 years (Supplementary Fig. 1F). But in spite of this, the complete life cycle of *U. maydis* remained mysterious until the sexual stage was discovered in 1927 (Stakman and Christensen, 1927). Genetic studies in *U. maydis* were initiated with the isolation of biochemical mutants by Perkins (1949). With the help of mutants and diploid strains in which homologous recombination could be induced, Holliday was able to deduce the formation of heteroduplex DNA during crossing over with a four-armed junction intermediate, later

termed the Holliday junction, yielding the first model for homologous recombination (Holliday, 1964).

With the advent of molecular genetics, a wide range of tools was developed allowing precise genome modifications (see Table 3). Research in *U. maydis* initially followed three main directions: (i) the characterization of genes involved in DNA recombination, (ii) the study of mating type loci and (iii) the so called Killer phenomenon, related to the presence of virus-like particles. Subsequently, researchers became interested in different aspects of the biology ranging from gene regulation, signaling and virulence to cell biology. Community efforts led to a high quality genome sequence that now serves as a blueprint for comparative studies (Kamper et al., 2006).

Nowadays, *U. maydis* is one of the best-characterized fungal plant pathogens, although it does not cause an economically important disease. Crop losses are usually below 2 % and crop rotation is used as control strategy (Dean et al., 2012). *U. maydis* exhibits a very narrow host range inducing disease only on corn and its progenitor teosinte (*Zea mays* ssp. *parviglumis*) (Table 1). During its life cycle, *U. maydis* undergoes a number of morphological transitions (Supplementary Fig. 2F). In the field, corn smut infections are spread by the air-borne diploid teliospores. During their germination, meiosis occurs and haploid progeny is produced. Haploid cells are yeast-like and divide by budding. Transition to the pathogenic form, the dikaryon, requires fusion of two haploid cells of opposite mating type (Feldbrugge et al., 2004). Cells differing in the *a* mating type locus recognize each other via lipopeptide pheromones, the cell cycle arrests in the G2 phase, budding is stopped and conjugation tubes are formed. These structures develop at one cell tip and grow towards each other guided through the pheromone gradient until they merge (Brefort et al., 2009). The resulting dikaryon switches to polar growth if the two mating partners carry different alleles of the *b* mating type locus. The *b* locus codes for a pair of homeodomain transcription factors that dimerize when derived from different alleles and control subsequent sexual and pathogenic development. In response to both chemical and physical signals of the plant surface, the dikaryotic filament forms poorly differentiated appressoria that penetrate the cuticle probably via the action of lytic enzymes (Brefort et al., 2009). After penetration, the cell cycle is reactivated concomitantly with the development of clamp-like structures that allow the correct sorting of nuclei to maintain the dikaryotic status (see Brefort et al., 2009). In this way, the fungus proliferates, forms a massive network of hyphae and induces plant tumors. Hyphal growth inside tumors is followed by sporogenesis, a poorly understood process that includes karyogamy, hyphal fragmentation and differentiation into melanized diploid teliospores. Eventually the tumors dry up, rupture and release the diploid spores, which are dispersed by air. This closes the life cycle.

7.1. Comparative genomics in U. maydis

The genome size of *U. maydis* is relatively small (20.5 Mb) in comparison to other plant pathogenic fungi (Table 2). An open source, manually curated MUMDB platform has been developed at Munich Information Center for Protein Sequences, MIPS (http://mips.helmholtz-muenchen.de/genre/proj/ustilago/). To date, a total of 6788 protein-encoding genes are annotated. The small genome size is attributed to few introns (70% of the genes contain no introns) and the absence of large scale DNA duplications. Non-functional transposon-derived sequences constitute only 1.1% of the genome assembly (Kamper et al., 2006). RNA interference (RNAi) components are absent and it has been hypothesized they may have been lost due to an incompatibility between RNAi and killer, a viral double stranded RNA system encoding a protein toxin providing a selective advantage (Drinnenberg et al., 2011). Signs of gene inactivation by repeat induced point mutation are also absent (Laurie et al., 2008).

The repertoire of genes coding for secreted hydrolytic enzymes is small and this has been correlated to the biotrophic lifestyle of the pathogen in which damage to the host that can trigger defense responses is minimized. By contrast, the genome contains a significant expansion in novel genes encoding secreted proteins, putative effectors which are involved in the control and reprogramming of the host during infection. Comparative analyses between *U. maydis*, *Sporisorium reilianum*, the causal agent of head smut of maize (Schirawski et al., 2010) and the barley covered smut *Ustilago hordei* (Laurie et al., 2012) demonstrated that smut fungi display largely syntenic chromosomes, although the overall sequence identity is only around 70%. Sequence identity with the related animal pathogens *Malassezia globosa* and *M. sympodialis* (Xu et al., 2007) and the biocontrol agent *Pseudozyma flocculosa* (Lefebvre et al., 2013) is significantly lower. The high level of synteny between *U. maydis* and *S. reilianum* allowed detection of 43 "divergence clusters" in which the sequence conservation is lower than average and many of these clusters encode effectors. This indicates that although both pathogens are able to infect the same host and about 90% of the putative secreted effectors are shared, many of these genes have undergone discrete specialization events during evolution (Schirawski et al., 2010). Moreover, the clusters of secreted proteins typical of *U. maydis* and *S. reilianum* are absent in the *Malassezia* and *Pseudozyma* genomes.

7.2. Assessing virulence of U. maydis

To assess virulence, artificial syringe inoculations are carried out on 7-day-old maize seedlings (Fig. 2L). Scoring for symptoms is done usually 12 days post injection following a disease rating developed by Kamper et al. (2006). The variety Early Golden Bantam is commonly used as host as symptoms are especially severe on sweet corn. For infections of tassel or ears, the dwarf variety Gaspe Flint has been adopted and other susceptible inbred maize lines have also been used (Skibbe et al., 2010).

The availability of haploid strains causing disease without a mating partner (termed solopathogenic) allows forward genetic approaches to analyze virulence, circumvents the need to introduce mutations into two compatible strains for virulence assays and allows to assess gene contributions to virulence independently of their involvement in mating (see Brefort et al., 2009).

7.3. Current research interests

7.3.1. Host perception and plant response

Surface recognition and penetration are among the most critical points during plant infection. In *U. maydis*, as well as in other fungal plant pathogens, surface perception involves the signaling mucin Msb2 and the tetraspanin protein Sho1 that regulate the activity of a downstream MAP kinase module via an as yet unknown mechanism (Lanver et al., 2010). For activity, Msb2 needs to be *O*-mannosylated by the mannosyltransferase Pmt4 (Fernandez-Alvarez et al., 2012). Already on the leaf surface, *U. maydis* is recognized by the plant via a non-specific PAMP-triggered reaction. However, with the establishment of biotrophy, plant defenses are suppressed. This includes various cell death-related processes such as ROS production and salicylic acid signaling (Djamei et al., 2011; Hemetsberger et al., 2012; Rabe et al., 2013). At later stages of the infection, photosynthesis and C_4 metabolism are significantly down-regulated (Brefort et al., 2009), illustrating metabolic reprogramming of the host.

7.3.2. Effector function

The *U. maydis* genome codes for more than 300 putatively secreted effector proteins mostly lacking any predicted enzymatic function or domain. Many effectors are encoded by gene clusters which are transcriptionally induced during plant colonization (Kamper et al., 2006). While some effectors show redundancy in function (Schirawski et al., 2010), others, such as *pep1* and *pit2*, are crucial virulence determinants. Pep1 acts as inhibitor of apoplastic plant peroxidases involved in H_2O_2 production (Hemetsberger et al., 2012), while Pit2 functions as an inhibitor of a set of apoplastic maize cysteine proteases, whose activity is directly linked with the salicylate pathway (Müller et al., 2013). The chorismate mutase Cmu1 was shown to enter plant cells after being secreted and re-routes the chorismate flow in maize cells towards phenylpropanoid compounds instead of salicylate (Djamei et al., 2011). How Cmu1 and probably additional effectors enter plant cells is currently unknown. A future challenge will be to elucidate the function of the *U. maydis* effector battery, which is likely to consist not only of conventionally secreted proteins but also of proteins secreted by unconventional routes (Reissmann S. and Kahmann R., unpublished) and to elucidate their mode of uptake by host cells.

7.3.3. Cell cycle, dimorphism and pathogenic development

A critical step in the life cycle of *U. maydis* is the morphological transition from the non-pathogenic haploid state to the dikaryotic infectious hypha. Many aspects of this transition remain poorly understood. Since pathogenic development is intimately coupled to cell cycle, several proteins controlling the cell cycle have been characterized. Among these are elements of the DNA damage response, such as Chk1 and Atr1 (de Sena-Tomas et al., 2011; Mielnichuk et al., 2009) and the protein Clp1 necessary for the correct nuclear distribution in the dikaryotic filament (Heimel et al., 2010). Recently, the cell wall in budding cells and filamentous hyphae has been analyzed. Proteins involved in β-1,6-glucan synthesis, N-glycosylation and glycosyl transfer seem to affect not only the morphological switch but also virulence (Robledo-Briones and Ruiz-Herrera, 2013). Finally, the production of antisense transcripts has been shown to have a regulatory role during pathogenic development (Donaldson and Saville, 2013). The detailed understanding of the function of proteins and RNAs controling cell cycle, dimorphism and virulence of *U. maydis* will be instrumental for the full understanding of the morpho-physiological transitions intimately coupled to discrete steps throughout the life cycle (Supplementary Fig. 1F).

7.3.4. Signaling and MAP kinase targets involved in virulence

Control of biotrophic development and virulence in *U. maydis* involves a complex cross-talk of regulatory and signaling mainly belonging to both the cAMP pathway and the pheromone MAPK cascade. The cAMP pathway is required for many aspects of the life cycle including nutritional sensing, morphogenesis and mating (Egan et al., 2009). The protein kinase A (PKA) activates among others the central regulator Prf1 which is responsible for expression of a large set of genes including the *a* and *b* mating type genes. Recently, two phosphodiesterases, UmPde1 and UmPde2, also acting on the c-AMP-dependent PKA pathway, have been implicated in pathogenic development as well as dimorphic growth control (Agarwal et al., 2013). cAMP signaling is connected with the pheromone responsive MAPK cascade, the best characterized signaling pathway in *U. maydis*. Mutants in components of this cascade are severely affected in their ability to infect corn plants. The core players in the MAPK pheromone pathway are the three hierarchical kinases Kpp4, Fuz7, and Kpp2. Two alternative MAPKs, Crk1 and Kpp6, necessary for full virulence, are also present (see Brefort et al., 2009).

As in other plant pathogenic fungi, two additional MAPK cascades exist in *U. maydis*: the cell wall integrity (CWI) pathway and the osmostress responsive MAPK cascade. The main components of these cascades, although not all characterized, are similar to those described in yeast as well as the plethora of stimuli to which they respond. In spite of the central role played by the CWI pathway in several pathogenic fungi, in *U. maydis* strains lacking elements of the CWI cascade, no apparent defects in the ability to infect maize plants have been detected (Carbo and Perez-Martin, 2010).

7.3.5. Understanding the molecular basis of fundamental eukaryotic processes

Due to its unexpected high level of genetic similarity shared with metazoans, *U. maydis* has recently been proposed as a model for the study of essential cellular processes of higher eukaryotes which are not present in *S. cerevisiae* or *Schizosaccharomyces pombe* (Steinberg and Perez-Martin, 2008). For instance, several homologues of human proteins involved in DNA repair, homologous recombination and genomic stability such as the Breast Cancer Type 2 susceptibility protein (BRCA2) or in long-distance transport such as kinesis-3 have been found in *U. maydis* (Kojic et al., 2002; Vollmeister et al., 2012). Surprisingly, *U. maydis* hyphae have similarities to mammalian neurons with respect to the elongation and orientation of microtubules and the transport of mRNA to the growth region (see Steinberg and Perez-Martin, 2008; Vollmeister et al., 2012). Moreover, the disassembly-assembly machinery of the nuclear pores during open mitosis appears conserved between *U. maydis* and animal cells (see Steinberg and Perez-Martin, 2008). Uniparental mitochondrial inheritance, a process dominating in sexual species including mammals, has also been described in *U. maydis* (Fedler et al., 2009). Furthermore, it has recently been demonstrated in *U. maydis* that several core enzymes for glycolysis reside in both the cytoplasm and in peroxisomes and that the peroxisomal targeting is achieved through post-transcriptional regulation. The presence of peroxisomal targeting sequences in glycolytic enzymes of mammals suggests that dual localization may be a general feature (Freitag et al., 2012).

7.4. Conclusions

In the years ahead we expect *U. maydis* to continue to serve as an attractive model for all aspects of microbial development where shape and morphological transitions are involved. We also expect this organism to continue to play a pioneering role for the study of complex biological processes involving genes for which no orthologs exist in yeast. And, last but not least, we expect the *U. maydis* system to reveal many more of the intricacies that have endowed this fungus with the ability to colonize a plant host and establish a biotrophic relationship. On the basis of comparative approaches we also expect to learn much more on the evolution of virulence traits and how they evolved to generate host specificity.

8. *Zymoseptoria tritici*

Zymoseptoria tritici (Desm.) is responsible for *Septoria tritici* leaf blotch (STB) on bread and durum wheat. The asexual form of the fungus was first described in 1842 by Desmazieres, while its sexual stage was only identified in 1972 (Quaedvlieg et al., 2011) (Supplementary Fig. 1G). Both asexual and sexual stages are currently reported in all wheat growing countries. STB has a severe economic impact in wheat-producing areas leading up to 30–50% of annual yield losses (Suffert et al., 2011). The disease is controlled mainly by fungicide treatments, however, *Z. tritici* populations resistant to fungicides have appeared worldwide and only a few resistant wheat cultivars are currently used to control this disease (Orton

et al., 2011). This might result from the fact that all (*Stb* 1-16) but one (*Stb16*) of the major resistance genes are overcome by virulent isolates (Ghaffary et al., 2012; Orton et al., 2011).

Z. tritici is a haploid ascomycete from the *Dothideomycetes* (order *Capnodiales*, family *Zymoseptoriaceae*) (Table 1). This heterothallic bipolar fungus has two alleles (*MAT1-1, MAT1-2*) at the mating type locus equally distributed in populations (Zhan et al., 2002). *Z. tritici* causes polycyclic epidemics on wheat leaves. Inocula are asexual spores (pycnidiospores) released from pycnidia found on leaves and sexual spores (ascospores) released from asci contained in fruiting bodies (pseudothecia) found on infected leaves and plant debris (Supplementary Fig. 2G). Wind-dispersed ascospores are the main source of primary inoculum and the peak of their release coincides with the growing season of wheat. Splash-dispersed pycnidiospores are the main source of secondary inoculum and spread of the disease from lower to upper leaves (Suffert et al., 2011). As a consequence of the high frequency of sexual reproduction of *Z. tritici* in wheat fields, its populations are characterized by a high level of genetic diversity at field, regional and continental scales (Orton et al., 2011).

Zymoseptoria tritici is a hemi-biotrophic pathogen with a biotrophic-like symptomless phase of about 10-days duration, followed by a necrotrophic phase (11–21 days). The fungus penetrates wheat leaves through stomata about 3 days after spore germination (Orton et al., 2011). Appressorium-like structures, dispensable for penetration, are formed at the point of stomatal entry. Once in the sub-stomatal cavities, the fungus develops through the intercellular space in close contact with mesophyll cells, without forming specialized feeding structures. During the asymptomatic phase, the fungal biomass is low and increases exponentially during the early stages of the necrotrophic phase. During the necrotrophic phase, the fungus differentiates black pycnidia in sub-stomatal cavities, which produce pycnodiospores that are released by the extrusion of mucilage (cirrus) through the stomata.

In the last decades, the development of genomics in this species and relatives has contributed to establishment of *Z. tritici* as a model organism for the study of effectors and their role in fungal infection and of evolutionary trends associated with speciation and host plant adaptation (Marshall et al., 2011; Stukenbrock, 2013).

8.1. Overview of the Z. tritici genome

Z. tritici IPO-323 has a genome size of approximately 40 Mb and 21 chromosomes, 13 of which are core chromosomes (CCs, 1–13) while the other 8 are dispensable (DCs, 14–21, referred to as "dispensosome") (Table 2). This dispensosome displays a high variation among isolates and a low density of genes among which only a few have paralogs on CCs (Croll et al., 2013; Goodwin et al., 2011). In particular, DCs carry only a few genes encoding secreted proteins (Morais do Amaral et al., 2012). The origin and evolutionary benefit of the DCs is still unclear although they have evolved differently from CCs (Goodwin et al., 2011). However, there are no natural isolates lacking all DCs, suggesting that they have an important role in nature. *Z. tritici* IPO-323 genome contains 10,933 genes and a significant level of repeated sequences (21%). Around 60% of the genes have a functional annotation, of which 4.5% encode proteins likely secreted (Morais do Amaral et al., 2012). Other particular aspects of *Z tritici* genome are the absence of methylation (Goodwin et al., 2011) and the reduced number of genes encoding plant cell wall degrading enzymes (Ohm et al., 2012).

8.2. Assessing virulence of Z. tritici

Several pathogenicity assays have been established for *Z. tritici*. Field experiments conducted to evaluate yield losses caused by *Z. tritici* are traditionally carried out under natural infection conditions, which, owing to the high diversity of *Z. tritici* populations, hide the effect of single fungal isolates (Fig. 2M). More recently, the development of protocols for field experiments using artificial inoculations with a single isolate have allowed a more precise evaluation of wheat resistance (Ghaffary et al., 2011) (Fig. 2N and O). Several assays have also been developed under controlled conditions either in greenhouses or in growth chambers. Such assays are necessary to standardize environmental parameters such as temperature, relative humidity and light, which strongly influence the outcome of wheat-*Z. tritici* interactions (Suffert et al., 2013) (Fig. 2P). Pathogenicity assays on young plants in controlled conditions are also used to rapidly characterize the virulence spectrum of *Z. tritici* isolates and identify candidate genes associated with pathogenicity (Ghaffary et al., 2011). A protocol to perform disease assays on detached leaves from adult plants is also available for cultivar resistance assessment (Orton et al., 2011) (Fig. 2Q1–2).

8.3. Current research interests

8.3.1. Signaling pathways and protein secretion

In recent years, genomics of the *Z. tritici*-wheat pathosystem has allowed a better understanding of its infectious process. Several studies have identified intracellular signaling pathways that are crucial for pathogenicity. Some components of the fungal mitogen-activated protein kinase (MAPK) signaling cascades have been functionally characterized in *Z. tritici*. They play a role either in the penetration of wheat leaves (*Mg*Fus3, *Mg*Hog1) or in leaf colonization (*Mg*Slt2) (Cousin et al., 2006; Mehrabi et al., 2006a, 2006b). These signaling pathways likely activate transcription factors regulating the expression of downstream genes essential for infection. For instance, the transcription factor Ste12, downstream of *Mg*Fus3 MAPK, is involved in fungal colonization of wheat leaves (Kramer et al., 2009). Other regulatory networks involved in pathogenicity have been identified in *Z. tritici*. *MgWOR1* encodes a transcription factor involved in morphological switches (yeast–mycelium) and pathogenicity that is orthologous to *WOR1* from *Candida albicans* and *SGE1* from *Fusarium oxysporum* (Mirzadi Gohari et al., 2014). *WOR1* null mutants display some of the phenotypes of *MgGPB1* null mutants (Mehrabi and Kema, 2006). *MgGPB1* belongs to the *G-proteins* complex, which acts upstream the cAMP-pathway. Components of the cAMP pathway, downstream of the adenylate cyclase, such as the protein kinase A catalytic subunit encoding gene *MgTPK2*, are also important for yeast–mycelium transition and infection. Inactivation of the cyclin *MgMCC1* orthologous to *Fusarium verticillioides FCC1* is associated with a reduced radial growth, a delayed filamentous growth, unusual hyphal swellings and increased melanin biosynthesis and stress tolerance as well as reduced pathogenicity (Choi and Goodwin, 2011a). Deletion of *MgMVE1* encoding one essential component of the regulatory complex Velvet leads to pleiotropic phenotypes including defects in yeast–mycelial transition and the effect of light on this process. However, it has no role in pathogenicity (Choi and Goodwin, 2011b). Several other genes important for infection have been functionally characterized. Deletion of *MgALG2,* involved in glycosylation of secreted proteins, led to a mutant unable to infect wheat. This mutant is also impaired in switching from yeast to filamentous growth and in protein secretion (Motteram et al., 2011). Among several ABC transporters studied, only *MgATR4* had a quantitative effect on pathogenicity (Stergiopoulos et al., 2003).

8.3.2. Plant-pathogen interaction: effectors

Currently, comparative genomic and proteomic studies are applied to identify secreted proteins (SPs). Within the putative secretome of *Z. tritici*, about 500 small SPs (<30 kD) have been

identified (Morais do Amaral et al., 2012). Two of the three *Z. tritici* LysM effector encoding genes (*Mg3LYSM* and *Mg1LYSM*), which bind to chitin, are strongly up-regulated during the symptomless phase of infection. One of them, *Mg3LysM,* is required for wheat leaf colonization and sporulation (Lee et al., 2014; Marshall et al., 2011). The secreted *MgNEP1* gene encoding a protein related to necrosis and ethylene-inducing peptide is up-regulated during the symptomless phase of disease. However, it is not essential for infection (Motteram et al., 2009). Tandem Repeat secreted Proteins (*Mg*TRPs) have been also characterized and shown to be expressed specifically during the symptomless phase of infection (Rudd et al., 2010).

8.3.3. Disease control

Molecular strategies have been used also to elucidate the mechanisms of resistance of the pathogen to most frequently applied fungicides. In the last years, European *Z. tritici* populations have become resistant to azoles by a combination of single point mutations in the *CYP51* gene and increased active efflux through ABC transporters (Cools and Fraaije, 2013). To circumvent these problems, a new-generation of carboxamide fungicides have been introduced for the control of STB (Fraaije et al., 2012). Major efforts are focused on identifying genetic determinants of resistance to *Z. tritici* in wheat. Quantitative trait loci (QTLs) implicated in partial resistance and susceptibility to STB, have been identified in addition to new *Stb* major resistance genes (Ghaffary et al., 2012; Ghaffary et al., 2011). Some *Stb* genes have been introduced in commercial bread wheat cultivars (*Stb*1, *Stb*5, *Stb*6 and *Stb*9), but all these resistance genes have been defeated by virulent isolates. Alternative strategies based on the pyramiding of *Stb* genes and/or resistance QTLs are being tested for their efficiency against *Z. tritici* (Orton et al., 2011).

8.3.4. Evolutionary genomics

In recent years, studies conducted on different populations of *Z. tritici* and related species has brought major inputs on the evolutionary trends of fungal pathogens (Stukenbrock, 2013). It has been shown, for example, that adaptation of *Zymoseptoria* species to different host plants is associated with positive selection signatures in a few genes, some of which encode candidate effectors (Stukenbrock et al., 2011, 2012), as well as plant cell wall degrading enzymes (Brunner et al., 2013). Due to its efficient sexual reproduction, *Z. tritici* is a good model for fungal genetics, and it has been successfully used to study the behavior of accessory chromosomes during meiosis (Croll et al., 2013; Wittenberg et al., 2009). Rapid identification of genes involved in adaptation to host plants will be aided by association genetics.

8.4. Conclusions

At present, *Z. tritici* is the main fungal disease of wheat. However, the molecular mechanisms involved in its pathogenicity on wheat are still poorly understood. Deciphering these mechanisms at different stages of infection will be of general interest for plant pathology and fungal development. To date, *Z. tritici* overcomes most control methods, even in a single growing season. For this reason, studying its biology, population dynamics and genetic structure is important to propose successful control methods.

9. General conclusions

The establishment of fungal model systems in the last 30 years has enabled researchers to begin to address fundamental questions of fungal pathogenesis. In this review, we have critically evaluated recent progress in seven fungal species which are well-studied models for elucidation of pathogenicity determinants of animal and plant hosts. We have presented an overview of the history, biology and current state of research for each model species in order to stimulate further interest and study of these pathosystems. In this context, Supplementary Fig. 1 provides a detailed account of the main steps relevant for establishment of each organism as a model system. We have also provided up-to-date representations of the life cycles of each of the species in Supplementary Fig. 2. Additionally, we have presented a comparative analysis of general biological features (Table 1), information gained from genome projects (Table 2) and available molecular tools (Table 3) for each of the seven fungal species. When considered together, this will provide a means of rapidly comparing some of the the most studied fungal pathogens.

Overall, our comparative analysis has highlighted a number of shared characteristics, but also key differences between each pathogen investigated. First of all, five of the seven species possess sexual (or parasexual) cycles, whereas no sexual cycle has yet been identified in *F. oxysporum* and mating is not required for sporulation in *A. gossyppi*. However, in spite of the ability to undertake sexual reproduction, the frequency with which this occurs also varies greatly between species, from being an absolute pre-requisite for plant infection, as in *Ustilago maydis*, to being almost absent from clonally propagating asexual populations of *M. oryzae*. The absence of sexually recombining populations clearly has consequences for the manner in which genetic variability is generated, as seen most obviously in *F. oxysporum* with its evidence of dispensable chromosomes and horizontal gene transfer. Comparative genome analysis, now possible on a very large-scale in populations of pathogens therefore promises new insight into the generation of variation in fungal populations, and the degree to which selective pressures, imposed since the onset of organized agriculture and monoculture has influenced plant pathogen populations, in comparison to the distinct pressures imposed on human pathogen populations.

Secondly, it is noteworthy that conserved signaling pathways, for instance those composed of cAMP-dependent protein kinase A and MAP kinases are essential in a very wide range of pathogens including *Magnaporthe oryzae*, *Ustilago maydis* and *Fusarium oxysporum* (Di Pietro et al., 2001; Hamer and Talbot, 1998; Kahmann et al., 1999) and for regulating morphological changes observed in human pathogens, such as *Candida albicans* (Sanchez-Martinez and Perez-Martin, 2001; Whiteway, 2000). Thus, although input signals, receptors and, indeed, downstream target genes differ very significantly among the pathogens we compared, there appears to be evidence of ancient, conserved signaling modules at the core of these pathways that underpin fungal pathogenesis and, in particular, the infection-associated morphogenetic transitions exhibited by the pathogens represented here. Other conserved traits include the importance of secondary metabolic pathways in pathogenic fungi, often in linked clusters in *Aspergillus* (Bergmann et al., 2007; Perrin et al., 2007) and *M. oryzae* (Dean et al., 2005), for example, and the considerable secreted proteomes associated with pathogens (Ma et al., 2010; Wilson and Talbot, 2009).

There are, however, also considerable differences among the pathogens explored in this review. It is striking, for instance, how the plant pathogens examined – which are highly diverse phylogenetically – all contain large repertoires of secreted effector-encoding genes. These effectors play diverse roles in the suppression and evasion of plant immune responses. LysM effectors can, for example, suppress PAMP-triggered immunity by competitively inhibiting pattern recognition receptors, such as those that perceive chitin monomer released from the walls of invading fungal pathogens such as *M. oryzae* (Mentlak et al., 2012) and *M. graminicola* (Lee et al., 2014), or modulate plant defense-associated metabolic pathways such as phenylpropanoid metabolism (Djamei et al.,

2011). Other effectors are delivered directly into host plant cells, where they appear to bind components of plant immune response pathways, thereby suppressing the plant's defenses (Giraldo et al., 2013), although the precise mechanisms are not yet clear in most fungal pathogens. The presence of large batteries of secreted effector proteins, encoded by gene specifically expressed during host invasion is, however, in marked contrast to the human pathogens studied here. Although evasion of host recognition may be a feature of some human pathogenic fungi and pattern recognition receptors of the innate immunity response of animals can perceive fungal PAMPs such as chitin, there is much less evidence of active effector-mediated suppression of immunity as exhibited by human pathogenic bacteria or plant pathogenic fungi. This may be a consequence of most human pathogens being opportunists that can only cause systemic infections in immuno-compromised hosts, as is the case for *A. fumigates*, *C. albicans* and the emerging pathogen *F. oxysporum*. It will therefore be interesting to investigate human pathogenic fungi of immune-competent hosts (such as *Histoplasma capsulatum*, for example) to determine if there are significant numbers of fungal effectors in such species.

An overriding consideration in compiling this review is the urgent need to identify novel targets for the development of antifungal agents that are broad-spectrum in terms of the diseases they control, but fungi-specific. This is a considerable challenge, but comparative genome analysis has already revealed fungal-specific genes and signaling pathways that are important in pathogenesis in diverse species, while targeting further intervention into fungal cell wall biogenesis also offers considerable promise. In human pathogens, there is a requirement for new antifungal drugs for treatment of fungal infections in both animal and human patients, showing less toxicity and better efficacy than current drugs. By contrast, in fungal plant pathogens, especially cereal crop pathogens, a deeper understanding of the host-pathogen relationship is going to be required in order to identify novel drug targets which do not adversely affect plant health and can lead to more efficient crop protection strategies. This is coupled with a requirement for any new fungicide to be rapidly degraded in the environment, such that residues do not accumulate in the environment, while being systemic and potent within plant tissue – a challenging combination. The powerful genetic systems developed for the fungal pathogens described in this review and elsewhere (see Potts et al., 2013) will be invaluable in discovering the next generation of chemical compounds for combating fungal diseases.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fgb.2014.06.011.

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