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Fungi causing diseases in human beings

Nupur Bhatnagar
Research Scholar, Department of Biotechnology, Mahatma Jyoti Rao Phoole University, Jaipur, Rajasthan, India

Abstract
Fungal infections today are among the most difficult diseases to manage in humans. Some fungi cause disease in healthy people, but most fungal infections occur in individuals already experiencing serious illness, and frequently jeopardize the success of the newest medical advances in cancer care, solid organ and hematopoietic stem cell transplantation, neonatal medicine, autoimmune disease therapies, trauma and intensive care, and sophisticated surgery. In fact, these medical advances themselves often make their beneficiaries vulnerable to fungal disease. Treatment can involve breaching normal immune functions, or susceptible patients, such as extremely premature newborns who survive long enough to become infected by a fungus.

Keywords: fungi, diseases, fungus, fungal disease

Introduction

The following discussion intends to touch on highlights of the evolutionary developments by which living humans became substrates for fungi. Given the tremendous wealth of recent findings on fungal evolution, phylogenetics, genomics, development, and pathogenesis, this overview will necessarily omit much work critical to our understanding of fungi, which the other articles in this collection will focus on in detail.

Among the estimated 1.5–5.0 million fungal species on planet Earth (O'Brien *et al*. 2005), only several hundred cause disease in humans, and very few are able to affect healthy people. Animals coevolved with fungi, and the sophisticated and potent human immune system arose from the constant challenge posed by the microbial world. Fungal pathogens likely adapted their pathogenic repertoire to other animal prey-mammals, insects, and even unicellular amoebae-before encountering and attacking humans. But unlike plants, insects, and ectothermic vertebrates (animals whose body temperature fluctuates with their surroundings), mammals are highly resistant to invasive fungal diseases, and evolution of endothermy and homeothermy enhanced antifungal immunity (Robert and Casadevall 2009; Bergman and Casadevall 2010) [13]. The remarkable resistance of mammals to fungal pathogens has been hypothesized to be responsible for emergence of mammals as the dominant land species, when proliferation of fungi at the end of the Cretaceous era created a "fungal filter" that selected for this animal group (Casadevall 2005, 2012).

For a fungus, parasitizing a human is a demanding strategy. Four criteria must be fulfilled. (1) It must be able to grow at a high temperature, at or above 37°C. (2) It must be able to reach the tissues it will parasitize, by penetrating host tissue barriers, or by circumventing them through small airborne cells that enter air-filled spaces of lungs and sinuses directly. (3) It must be able to digest and absorb components of human tissues. (4) Finally, it must be able to withstand the human immune system.

Virulence factors can be divided into aspects of physiology that allow a fungus to fulfill these four criteria. Growth at high temperature is a stringent criterion, because land-colonizing fungi likely evolved in association with plants (Humphreys *et al*. 2010), whose nighttime temperature must be cool enough to minimize the ratio of carbon-expending respiration to carbon-assimilating photosynthesis. Casadevall and colleagues have discussed how the stable elevated temperature of endothermic (warm-blooded) animals may have been one of the most potent developments in antifungal immunity (Robert and Casadevall 2009; Bergman and Casadevall 2010; Garcia-Solache and Casadevall 2010) [13]. The energy cost of the human fever response to infection, which is tightly regulated by cytokines like TNF-α, represents another testimony to the evolutionary importance of temperature as a major host defense against fungi.

The role of fungal heat intolerance is illustrated by the different clinical scenarios of disseminated *Fusarium* infection versus disseminated candidiasis. *Fusarium* spp. are important plant pathogens that can infect neutropenic patients. As filamentous *Fusarium* spp. produce cells resembling yeast in the human host, which readily float within the bloodstream, a hallmark of their dissemination is development of numerous metastatic foci in the skin, presumably because the skin is the coolest organ. The development of metastatic lesions in warm organs distant from the primary focus, like spleen and kidneys, is much more unusual in fusariosis. In contrast, invasive infections with human temperature-adapted *Candida* spp., which also use yeast to disseminate through the bloodstream, frequently lead to innumerable metastatic foci in deep organs.

Morphogenesis is an important virulence factor related to fungal locomotion. Although fungal hyphae can pierce tissue barriers owing to the turgor pressure at their tips, yeast can readily disseminate to distant sites in a large animal. Fungi that infect healthy humans do so almost exclusively in their yeast form. But for most fungi, the ability to assume various

shapes is critical for infecting humans, because many enter the body in the form of small, round airborne dispersal propagules, sporangiospores or conidia, which are produced from hyphal cells. In the oceans, fungi originated as unicellular, oval cells propelled by flagella, so growth as a round or oval cell was a fundamental fungal trait. Lucking *et al*. (2009), in their comparison of fossil and molecular dating systems of fungal evolution, state that "all dating estimates show that the evolution of filamentous fungi occurred much later than the origin of the fungal lineage itself, suggesting that for a long time after their origin, fungi were heterotrophic, unicellular, flagellate, aquatic organisms." An option for the hyphal form, whose adaptive utility is shown by convergent evolution of hyphae in fungi and oomycete water molds (Money *et al*. 2004), may have evolved when fungi colonized land as plant symbionts (Redecker *et al*. 2000; Heckman *et al*. 2001; Lucking *et al*. 2009). Morphogenesis also contributes to protection against amoeboid cell predation. Filamentous forms of *Cryptococcus neoformans* are resistant to amoeba (Neilson *et al*. 1978), and incubation of *Histoplasma capsulatum* and *Blastomyces dermatitidis* yeast with amoeba at 37°C triggers transition to filamentous growth (Steenbergen *et al*. 2004).

Tremendous variability of cell forms and the ability to switch between them continues in extant fungal phyla, and few lineages have completely lost a round or a filamentous cell form. A highly successful human-host-associated fungus, *Candida albicans*, is notable for the facility and frequency with which it switches between a broad spectrum of growth forms in the host, between round yeast, elongated pseudohyphae, and filamentous hyphae (Sudbery *et al*. 2004). Adhesion molecules are virulence factors related to fungal locomotion strategies because a hypha must anchor itself to host tissue to exert tissue-penetrating pressure at its tip, and because once a fungal cell has reached a favorable location, it needs to be able to stay at the site.

Secretion of digestive enzymes appropriate to dissolve host tissue is the first step for a fungus to use the host as a nutritious substrate. The next step is to absorb the small molecules released by digestion of host tissues; fungal cells have transporters of nitrogen and carbon sources as well as metal ions and other micronutrients. Iron acquisition plays a special role because of its importance in critical enzymatic processes including respiration, and because the human host actively sequesters iron during infection to withhold it from the pathogen (Boelaert *et al*. 1993; Ibrahim *et al*. 2006; Kronstad *et al*. 2013; Noble 2013) [18]. The importance of iron sequestration in the host to defend against fungal growth was dramatically illustrated by disseminated Mucorales infections in patients receiving the iron chelator, deferoxamine (Windus *et al*. 1987).

Most fungi, even with these capabilities, cannot withstand the phagocytes unleashed against them by an immunocompetent human, which have help from opsonins such as complement and specific antibody, and from activating T lymphocytes. Fungi that infect healthy humans devote a large portion of their physiology to withstanding or evading the immune system. To this end they use an amazingly diverse array of strategies, which sometimes differ even between lineages of a single species. In contrast, many species that grow at 37°C can readily infect a severely immunocompromised patient.

Last, coordinating the processes to fulfill these four criteria of pathogenicity demands a complex network of sensing and signaling systems, which inform the fungal cell of external conditions and set in motion its appropriate responses. Loss of virulence was shown many times in many fungal pathogens, when just a single element of a sensing and signaling network was genetically or pharmacologically disturbed, such as mechanosensor, calcineurin, protein kinase A, MAP kinase, Tor, and high osmolarity signaling pathways (Rohde and Cardenas 2004; Cramer *et al*. 2008; Kumamoto 2008; Bastidas and Heitman 2009; Argimon *et al*. 2011; Ernst and Pla 2011; Hogan and Muhlschlegel 2011; Shapiro *et al*. 2012; Chen *et al*. 2013) [11, 7].

Fungi that infect healthy humans

The capability to infect an immunocompetent human has arisen independently multiple times among three major fungal phyla: the Entomophthoromycota (Kwon-Chung 2012), the Ascomycota, and the Basidiomycota.

Entomophthoromycota

Entomophthoromycota contains effective pathogens of insects. They occur worldwide, but have been found as agents of invasive human infection only in subtropical and tropical regions. The species of genera pathogenic for humans, *Basidiobolus* and *Conidiobolus*, can be isolated from plant debris and soil especially during rainy months (Bittencourt 1988) [15]. As emphasized recently by Kwon-Chung (2012), these genera are evolutionarily distant from each other as well as from the Mucorales, with whom they traditionally were classified in Zygomycota.

Conidiobolus spp. cause submucosal disease of the nose, sinuses, and central face. In most cases, gradual progression causes swelling of submucosal and subcutaneous tissue, disfigurement, breathing difficulties, and chronic bacterial sinusitis caused by the blockage of ostia. Unlike the Mucorales causing sinus disease, *Conidiobolus* does not usually invade blood vessels nor penetrate into the central nervous system (CNS) (Prabhu and Patel 2004).

Two species, *Conidiobolus coronatus* and *Conidiobolus incongruus*, have been isolated from central facial disease. Presumably, the route of infection is inhalation of large ballistospores forcibly launched from the sporangiophores on which they are produced singly to distances up to 30 cm (Isa-Isa *et al*. 2012). Owing to their large size of 30–38 µm (Isa-Isa *et al*. 2012), spores presumably land on the mucosa of nasal air passages and fail to reach the distal airways (Bittencourt *et al*. 2006) [16].

Conidiobolus spp. infect arthropods including mites, spiders, and insects (Isa-Isa *et al*. 2012). They elaborate lytic enzymes including elastases, collagenases, and lipases, likely to parasitize and kill arthropods (Comerio *et al*. 2008). These enzymes enable *Conidiobolus* to digest human tissue, growing by extension from nasal submucosa through the facial soft and bony tissue (Gugnani 1992). Metabolites toxic to insects have also been identified but because the fungus is highly lethal to arthropods and only of moderate virulence in mammals, it remains to be seen whether these metabolites play any role in human infection (Wieloch *et al*. 2011).

The host sends neutrophils, eosinophils, and histiocytes to

contain the fungus, and granulomas (structured assemblies of histiocytes) develop. Histopathology can show dense eosinophilic material surrounding the hyphae, the Splendore-Hoeppli phenomenon, which has been suggested to consist of antigen–antibody precipitate (Isa-Isa *et al*. 2012).

Basidiobolus ranarum causes subcutaneous disease primarily in children of tropical and subtropical Africa, Asia, and the Americas. Nodules appear at sites of inoculation through insect bites, scratches, or small wounds (Ribes *et al*. 2000). In addition, *Basidiobolus* has caused gastrointestinal infections, presumably after ingestion of large inocula, and symptoms resemble inflammatory bowel disease (Zavasky *et al*. 1999; Vikram *et al*. 2012). When insects infected by *Basidiobolus* are eaten by amphibians and reptiles, the fungus is shed in the feces (Ribes *et al*. 2000) and attaches to plant matter with which it is accidentally inoculated under the skin, for instance, by thorns, leaves used for cleansing, etc. As an insect pathogen, it also elaborates proteases and lipases (Echetebu and Ononogbu 1982; Okafor *et al*. 1987; Okafor and Gugnani 1990; Okafor 1994). Its inability to invade deep organs is likely related to limited thermotolerance; 37°C may be its maximal growth temperature (Ribes *et al*. 2000).

Ascomycota

Pathogenic Onygenales

Several soil-inhabiting members of the ascomycete order Onygenales have evolved to parasitize mammals and cause systemic infection. They have been classified in the family Ajellomycetaceae (Untereiner *et al*. 2004; Bagagli *et al*. 2008) [9], which include *B. dermatitidis* (teleomorph, *Ajellomyces dermatitidis*), *H. capsulatum* (teleomorph, *Ajellomyces capsulatus*), *Paracoccidioides brasiliensis, Paracoccidioides lutzii, Lacazia loboi, Coccidioides immitis*, *Coccidioides posadasii*, and related fungi with similar properties. The intracellular pathogenic strategy of some of these organisms is similar in protozoa and macrophages (Steenbergen *et al*. 2001, 2003), raising the possibility that their capacity for virulence arose accidentally as a result of environmental pressures (Casadevall and Pirofski 2007).

Disease caused by these fungi begins asymptomatically in the lungs and progresses to an influenza-like illness or frank pneumonia. They can disseminate to distant sites, can persist and reactivate, with different organ predilections among different genera: *Paracoccidioides* in oral and respiratory mucous membranes (Queiroz-Telles and Escuissato 2011; Marques 2012), *Blastomyces* in bones, joints, and skin (Bradsher *et al*. 2003), and *Histoplasma* in multiple organs including the gastrointestinal tract and adrenals, as well as bones and skin (Kauffman 2007).

Their primary lifestyle is saprobic. Most genera occur in defined geographic areas, which follow particular soil consistencies, for instance, dry and alkaline for the *Coccidioides* spp. and acidic for *Histoplasma*. It has not been ruled out that their geographic distribution follows that of specific host mammals. In the soil, they grow as a branched network of hyphal filaments, a mycelium, and saprobically use organic matter. They produce airborne dispersal cells: Conidia produced from specialized hyphae, as *Histoplasma* and *Blastomyces* do, or arthrospores from regulated fragmentation of hyphal compartments, as *Coccidioides* and *Paracoccidioides*do. *Paracoccidioides* produces both arthroconidia and aleuriconidia (conidia directly produced along the hyphae). Once conidia or arthrospores are taken up by a mammal, usually by inhalation, they convert into parasitic yeast (or spherules, in the case of *Coccidioides* spp.) and initiate an infection. The principal signal for their conversion to the yeast form is the elevated mammalian body temperature, so that this switch can be induced in vitro when they are cultured at 37°C or above. For this reason they are grouped as thermal dimorphs. These fungi infect, persist, and cause disease in healthy hosts. In immunocompromised hosts, the infections are more clinically apparent, more severe, and more likely to disseminate to multiple organs.

Adaptive cellular immunity is required to control the infection with these fungi and can be detected by skin tests with purified antigens. For this reason, clinically apparent infections with some members of this group, such as *H. capsulatum*, became much more common with the rising prevalence of AIDS. These Onygenales (Ajellomycetaceae) are the only phylogenetically related group whose members all cause systemic disease in otherwise healthy people.

Other thermally dimorphic fungi are *Sporothrix schenckii* (order Ophiostomatales), which is primarily introduced into the host through skin injuries, and *Talaromyces* (*Penicillium) marneffei* (order Eurotiales), which does not infect healthy hosts but because of the AIDS epidemic has become a prevalent and important invasive pathogen in Southeast Asia. These fungi coevolved with higher vertebrates, as it is the adaptive immune system by which they are controlled. The fact that these evolutionarily distant fungi convert from saprobic hyphal to pathogenic yeast growth at the temperatures of endothermic animals suggests that an ability to access large nitrogen and other nutrient stores of an animal host is an advantageous lifestyle option for a soil dweller, and a result of convergent evolution in several phyla.

H. capsulatum will be discussed at greater length because it is well studied and also because it exemplifies important principles for other thermally dimorphic Onygenales. This fungus inhabits soil and other organic matter such as decaying wood enriched with bat and bird droppings. Its hyphae produce macro- and microconidia, and the latter are small enough, <5 μm, to penetrate into the alveoli of mammalian lungs. There they are phagocytized by alveolar macrophages and converted to yeast that is able to proliferate in the hostile environment of the phagolysosome.

Aspects of histoplasmosis resemble infections with a well-adapted bacterial intracellular pathogen, *Mycobacterium tuberculosis*. Depending on inoculum size, infection is most often inapparent or leads to a self-limited influenza-like illness. The pathogen can persist indefinitely until immune decline allows it to reactivate. Lung disease resembling TB can ensue immediately after infection, and dissemination to all organs occurs depending on host immune status and age. The response of an immunocompetent host is granuloma formation.

Histoplasma occurs on all continents, although it is rare in Eurasia and most common in North and South America. Two studies of *H. capsulatum* showed that its growth and clinical phenotypes, which had traditionally been the basis for its division into varieties (*capsulatum*, *duboisii*, and

farciminosum), did not align with the divisions of its seven phylogenetic clades (Kasuga *et al*. 1999, 2003), and that the different clinical disease states attributed to these (now obsolete) varieties were caused by the same clades.

Phylogenetic analyses suggest that *H. capsulatum* arose in South America 3.2–13.0 million years ago and its present distribution reflects a period of rapid spread to all continents except Eurasia (where it was later introduced), followed by restriction to the warmest areas during a period of intense cold 1.8 million years ago (Kasuga *et al*. 1999, 2003). From there, it spread to temperate regions as the earth warmed again. This idea is consistent with its low genetic diversity in temperate regions and high diversity in tropical areas (Kasuga *et al*. 1999), mirroring the diversity of plant species in areas of temperate and tropical forest, which are its geographic areas of distribution.

The two North American clades are not closely related and are thought to have been genetically isolated for the past ~20 million years (Kasuga *et al*. 1999), despite overlapping territories. These two clades differ in virulence, with North American clade 1 having been isolated predominantly from AIDS patients, and clade 2 infecting immunocompetent hosts (Kasuga *et al*. 1999). A clade 2 isolate and a representative of the Panama clade have been used in genetic studies of virulence factors, and surprising differences were found, as discussed below.

The habitat of *Histoplasma* is soil enriched with bird and bat droppings, in specific geographic locations including the Ohio and Mississippi valleys of North America and moist regions of Central and South America. Whereas birds are very rarely infected with *H. capsulatum* (Quist *et al*. 2011), bats are frequently infected but respond with minimal inflammation (Taylor *et al*. 1999), suggesting active repression of the host immune response by the fungus. It was suggested that, "in at least some bat species natural exposure may result in a chronic, controlled infection, which allows the bat to excrete viable fungi over a long period" (Hoff and Bigler 1981). Experimentally infected bats also had a minimal inflammatory response (McMurray and Greer 1979).

A broad variety of other infected mammals such as cats (Aulakh *et al*. 2012), dogs (Brömel and Sykes 2005), sea otters (Morita *et al*. 2001), and badgers (Bauder *et al*. 2000) do respond with granulomatous inflammation to the presence of *Histoplasma* yeast. Perhaps *H. capsulatum* has evolved to infect bats, in which it avoids activation of host responses, by mechanisms only partially adapted to other immunocompetent mammals. If this is the case, the fungus has established an efficient amplification cycle by which its mycelial form grows in droppings under bat roosting places, for instance, in caves, and its microconidia are released into the air to be inhaled by the bats that fertilize its soil substrate (Taylor *et al*. 1999).

Several mechanisms by which *H. capsulatum* yeast successfully interacts with mammalian hosts have been identified. Surprisingly, different clades use different strategies to achieve an intracellular lifestyle in macrophages, and to suppress macrophage activation. For example, macrophages recognize the important pathogen associated molecular pattern (PAMP) β(1,3)-d-glucan with the receptor dectin-1 (Rappleye *et al*. 2007), and ligand binding triggers responses that enhance phagocyte activation responses (Underhill *et al*. 2005). β(1,3)-d-glucan is the main structural polymer of ascomycete cell walls, so Histoplasma cannot forgo its use to avoid activating their hosts' macrophages. Instead, Histoplasma yeast of most clades hide it by coating their cell surface with α(1,3)-d-glucan. Perturbation of genes whose products are required for α(1,3)-d-glucan synthesis renders cells of the Panama clade avirulent (Rappleye *et al*. 2004). Notably, this α(1,3)-d-glucan coat is lacking in a North American clade 2 strain (Edwards *et al*. 2011), and an α(1,3)-d-glucan synthase mutant in this strain background is fully virulent (Edwards *et al*. 2011).

Another cell surface protein, Yps3, is expressed to a significant level only in the more virulent North American clade 2 (Bohse and Woods 2007). This protein, a homolog of *B. dermatitidis* BAD1, contributes to dissemination of *H. capsulatum* to extrapulmonary foci (Holbrook and Rappleye 2008). Its gene is present in most strains but substantial transcription is known to occur only in North American clade 2, the clade lacking α(1,3)-d-glucan. Yps3, like BAD1, may directly suppress TNF-α production, circumventing the need for this clade to use α(1,3)-d-glucan to hide the stimulatory PAMP β(1,3)-d-glucan (Holbrook and Rappleye 2008). The known variations in their pathogenic repertoires among clades of *Histoplasma* are based in differences of regulatory rather than coding regions between the sequenced strains (Edwards *et al*. 2013).

H. capsulatum yeast have evolved a repertoire of manipulative molecules to turn the host compartment specifically evolved to kill pathogens, the phagolysosome, into a habitat in which they thrive (Sebghati *et al*. 2000; Youseff *et al*. 2009). Remarkably, within the teleomorph genus Ajellomyces, originally comprising the anamorph sister species *H. capsulatum* and *B. dermatitidis*, one species *H. capsulatum* chose this route of intracellular life, whereas the other, *B. dermatitidis*, is a successful extracellular pathogen.

Distinct *H. capsulatum* clades differ in specific details of the pathogenic program of their yeast, but they have in common that their suite of virulence factors is turned on simultaneously with the switch from hyphal to yeast growth. Mammalian body temperature of 37°C or above is the main signal to trigger conversion from the mycelium to yeast. This conversion entails differential transcription of ~20% of the genome (Nguyen and Sil 2008), and is controlled by a network of transcriptional regulators apparently responding to a histidine kinase signal (Nemecek *et al*. 2006; Webster and Sil 2008; Cain *et al*. 2012; Beyhan *et al*. 2013) [14]. Mutants in these regulators are incapable of the transition to yeast growth, and are avirulent (Nemecek *et al*. 2006; Webster and Sil 2008; Cain *et al*. 2012; Beyhan *et al*. 2013) [14].

Other members of the Ajellomycetaceae, all of which associate with mammals, have equally distinctive ecology and pathobiology. Mycelia of the *Coccidioides* spp., growing near rodent burrows in arid regions of the Americas (Nguyen *et al*. 2013), produce arthroconidia. When arthroconidia are inhaled by a mammal, they give rise to round cells-spherules-in which growth and mitosis occur to eventually fill the enlarging mother cell with hundreds of endospores. On maturity, the spherule ruptures and releases the endospores into host tissue. Newly released endospores repeat the growth cycle locally or disseminate hematogenously. Phylogenetic analysis shows

that the fungus evolved in North America before that continent's geologic connection with South America 2.5–3.5 million years ago (Fisher *et al*. 2001), and that the genetically homogeneous South American strains, derived from populations in present-day Texas, reached their current distribution 8940–134,000 years ago, possibly with the migration of humans from North into South America (Fisher *et al*. 2001). Another unique member of the Ajellomycetaceae, *L. loboi*, shares an important feature with a distant member of its phylum Ascomycota, *Pneumocystis jirovecii*. They are the only human fungal pathogens not grown in culture (Vilela *et al*. 2009). *Lacazia*, a sister genus to *Paracoccidioides*, has been found only in subcutaneous infections of previously healthy humans in the Amazon basin and in dolphins of the Atlantic ocean and of the Amazon (Vilela *et al*. 2009; Theodoro *et al*. 2012), and (analogous to *Mycobacterium leprae*) can be amplified only in a mouse model. In contrast, *Paracoccidioides* spp., prevalent fungal pathogens that cause pneumonia, systemic disease of the monocyte–macrophage system, and destructive lesions of skin and oral mucous membranes (Fortes *et al*. 2011; Queiroz-Telles and Escuissato 2011; Marques 2012, 2013), occur in Central and South America mainly outside the Amazon region (Theodoro *et al*. 2012). *Paracoccidioides* causes clinical disease in many more men than women, in a ratio of 13:1, possibly because estrogen blocks the conversion of inhaled arthroconidia to the tissue-invasive yeast form (Shankar *et al*. 2011). This fungus frequently infects armadillos, and its virulence factors such as the immunodominant adhesin glycoprotein 43 (Puccia *et al*. 1999, 2008, 2011;Fortes *et al*. 2011) may have evolved in its coevolution with these ancient mammals (Bagagli *et al*. 2006; Richini-Pereira *et al*. 2009) [10].

Some members of the Onygenales infect healthy humans, but very rarely cause invasive disease. Dermatophytes of the genus Arthrodermataceae specialize in degrading keratins, important structural proteins in vertebrate skin whose extensive cross-linking by disulfide bonds makes them proteinase resistant (Gradisar *et al*. 2005). With the evolution of animals with substantial keratin appendages such as scales, feathers, nails, and hair, specializing in use of these nutrient sources must have been a worthwhile niche for the presumed ancestor of the dermatophytes (Weitzman and Summerbell 1995).

Reptilian amniotes had evolved keratin, so that the basis for this specialization was available as a fungal substrate 300 million years ago (Eckhart *et al*. 2008). Recent analysis of mitochondrial genomes of six dermatophytes comprising the three morphologically defined anamorphic genera, *Trichophyton*, *Microsporum*, and *Epidermophyton*, together with 29 other members of Ascomycota, confirmed that the dermatophytes are descended from a common ancestor (are monophyletic) and separated from other fungi only 32–50 million years ago (Wu *et al*. 2009). This study also found that classification of species by molecular characters frequently contradicted the traditional classifications based on morphologic traits (Wu *et al*. 2009), emphasizing the fact that fungal morphology is enormously flexible and convergent evolution is common, so that only limited predictions of their physiology can be made from the appearance of fungal structures. In contrast, molecular phylogenetic studies have confirmed the grouping of dermatophytes by their ecologic and clinical features, including the distinct sites of the human body they tend to infect (Cafarchia *et al*. 2013).

Anthropophilic dermatophytes travel over the world with their human hosts. Although many species are endemic to specific geographic regions, some species occur worldwide. Their prevalence varies with the lifestyle and socioeconomic conditions of their human hosts, and is undergoing continuous epidemiologic changes (Ameen 2010). Unlike their thermal dimorphic Onygenales relatives, they can reach a new host by person-to-person transmission, and their access to new human substrate is made even easier by the ability of their arthroconidia to persist for years in fomites (Weitzman and Summerbell 1995). Anthropophilic dermatophytes, relying on person-to-person access to new hosts, can forgo long-distance travel via airborne conidia, which their geophilic relatives produce in larger profusion to reach distant deposits of animal keratin (Weitzman and Summerbell 1995).

Coevolution with their animal hosts presumably allowed dermatophytes to evade or resist the evolutionarily more ancient innate immune mechanisms, so that today their control requires adaptive cellular immunity (Dahl 1993). By restricting their usual habitat to the most superficial keratinized layer of the skin and its appendages, dermatophytes reduce their contact with immune cells. Human-specific dermatophytes (anthropophiles) are able to down-regulate host inflammation to establish a chronic infection (Blake *et al*. 1991; Shiraki *et al*. 2006). In contrast, soil-dwelling (geophilic) and animal-specific (zoophilic) dermatophytes are unable to manipulate host immunity and are eliminated by a vigorous inflammatory response, illustrating the principle that a well-adapted parasite carefully calibrates its virulence.

Basidiomycota

Cryptococci

Basidiomycete yeast with a worldwide distribution, cryptococci, in the past century, infected humans only rarely (Molez 1998). In the 1950s, increasing numbers of cryptococcal meningoencephalitis were reported from central Africa, viewed in retrospect as sentinels of the emergence of AIDS around the Congo River (Molez 1998). AIDS is still the setting in which the vast majority of cryptococcosis occurs (Mitchell and Perfect 1995; Pukkila-Worley and Mylonakis 2008); for 2006, 957,900 cases of cryptococcal meningitis associated with AIDS were estimated, resulting in 624,700 deaths (Park *et al*. 2009). Furthermore, recent outbreaks of *Cryptococcus gattii* infections with significant mortality on Vancouver Island and the northwestern United States raise the concern that the fungus may be evolving to become virulent for healthy humans (Fraser *et al*. 2005; Byrnes *et al*. 2010; Springer *et al*. 2012).

The pathogenic cryptococci, *C. neoformans* and its sister species *C. gattii*, enter a human by inhalation of infectious cells: dried yeast or possibly basidiospores, the products of meiosis after mating. When these small airborne cells, <5 µm in size, are inhaled, pneumonia can ensue in susceptible hosts, and on gaining access to the bloodstream, the yeast can disseminate to all organs. *Cryptococcus* has a special predilection for the CNS and causes subacute

meningoencephalitis, in which high intracranial pressure plays an especially deleterious role, which, if left untreated, is lethal. The fungus can persist for years in the lung or in sites of previous dissemination and reactivate only on weakened immune surveillance.

C. gattii causes invasive disease and death in wild and domestic land and ocean mammals and birds and pneumonia and meningoencephalitis in previously healthy people (Krockenberger *et al*. 2002; Miller *et al*. 2002; Raso *et al*. 2004; Santos *et al*. 2008; McGill *et al*. 2009). A wealth of analyses of pathogenic cryptococci over the past several decades have shown that *C. gattii* in many settings is a more common primary pathogen than *C. neoformans* (Speed and Dunt 1995; Springer *et al*. 2012), but new reports continue to blur the boundaries between primary and opportunistic pathogens among the *Cryptococcus* species (Chen *et al*. 2008; Chau *et al*. 2010; Choi *et al*. 2010), illustrating the limited utility of these terms (Casadevall and Pirofski 2001). A recent report has indicated that the presence of anti-GM-CSF autoantibody is a risk factor for *C. gattii* CNS infection in otherwise healthy individuals (Saijo *et al*. 2014). It suggests that patients with cryptococcal CNS infection considered "immunocompetent" may carry immune defects that can yet not be identified by routine immunological screening. An important condition for a clearer understanding of ecology, epidemiology, and evolution of cryptococci is that more resources for microbiologic analysis become available in poor countries. Cultures of blood and cerebrospinal fluid currently cannot be performed in medical settings in which most patients with cryptococcosis will seek help.

The separation of the sister species *C. gattii* and *C. neoformans* is thought to have occurred 45 million years ago (Simwami *et al*. 2011). Molecular typing recapitulates traditional serologic classification of varieties into *C. neoformans* var. *grubii* (serotype A, VNI, VNII, VNB) and var. *neoformans* (serotype D, VNIV) and their hybrids (serotype AD, VNIII), as well as *C. gattii* (serotypes B and C) (Mitchell and Perfect 1995; Meyer *et al*. 2003). *C. gattii* has been subdivided into molecular varieties or cryptic species VGI through VGIV, among which further subdivisions correlate with geographic location and virulence (Kidd *et al*. 2004; Fraser *et al*. 2005; Byrnes *et al*. 2010).

In the environment, *C. gattii* has been isolated from numerous tree species in tropical and more recently in temperate regions, particularly from sites of wood decay and insect consumption of vegetable matter (Ellis and Pfeiffer 1990; Fortes *et al*. 2001; Kidd *et al*. 2003); although it was first found on *Eucalyptus* trees and believed to follow their worldwide distribution (Ellis and Pfeiffer 1990), its isolation from the midst of pristine Amazon rainforest revises the interpretation of its origin (Fortes *et al*. 2001). Environmental sampling on Vancouver Island to define the reservoirs of the outbreak that began in 1999 found more than 10 species of trees to yield *C. gattii*, which comprised ~10% of the trees sampled (Kidd *et al*. 2007), and it survives for at least 1 yr in fresh- and seawater, suggesting it could spread with ocean currents (Kidd *et al*. 2007).

Supporting the idea of an origin of pathogenic *C. gattii* in South America is the uniquely symmetric distribution of mating types in haploid isolates from that continent. Although clinical and environmental isolates of the α mating type vastly predominate in other parts of the world, and isolates of the a type are almost never encountered, the South American distribution is 0.8a–1.0α (Hagen *et al*. 2013).

C. neoformans var. *grubii*, which is responsible for ~95% of cryptococcal infections worldwide and 98% of infections among AIDS patients (Simwami *et al*. 2011), is genotypically most diverse in southern Africa; and a collection of strains from Botswana contained 12% isolates of the a mating type, which in other sites is extremely rare (Litvintseva and Mitchell 2012). In that study, distinct genotypes were thought to have diverged 5000 years ago; the investigators speculate that pigeons contributed to the worldwide spread of one specific strain of *C. neoformans* var. *grubii* (Litvintseva and Mitchell 2012) because *C. neoformans* is enriched in bird droppings and has been shown to mate on pigeon droppings (Nielsen *et al*. 2007). A study from Thailand supports the concept of an origin of *C. neoformans* var. *grubii*in Africa (Simwami *et al*. 2011).

Like *C. neoformans*, with which it shares virulence traits, and unlike nonpathogenic cryptococcal species (Findley *et al*. 2009; Araujo Gde *et al*. 2012), *C. gattii* possesses a thick polysaccharide capsule composed of glucurono- and galactoxylomannan, which, analogous to encapsulated bacteria like *Streptococcus pneumoniae*, blocks phagocytosis of the organism unless it is opsonized (Casadevall *et al*. 1998). Presumably, the dense capsule also provides some protection against hydrolytic enzymes exocytosed by neutrophils. Capsular glucuronoxylomannan and other macromolecules, as well as virulence-associated proteins, are carried through the cell wall in an amazing process involving vesicles that originated from the late endosome (multivesicular body) (Rodrigues *et al*. 2007, 2008). This process, first discovered in *Cryptococcus*, subsequently was found in other pathogenic fungi like*Paracoccidioides* (Vallejo *et al*. 2011, 2012), and in the model yeast *Saccharomyces cerevisiae* (Oliveira *et al*. 2010). Like other fungi, cryptococci can diversify morphologically in the host, producing a population of Titan cells, giant cells of up to 50 μm in diameter (Okagaki *et al*. 2010; Zaragoza *et al*. 2010) created by DNA replication and growth not followed by mitosis (Okagaki *et al*. 2010). Other antiphagocytic mechanisms, independent of the capsule, are controlled by two GATA transcription factors and are likely to involve coordinated regulation of numerous physiologic processes because hundreds of genes are differentially expressed in their mutants (Liu *et al*. 2008; Chun *et al*. 2011).

Once ingested by alveolar macrophages not activated by T lymphocytes, the fungus can survive and proliferate, and it is thought that some cryptococci may enter the brain within macrophages crossing the blood–brain barrier (a Trojan horse-like mechanism), whereas free yeast in the bloodstream cross the blood–brain barrier by transcytosis (Casadevall 2010; Shi *et al*. 2010) [13]. To survive in phagocytes, it buffers oxidative and nitrosative stress with melanin deposited in its cell wall (Wang *et al*. 1995), and with neutralizing enzymes like superoxide dismutase, glutathione reductase, and thioredoxins (Kronstad *et al*. 2011). Both in macrophages and in the free-living amoeba *Acanthamoeba castellanii*, it disrupts phagolysosome membranes to prevent acidification of this compartment and to dilute the lytic enzymes it contains; it also

sheds large amounts of capsular polysaccharide to fill the phagocytic cells' cytoplasm (Steenbergen *et al*. 2001; Tucker and Casadevall 2002). Virulent strains not only resist killing but proliferate in the amoebae (Steenbergen *et al*. 2001) so that the predator finds itself prey.

How did some cryptococci evolve from survivors of amoeba attacks to occasional parasites of humans? A recent study examined evolutionary relationships and virulence traits of *C. gattii*and *C. neoformans* strains, and related fungi belonging to the order Tremellales, which do not infect mammals (Findley *et al*. 2009). Other Tremellales parasitize fungi by attaching to the host fungus with a specialized hypha and accessing its cytoplasm through a newly created pore (Zugmaier *et al*. 1994; Millanes *et al*. 2014). An evolutionary trajectory from parasitizing another live fungus to parasitizing an animal host may have proceeded through association with and parasitism of insects, and through selection in soils by amoebae for traits that can also function in mammalian virulence. The human-pathogenic *Cryptococcus* species killed larvae of the wax moth *Galleria mellonella* in one study, whereas their Tremellales relatives partially or completely lacked virulence in this insect model (Findley *et al*. 2009). Of note, insect cellular immune defenses consist of amoeboid phagocytes (Williams 2007; Browne *et al*. 2013), so that resistance to free-living amoebae may transfer well to resistance to insect plasmatocytes. However, in nature, cryptococci so far have only been found in association with insect frass (the excreta of plant-eating insects), not as insect parasites (unlike, e.g., the entomophthorales and the ascomycetous *Cordyceps* spp.). Further ecologic research will have to show whether insect parasitic cryptococcal relatives can be identified, perhaps in their ancestral tropical forest habitats. The role of its versatile mating system in virulence evolution of *Cryptococcus* will be discussed in the dedicated articles in this collection.

Fungi that infect immunocompromised humans

A sufficiently immunocompromised host can be infected by hundreds of environmental fungal species that grow at human core temperatures. However, a predictable set of actors is known to cause the most common invasive infections in immunocompromised individuals, and they will be discussed next according to their phylogenetic affiliations, which predict important parameters of their physiology.

Ascomycota

Candida

Candida species are a polyphyletic group of the order Saccharomycotina, which live as commensals on mammalian mucous membranes, particularly of the gastrointestinal tract (Wrobel *et al*. 2008). They have rarely been found in the soil (Marples and Di Menna 1952;Skinner and Fletcher 1960). A limited number of species are commonly associated with humans as colonizers and opportunistic pathogens: *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida lusitaniae*, and *Candida krusei*. Of these, *C. albicans* is the most commonly isolated human commensal and pathogen (Odds 1988; Kam and Xu 2002; Krcmery and Barnes 2002). The frequency of colonization with nonalbicans*Candida* species shifts according to age of the host, with *C. parapsilosis* being more prevalent among children and *C. glabrata* among older adults (Soll 2002).

Invasive candidiasis takes many forms, depending on the setting in which a host became susceptible to this opportunist, and depletion of normally competing bacterial flora by antibiotics often plays a role. For example, in cancer patients receiving chemotherapy, candidiasis is often caused by fungal transmigration through disrupted intestinal epithelium, and the organs with the heaviest fungal burden are those connected by the portal circulation—liver and spleen—but not the brain. In contrast, premature newborns with candidiasis often have abscesses in the brain, because their blood–brain barrier may be immature and because their brain receives such a large fraction of cardiac blood output.

C. albicans is the human companion fungus par excellence. Many healthy humans carry *C. albicans*, which harmlessly colonizes mucous membranes to high numbers at different anatomic sites of a single individual (Odds 1984, 1988; Soll *et al*. 1991). It has evolved to flourish in a wide range of environmental conditions: high pH in the intestine versus low pH in the vagina, feast and famine nutritional conditions in the gastrointestinal tract according to its host's mealtimes versus steady glycogen supply in the vagina, and aerobic conditions on oral surfaces versus anaerobic conditions in the intestine. Unlike the dermatophytes, in which different clades inhabit different anatomic sites, *C. albicans* biotypes are generalists and each can adapt to all sites (Odds 1984).

Not only can *C. albicans* survive and thrive in highly disparate microenvironments of the human host, but as part of the normal flora it has also honed its ability to avoid triggering human immune defenses, for instance, by covering its main structural cell wall component, β(1,3)-d-glucan, with glycoproteins to avoid engaging the macrophage dectin-1 receptor (Wheeler and Fink 2006).

Analysis of its populations, which comprise at least five clades, suggests that *C. albicans*migrated throughout the world with its human hosts (Lott *et al*. 2005). It is unknown whether the fungus accompanied the mammalian lineages that led to primates throughout evolution, or whether it made the jump to mammalian commensal at a more recent time before emergence of modern humans (Lott *et al*. 2005).

Perhaps in its long cohabitation with the human, which as to host numbers proved to be a winning strategy, *C. albicans* became so closely hostadapted that major genomic rearrangements, as facilitated by sex and meiotic recombination, became less advantageous (Goddard *et al*. 2005). In a stable and benign environment, such as the equilibrium between thriving hosts and commensals, the expenditure of time and energy required for sexual reproduction may cost more than it benefits. *C. albicans* appears to consist of stable clonal diploid populations (Bougnoux *et al*. 2008), and its mating and parasexual cycle seem to be such rare events that their discoveries were sensational paradigm shifts in the community of *Candida* researchers (Hull and Johnson 1999; Magee and Magee 2000; Miller and Johnson 2002; Alby *et al*. 2009; Hickman *et al*. 2013). Although the parasexual cycle occurs infrequently, it can generate progeny with extensive genetic diversity by shuffled combinations of eight chromosomes or by recombination between homologous chromosomes mediated by the conserved Spo11 protein

integral to meiotic recombination (Forche *et al*. 2008). More discoveries are sure to follow on the role of mating and various ploidy states in *Candida*ecology today, and virulence associated with the mating loci (Lockhart *et al*. 2005; Wu *et al*. 2007) may become more important as *Candida* habitat changes with current changes in human epidemiology.

In the millions of years of coevolution, human predecessors and humans survived only briefly with diseases during which *C. albicans* could significantly invade the host. Invasive candidiasis was very rare (Browne 1954; Zimmerman 1955). *C. albicans* is an efficient invasive pathogen, causing mucous membrane infections in individuals with ineffective adaptive cellular immunity and fatal disseminated infections in patients lacking functional innate immune cells, neutrophils. For a microorganism that relies on person-to-person transmission and does not have a significant soil reservoir (or mode of locomotion back from the soil to its primary host), the benefit of multiplying to the point of host death seems mysterious. One idea might be that the *Candida* pathogenic repertoire evolved to take advantage of a constant large number of temporarily susceptible humans, infants, who develop oral candidiasis because of immature adaptive immunity. Human infants' propensity to widely distribute their oral secretions (to slobber) may allow infecting *Candida* to launch its increased numbers from infected mucous membranes of these hosts and to colonize more of their contacts. If this is the case, *Candida* virulence factors evolved to efficiently infect mucous membranes, and invasive disease is for it an accidental dead end.

The pathogenic repertoire of *Candida* comprises all features needed for a human fungal pathogen. It is able to grow well at human febrile temperatures of 39°C–40°C. It can penetrate host tissues with hyphal cells and it has multiple adhesion molecules to facilitate the drilling action of the hyphal tip (Staab *et al*. 1999; Sundstrom 2002; Hoyer *et al*. 2008; Liu and Filler 2011). Its hyphae constitutively produce yeast, constantly diversifying the population with more mobile cells (Shen *et al*. 2008). An array of lytic enzymes suitable for digesting human macromolecules is induced during tissue invasion (Ghannoum 2000; Staib *et al*. 2000; Calderone and Fonzi 2001; Schaller *et al*. 2005; Albrecht *et al*. 2006; Trofa *et al*. 2011), and transporters are regulated coordinately to absorb the released monomers into the fungal cell, as reviewed by (Morschhäuser 2011), and to mobilize micronutrients from the host (Weissman *et al*. 2008; Citiulo *et al*. 2012; Noble 2013). Unbiased genetic screening yielded novel virulence factors like glucosylceramide synthesis (Noble *et al*. 2010) and is expected to reveal new aspects of *Candida* invasive lifestyle (Holland and Summers 2008).

Resistance of *Candida* to the human immune system is limited. Features increasing its virulence were likely selected against in its long coevolution with us as a commensal. Yet today we are saving, and improving the quality of, human lives that are vulnerable to this opportunist, and any line of investigation that might result in better control of invasive candidiasis has the potential worldwide to save hundreds of thousands of lives every year (Pfaller and Diekema 2007).

Aspergillus

Aspergillus fumigatus, the species responsible for ~90% of invasive aspergillosis (Schmitt *et al*. 1990), causes relentless pneumonia, sinusitis progressing through tissue planes, and brain abscesses in neutropenic patients and those with phagocyte defects like chronic granulomatous disease. It is feared in immunosuppressed individuals because its susceptibility to antifungals is limited. This organism also causes disease at another extreme of immune function: allergic reactions.

Normal human innate immunity controls *A. fumigatus*, a versatile thermophilic plant saprobe. It is well adapted to the high temperatures that occur during bacterial decomposition of dead plants and tolerates thermophilic bacteria's optimum of around 55°C (Latgé 1999). At human febrile temperatures of 39°C–40°C, it grows well. Its secretion of a very broad and redundant range of hydrolases (Kothary *et al*. 1984; Kwon-Chung and Sugui 2013), evolved in the tumultuous competition for nutrients among the microbiome of decaying plants, allows it to easily access human tissues as sources of nutrition (Abad *et al*. 2010).

The mycelia of *A. fumigatus* give rise to conidiophores, specialized hyphae that produce small (~3 μm) airborne dispersal cells, conidia. Their small size allows them to remain airborne for long periods and, incidentally, to enter human alveoli. On average, a person is estimated to inhale several hundred *A. fumigatus* conidia each day (Hospenthal *et al*. 1998); with exposure to grass cuttings, leaf litter, or compost, this number may be orders of magnitude higher (Mullins *et al*. 1976; Poole and Wong 2013). Conidia are coated with hydrophobic proteins (Thau *et al*. 1994; Paris *et al*. 2003) and with the chemoprotectant melanin (Pihet *et al*. 2009) to withstand harsh environmental stressors like freezing, sunlight, and desiccation (Kwon-Chung and Sugui 2013). They are recognized by innate immune cells of a human host only when they begin to germinate to produce a growing hypha (Levitz and Diamond 1985; Aimanianda *et al*. 2009) after landing in an air-filled space like a paranasal sinus or lung. *Aspergillus* conidia can give rise to allergic lung disease, and the fungus can colonize the bronchiectasis of cystic fibrosis or chronic obstructive lung disease; but lethal invasive infection occurs when there is a dearth of functional neutrophils and macrophages to control germinating conidia. Hyphae proliferate in lung parenchyma or sinuses, and hyphal fragments occasionally are carried to highly perfused organs like the brain in which they initiate foci of metastatic infection. On encountering blood vessels, *Aspergillus* hyphae tend to enter and follow their course, clogging the vessel and causing infarction of downstream tissue. This angioinvasive behavior may be attributable to thigmotropism, the ability to sense and follow contours, which aspergilli have in common with fungi of diverse phyla (Perera *et al*. 1997; Bowen *et al*. 2007).

Pathogenicity studies of *A. fumigatus* highlight general themes of innate immunity (Morton *et al*. 2012). For example, specialized alveolar epithelial cells, type II pneumocytes, phagocytose and kill conidia of *A. fumigatus* in their lysosomes, unless the conidia manage to germinate before their death (Wasylnka and Moore 2003). Neutrophils are attracted by the chemokines secreted by resident alveolar macrophages encountering *A. fumigatus* during their patrols of

the alveolar space. Whereas small hyphae are engulfed and killed by neutrophils, larger hyphae are killed by their extracellular release of reactive oxygen and nitrogen species and antimicrobial peptides, in dependence on the pathogen recognition receptors (PRRs) TLR2 and 4, and dectin-1 (Netea *et al*. 2003; Kennedy *et al*. 2007; Werner *et al*. 2009). Neutrophil extracellular traps (NETs), critical to control polymicrobial infection in appendicitis (Brinkmann *et al*. 2004), nutritionally constrain *A. fumigatus* hyphae (McCormick *et al*. 2010) and down-regulate excessive inflammation elicited by their presence (Rohm *et al*. 2014).

Non-fumigatus aspergilli, fusarium, pseudoallescheria, and other opportunistic ascomycetous fungal pathogens

Profound immunosuppression, for instance, during prolonged neutropenia, graft-versus-host disease, or severe rejection episodes of transplanted solid organs, permits invasive disease of many other types of environmental filamentous ascomycetes. Among them are the non-*fumigatus* species of *Aspergillus* (Torres *et al*. 2003), *Fusarium solanum*, *Fusarium oxysporum*, and other *Fusarium* spp., and their teleomorph *Nectria* spp. (Nucci and Anaissie 2007), *Pseudoallescheria boydii*, and its *Scedosporium anamorphs* (Quan and Spellberg 2010). Infections with these fungi are often lethal because the hosts they usually infect are incapable of mounting an effective immune response, and because they tend to be more resistant to currently available antifungals.

Fusarium spp. have evolved to infect plants, and the genomes of certain lineages contain one or more entire chromosomes encoding plant pathogenicity factors, which may be horizontally transferred within the genus (Ma *et al*. 2010). Encoded on pathogenicity chromosomes, and other genomic clusters reminiscent of bacterial pathogenicity islands, are secreted hydrolytic enzymes and signaling molecules expressed during early plant infection (Ma *et al*. 2010; Rep and Kistler 2010). Whether possession of a pathogenicity chromosome corresponds to virulence for humans, for instance, in the *F. oxysporum* lineage identified to have caused 70% of invasive fusariosis in a San Antonio hospital (O'Donnell *et al*. 2004), is not yet known. In *Fusarium*, morphogenesis contributes to virulence for humans, because yeast-like cells are produced from hyphae in the host, and typically spread widely through the bloodstream to cause numerous foci of infection in the skin.

Pigmented filamentous ascomycetes, also called dematiaceous fungi, infect immunocompromised and, rarely, immunocompetent individuals to cause phaeohyphomycosis. Their pathobiology is diverse including brain abscesses (*Cladophialphora bantianum*, *Ramichloridium* spp., and *Dactylaria gallopava*), keratitis, sinus or soft tissue infections, ulcers, and cysts (*Exophiala jeanselmei*, *Exophiala dermatitidis*, *Curvularia* spp., *Bipolaris*, or *Alternaria* spp.). When directly inoculated into tissue, these fungi can cause devastating disease, as emphasized in a tragic outbreak caused by contaminated injectable steroids (Smith *et al*. 2013).

Another epidemiologically, biologically, and profoundly important ascomycete, *P. jirovecii*, is so distinctive in its biology, that only a reference to the related article in this collection can be made (Gigliotti *et al*. 2014).

Basidiomycota

In addition to the *Cryptococcus* spp. discussed above, other Basidiomycota growing predominantly as yeast in the host, *Malassezia furfur*, *Trichosporon asahii*, and other members of human skin flora, are opportunists in patients with venous catheters and in immunosuppressed patients.

Mucorales

Profoundly immunocompromised patients at risk for the environmental fungi described above are also at risk for Mucorales infections. Additionally, Mucorales cause severe disease in diabetic patients especially at times of uncontrolled blood glucose, and patients with elevated serum iron (e.g., attributed to hemosiderosis), as described in the excellent series by Roden *et al*. (2005). Like the aspergilli, these fungi cause disease where airborne dispersal cells, sporangiospores, enter air-filled spaces (i.e., in sinuses and lungs). Infections with Mucorales progress rapidly as the fungus grows fast and relentlessly through tissue planes and bone, penetrating the eye and the brain when originating in sinuses, and causing widespread infarction because of its angioinvasive behavior. Depending on the underlying condition, such infections are often fatal (Roden *et al*. 2005), especially because these fungi tend toward resistance to current antifungals.

The Mucorales are an ubiquitous, ancient group mostly of saprotrophs, which, unlike the Ascomycota and Basidiomycota (the Dikarya), do not manifest sophisticated adaptations to diverse substrates, but rapidly use up available sugars and then move on via their sporangiospores (Hoffmann *et al*. 2013). Their evolutionary distance from the phyla of other human parasitic fungi is apparent in the structure of their hyphae, which are fragile, thin walled, and lack septa, and in the composition of their cell walls in which chitin and chitosan play the structural roles that glucans fulfill in the Dikarya (Dijksterhuis and Samson 2006). Some species like *Mucor circillenoides* are dimorphic and form yeast in the host (Dizbay *et al*. 2009; Khan *et al*. 2009; Lee *et al*. 2013), contributing to dissemination.

Among the *Rhizopus* spp., *Rhizopus oryzae* is responsible for ~70% of human disease (Spellberg *et al*. 2005). Several of its virulence factors are areas of active investigation: its iron scavenging from the host (Fu *et al*. 2004; Ibrahim *et al*. 2008, 2010; Spellberg *et al*. 2009; Ibrahim 2011) and its ability to bind GRP78/BiP, a Kar2 homolog, on the surface of endothelial cells with its spore coat protein homolog CotH3 (Liu *et al*. 2010; Gebremariam *et al*. 2014). Like a broad phylogenetic variety of fungi, Mucorales are angioinvasive. Thigmotropism, the proclivity to sense and follow curvatures of a surface, has been shown for one species, *Mucor mucedo* (Perera *et al*. 1997), and is likely to be common to all. Much remains to be learned about the biology of these evolutionarily old fungi and historically new human pathogens.

Conclusion

Immunologically intact humans manifest robust defenses against fungal diseases. Recently, human social evolution produced scientific medicine, whose progress has rendered a large population susceptible to infections with fungi not considered human pathogens as recently as a hundred years

ago. Human social evolution may have reached a stage where prioritization of the resources needed for understanding fungal biology and for successful development of multiple new classes of antifungals is possible, as the example of AIDS shows. Effective antiretroviral therapy was developed with a massive research effort less than two decades ago, and if human society evolves to a point of valuing all human lives equally, near elimination of AIDS is as feasible as the quasi elimination of HIV mother-to-child transmission has been in wealthy countries (Siegfried *et al*. 2011; Cohen *et al*. 2013; Nicol *et al*. 2013). Unlike with HIV, which has no reservoir in nature, humans will always have to cope with fungal infections, because potential fungal pathogens are part of our normal flora and of soil, water, and air. But building on the tremendous efforts and findings of the past decades, new low-toxicity therapies and novel preventative measures of fungal infections can be within our reach.

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