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Fungal Pathogens: Shape-Shifting Invaders

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Abstract

Fungal infections are on rise due to new medical procedures that have increased the number of immune compromised patients, antibacterial antibiotics that disrupt the microbiome, and increased use of indwelling medical devices that provide sites for biofilm formation. Key to understanding the mechanisms of pathogenesis is to determine how fungal morphology impacts virulence strategies. For example, small budding cells use very different strategies to disseminate than long hyphal filaments. Furthermore, cell morphology must be monitored in the host, as many fungal pathogens change their shape to disseminate into new areas, acquire nutrients, and avoid attack by the immune system. The shape shifting alterations in morphogenesis of human fungal pathogens and how they influence virulence strategies will be described in this review.

Keywords

fungal; yeast; pseudohyphae; hyphae; morphogenesis; conidia; capsule

Fungal pathogens alter cell shape as part of their virulence strategy

Most fungi play beneficial roles in the environment, but a small subset can cause lethal infections in humans [1]. These pathogenic species grow in a wide range of different morphologies that provide distinct advantages for virulence [2–4] (Key Fig. 1). Many fungal pathogens grow as yeast (unicellular organisms) which are about the size of a red blood cell and are therefore well suited for bloodstream dissemination. In contrast, molds (multicellular chains of filamentous hyphal cells) are better able to grow invasively and disseminate into tissues. Additional morphological forms can be observed in some fungi, such as large spherules containing hundreds of endospores (Key Fig. 1). Cell morphology also impacts how fungi interact with the immune system [5]. Small yeast cells can be phagocytosed by leukocytes, but long hyphal filaments are too big to be attacked in this manner. Interestingly, some yeasts have developed strategies for surviving in the phagosome, and therefore use this “Trojan Horse” mechanism to their advantage to disseminate within the host.

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Fungi are not locked into one cell morphology; they change shapes as one of the mechanisms they use to acquire new virulence properties in the host. The power of shape shifting has long been recognized in fiction ranging from old tales of Loki to new science fiction stories. Just as fictional shape shifters transform to avoid detection or gain new powers, so do human fungal pathogens. Some species undergo a dramatic shift when they enter the host, such as switching from filamentous hyphae to budding yeast. Others shift the opposite way and transform from a small conidia (spore) into a long hyphal filament (Key Fig. 1) [3, 4]. In addition, some species undergo changes in size during the course of an infection, such as forming giant cells that are too big to be phagocytosed. This review will therefore describe how shape shifting influences the virulence strategies of some of the most common lethal fungal pathogens. We will also make some comparisons to analogous ways in which cell shape affects the virulence of bacterial pathogens, as there are common themes even though bacteria are much smaller than fungi [6]. It is critical to understand the role of shape shifting in fungal virulence as new insights are needed in order to develop novel therapies as the potential for health problems associated with fungal infections are increasing [1].

***Cryptococcus*: Yeasts that cloak themselves in a protective capsule and shift into giant cells.**

Cryptococcus neoformans is one of the best studied fungi because it is a major cause of lethal fungal infections worldwide due to its ability to infect AIDS patients. The related species *C. gattii* has also gained attention recently for causing clusters of infections in seemingly healthy individuals [1, 4, 7]. *Cryptococcus* species grow by budding (Fig. 2A) and by forming filamentous cells in nature, but shift primarily into the budding mode of replication in the host [8]. To get into the host, *C. neoformans* cells undergo a series of morphological shifts [8]. First, opposite mating type cells, or in some cases same-sex cells, conjugate to create a diploid cell that then undergoes meiosis to form small spores that can be aerosolized (Fig. 2B) [8]. Inhaled spores start to grow by budding and then undergo two other morphological changes (Fig. 2C) [9]. One is the formation of a protective polysaccharide capsule, a unique feature of *C. neoformans* and its close relatives not seen in other human fungal pathogens. The other change in the host is that *C. neoformans* cells start to grow in a wide range of sizes.

***Cryptococcus* capsule protects against stress and immune attack.**

Early after infection *C. neoformans* cells form a thick polysaccharide capsule around the cell wall that provides protection from phagocytosis and stress [10]. Capsule thickness varies depending on environmental influences, but can be up to 30 μm thick, which is quite large compared to the typical cell diameter of about 5 μm [11, 12]. The capsule is mainly composed of glucuronoxylomannan (GXM), with about 10% galactoxylomannan (GXMGal) and 1% mannoproteins [10, 13]. This barrier provides protection from stress, such as reactive oxygen species, and also acts in several ways to protect against the immune system. In particular, the capsule has antiphagocytic properties, which are due in part to masking of pathogen-associated molecular patterns (PAMPS) and remodeling of the surface in ways that alter the type of opsonin needed to promote phagocytosis [14, 15]. In addition,

the major capsular components GXM and GXMGal are thought to modulate the host immune response by inhibiting immune recognition and activation [16, 17]. Melanin production in the cell wall also confers resistance to stress [7].

***C. neoformans* forms large titan cells that persist in the host.**

At later times of infection, *C. neoformans* cells display extreme size variation, ranging from 1 to 100 μm in diameter [12, 18]. The role of the smaller cells is unclear, but they may disseminate better in the host [19]. However, the large cells, known as giant or titan cells, are thought to be better able to persist in the host because they are more resilient. They are too large to be phagocytosed and they have a thicker cell wall and a denser capsule, which makes them more stress resistant [18]. The large size of the titan cells is thought to be achieved by endoreduplication, a process known to create large cells [20, 21]. However, titan cells grow slowly, and their large size is thought to limit their ability to disseminate. Thus, titan cells are mainly a concern because they can persist in the host, and potentially reinstate an infection. In addition, older cells that have divided several times are more resistant to stress, which has been linked to changes in the cell wall [22]. Analogous morphological changes have been seen for some bacterial pathogens where small cells are thought to be advantageous for entering special niches and by providing a smaller target for complement deposition, whereas large cells are better able to avoid phagocytosis [6, 23].

***Histoplasma capsulatum*: A dimorphic fungal pathogen that grows as hyphal filaments in the environment and shifts to yeast in the host.**

In spite of its species name *Histoplasma capsulatum* does not form a capsule. It is part of a group known as thermally dimorphic fungi because it forms filamentous hyphae at low temperatures in the environment, but shifts to yeast growth at the elevated temperature of the human body [24, 25]. Infection is often initiated when hyphal filaments transition to forming structures known as conidiophores that release small asexual conidia (spores) that are subsequently inhaled into lung [26]. Thus, an extensive series of morphological shifts must occur for *H. capsulatum* to cause an infection as it shifts from hyphal filaments to small conidia and then resumes growth as a budding yeast in the host (Fig. 3A).

***H. capsulatum* transforms its cell surface to promote phagocytosis without immune stimulation.**

H. capsulatum is distinct from most other pathogens in that it actively employs strategies to facilitate entry into the macrophage phagosome [27, 28]. This contrasts with *C. neoformans*, which, even though it can survive in the phagosome, uses a capsule and large cell size to avoid phagocytosis. Although *H. capsulatum* seeks to be phagocytosed, it does so in a “stealth” mode to avoid activating macrophages [27]. One strategy is to secrete glucanase enzymes to trim immunostimulatory β -glucans sugar chains from the surface of the cell wall [29]. Another strategy used by the chemotype II group of *H. capsulatum* is to cloak itself by covering the β -glucans with a nonstimulatory layer of α -(1,3)-glucan [30]. These cell wall modifications reduce activation of pattern recognition receptors on innate immune cells, such as Dectin-1, thereby reducing inflammatory cytokine production. This allows *H. capsulatum* to replicate safely within the phagosomes of macrophages without further attack

by the immune system [26, 27]. The ability to survive in the phagosome also allows both *C. neoformans* and *H. capsulatum* to disseminate in the host as the macrophage moves about in a Trojan Horse mechanism. In this way they are similar to some bacterial intracellular pathogens [6].

***Aspergillus fumigatus*: Inhaled conidia transform into invasive hyphae that further shift into an interconnected mycelial network**

Filamentous hyphal growth has several virulence advantages including the ability to invade into tissues and that the large size of hyphae prevents phagocytosis. *Aspergillus fumigatus*, the primary focus of this section, is one of the most common lethal fungal pathogens [31]. It has been a particularly severe problem in immunocompromised individuals, such as bone marrow transplant patients [1]. For comparison, we will also describe *Rhizopus oryzae*, which differs from *A. fumigatus* in that it forms multinucleate hyphal cells that lack a septum between each nucleus (known as coenocytic or aseptate hyphae) (Fig. 3B,C). *R. oryzae* more rarely infects humans, but it is a major concern because current therapies are not effective [32].

Hyphal branching and fusion promote dissemination and nutrient acquisition.

Inhaled conidia that survive to germinate in the lung shift to forming tubes of growth (germ tubes) that expand into hyphal filaments [33, 34] (Fig. 3B). Dissemination to form a large mass of cells (mycelium) is further advanced by hyphal branching, which can occur either when hyphal tip growth splits into two sites of polarized growth or by formation of a new site of growth at a subapical region (Fig. 3D) [35]. In addition, some hyphal tips are thought to fuse to expand the interconnected mycelial network (Fig. 3E). *A. fumigatus* hyphal fusion has not been well studied, in part because it is not efficient under most in vitro conditions [36, 37], so it has been studied in more detail in nonpathogenic model organisms like *Neurospora crassa* [37]. More research needs to be done to define the spatial interrelationships of hyphae in the mycelia and how they are influenced by ambient conditions in the host [38].

The mycelial network promotes pathogenesis by facilitating the exchange of nutrients, water, signal molecules, and genetic material between filaments. Although *A. fumigatus* hyphae are composed of individual cell compartments, the cells can share molecules with their neighbors because they are not completely walled off. The septum that forms between cells is only partially closed, leaving an opening known as a septal pore [33, 34]. The septal pores can be blocked if necessary, for example due to injury, by structures known as Woronin bodies. In contrast, *R. oryzae* forms aseptate hyphae that readily share nutrients because they form few septal barriers. Comparison with bacterial pathogens suggests that aseptate hyphae could have advantages under conditions of genotoxic stress because the greater number of nuclei may facilitate sharing of templates for healing DNA damage [6].

Hyphal elongation, branching, and mycelium formation help to subvert attack by phagocytes.

Inhaled spores are small enough to be readily phagocytosed by macrophages, so an important advantage of the switch to filamentous growth is that the emerging hyphae can burst out of macrophages and are then too big to be phagocytosed. Interestingly, *in vitro* studies have shown that interaction with neutrophils can stimulate hyphal branching as an evasive maneuver [39]. Typically when hyphae are too big to be phagocytosed, neutrophil extracellular traps (NETs) are formed in which neutrophil DNA is used to entangle the hyphal filaments and provide a platform in which antimicrobial peptides and other toxic compounds are directed at the fungus [40]. However, it is not clear that NETs play an important role against *A. fumigatus* *in vivo* [41, 42], so other aspects of immune system must come into play.

Multimorphic *Candida* species form cell aggregates and biofilms

Candida species are distinct from most other fungal pathogens in that they are commensal organisms that colonize the skin or GI tract. Thus, rather than inhaling conidia, patients are usually infected by endogenous organisms that, under conditions such as immune deficiency or biofilm formation, are able to overwhelm host defenses and disseminate. *Candida* species are also distinct in that most are multimorphic in the host where they transition between budding and filamentous growth [43]. *C. albicans* has a wide range of morphologies as it can form buds, pseudohyphae, and true hyphae in the host (Fig 4A, B) [44]. Most other *Candida* species, such as *C. tropicalis* and *C. parapsilosis*, can form pseudohyphae, although some species do this more rarely (e.g. *C. glabrata* and *C. auris*) [45, 46]. As described above for *A. fumigatus*, the filamentous forms provide advantages for dissemination, although *Candida* species are not known to form septal pores or undergo hyphal fusion. However, *Candida* species can shift from filamentous growth back to budding, which is thought to be an advantage for dissemination in the bloodstream. The budding form of *C. albicans* has also been shown to be advantageous for growth in certain niches, such as the gastrointestinal tract, where it is thought that the budding form colonizes better since it has fewer immunostimulatory molecules on the surface than hyphal cells [47, 48].

***Candida* species form large multicellular clusters and biofilms.**

A common feature of the *Candida* species is the ability to form large multicellular clusters (Fig. 4C). One important example of this is biofilm formation on abiotic surfaces, such as catheters and implanted devices, that can lead to invasive infection even in immunocompetent patients [49]. Mutational studies on *C. albicans* indicate that shifting to hyphal morphogenesis is needed to create a biofilm [50]. However, true hyphae are not always essential since other species that only form pseudohyphae can form biofilms. Some *Candida* species, such as *C. glabrata* and *C. auris*, a newly emerging pathogen, appear to grow primarily or possibly only as budding cells in the host. However, both of these species are often detected in cell aggregates or in biofilms [46, 51, 52]. Thus, the *Candida* species are generally distinct from the budding species described above (*C. neoformans* and *H. capsulatum*), in their propensity to form aggregates in the host. The ability to form large filaments or clusters of budding cells that are too big to be phagocytosed confers advantages

for survival in the host. Formation of analogous types of aggregates has also been seen for some bacterial pathogens [53].

Hyphal formation contributes to the ability of *C. albicans* to escape from the phagosome.

An additional advantage of multimorphic *C. albicans* is its ability to shape shift to escape from macrophages. Budding cells that are phagocytosed by macrophages respond to signals in this environment that cause a shift to filamentous hyphal growth [54]. It was initially thought that *C. albicans* escaped the macrophage because elongating hyphal cells physically pierced the macrophage. More recent studies have indicated that hyphal growth is not needed to kill macrophages, as phagocytosed *C. albicans* that are defective in switching to filamentous growth can still kill macrophages [55, 56]. Subsequent studies revealed that *C. albicans* stimulates a cell death pathway in macrophages (pyroptosis) by activating the NLRP3 inflammasome [57, 58]. However, hyphal growth is still thought to contribute to escape from the phagosome because the elongating filamentous growth can stretch the phagosomal membrane, causing neutral pH from the cytoplasm to prevent the acidification of the phagosome which weakens attack by the macrophage [59].

***Coccidioides* and *Pneumocystis*: spore-forming in the host**

***Coccidioides* species form giant spherules in the host containing hundreds of endospores.**

Coccidioides immitis and *Coccidioides posadasii* are found in the soil in arid regions of the southwestern U.S. and northwestern Mexico [60]. Although *Coccidioides* are thermally dimorphic fungi evolutionarily related to *Histoplasma*, they undergo very distinct morphological shifts. In soil, they grow as hyphal filaments and then alternating cells in the hyphae transform into arthrospores, which persist in the soil (Fig. 5 A). When the soil is disturbed, aerosolized arthrospores infect humans by inhalation [61]. In the lung, the arthrospores expand into large, multinucleate cells called spherules. Hyphae and arthrospores have also been observed in patient samples [62], so whether the germinating arthrospores develop directly into spherules or transit through a hyphal phase is unclear.

During the initial stages of spherule growth, nuclei proliferate by mitosis until a single spherule contains hundreds of nuclei. The spherule then undergoes endosporulation in which each nucleus is packaged into an individual cell termed an endospore that is 2 to 4 μm in diameter [61]. The spherule then ruptures to release hundreds of endospores, which spread in the host and form new spherules. Though little is known about the molecular events leading to endospore formation, the process through which they form - the generation of a multinucleate syncytium that subsequently forms individual cell walls around each nucleus - resembles the process of ascospore formation that occurs after meiosis in ascomycete fungi that enclose their spores in a cell termed an ascus [63].

Formation of spherules has been proposed to help avoid immune attack by creating a protective environment to allow for proliferation of endospores [62]. In addition, spherules can be as large as 100 μm in diameter, or about the size of a *C. neoformans* titan cell, which makes them too large to be phagocytosed. The spherule cell wall is enriched in the Spherule

Outer Wall glycoprotein (SOWgp) that mediates adhesion and is important for virulence [64]. SOWgp is the dominant antigen of the spherule and sequence variability in repeated elements of this protein may play a role in evasion of the host immune response [65]. In addition, the walls of the endospores are distinct from the wall of the spherule. In particular, SOWgp is removed from the endospore surface by proteolysis, and this shift in cell surface antigens may assist in avoiding immune detection during dissemination [66].

Pneumocystis undergoes meiosis and sporulation in the host that may help in immune evasion

Pneumocystis species are specific to their host. The species associated with humans, *Pneumocystis jirovecii*, causes pneumocystic pneumonia in immune-compromised patients [67]. A distinctive feature of *P. jirovecii* is that it has been described to transition between two different cell types in the host: a trophic form and a cystic form [68] (Fig. 5B). The trophic form is haploid and is thought to reproduce by fission. However, two trophic cells can undergo mating and fuse to form a cyst. Within the cyst, the haploid nuclei fuse, and then undergo meiosis and one round of mitosis to generate eight haploid nuclei. These nuclei are then packaged into individual cells within the cyst [69–71]. This pattern exactly matches the formation of eight spores packed into an ascus in hyphal ascomycetes [63], so trophic cells and cysts likely represent the vegetative and ascus forms described in other fungi.

Ascospore formation could provide advantages in immune evasion similar to endosporulation in *Coccidioides*: an opportunity for proliferation in the protected intracellular environment of the cyst and (potentially) a distinct cell wall on the trophic cells formed within the cyst [68, 72]. Furthermore, it has been suggested that the process of meiotic recombination during encystation might provide for allelic diversity of cell surface antigens to promote immune evasion [72]. Although the role of these morphological transitions is understudied because *P. jirovecii* cannot be grown in vitro, the parallels to meiosis and ascospore formation in other fungi provide a useful model for encystation and suggests candidate genes that could be important for morphogenesis and pathogenesis.

Concluding Remarks:

This review covers a sampling of the major human pathogens, but there is still much to be learned about shape-shifting fungi (see Outstanding Questions). One is that we have only a limited view of fungal morphology in the host from histology. However, fungal strains that produce fluorescent proteins are making it possible to identify new morphological shape transitions in different host niches and after stress conditions such as hypoxia and antifungal therapy [73]. There are also many more fungi with different morphogenesis programs that need to be studied. Comparisons with a broader range of fungi, including pathogens of plants and insects, will be helpful for identifying the fuller range of virulence strategies based on shape-shifting. For example, many plant pathogens form specialized invasion structures. In contrast, we are only aware of one type of human fungal pathogen, the obligate intracellular *Microsporidia*, that forms special invasion structures [74]. Insect fungal pathogens also use specialized morphogenesis, such as *Ophiocordyceps unilateralis* that turns ants into zombies to disseminate to other ants [75]. Ultimately, a major challenge for

the future is to determine the mechanisms that regulate morphological changes in order to help develop novel therapeutic approaches for controlling lethal fungal infections.

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Glossary

Ascus	A cell structure containing sexual spores produced following meiosis by <i>Ascomycete</i> fungi
Capsule	A protective layer composed of polysaccharide chains that surrounds the cell wall
Cyst	A term used to describe an ascus that contains the spores (ascospores) that form after mating of <i>Pneumocystis jirovecii</i> . This term is a holdover from when <i>P. jirovecii</i> was thought to be a parasite. (DNA sequencing revealed it belongs in the fungal kingdom)
Conidia	Asexual spores produced by molds. Also called conidiospores
Hyphae	Chains of elongated filamentous cells with parallel cell walls. Hyphae can be septate, in which one or a few nuclei is walled off into a separate cell compartment, or coenocytic (aseptate) in which the cells have multiple nuclei and few septae
Mold	Multicellular fungus that grows as long hyphal filamentous cells
Pseudohyphae	Filamentous multicellular structures comprised of a chain of connected cells in which there is an obvious narrowing at the site of the septae. Formed by unidirectional budding
Spherule	A large structure formed in the host by <i>Coccidioides</i> species which contain hundreds of endospores
Spore	A resistant type of cell produced asexually or sexually through mating and meiosis. Inhalation of spores is the typical route of infection for most of the serious human fungal pathogens
Trophic form	Term for the form of <i>P. jirovecii</i> that reproduces by mitosis
Yeast	Unicellular form of fungi. Yeasts can divide by budding or fission

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Outstanding Questions Box

- Macro-scale changes in morphology are easily seen, but new approaches are needed to map out smaller scale changes in the cell wall and capsule that affect stress resistance or immune stimulation. Approaches for analyzing cells isolated in vivo will be important to define changes that occur in the host.
- What are the signaling pathways that control shifts in morphology? It is currently not well understood what regulates important shifts in morphology such as the switch to hyphal growth for *C. albicans* or spherule formation by *Coccidioides*.
- How do the different morphologies of *Pneumocystis* contribute to virulence? It will also be important to develop in vitro culture conditions for *Pneumocystis* in order to better define different stages of morphogenesis during growth.
- How does interspecies communication with bacteria alter fungal morphology? Fungal pathogens are often present in mixed infections with bacteria. Although it is known that some bacteria can alter the morphology and virulence properties of certain fungal pathogens, more work has to be done to more fully understand how mixed infections alter pathogenesis.
- It will be interesting to determine how ageing affects cell morphology. This may help to explain why older cells *C. neoformans* cells persist better in the host.
- How are cells organized in vivo to promote virulence? In addition to studying the morphology of individual cells, it will be important to define the spatial relationships of cells in vivo to determine how this can affect nutrient sharing and acquisition, and also avoidance of attack by the immune system cells.

Highlights

- Fungal pathogens grow in different shapes that influence their ability to disseminate an infection. For example, small spores (conidia) penetrate deep into the lung, budding cells that are about the size of a red blood cell disseminate in the bloodstream, and long hyphal filaments grow invasively into solid tissues.
- Some fungi undergo shape shifting in the host to form larger cells, or clusters of cells, that are too big to be eaten (phagocytosed) by leukocytes.
- Other fungi form small yeasts that have developed strategies for surviving inside the phagosome where they are safe from attack by other immune cells. This also allows them to disseminate throughout the host in a Trojan Horse mechanism.
- Fungal shape shifting can also enhance growth in the host by creating interconnected networks of hyphal filaments (mycelia) that promote sharing of nutrients.

Key Figure 1. Shape shifting fungal pathogens.

Morphology changes seen for some of the human fungal pathogens known to cause lethal systemic disease. Morphological forms on the left correspond to those seen in the environment (or at room temperature in the lab). The top four species all undergo a morphological shift to form a type of small spore that can be aerosolized and inhaled deeply into the lung. The spore germinates in the host to shape shift again into the forms shown on the right. *C. albicans* is a commensal organism that is commonly found growing on human skin or mucosa, so it does not require a shift to a spore form to infect a host. *P. jirovecii* forms cysts with sexual spores, but it is not clear they play a role in the spread of infection to new hosts. The environmental form of *P. jirovecii* is not known since it cannot be grown in vitro, and it is not known if there is an environmental reservoir.

Figure 2. *Cryptococcus neoformans* undergoes changes in size and outer capsule formation.

(A) Typical sequence of morphological changes during the budding mode of replication seen for *C. neoformans*.

(B) Cells of opposite mating type (a and α) undergo mating to create a diploid cell, which then undergoes meiosis to create haploid nuclei that are each encased in a spore [8]. Haploid spores of the α mating type account for more than 99% of human infections.

(C) Spores inhaled into a host shift back to a budding mode of replication. A thick polysaccharide capsule forms to protect the cell against stress, and large cells form which are resistant to phagocytosis by immune cells.

Figure 3. Molds in the environment produce spores that shift back to filamentous growth forms in the host.

(A-C) Molds growing as filamentous hyphae in the environment undergo morphological changes to produce distinct types of spore-forming structures. The small spores are aerosolized and then inhaled into the lung.

(A) *H. capsulatum* produces micro- and macroconidia, which differ in size and surface structure. The smaller microconidia are thought to be more easily inhaled deep into the lung where they shift to budding to initiate an infection. Immunogenic surface molecules are cloaked, such as by the production of an outer layer of a polymer of the sugar α -glucan (green).

(B) *A. fumigatus* produces conidia that shift back to forming septate hyphae in the host.

(C) *R. oryzae* spores revert back to growing as coenocytic (aseptate) hyphae in the host that produce very few septa.

Filamentous growth can disseminate to form a complex interconnected mycelium by (D) hyphal branching and (E) fusion of hyphal tips.

(A) Pseudohyphal growth

(B) Hyphal growth

(C) Shift to multicellular states or cell aggregates in the host

Figure 4. *Candida* species form multicellular structures.

Candida species divide by budding, but under certain conditions some can form

(A) pseudohyphae which are multicellular chains of created by sequential budding in the same direction.

(B) *C. albicans* can also undergo a shift to forming true hyphae characterized by smooth parallel walls.

(C) *Candida* species form multicellular structures to avoid attack by the immune system. As described in the text, *C. albicans* can form multicellular hyphae and pseudohyphae that are too large to be phagocytosed. Other species that more rarely form hyphae or pseudohyphae often form cell aggregates in the host or biofilms surrounded by a matrix that resist attack by the immune system. *C. albicans* is also very good at forming biofilms.

Figure 5. *Coccidioides* and *Pneumocystis* form spores in the host.

Although many fungal species form spores in the environment, two human pathogens are worth noting for their ability to form a type of spore in the host.

(A) *Coccidioides* species (*immitis* and *posadasii*) convert hyphal cells to arthrospores in the environment. Inhaled arthrospores shift to forming large spherules containing hundreds of endospores. Rupture of the mature spherules releases the endospores, which go on to form more spherules.

(B) Haploid cells of the trophic form of *P. jirovecii* divide by fission. However, mating can occur between opposite mating type followed by meiosis to create a cyst form with 8 haploid nuclei.