

15 Pathogenic Fungi

15.1 Diseases Caused by Fungi

Though *S. cerevisiae* is normally considered a non-pathogenic micro-organism, occasional infections may occur. Furthermore, budding yeast can serve as a model to learn more about pathogenic fungi, in particular with regard to regulatory features and drug therapy, because yeast as a fungal species shares many characteristics with its pathogenic relatives.

Fungal infections or mycoses are classified depending on the degree of tissue involvement and mode of entry into the host. These are:

Superficial - localised to the skin, the hair, and the nails.

Subcutaneous - infection confined to the dermis, subcutaneous tissue or adjacent structures.

Systemic - deep infections of the internal organs.

Opportunistic - cause infection only in the immunocompromised.

Human fungal infections in Europe and large parts of the world are uncommon in normally healthy persons, being confined to conditions such as candidiasis (thrush) and dermatophyte skin infections such as athlete's foot. However, in the immunocompromised host, a variety of normally mild or nonpathogenic fungi can cause potentially fatal infections. Furthermore, the relative ease with which people can now visit "exotic" countries provides the means for unusual fungal infections to be imported into Western countries.

15.1.1 Superficial Mycoses

In superficial mycoses, infection is localised to the skin, the hair, and the nails. An example is "ringworm" or "tinea", an infection of the skin by a dermatophyte. Ringworm refers to the characteristic central clearing that often occurs in dermatophyte infections of the skin. Dermatophyte members of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are responsible for the disease. Tinea can infect various sites of the body, including the scalp (tinea capitis), the beard (tinea barbae) the foot (tinea pedis: "athlete's foot") and the groin (tinea cruris). All occur in Europe although tinea infections, other than pedis, are now rare. *Candida albicans* is a yeast causing candidiasis or "thrush" in humans. As a superficial mycoses, candidiasis typically infects the mouth or vagina. *C. albicans* is part of the normal flora of the vagina and gastrointestinal tract and is termed a "commensal". However, during times of ill health or impaired immunity, the balance can alter and the organism multiplies to cause disease. Antibiotic treatment can also alter the normal bacterial flora allowing *C. albicans* to flourish.

15.1.2 Subcutaneous Mycoses

These are infections confined to the dermis, subcutaneous tissue or adjacent structures. Infection may arise following the wounding of the skin and the introduction of vegetable matter. These mycoses are rare and confined mainly to tropical regions. They tend to be slow in onset and chronic in duration. An example is sporotrichosis caused by *Sporothrix schenckii*. The fungus is dimorphic, being a mould that can convert to a yeast form at 37°C on rich laboratory media or in infection. Sporotrichosis was once common in Europe but cases are now rare. The disease is most prevalent the Americas, South Africa and Australia. Infection usually follows an insect bite, thorn prick or scratch from a fish spine. Certain occupation groups appear to have increased risk from infection. These include florists, farm workers

and others who handle hay and moss. The most common symptom is an ulcerative lesion that may develop into lymphangitis.

15.1.3 Systemic Mycoses (primary and opportunistic)

These are invasive infections of the internal organs with the organism gaining entry by the lungs, gastrointestinal tract or through intravenous lines. They may be caused by: (i) primary pathogenic fungi or (ii) by opportunistic fungi that are of marginal pathogenicity but can infect the immunocompromised host.

Primary Pathogenic Fungi

Infection occurs in previously healthy persons and arises through the respiratory route. Examples include histoplasmosis, blastomycosis, coccidiomycosis and paracoccidioidomycosis. The fungi occur throughout the world but not in large parts of Europe.

Histoplasmosis. This is caused by *Histoplasma capsulatum*. The organism is dimorphic (being a mould that can convert to a yeast form). *H. capsulatum* is endemic in many parts of the world including North and South America. It is found in the soil and growth is enhanced by the presence of bird and bat excreta. Environments containing such material are often implicated as sources of human infection. The lungs are the main site of infection but dissemination to the liver, heart and central nervous system can occur. Pulmonary infection can resemble symptoms seen in tuberculosis.

Opportunistic Fungi

Here, patients usually have some serious immune or metabolic defect, or have undergone surgery. The diseases include aspergillosis, systemic candidosis and cryptococcosis. Exceptionally, other fungi that are normally not pathogenic, such as *Trichosporon*, *Fusarium* or *Penicillium*, may cause systemic infections.

Aspergillosis. This is the name given to a number of different diseases caused by the mould *Aspergillus*. It produces large numbers of spores and occurs world-wide. In Europe, *A. fumigatus* is the most common species causing disease. The organism can infect the lungs, inner ear, sinuses and, rarely, the eye of previously healthy persons. In the immunosuppressed host, *Aspergillus* can disseminate throughout the body.

Candidosis. In severely immunocompromised patients (e.g. those receiving chemotherapy) *C. albicans*, that is part of the normal human flora (see above), can proliferate and disseminate throughout the body.

Cryptococcosis. This is a systemic infection caused by the yeast *Cryptococcus neoformans*. The commonest manifestation is a subacute or chronic form of meningitis resulting from the inhalation of the organism. Pulmonary infection can also occur. The disease affects both healthy and immunosuppressed individuals and occurs world-wide. *C. neoformans* can be isolated in large numbers from pigeon droppings in the environment, although such birds do not appear to harbour the yeast.

Other Fungal Related Disease


Constant exposure to fungal spores in the atmosphere can induce respiratory allergies. Elevated antibodies to a range of common spore forming fungi have been demonstrated in occupational diseases such as Humidifier fever, Malt workers' lung and Wheat threshers' disease. Certain fungi, such as mushrooms, can produce poisonous toxins that may prove fatal if ingested (e.g. *Amanita phalloides*: "death cap"). Others (*Psilocybe*) affect the central nervous system inducing hallucinogenic responses. Many moulds produce secondary metabolites (mycotoxins) that are highly toxic to humans. Ergotism is caused by eating bread prepared from rye infected with the fungus *Claviceps purpurea*. Historically, several large scale outbreaks of madness in local populations have been attributed to ergotism.

Pneumocystis. This is an infection of the lung caused by *Pneumocystis carinii*. The organism is a common cause of fatal pneumonia in AIDS patients. An intracellular parasite, with a life cycle of trophozoite and cyst, it was formerly considered to be a protozoan. However, comparison of DNA and RNA sequences have established that it is one of the group of uromyctetous red yeast fungi. The cysts contain 8 nuclei which can be seen in smears of pulmonary aspirates. *P. carinii* is a commensal of many wild and domestic animals and evidence suggests that human infection is commonly derived from dogs.

15.2 Examples and Potential Therapy

Figure 15-1 summarizes some examples of pathogenic fungi. A characteristic of these species is that they are dimorphic: they can propagate as singular cells, the yeast form, or grow as large filaments (hyphae or pseudo-hyphae) of cells connected to each other. Many yeasts can switch between the two forms, and in most cases, infectivity is mediated by the hyphal or pseudo-hyphal variant.

Dimorphic: Unicellular (yeast form) - Pseudohyphal or hyphal growth form



***Aspergillus* species**
Candida albicans/tropicalis
Cryptococcus neoformans
Histoplasma capsulatum
Coccidioides immitis
Paracoccidioides brasiliensis
Pneumocystis carinii

Superficial mycoses (skin, hair, nails)
 Subcutaneous mycoses (dermis, subcutaneous tissue)
 Systemic mycoses (deep infections of internal organs)
 Opportunistic infections in mammals -
 airborne or contact modes of transmission

Higher risk of infections (life-threatening)

- AIDS and immune-suppressed patients
- extensive surgery or prosthetic devices
- treatment with broad-spectrum antibiotics
- dialysis patients

Infectivity

Cells penetrate epithelial and endothelial cell layers

Virulence

Different mechanisms. Toxins may not be involved (only in moulds)

Therapy:

Treatment difficult, because eukaryotic organisms
 Drugs that inhibit cell wall or membrane synthesis

Figure 15-1: Characteristics of some pathogenic fungi.

Drug treatment to cure mycoses has turned out to be still difficult. Being eukaryotes, fungi are not amenable to treatment with antibiotics. The major points of attack are the rigid cell wall and peculiarities of their sterol metabolism that distinguishes all fungi from cells of their potential animal hosts (Figure 15-2). A variety of drugs interfering with the synthesis of ergosterol or components of the cell wall have been developed but are not successful in many cases. Budding yeast has proven to be a useful system to test the efficacy of newly developed drugs.

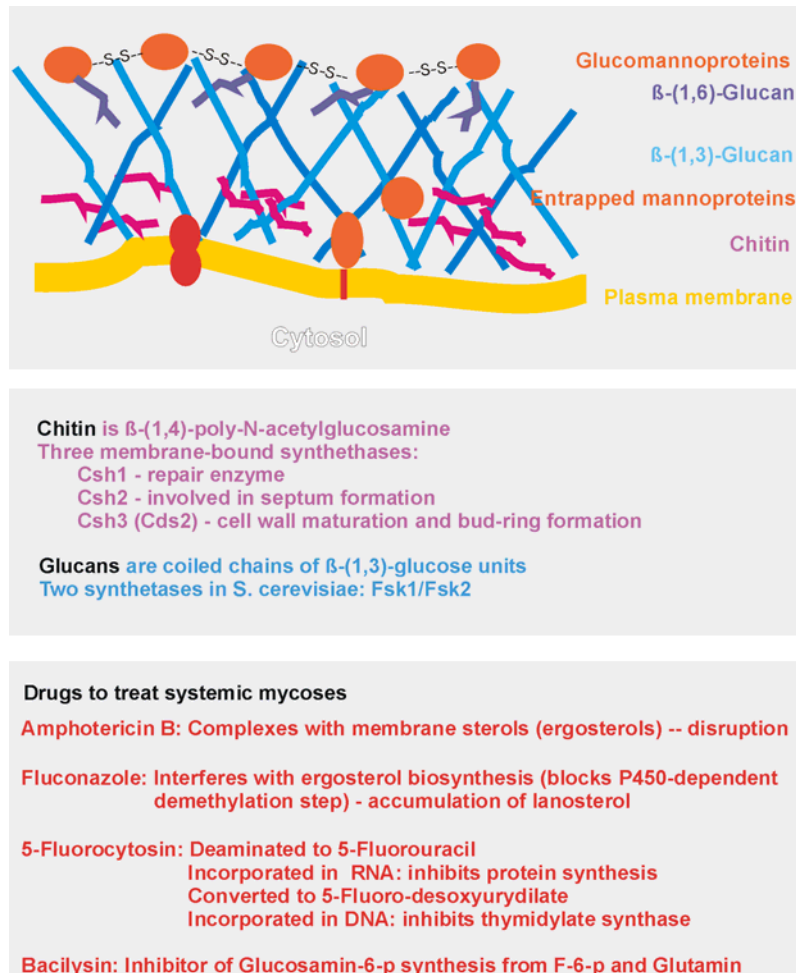


Figure 15-2: Drug treatment of systemic mycoses.

15.2.1 Pseudohyphal Growth in Yeasts

Pseudohyphal growth in budding yeast is induced by nitrogen starvation, which allows the cells to penetrate into soft agar. The process has been thoroughly investigated, and the signalling mechanism leading to pseudohyphal growth (by a specialized MAP kinase pathway) has been discussed in chapter 13.

15.2.2 *Candida albicans*

A system that also has been widely studied is the pathogenic fungus *Candida albicans* (belonging to the class of the Hemiascomycetes yeasts), because this organism is responsible for most of the fungal infections that occur in immuno-compromised individuals (Figure 15-3). *Candida* species are the fourth-leading cause of nosocomial infections, and in those patients with candidemia, the

attributable mortality rate is 35%. Furthermore, oropharyngeal candidiasis occurs in approximately 70% of patients with AIDS, ca. 70% of all women (with or without AIDS) will experience at least one episode of vaginitis caused by *Candida* and 20% will experience recurrent disease.

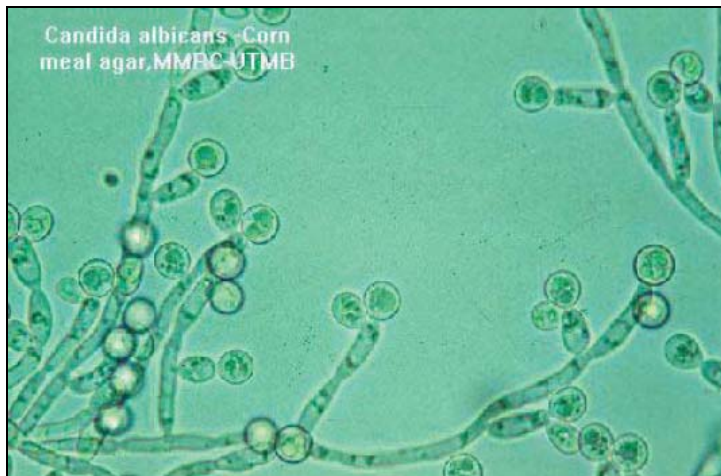


Figure 15-3: Characteristics of *Candida albicans*.

C. albicans is a normal member of the intestinal flora (commensal fungus). It is pleiomorphic and undergoes reversible morphogenic transitions between budding, pseudohyphal and hyphal growth forms. Like *S. cerevisiae*, it can form diploid cells, but no sexual cycle is known.

It should be mentioned here that pseudohyphal growth in *S. cerevisiae* is induced upon N-starvation conditions upon which the cells show invasive growth in agar plates. This process is dependent on Ste7p, Ste11p, Ste2p, Ste20p, and Tec1p, components of the mating type. The essential genes are *STE12* and *PDH1*. The yeast-to-hypha transition has been shown to be one of several virulence attributes that enable *C. albicans* to invade human tissues (Figure 15-4). Hyphal growth is blocked by inactivation of the transcription factors Cph1p (paralog of Ste12p) and Efg1p (paralog of Pdh1p), which belong to the mitogen-activated protein kinase (MAPK) and Ras-cAMP pathways. Efg1p is a bHLH protein with homology to Myc and MyoD. The only pathogenic form is the hyphal form. It invades macrophages, propagates and kills the cells upon exit. Significantly, a double mutant (*cph1 efg1*) displays reduced virulence in animal models of systemic candidosis. The double mutants fail to switch to the hyphal form, they are recognized by macrophages equally to wild-type, but they will not kill the cells, which can be filled with up to 80 invading *Candida* cells.

The signals that promote morphogenesis in *C. albicans* often impose stresses. For example, simultaneous changes in temperature and pH, activate hyphal development. To adapt to changes of pH (e.g. neutral pH in the bloodstream or acidic pH in the vagina), *C. albicans* induces or represses, respectively, particular pH-sensitive genes (*PHR1* and *PHR2*).

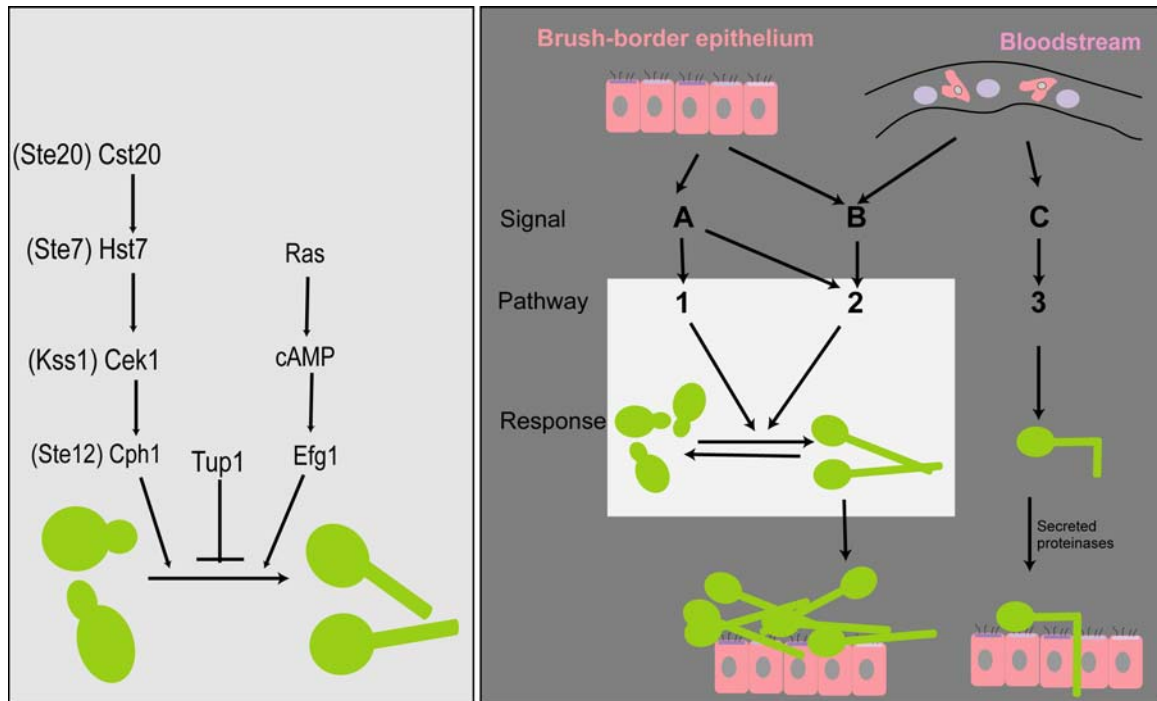


Figure 15-4: Morphogenetic control in *C. albicans*.

A number of additional virulence factors have been identified. Like other pathogens, virulence in *C. albicans* includes host recognition. Binding of the organism to host cell proteins (or microbial competitors) more than likely prevents or at least reduces the extent of clearance by the host. Additionally, several degradative enzymes have been shown to promote virulence. Molecules involved in host cell recognition found in *Candida* are adhesins that bind to several extracellular matrix proteins of mammalian cells, such as fibronectin, laminin, fibrinogen and collagen. Adhesion function (e.g. to human buccal epithelial cells) is provided by agglutinin-like sequences of *Candida*, resembling the *S. cerevisiae* α -agglutinin protein that is required for cell-cell recognition during mating. A further biomolecule important in adherence is a hyphal- and germ-tube-specific protein, an outer surface mannoprotein in *Candida*, that is believed to be oriented with its amino-terminal domain surface-exposed and the C-terminus most probably covalently integrated with cell wall β -glucan. Another potential adherence protein of *Candida* is reminiscent of the integrin family of mammalian cell receptors. Additionally, a membrane protein (α -1,2-mannosyltransferase) that is required for both O- and N-mannosylation in fungi might be required for host recognition. Enzymes that contribute to invasiveness are the secreted aspartyl proteinases and phospholipases which form rather large families in *C. albicans*.

15.3 Other Pathogenic Fungi

A brief survey of pathogenic fungi that are 'prominent' inducers of severe diseases, mostly outside Europe, is given in Figures 15-5 to 15-8. Some virulence factors in these pathogens have been described, but more detailed information will be available when genome sequences will have been accomplished in the near future.

15.3.1 *Histoplasma capsulatum*

Histoplasma is a primary pathogenic dimorphic fungus forming macroconidia and microconidia. The yeast form is required for propagation in human tissues. The fungus is endemic world-wide (mainly in North and South America) and is found in soil. It enters macrophages or trachea epithelial cells, whereby the cell surface of the fungus is modified. The proliferation occurs in phagolysosomes. This causes pneumonia similar to tuberculosis and affects the renal cortex and nervous system.

15.3.2 *Cryptococcus performans*

Cryptococcus cells are encapsulated by polysaccharide layers (mannose) like bacteria. The negative charge contributed by N-acetyl-neuramic acid and sialic acid prevents phagocytosis of the infecting agent. Virulence is caused by secreted phospholipase B, and possibly by melanine produced by the fungus, which protects against heat and cold. Also calcineurin is required for virulence. The synthesis of melanin and capsula are regulated by a transduction pathway involving a G-protein (GPA1) and cAMP. By knocking out the GPA1 gene, *Cryptococcus* remains avirulent.

By inhalation, *Cryptococcus* cause a chronic, subacute to acute pulmonary, systemic or meningitic disease. Infection suppresses the immune response and gives a poor inflammatory response. The mannoproteins of *C. performans* induce a proliferative response in human peripheral blood mononuclear cells thereby enhancing HIV replication.

15.3.3 *Blastomyces dermatitidis*

B. dermatitidis is the causative agent of the 'Chicago disease'. Blastomycosis may be a benign infection or a chronic granulomatous mycosis. Primary infection occurs in the lungs, causes an influenza-like pneumonia, later affecting bones and skin. The virulence is probably caused by a 120 kDa antigen, adhesion WI-1, which is homologous to bacterial adhesins from *Yersinia*. The adhesion binds CD11b/CD18 (=CR3) and CD14 on host cells, thereby interacting with macrophages.

15.3.4 *Coccidioides*

Coccidioides immitis produces mycoses ('Valley fever') that can become acute, chronic, severe or fatal and is manifest in lung, bone and joints, or may disseminate to meningitis.

Paracoccidioides brasiliensis causes a granulomatous disease that originates as a pulmonary infection. Dissemination occurs resulting in ulcerative granulomatoma in the nasal and buccal, occasionally in the gastrointestinal mucosa or lymph nodes.

References

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