Medical Mycology
When Fungi Go Bad

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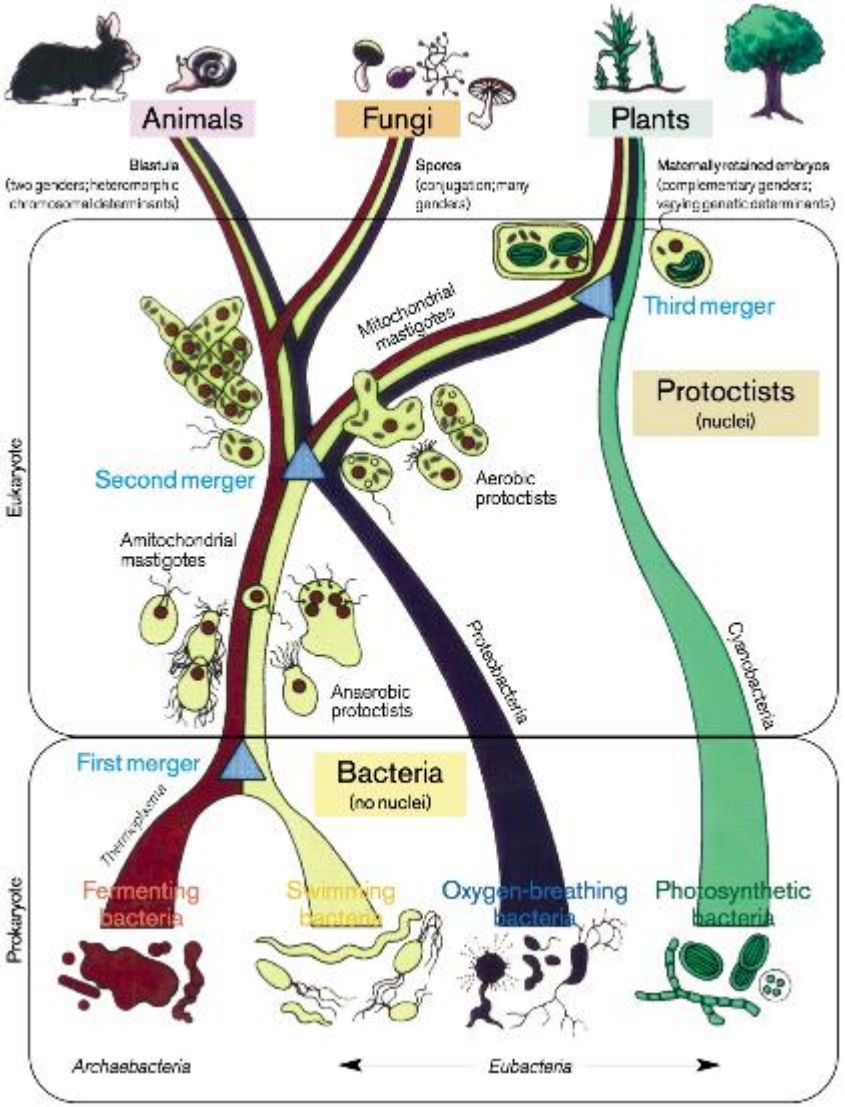
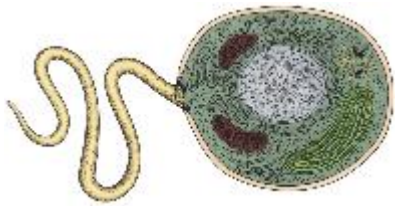
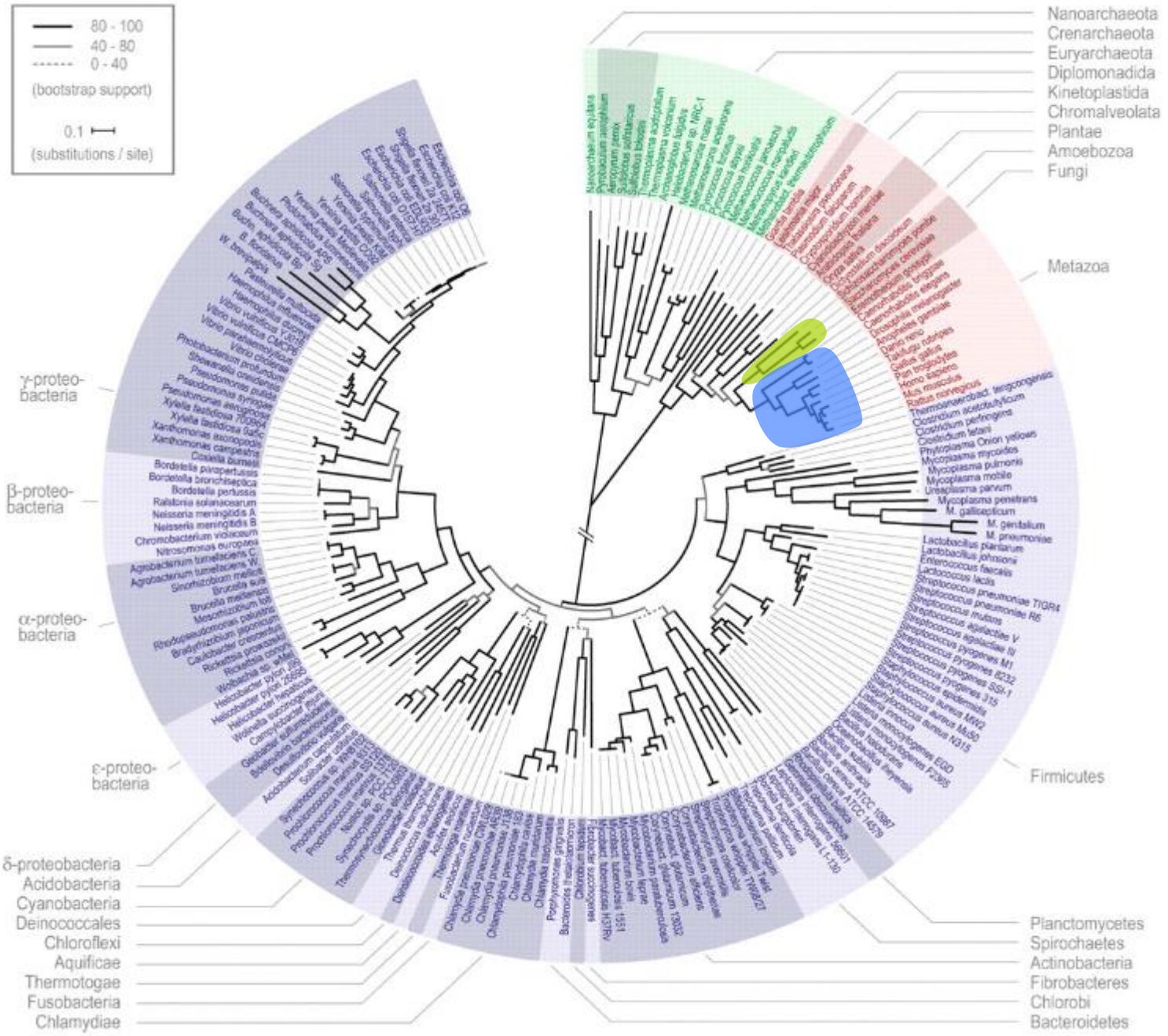
Fungi are Closely Related to Animals



- A broad group of eukaryotes, the opisthokonts include the animal and fungal kingdoms
- Cells that propel themselves with a single posterior flagellum
- Until proposed by Cavalier-Smith in 1987, fungi were believed to be more closely related to plants

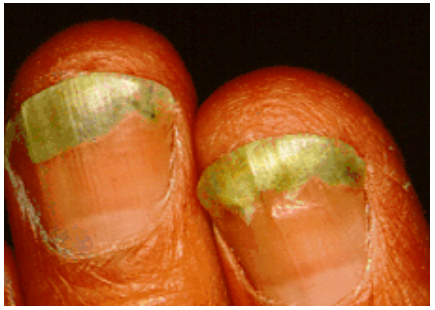


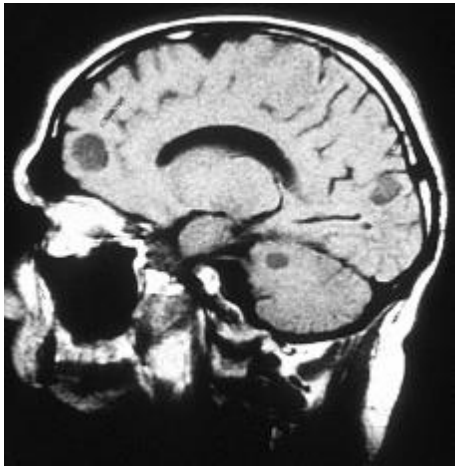
Margulis, 2004



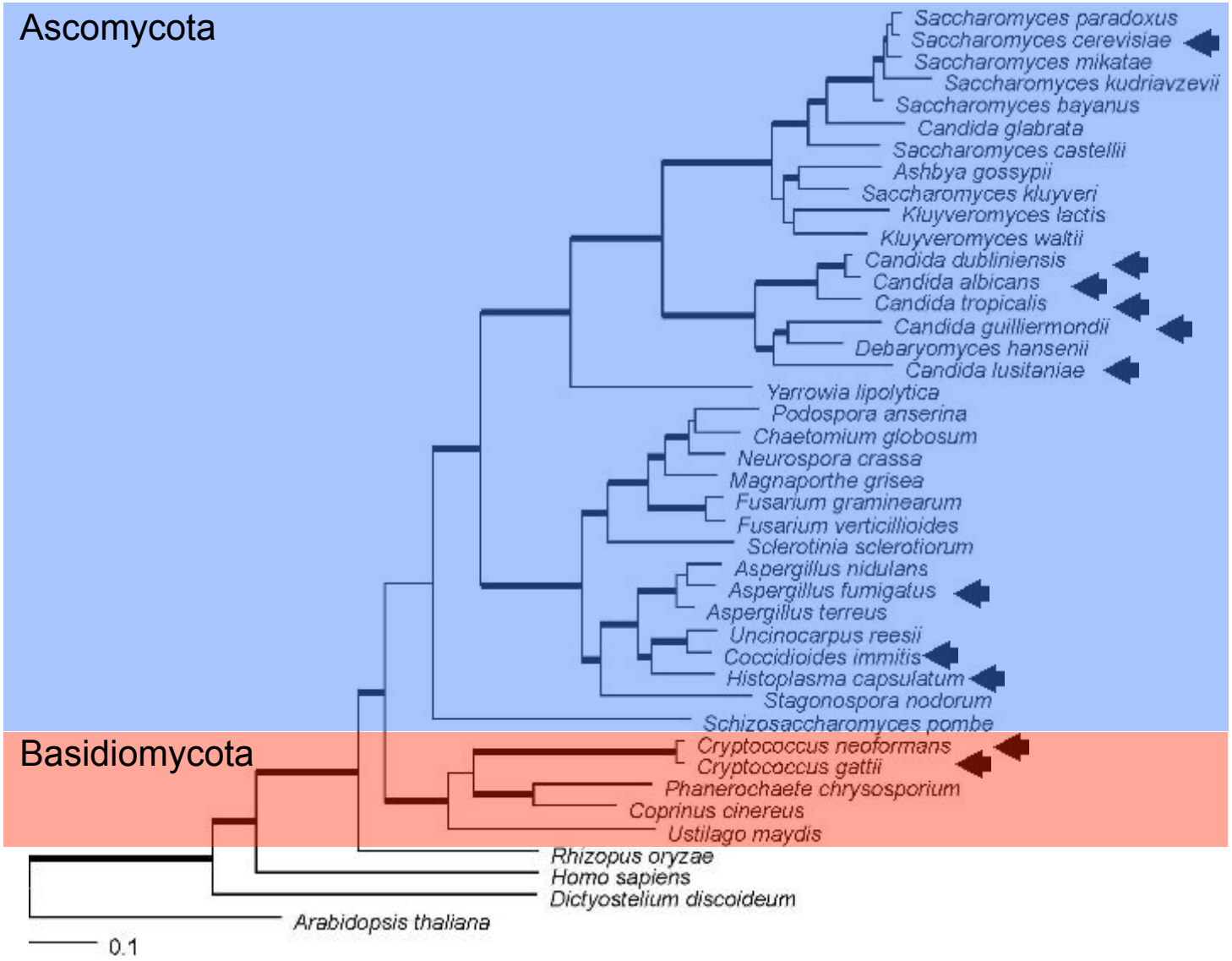
Ciccerelli *et al*. 2006

Fungi as Pathogens of Humans





Phylogeny of Common Fungal Species



Types of Fungal Infections

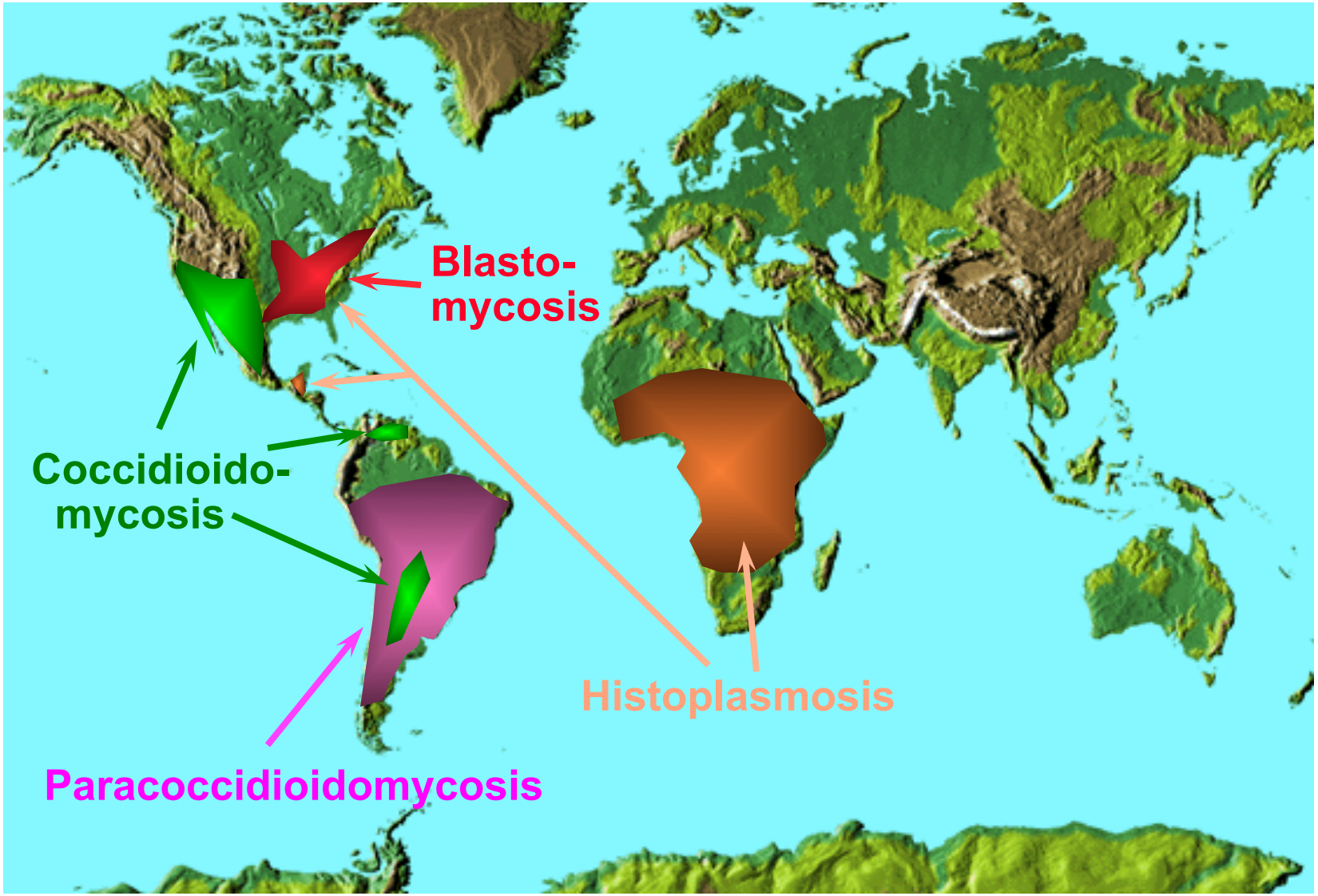
Superficial mycoses:

- Tinea/athlete's foot, ringworm (*Malassezia furfur, Trichophyton sp., Microsporum sp.)*
- Oral thrush/vaginitis (*Candida sp.*)

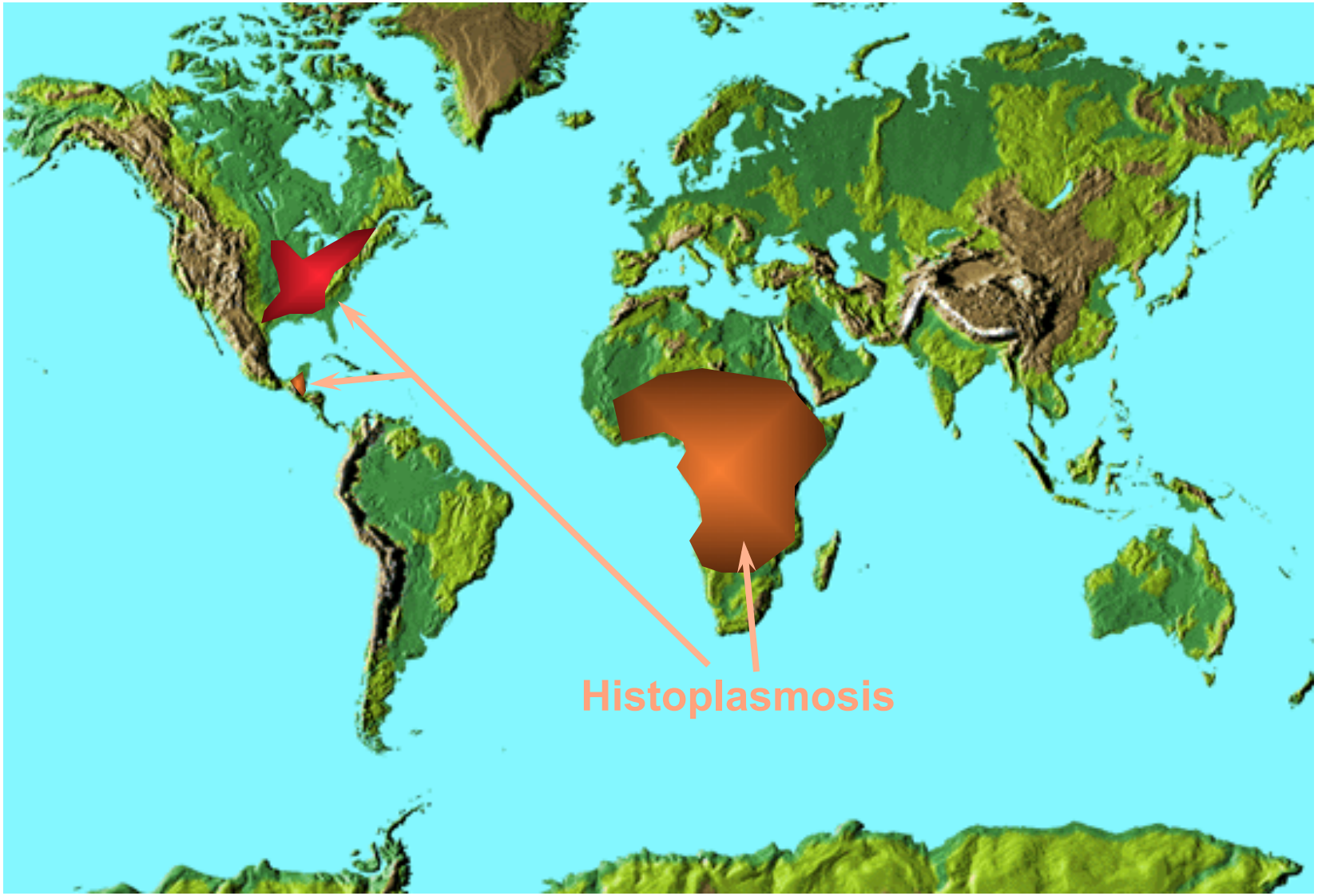
Systemic mycoses:

- Histoplasmosis (*Histoplasma capsulatum*)
- Coccidiomycosis (*Coccidioides immitis*)
- Paracoccidiomycosis (*Paracoccidiodes brasiliensis*)
- Blastomycosis (*Blastomyces dermatitidis*)
- Candidiasis (*Candida sp.*)
- Aspergillosis (*Aspergillus sp.*)
- Cryptococcosis (*Cryptococcus neoformans* & *Cryptococcus gattii*)

Endemic Zones for the Dimorphic Fungal Pathogens (Endemic Mycoses)



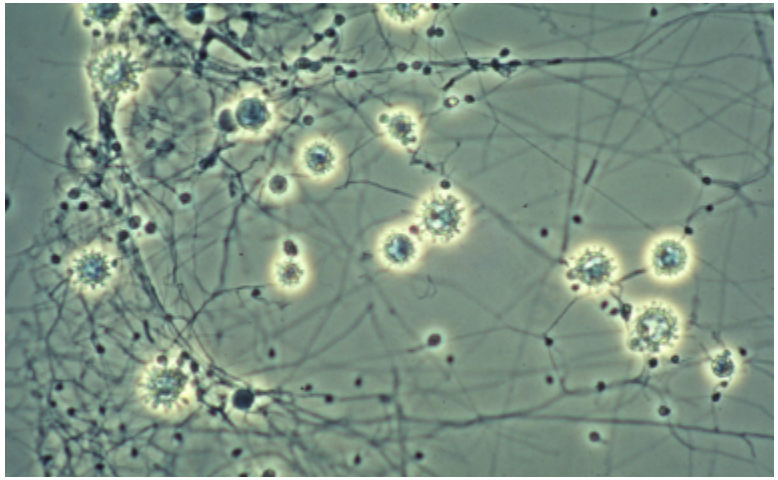
Endemic Zones for the Dimorphic Fungal Pathogens (Endemic Mycoses)

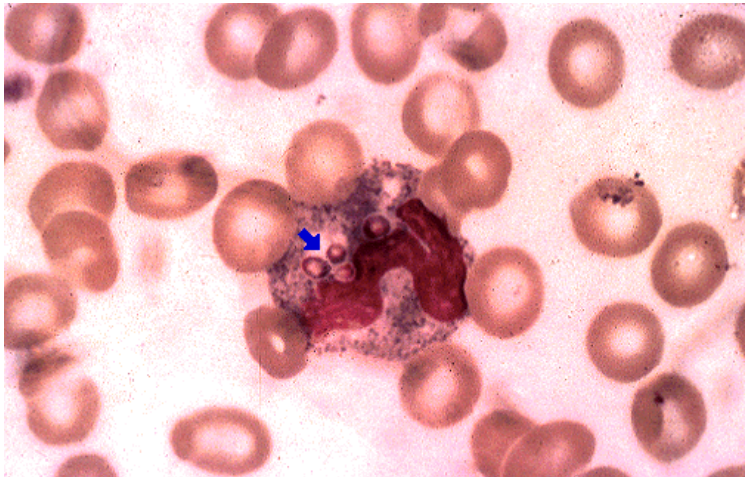


Histoplasmosis

Causative species: *Histoplasma capsulatum*

- A thermally dimorphic haploid fungus that adopts a yeast form in tissues, it is an intracellular parasite that survives within phagolysosomes by modulating pH
- Several varieties (species?) found around the world
- Affinity for high nitrogen content earth
- Infection (common) confers immunity; disease can be reactivated

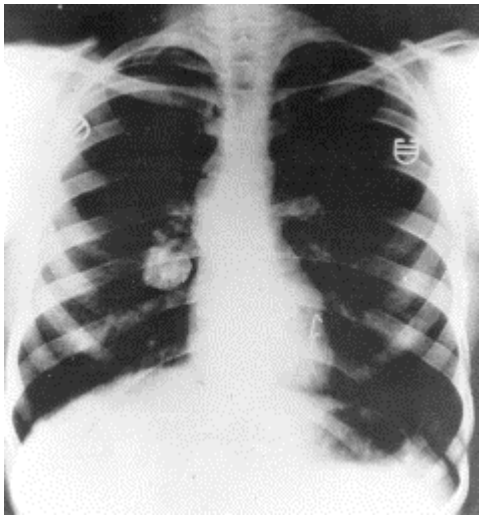




Histoplasmosis

Types of disease:

- Mild (flu-like) respiratory infection
- Serious pulmonary infection (similar to TB)
- Disseminated (often fatal) infection, can include skin



Route of infection:

- Inhalation of conidia

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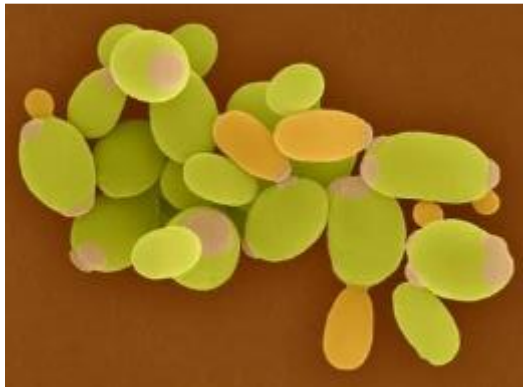
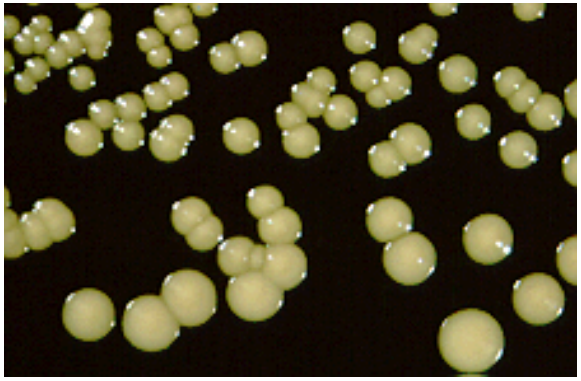
- Aspergillosis (*Aspergillus sp.*)
- Cryptococcosis (*Cryptococcus neoformans* & *Cryptococcus gattii*)

Candidiasis

Causative species: Most commonly *Candida albicans*

- A **diploid** yeast
- Can grow in a yeast, pseudohyphal or true hyphal form in response to appropriate environmental stimuli
- A commensal yeast that inhabits mucosal surfaces
- Sometimes caused by *C. tropicalis, C. glabrata, C. parapsilosis, C. dubliniensis, C. lusitaniae* or *C. krusei*

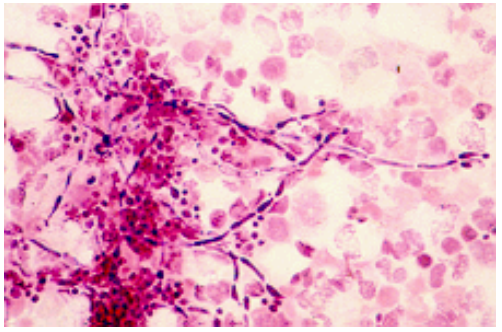




Candidiasis

Types of disease:

- Oral thrush
- Vaginitis
- Many other body parts:
  - nail infections
  - skin infections
  - oesophageal infections
  - urinary tract infections
- Disseminated infection in seriously immunocompromised patients



Aspergillosis

Causative species: Most commonly *Aspergillus fumigatus*

- A haploid mould
- Normally a saprophytic lifestyle, recycling carbon & nitrogen
- 1-100 conidia /m3; continuously inhaled but eliminated easily
- Infections also caused by *A. terreus, A. niger, A. lentulus*

Aspergillosis

Types of disease:

- Many body parts:
  - nail infections
  - sinus infections
  - ear infections
  - eye infections
- Disseminated infection in seriously immunocompromised patients:
  - Angioinvasive pulmonary aspergillosis
  - Cerebral aspergillosis

Cryptococcosis

Causative species: *C. neoformans, C. gattii*

- Haploid basidiomycete yeasts that preferentially disseminate to the central nervous system
- *C. neoformans* found around the world, *C. gattii* more common in sub-tropics (Vancouver Island)
- Disease can be reactivated, relapse common

Types of disease:

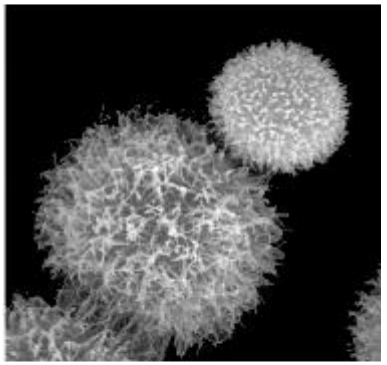
- Cutaneous infection
- Respiratory infection (pneumonia)
- Meningoencephalitis (fatal if untreated, ~20% mortality in Australia)

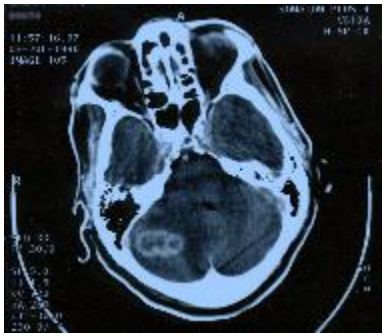
Route of infection:

- Inhalation of sexually produced basidiospores or dessicated yeast

Cryptococcus neoformans

- Haploid basidiomycete yeast
- Worldwide distribution
- Most common life-threatening fungal infection in AIDS patients
- Causes systemic mycoses, commonly meningoencephalitis
- Up to a quarter of a million deaths per year
- Fatal if untreated
- Relapse common

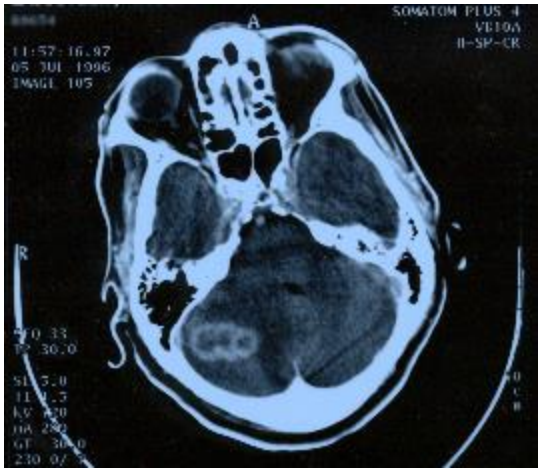


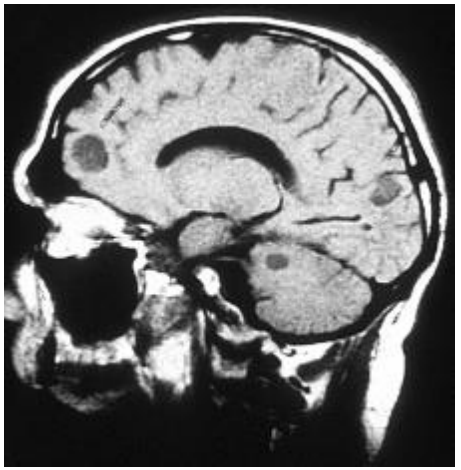


Cryptococcus - Clinical Manifestations

Cutaneous infection

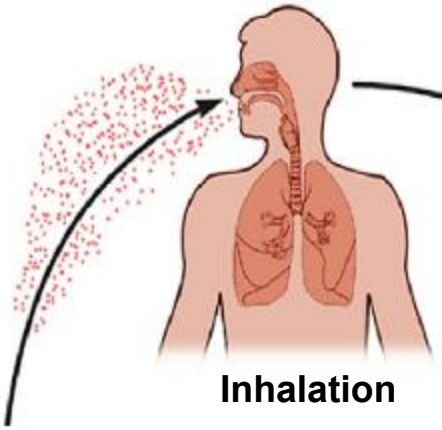
Pneumonia



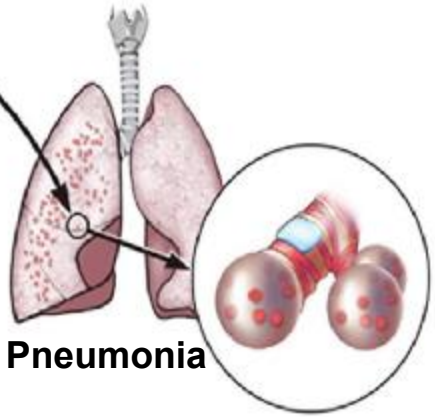


Meningoencephalitis

Route of Infection

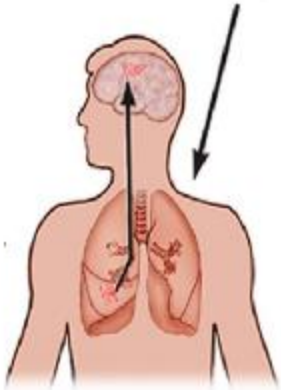


Inhalation

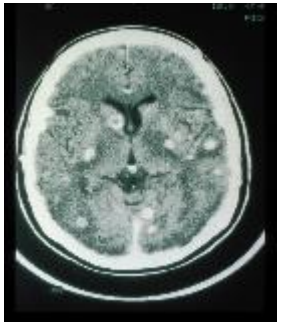


Pneumonia

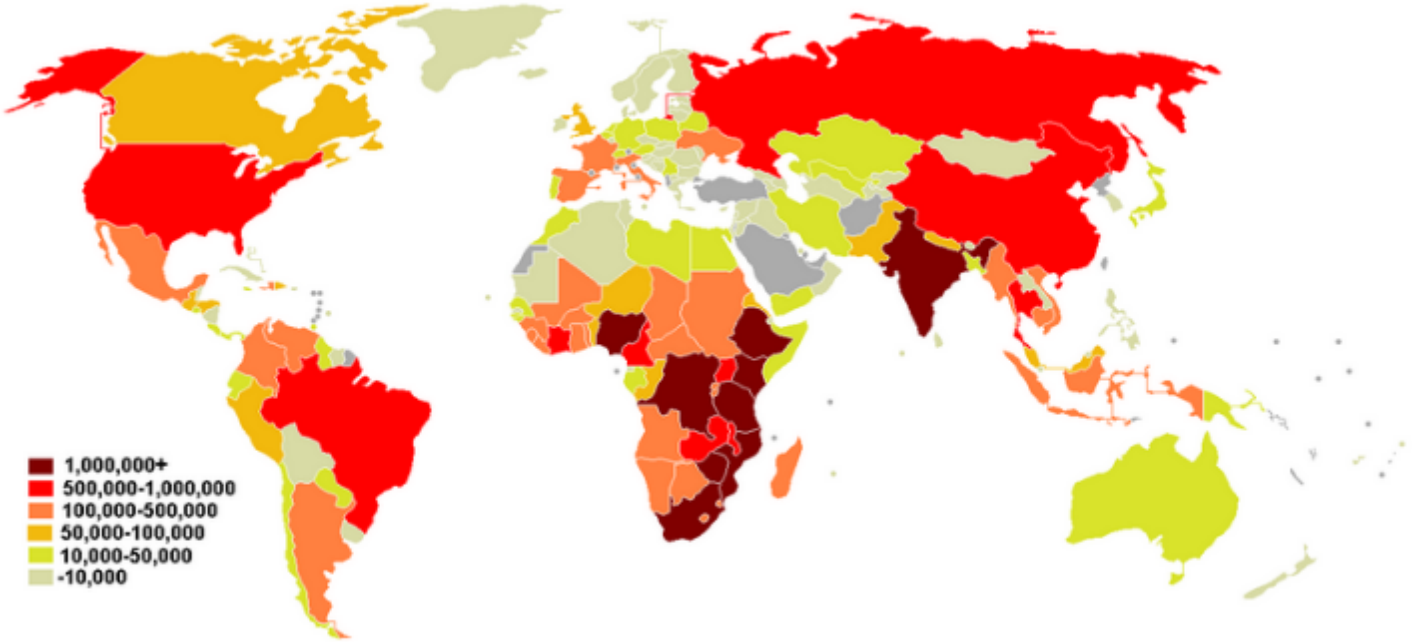
Nitrogen-rich pigeon guano



Meningoencephalitis



The AIDS Pandemic



- WHO estimates that there are currently approximately 33 – 46 million people infected with HIV
- 21 – 27 million of these are in Sub-Saharan Africa
- ~40% will become infected with *C. neoformans*

Treatment of Cryptococcal Meningoencephalitis

Dependent on site of infection and patient immune status.
For infected AIDS patients suffering meningoencephalitis:

Induction therapy

- Treatment when the patient is first diagnosed
- Lasts ~6-10 weeks
- Combination therapy of Flucytosine and Amphotericin B

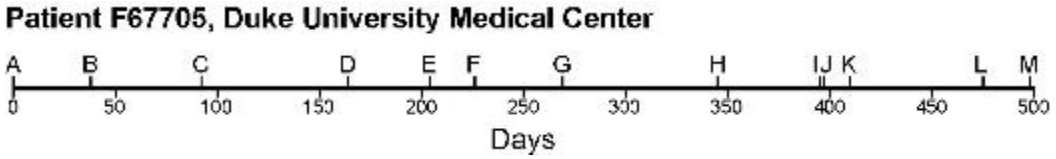
Maintenance therapy

- Treatment following induction
- Lifelong for AIDS patients*
- Fluconazole

Treatment Complications

Treatment of patients is complicated by:

- A delay with the initial diagnosis
- Elevated intracranial pressure and associated manifestations i.e. papilloedema, hearing loss, loss of visual acuity, severe headache and abnormal mental activity
- The presence of enlarging and/or the appearance of new cryptococcomas during therapy
- Prolonged treatment times
- Relapse (sometimes due to noncompliance)

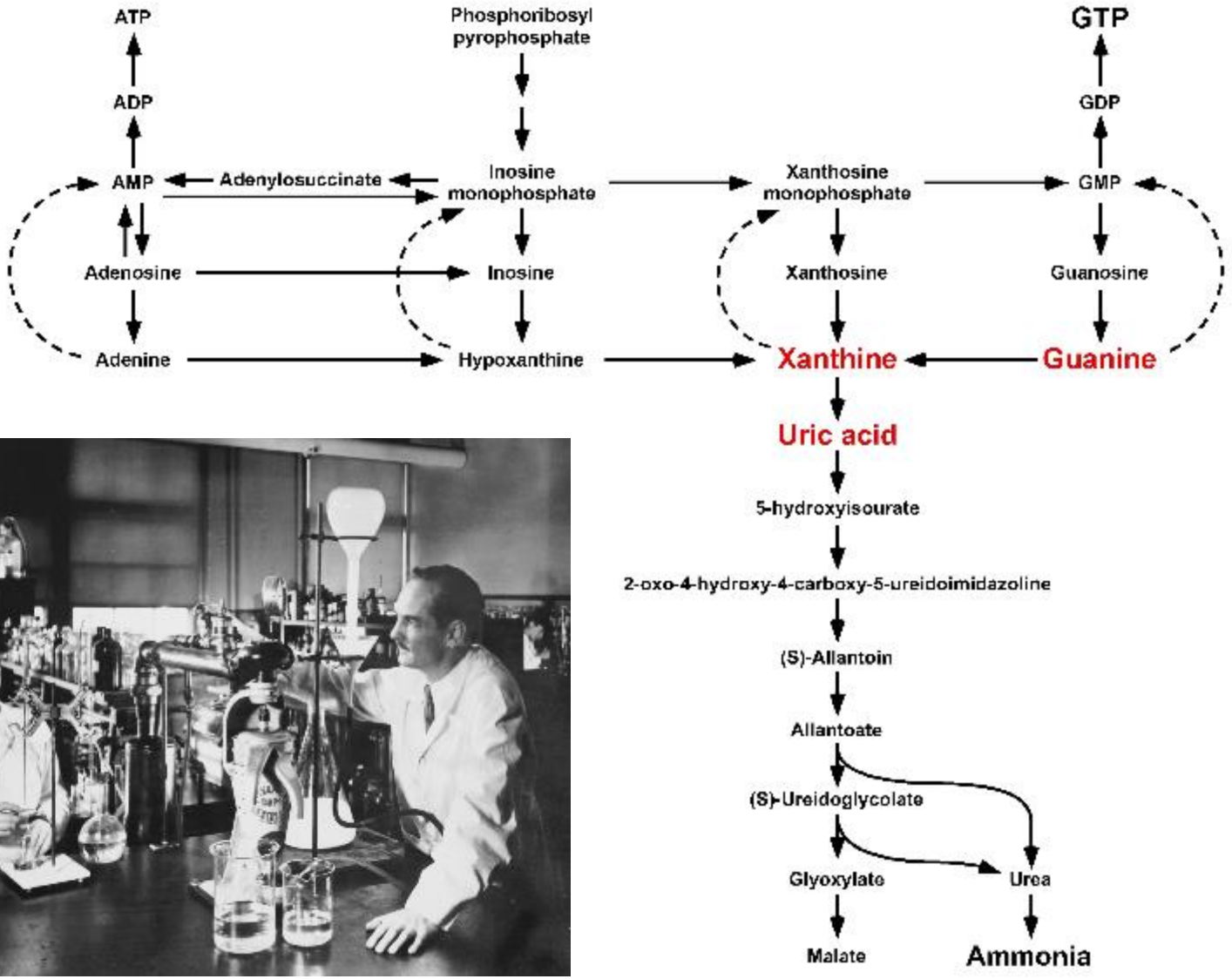


Nitrogen Metabolism of *C. neoformans*

- Bird guano is extremely purine rich, the human host is not (500x adenine, 2000x guanine)
- The virulence factor urease is the final step in the degradation of purines as a nitrogen source
- Production of capsule, melanin and infectious basidiospores are regulated in response to nitrogen availability

Is purine metabolism a potential antifungal target?

Purine Metabolsim as a Platform for Rational Drug Design



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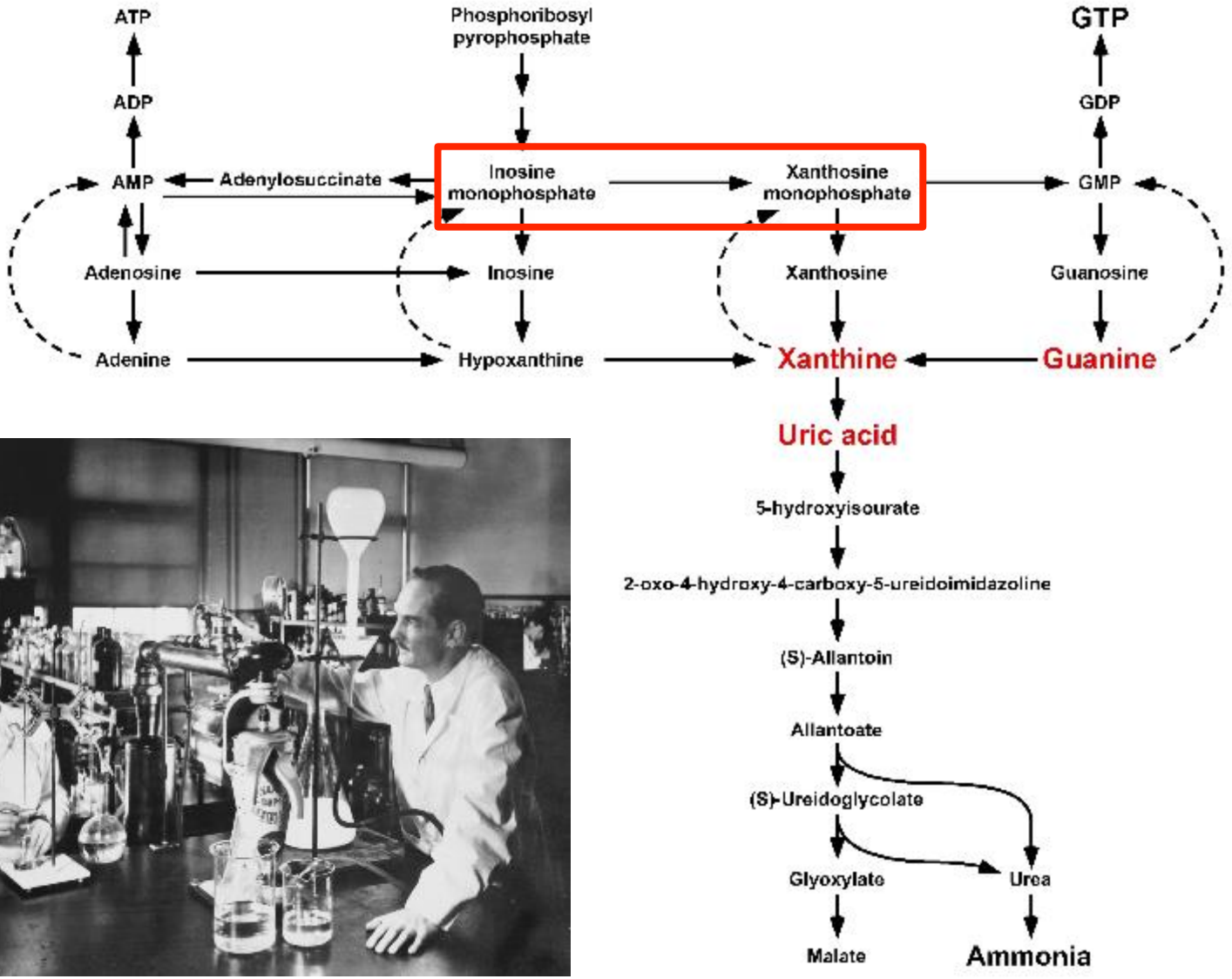
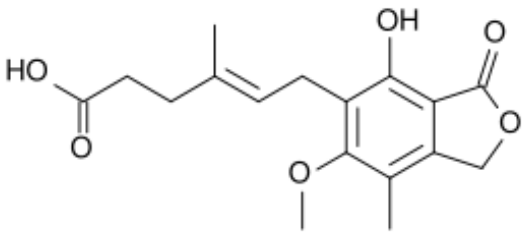
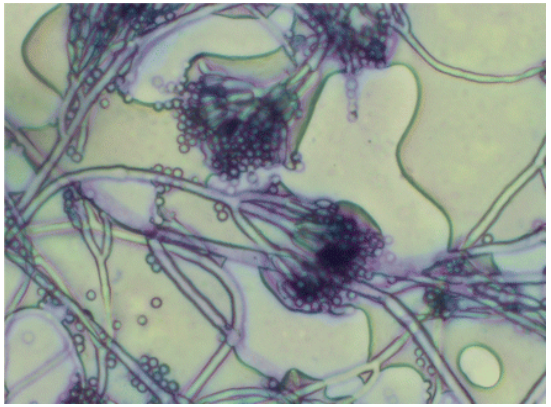


Mycophenolic Acid is an Inhibitor of *De Novo* GTP Biosynthesis

- Secondary metabolite produced by *Penicillium brevicompactum* first identified in 1893
- Activity ranging from bacteria to humans
- Immunosuppressant in transplant patients
- Uncompetitive inhibitor of IMP dehydrogenase, the first committed step in *de novo* GTP biosynthesis

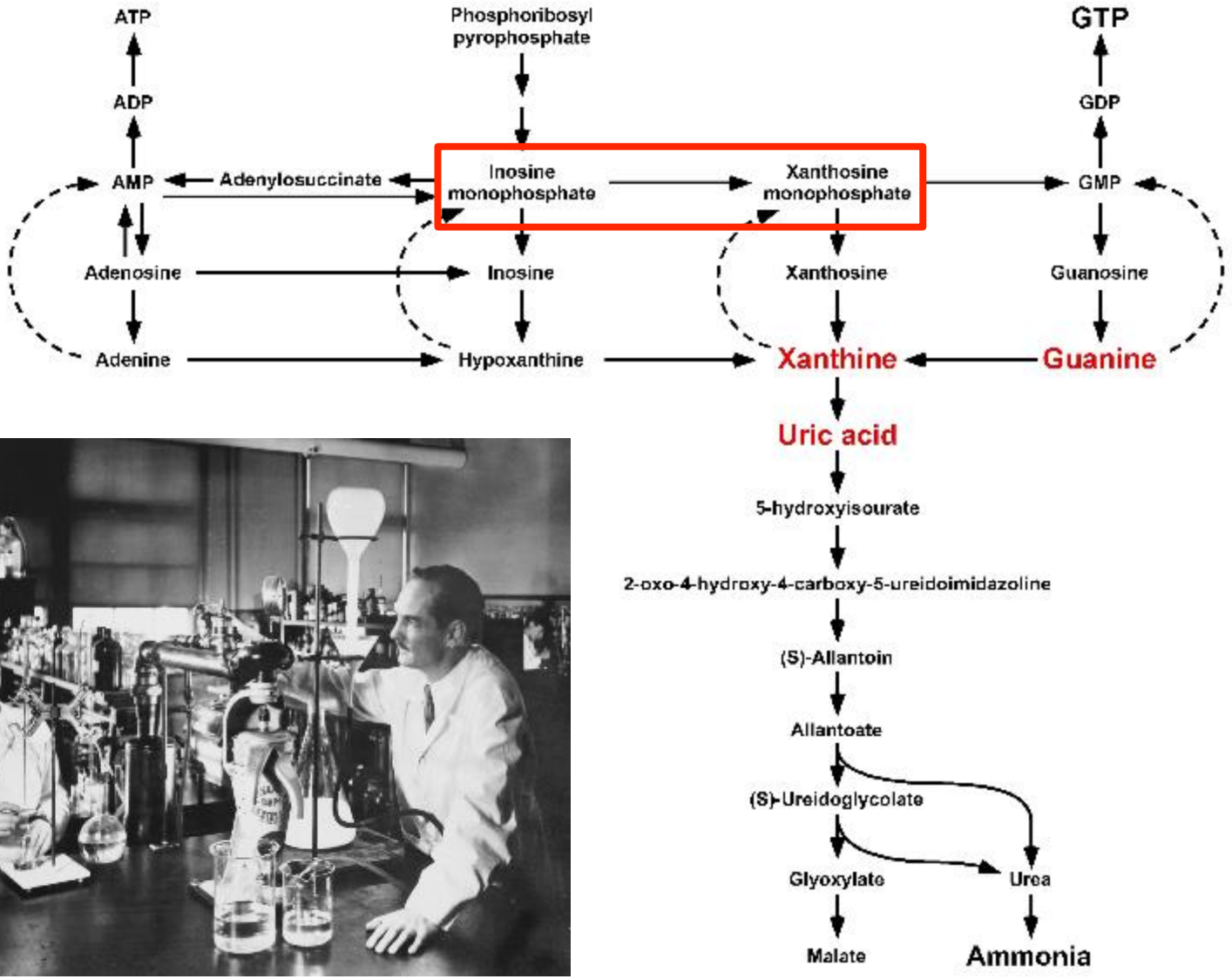


Mycophenolic Acid



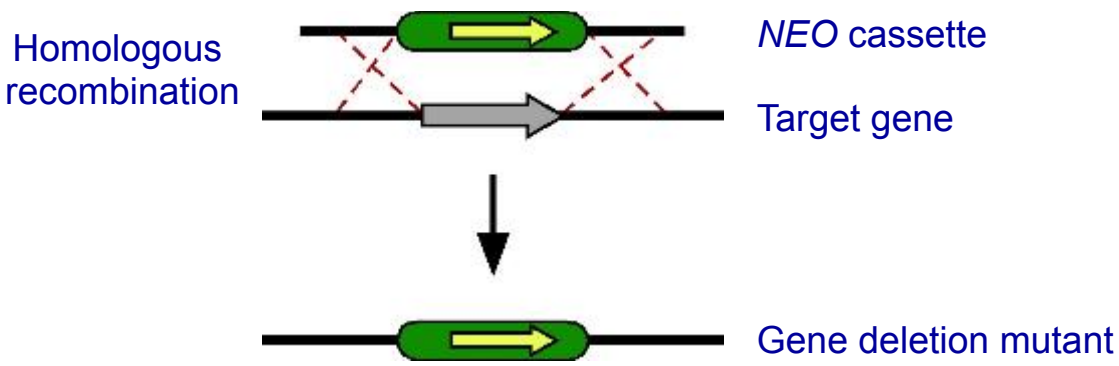
Penicillium brevicompactum

Purine Metabolsim as a Platform for Rational Drug Design

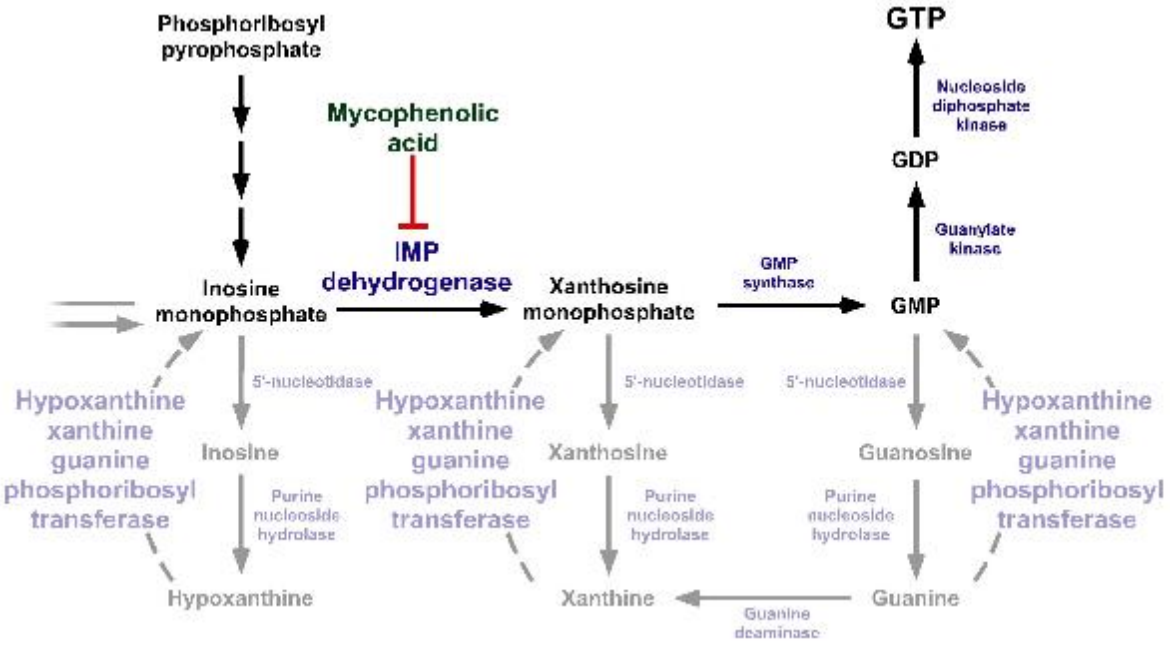


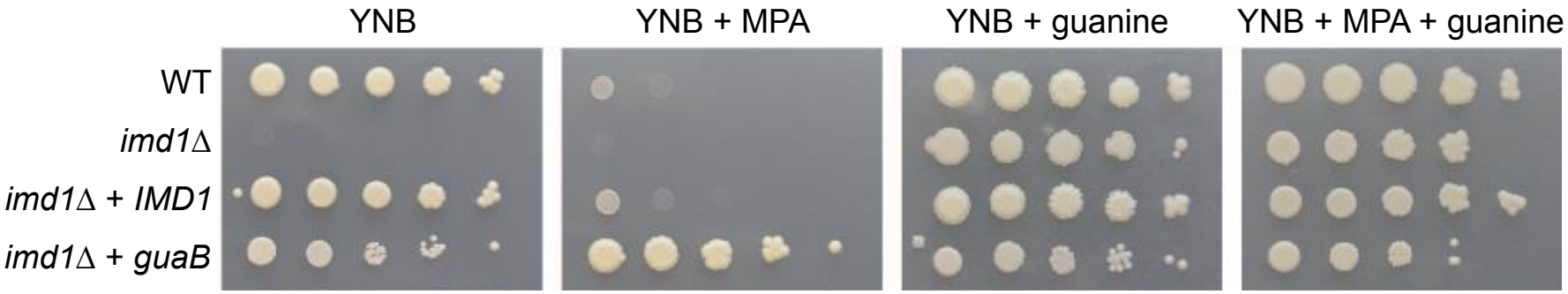
Validating the Role of Genes in Infection

- 20 Mb genome, ~6,000 genes
- Potential virulence genes can be identified using a candidate approach, forward genetics, transcriptome data
- Create gene deletion mutants via gene gun-mediated biolistic transformation
- Infect mice via intranasal inoculation

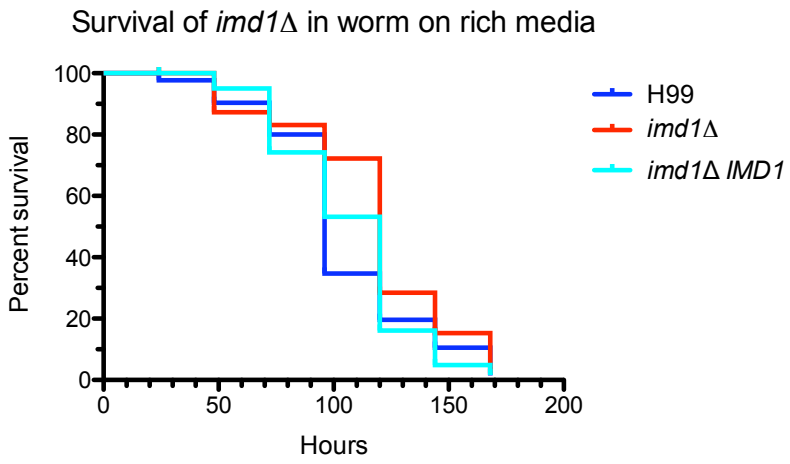


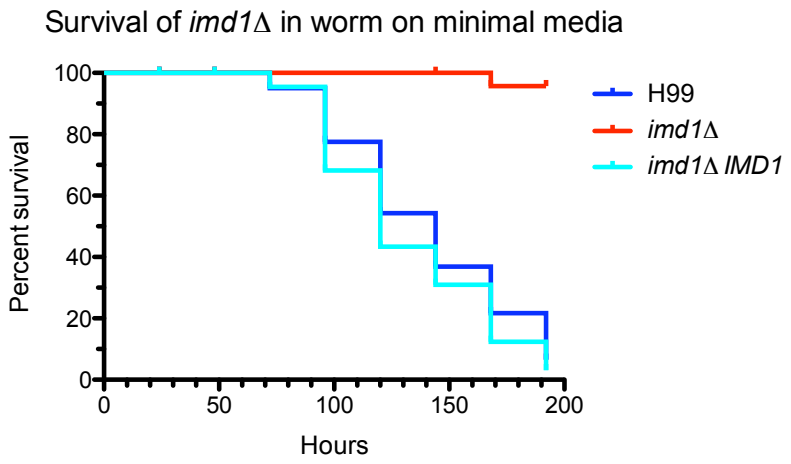
IMD1-encoded IMP Dehydrogenase is Essential for *De Novo* GTP Biosynthesis

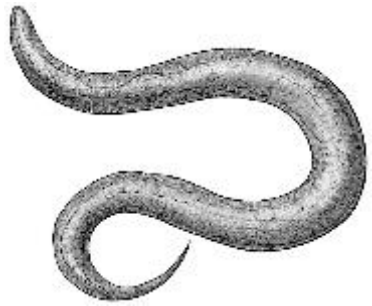




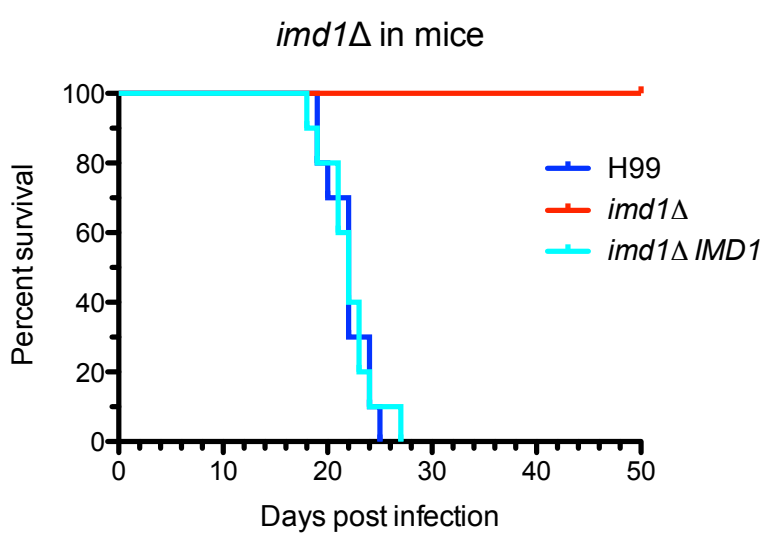
De Novo GTP Biosynthesis is Essential During Purine-Limited Worm Infection

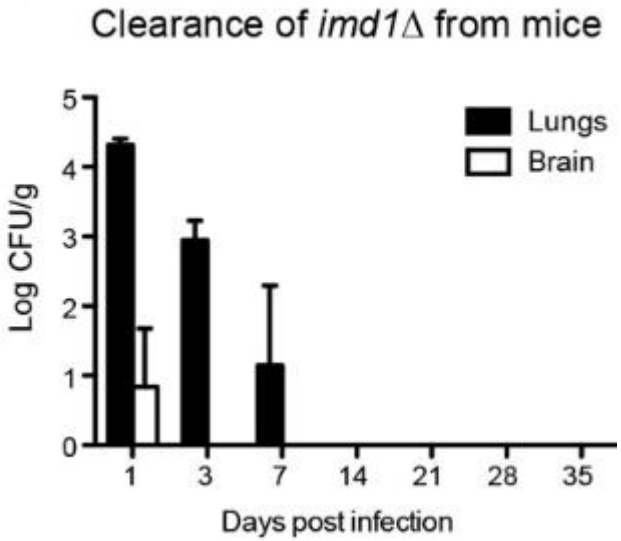






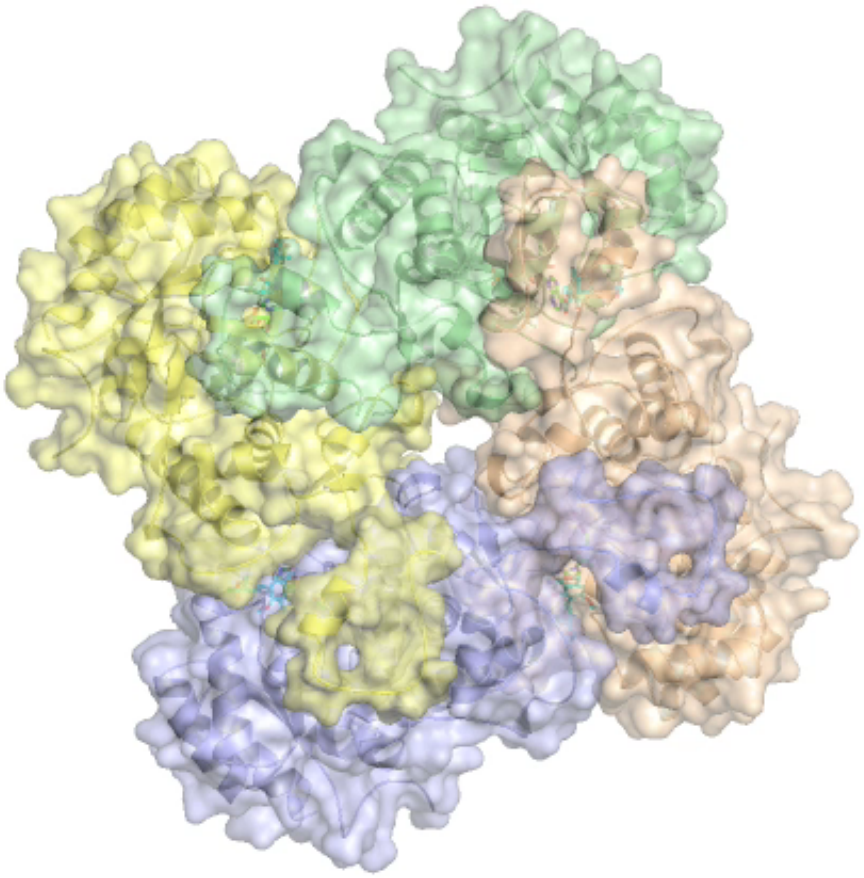
De Novo GTP Biosynthesis is Essential During Infection of Mice





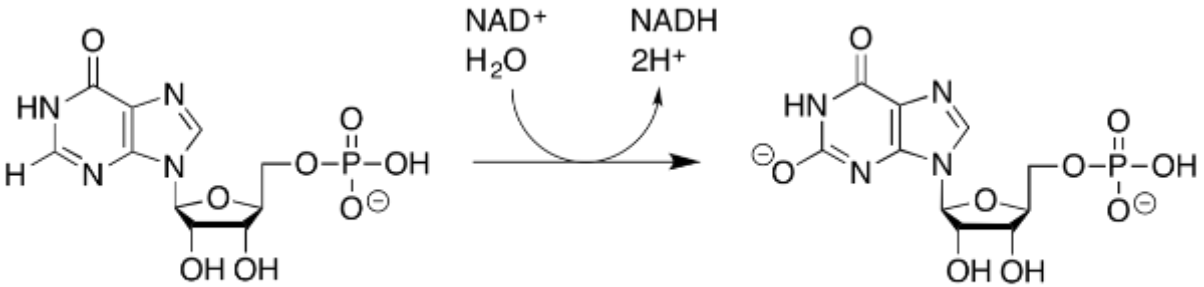
- IMP dehydrogenase mutants are avirulent in a murine intranasal model of cryptococcosis
- Is cryptococcosis treatable with a drug that targets IMP dehydrogenase?

Cryptococcus IMP Dehydrogenase Structure (with IMP & MPA bound)



- 2.2 Å resolution
- Very stable tetramer due to many intramolecular contacts
- 53% identity to human IMPDH
- Active site exists at interface of two subunits, producing four active sites per tetramer
- Differences from animal structure in active site

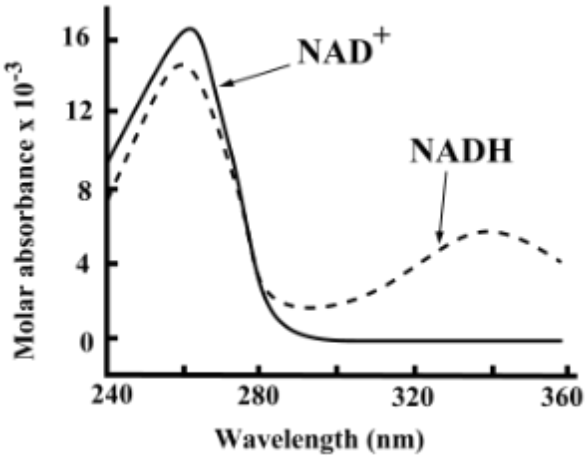
Assaying IMP Dehydrogenase Activity



IMP XMP

IC_{50} determination assay

- Monitor rate of conversion of NAD^+ to NADH; NADH absorbs light at 340 nm
- Enzyme assay transferred from cuvette to 384 well plate format for high throughput screening



UV absorption spectra of NAD^+ and NADH

High-Throughput Screening for Inhibitors of IMP Dehydrogenase

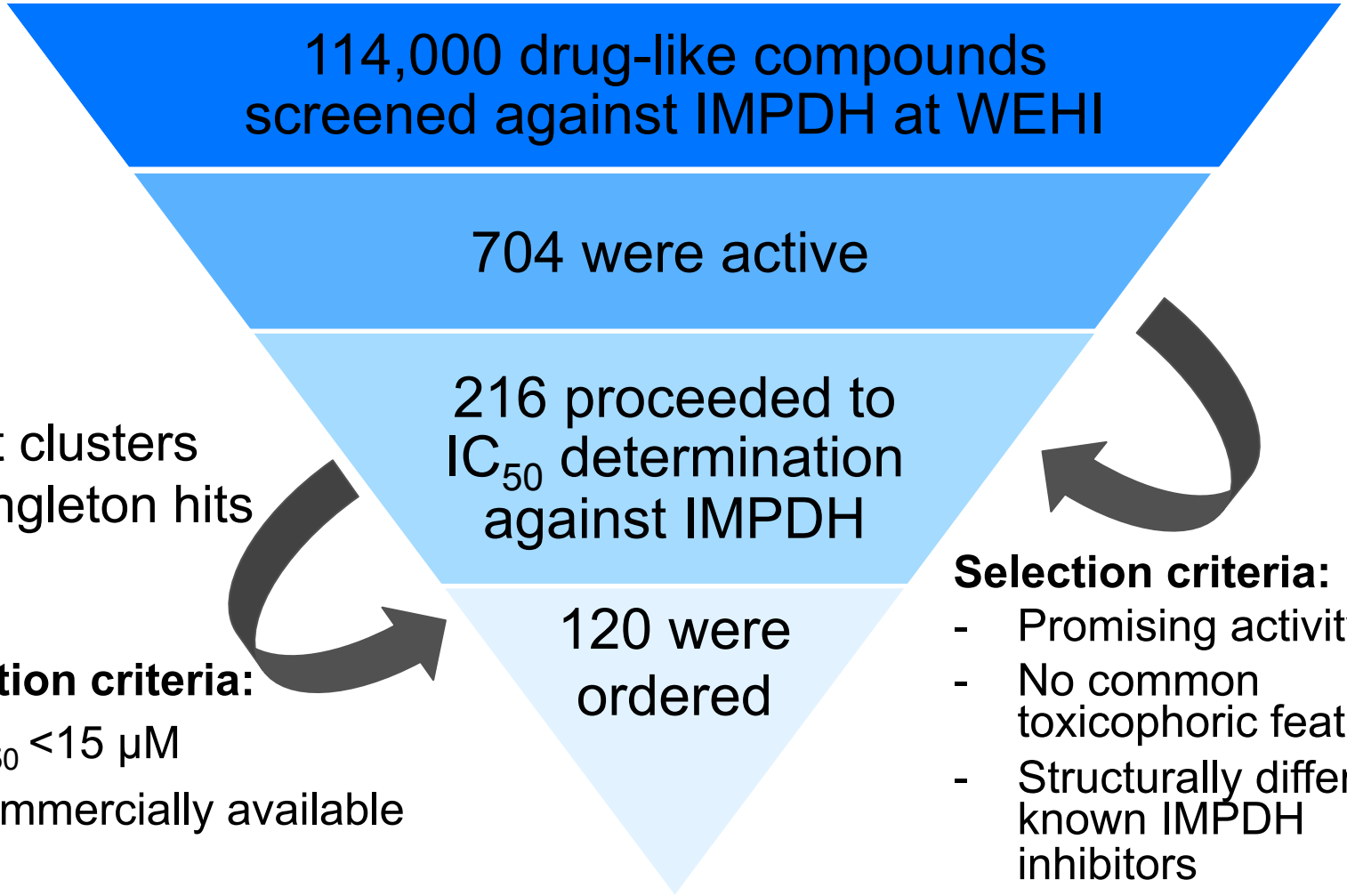
THERAPEUTIC INNOVATION AUSTRALIA QUEENSLAND

FIAT LUX Walter+Eliza Hall Institute of Medical Research

- Enzyme Inhibition Assay screen of 114,000 compounds
- 704 confirmed hits from primary screening at 10 µM
- 216 progressed to 11 point titration

- SPR screen of 1,600 fragments
- 148 potential binders identified

High-Throughput Screening for Inhibitors of IMP Dehydrogenase



26 hit clusters
24 singleton hits

Selection criteria:

- IC_{50} <15 μM
- Commercially available

Selection criteria:

- Promising activity
- No common toxicophoric features
- Structurally different to known IMPDH inhibitors

16 clusters and 6 singletons with antifungal activity

A Pathway to the Development of New Therapeutic Agents

- Fungi are closely related to humans, making the identification of effective and safe antifungals difficult
- *De novo* GTP biosynthesis is essential to *C. neoformans* during infection
- After screening 114,000 drug-like molecules and 1,600 fragments we are pursuing novel lead compounds in the development of fungal-specific IMP dehydrogenase inhibitors
- Moving beyond IMP dehydrogenase, we are making excellent progress investigating other GTP and ATP *de novo* biosynthesis enzymes as alternate drug targets

Ross Blundell
Jessica Chitty
Russel Lee
Carl Morrow
Tayla Tatzenko
Kirsten Blake

Bostjan Kobe
Simon Williams
Daniel Ericsson
Anna Stamp
Eugene Valkov

Centre for Drug Discovery and Design

Matt Cooper
Mark Butler
Avril Robertson
Emily Furlong
Alberto Boucas Da Silva
Johannes Zuegg

Walter+Eliza Hall Institute of Medical Research

Kurt Lackovic
Kate Jarman
Kym Lowes
Hong Yang

Ulrike Kappler
Justine Hill
Julie Djordjevic

AID Australian Infectious Diseases research centre

The University Of Queensland AUSTRALIA

Australian Government
National Health and Medical Research Council
NHMRC

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