Otago Spotlight Series

Infectious Disease Research

The hidden danger of fungal infections

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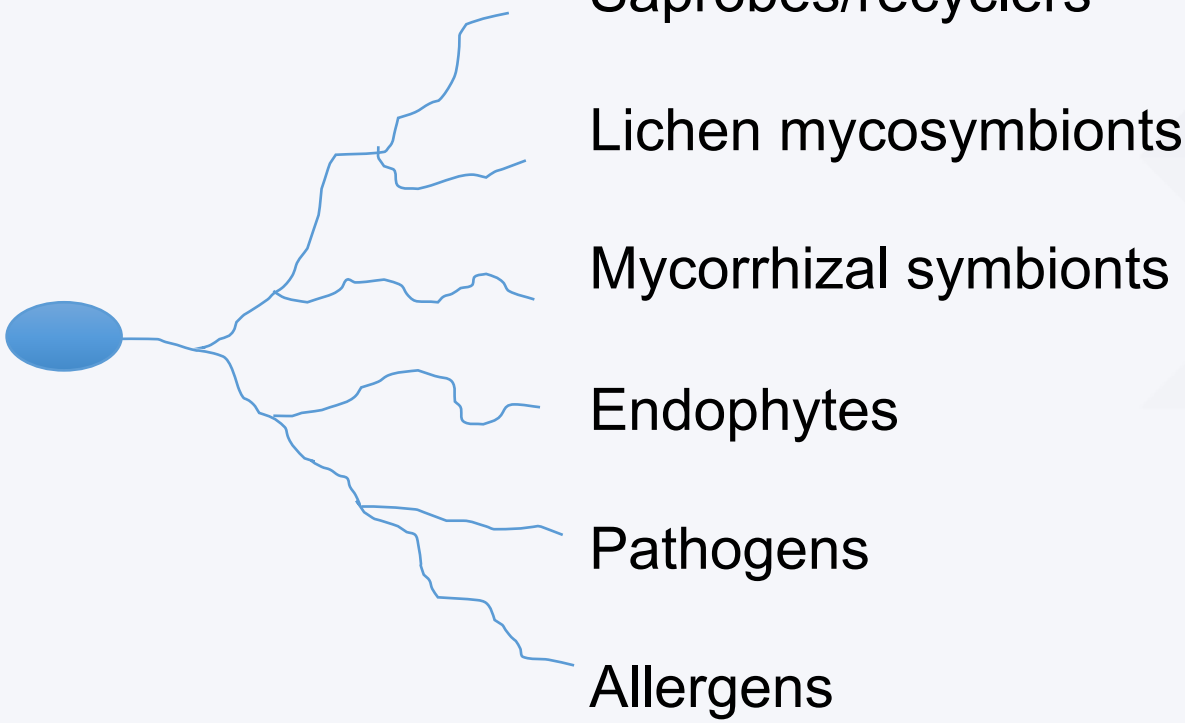
The mycosphere



Saprobes/recyclers

Lichen mycosymbionts

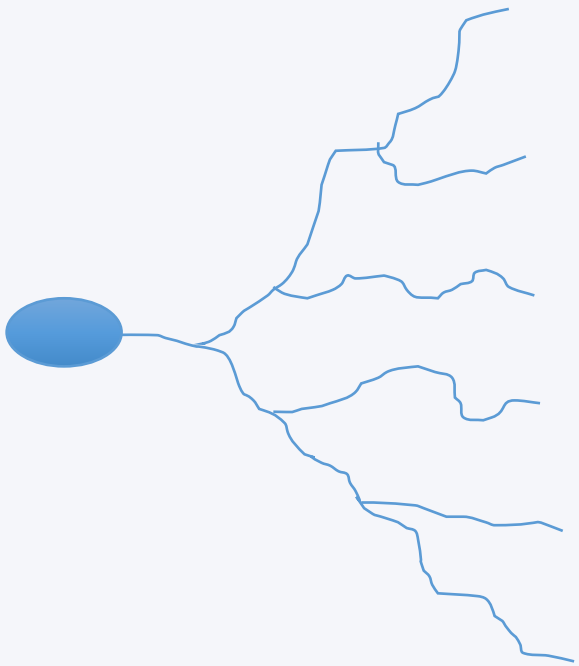
Mycorrhizal symbionts

Endophytes

Pathogens

Allergens

The mycosphere in the anthropocene



Foods

Metabolite production

Fermentation

Enzyme production

Pharmaceutical production

Biocontrol agents

Enablers of fungal infection

Fungi - *"A mutable and treacherous tribe"*

Albrecht von Haller in a letter to Carolus Linnaeus ca 1745

Loss of natural barriers to infection

- Migration into naive ecosystems
- Intensive farming of crops with limited genetic diversity
- "Hidden killers" of humans – especially the immune deficient and immunocompromised (*Candida*, *Aspergillus*, *Cryptococcus* species)
- Poverty - poor living conditions (*Stachybotris*)

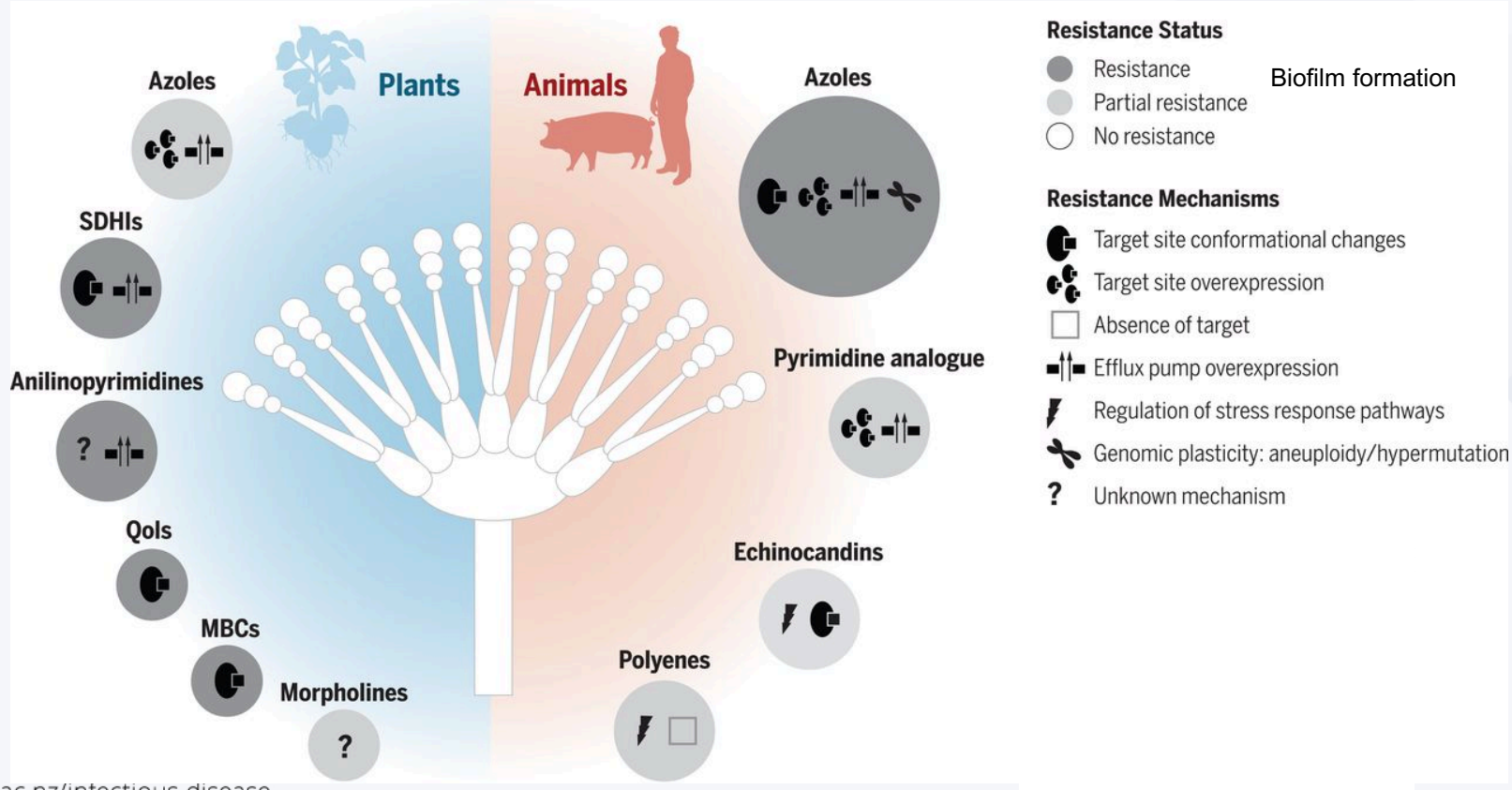
Antifungal exposure selects for antifungal resistance

"Resistance follows chemotherapy as a faithful shadow" Paul Ehrlich

- "Preventative" spraying of crops and growth enhancement
- Extended/recurring antifungal treatments or prophylaxis in humans

Antifungals used against plant and animal infections

Agricultural “fungicide” market ~US$11.2 billion (2014)
Medical antifungal market ~ US$13.1 billion (2016).
General inhibitors: Copper and sulphur - used extensively and successfully against plant pathogens (phytopathogens) for >150 years.



otago.ac.nz/infectious-disease

Matthew C. Fisher et al. Science 2018;360:739-742

Mechanisms of antifungal resistance

- Biofilm formation
- Drug tolerance
- Target-based intrinsic and acquired resistance –
  target amino acid substitutions/mutations and
  target overexpression due to genome plasticity.
- Efflux-based resistance –
  gain-of-function mutation in transcription factors
  causing overexpression of drug efflux pumps
- Conferral of multidrug resistance –
  mutator gene phenotype in *Candida glabrata* and
  Cryptococcus deuterogattii

Medical or agrochemical exposure give azole resistant *A. fumigatus*

- Long term or repeated exposure to azole drugs give rise to resistant Cyp51A lanosterol 14α-demethylase (LDM) mutants of *A. fumigatus*.

- Patients naïve to medical triazoles were found with azole resistant (e.g. to voriconazole) aspergillosis in areas exposed to agrochemical triazoles not used in medicine. Initially detected in the Netherlands, these mutations are found in overexpressed CYP51A LDM. They appear to come from a single genetic background and are now found in all continents.

Fungal infections in NZ
Why we should be concerned

- 2011 - 92,000 serious fungal infections in NZ
- Recurrent candida vaginitis - 61,000
- Candidemia, invasive aspergillosis, chronic pulmonary aspergillosis – ~200 each
- Cryptococcus meningitis ~100
- Severe asthma with with fungal sensitisation ~15,000
- Allergic bronchopulmonary aspergillosis ~17,000 (3.5 % of 500,000 asthma patients)
- ABPA and chronic pulmonary aspergillosis are partially (60%) preventable with azole drugs. Consider use of azole drugs other than voriconazole e.g. posaconazole or isavuconazole
- Fungal keratinitis of the eye (~300) caused by several fungal species including Aspergillus species and the mucormycetes (*Mucor* and *Rhizopus* species), which are innately resistant to fluconazole and voriconazole due to CYP51 substitutions.

Problems on the horizon

- Rapid international spread of *Candida aureus.*

From an ear infection in Japan to major threat worldwide due to multidrug resistance phenotype involving mutations affecting LDM and drug efflux pumps

- Skin organisms spread by hand contact

C. parapsilosis a major pathogen in hospitals and elderly care facilities. Mutations in LDM have been identified.

Can we make better antifungals?

- Problems –

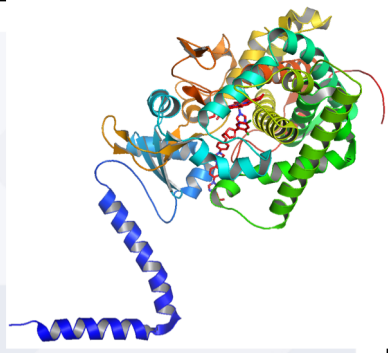
  similarity to hosts

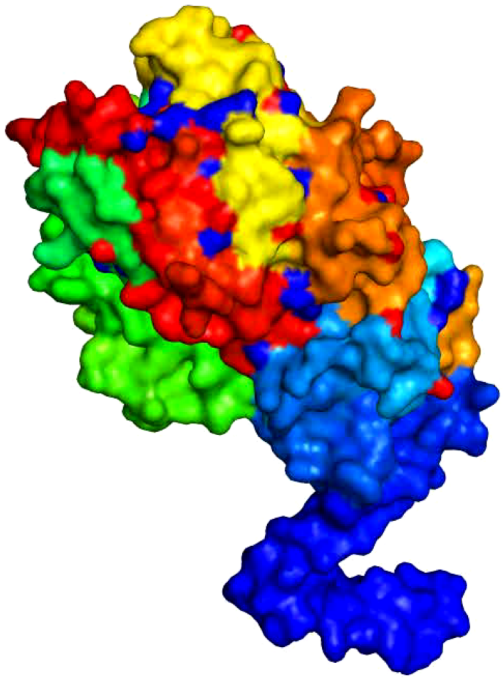
  diversity of fungal pathogens

- Structural biology?

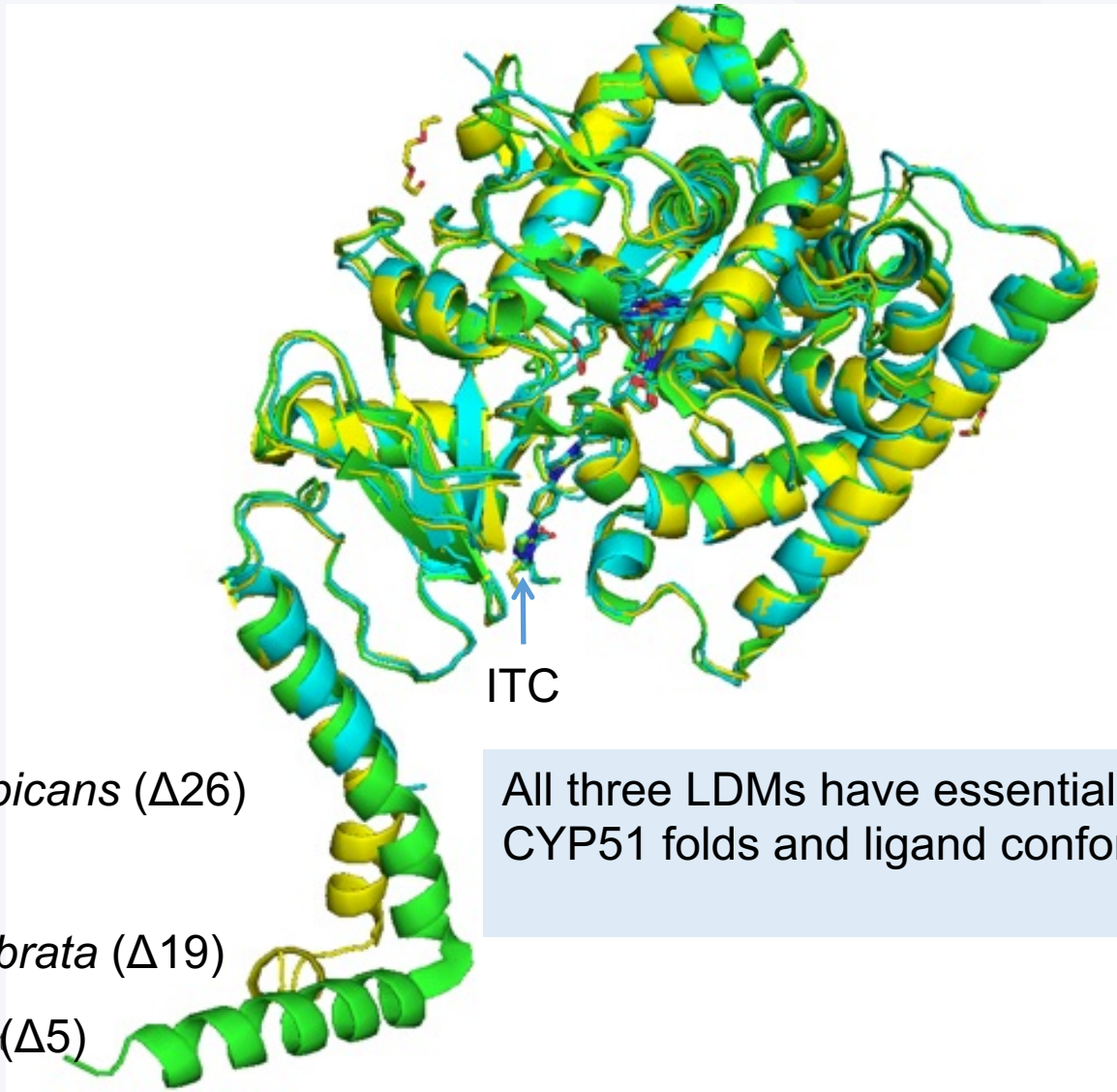
  the main protein targets used in the treatment of fungal infections of humans are membrane proteins

Using structural biology to identify new antifungals





Crystal structures of LDM from *S. cerevisiae*, *C. albicans* and *C. glabrata*



All three LDMs have essentially identical CYP51 folds and ligand conformations.

Dividends from structure-function analysis of lanosterol 14α-demethylase

- *High-resolution structures improve understanding of LDM function*

Visualize key biochemical functions and relationships with the membrane, substrates, products, and inhibitors - including hydrogen bond networks and mutations affecting drug binding

- *High-resolution structures enable targeting of fungal LDMs*

Use structure-informed bioinformatics to identify potential binding sites for broad-spectrum, fungal-specific inhibitors

Use *in silico* screens and homology models of fungal pathogen LDMs

- *Expression of functional LDMs in yeast enables surrogate screens*

Phenotypic screening of compound libraries

Ways to improve therapy?

- Identify antifungals that circumvent resistance mechanisms
- Use mixtures of antifungals with different targets
- Employ different targets for medical and agrochemicals antifungals
- Consider antifungal impacts on the mycosphere and the human mycobiome

Key messages

- Fungi contribute vitally to the biosphere and our well-being.
- Better diagnosis, improved surveillance of fungal infections and their outcomes, and optimal stewardship of the existing antifungals are all needed.
- Fungal pathogens, especially those resistant to antifungal drugs/agrochemicals, provide challenges in developing more effective antifungals, without unintended consequences.
- Molecular understanding of antifungal action is helping discovery of potent next-generation antifungals designed to overcome drug resistance.

Collaboration and funding

Collaborators

- *Molecular Biosciences Laboratory, Otago* – Mikhail Keniya, Alia Sagatova, Matthew Woods, Rajni Wilson, Manya Sabherwal Franziska Huschmann, Danyon Graham, Harith Hassan, Danni Chen, Yasmeen Ruma, Parham Hosseini and Richard Cannon
- *School of Pharmacy, Otago* – Joel Tyndall
- *Stroud Laboratory UCSF* – Robert Stroud, Thomas Tomasiak, Janet Finer-Moore, Joseph O'Connell, Laura Caboni.
- *Goffeau group, UCL, Belgium* – Andre Goffeau, Anabelle Decottignies
- *MicroCombiChem* – Anette Klinger, Edmond Fleischer
- *PHRI Rutgers* – David Perlin
- *MU Innsbruck* – Michaela Lackner

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