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FUNGAL PATHOGENESIS

Divide and conquer

> the intracellular environment of macrophages induces a 'division of labour' in *C. gattii*

The intracellular fungal pathogen *Cryptococcus gattii* infects the lungs and nervous system of otherwise healthy individuals, but the molecular mechanisms that drive disease pathogenesis are poorly understood. Now, Voelz *et al.* show that exposure to reactive oxygen species (ROS) in host macrophages results in a 'division of labour' in *C. gattii* populations, in which a non-replicative subpopulation of *C. gattii* emerges and facilitates the rapid growth of neighbouring fungal cells, thereby promoting the establishment of the infection.

In contrast to the closely related pathogen *Cryptococcus neoformans*, which primarily infects immunocompromised individuals, *C. gattii* can infect otherwise healthy individuals and is responsible for a large ongoing outbreak in North America. Notably, outbreak isolates of *C. gattii* have an increased propensity to form tubular mitochondria — a physiological response that is associated with increased cell viability under stress conditions in other fungal and mammalian cells — and this phenotype is associated with an increased ability to proliferate within macrophages. To determine whether this trait is specific to *C. gattii* outbreak strains, Voelz *et al.* examined 24 *C. gattii* and 14 *C. neoformans* clinical and environmental isolates for their ability to replicate inside macrophages. They found a positive correlation between mitochondrial tubularization and the intracellular replication rate, which was exclusive to the outbreak isolates of *C. gattii*.

Using time-lapse microscopy to track individual cryptococci in which the mitochondria had been tagged with GFP, the authors found that mitochondrial tubularization is induced rapidly following phagocytosis. Importantly, cells with tubular mitochondria were more resistant to host-mediated killing than cells with non-tubular mitochondria, but surprisingly they also replicated more slowly. This suggested that the intracellular environment of macrophages induces a 'division of labour' in *C. gattii*, with some cells developing tubular mitochondria and differentiating into a resistant, non-replicative state, whereas other cells remain susceptible to host-mediated killing but divide more rapidly. As mitochondrial tubularization correlates with an overall increase in the replication rate of the *C. gattii* population, the authors postulated that the non-replicative subpopulation might promote replication of the susceptible subpopulation of cells with non-tubular mitochondria. Consistent with this hypothesis, co-infections using outbreak strains and environmental strains revealed that the outbreak strains increase the intracellular replication of the environmental strains.

But what triggers this 'division of labour' inside macrophages? *In vitro* exposure of outbreak strains to individual phagocyte stresses revealed that a non-lethal dose of H_2O_2 (which mimics the oxidative stress environment inside macrophages) triggers mitochondrial tubularization. Similarly, treatment of infected macrophages with apocynin — an NADPH oxidase inhibitor that reduces the levels of ROS — reduced the frequency of *C. gattii* cells with tubular mitochondria, leading to an overall reduction in the proliferation rate of the cryptococcal population.

This study provides a new model for *C. gattii* pathogenesis, in which exposure of intracellular cryptococci to ROS triggers the formation of a quiescent subpopulation that are resistant to host-mediated killing, which promotes replication of the remaining, highly proliferative population. Future studies are needed to understand how mitochondrial tubularization mediates this mechanism of fungal virulence.

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ORIGINAL RESEARCH PAPER Voelz, K. *et al.* 'Division of labour' in response to host oxidative burst drives a fatal *Cryptococcus gattii* outbreak. *Nature Commun.* http://dx.doi.org/10.1038/ncomms6194 (2014)