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Prison break – How fungi escape from a hostile environment inside immune cells

Candida albicans is a commensal yeast of humans and colonizes ≈70% of the human population. It is common for *C. albicans* to cause self-limiting infections, but severe infections can be life-threatening. Invasive candidiasis occurs in patients with a compromised immune system, such as anti-cancer therapies. Even if antifungal drugs exist, ≈3 million people acquire life-threatening fungal infections every year, 700,000 of which are invasive candidiasis. Despite this, fungi remain underestimated as pathogens.

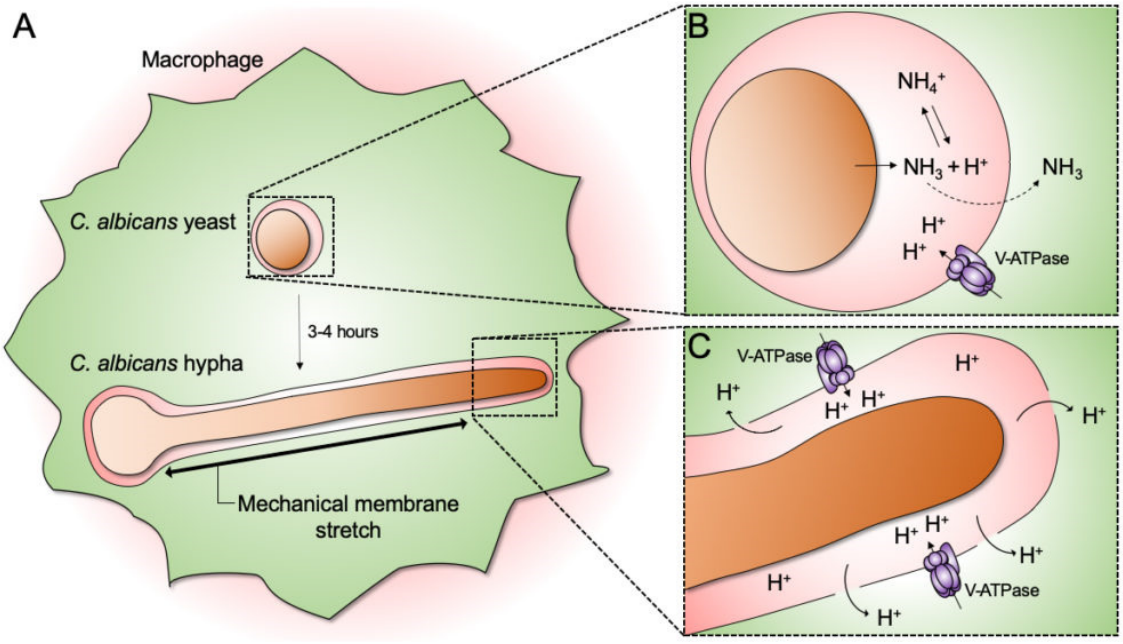

Fig. 1. (A) *C. albicans* undergoes yeast-to-hyphal transition inside the macrophage phagosome.
(B) *C. albicans* secretes NH3 into the acidic phagosome. However, the influx of H+ via V-ATPases exceeds NH3 production by ~100-fold, and NH3 leakage from the phagosome exceeded the rate of NH3 production by ~10,000-fold.
(C) We propose that the observed increase in phagosomal pH is a consequence of membrane rupture and H+ leakage, not NH3 production.

Unlike many other pathogenic microbes, *C. albicans* is polymorphic and grows as budding yeast or filamentous hypha (Fig. 1A). This switch in morphology, termed yeast-to-hypha transition, occurs when the fungus is confronted by stressful conditions, including contact with immune cells, elevated temperatures, and changes in nutrient availability. Engulfment and destruction of fungi by professional phagocytes (Greek for "cells that eat") is critical to the innate immune response. One important phagocyte known as "macrophage" is a type of white blood cell patrolling the body. As the name describes (macrophage meaning "large eater"), macrophages can effectively sense and internalize fungi by a process termed phagocytosis. The newly formed phagosome contains the engulfed *C. albicans* yeast, and thereafter undergoes a series of events termed "phagosome maturation", where the phagosome acquires a hostile, acidic environment with low nutrient

availability. The acidic phagosomal environment is critical to limit fungal growth. Many enzymes inside this fungus-containing phagosome require low pH to function properly. Phagosome acidification is achieved by influx of protons (H^+) via pumps named V-ATPases (Fig. 1B). It is currently believed that the yeast-to-hypha transition is inhibited within acidic phagosomes. Consequently, *C. albicans* is thought to increase the phagosomal pH prior to yeast-to-hypha transition. Thus, the ability of *C. albicans* to increase pH inside the phagosome is considered crucial for its survival and escape from macrophages.

C. albicans was shown to form hyphal filaments inside the macrophage phagosome, which leads to its escape and sustained infection. Recent studies have proposed that *C. albicans* increases the phagosomal pH by producing and secreting ammonia (NH_3) inside the phagosomal space. NH_3 can in principle elevate the pH of the phagosome by binding free H^+ inside the phagosome to form ammonium (NH_4^+) (Fig. 1B). We revisited this hypothesis with the following argumentation: to effectively increase phagosomal pH, NH_3 production by *C. albicans* has to exceed the rate of H^+ pumping into the phagosome by the V-ATPases. Most importantly, the rate of fungal NH_3 generation has to exceed the rate at which NH_3diffuses out of the phagosome (Fig. 1B). In this regard, it is noteworthy that NH_3 diffuses rapidly through most mammalian membranes.

We used a quantitative pH imaging approach to measure the rate of H^+ pumping into the phagosome, and NH_3 leakage across the phagosomal membrane. Our results indicate that the influx of H^+ into the phagosome exceeded NH_3 production by ≈100-fold, and that NH_3 leakage from the phagosome exceeded the rate of NH_3 production by ≈10,000-fold. This suggests that NH_3 production cannot account for the observed changes in phagosomal pH. *C. albicans* would have to produce at least 10^6 times more NH_3 to alkalinize the phagosome. Instead, we show that the yeast-to-hyphal transition occurs inside acidic phagosomes. *C. albicans* grows for 3-4 hours before the extending hypha ruptures the phagosomal membrane, leading to H^+ leakage out of the phagosome and consequent phagosomal alkalinization (Fig. 1C). In conclusion, we propose that the yeast-to-hypha transition is initiated (at a reduced rate) inside acidic phagosomes and that alkalinization results from phagosomal membrane rupture.

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Publication

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