

***Candida albicans* hyphal expansion causes phagosomal membrane damage and luminal alkalinization.**

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Abstract

Macrophages rely on phagosomal acidity to destroy engulfed microorganisms. To survive this hostile response, opportunistic fungi such as *Candida albicans* developed strategies to evade the acidic environment. *C. albicans* is polymorphic and able to convert from yeast to hyphae, and this transition is required to subvert the microbicidal activity of the phagosome. However, the phagosomal lumen, which is acidic and nutrient deprived, is believed to inhibit the yeast-to-hypha transition. To account for this apparent paradox, it was recently proposed that *C. albicans* produces ammonia that alkalinizes the phagosome, thus facilitating yeast-to-hypha transition. We reexamined the mechanism underlying phagosomal alkalinization by applying dual-wavelength ratiometric pH measurements. The phagosomal membrane was found to be highly permeable to ammonia, which is therefore unlikely to account for the pH elevation. Instead, we find that yeast-to-

hypha transition begins within acidic phagosomes and that alkalinization is a consequence of proton leakage induced by excessive membrane distension caused by the expanding hypha. **IMPORTANCE** *C. albicans* is the most common cause of nosocomial fungal infection, and over 3 million people acquire life-threatening invasive fungal infections every year. Even if antifungal drugs exist, almost half of these patients will die. Despite this, fungi remain underestimated as pathogens. Our study uses quantitative biophysical approaches to demonstrate that yeast-to-hypha transition occurs within the nutrient-deprived, acidic phagosome and that alkalinization is a consequence, as opposed to the cause, of hyphal growth.

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