

***Candida* species rewired hyphae developmental programs for chlamydospore formation.**

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Abstract

Chlamydospore formation is a characteristic of many fungal species, among them the closely related human-pathogenic dimorphic yeasts *Candida albicans* and *C. dubliniensis*. Whereas function and regulation of filamentation are well-studied in these species, the basis of chlamydospore formation is mostly unknown. Here, we investigate the contribution of environmental and genetic factors and identified central proteins involved in species-specific regulation of chlamydosporulation. We show that specific nutrient levels strongly impact chlamydospore initiation, with starvation favoring sporulation and elevated levels of saccharides or peptone inhibiting it. Thresholds for these nutritional effects differ between *C. albicans* and *C. dubliniensis*, which explain species-specific chlamydospore formation on certain diagnostic media. A *C. albicans* *nrg1*Δ mutant phenocopied *C. dubliniensis*, putting Nrg1 regulation at the basis of species-specific chlamydospore formation under various conditions. By screening a series of potential chlamydospore regulators, we identified the TOR and cAMP pathways as crucial for sporulation. As rapamycin treatment blocked chlamydosporulation, a low basal Tor1 activity

seems to be essential. In addition, TOR effector pathways play an important role, and loss of the NCR (nitrogen catabolite repression) gene regulators Gat1 and Gln3 reduced chlamydospore formation. A severe reduction was seen for a *C. albicans* *gcn4Δ* deletion strain, implicating a link between regulation of amino acid biosynthesis and chlamydospore development. On the other hand, deletion of the GTPase gene *RAS1* and the adenylyl cyclase gene *CYR1* caused a defect in chlamydospore formation that was mostly rescued by cAMP supplementation. Thus, cAMP-signaling is a second major pathway to control chlamydospore production. Finally, we confirmed light exposure to have a repressive effect on chlamydosporulation. However, permanent illumination only reduced, but not abolished chlamydospore production of *C. albicans* whereas *C. dubliniensis* sporulation was unaffected. In summary, we describe novel environmental factors which determine chlamydosporulation and propose a first model for the regulatory network of chlamydospore formation by different *Candida* species.

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