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Original Article

The roles of actin cytoskeleton and actin-associated protein Crn1p in trap formation of *Arthrobotrys oligospora*

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Abstract

Nematode-trapping fungi include a variety of species capable of generating specific trapping devices to capture nematodes and the production of devices is also an indicator of a switch from saprophytic to predacious lifestyles. Traps are developed from vegetative mycelia, but they are quite different from hyphae in both morphological and physiological characteristics. Therefore, the molecular mechanisms underlying their formation have attracted much attention. In this investigation, *Arthrobotrys oligospora*, a nematode-trapping fungus, has three-dimensional networks and genomics and proteomics were recently performed, so as to reveal the relationship between actin cytoskeleton and trap formation. Both actin staining via FITC-phalloidin and treatment of actin polymerization inhibitor Lat-B illustrated that the actin cytoskeleton played an important role in trap development. Furthermore, absence of the conserved actin-associated protein Crn1p caused a structural defect of traps and failure to infect nematodes. It was observed that mutant Δ*crn1* represented a reduced number of rings and a lower complexity of three-dimensional networks, likely due to the disturbance of actin branching. Collectively, our study confirmed the involvement of the actin cytoskeleton as well as the conserved actin-associated protein Crn1p in trap formation. It further suggested the manners in which Crn1p influences the development of three-dimensional networks in *A. oligospora*.
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Keywords: Nematode-trapping fungi; *Arthrobotrys oligospora*; Trapping devices; Three-dimensional networks; Actin cytoskeleton; Crn1p

1. Introduction

Some microorganisms broadly distributed in natural ecosystems can generate a series of virulence-related factors to infect nematodes and thus function as antagonists against nematodes. Among them, nematophagous fungi have been the most frequently investigated. A nematode-trapping fungus is defined as a type of fungus that catches nematodes by producing sophisticated mycelial trapping devices, such as adhesive networks, adhesive knobs and constricting rings [1]. When their vegetative mycelia are transitioned into trapping devices, nematode-trapping fungi can attract, adhere, penetrate and digest nematodes efficiently, which helps those fungi assimilate nutrients from their host. Therefore, this "predatory" phase is considered to be beneficial for fungi to obtain nitrogen under stressful environments [2].

Nematode-trapping fungi comprise a great variety of fungi belonging to widely divergent orders and families. Based on morphological and molecular studies, most of them can be placed within the asexual family Orbiliaceae. The family includes 95 species and is currently assigned in three anamorph genera *Arthrobotrys*, *Dactylellina* and *Drechslerella* [3,4]. *Arthrobotrys oligospora* is a model species in the genus *Arthrobotrys*. Similar to other nematode-trapping fungi,

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A. oligospora generally lives as a saprophyte and produces the sophisticated three-dimensional networks in the presence of some specific signals including nematodes. Though the trapping devices are developed from vegetative mycelia, they represent a distinguished phase with the morphological and physiological characters different from those of regular hyphae. Thus, to reveal the potential mechanism underlying trap formation, several gene expressional profiles between trap cells and vegetative hyphae have been carried out, for example knobs versus mycelia in *Monacrosporium haptotylum* [5] and *Monacrosporium lysipagum* [6]. Recently, an investigation on genomics and proteomics in *A. oligospora* in our lab also proposed a model on how the three-dimensional networks were generated [7]. Besides the analysis between trap cells and vegetative hyphae, the comparative genomics and transcriptomics have also been performed among different nematode-trapping fungi with different traps for the similar purpose [8,9]. However, despite of the availability of large datasets from the high throughput investigations, the detailed molecular mechanism remains little known.

Actin, one of the most important components of cell cytoskeleton, undergoes rapid remodeling in a variety of cellular processes such as cell locomotion, endocytosis, vesicle and organelle transport, cytokinesis, polarized cell growth, vegetative reproduction through budding and sexual reproduction through mating and meiosis [10,11]. Actin cytoskeleton also participates in the infectious process of pathogenic fungi. In the study of *Ustilago maydis*, the infectious process was seriously compromised when LatA, an inhibitor of F-actin, was added to the medium [12]. Appressorium, the main infection structure in the rice pathogen *Magnaporthe oryzae*, can generate extreme turgor pressure and help the fungus mechanically penetrate the tough leaf cuticle of rice. Indeed, this structure is believed to be required for successful infection of many fungal pathogens, and is closely associated with F-actin. It has been shown that production of appressorium requires polar growth of cells, and that actin dynamics play a crucial role in shaping cell polarity [13]. Furthermore, septin ring assembly is needed in order to scaffold F-actin into a toroidal network at the base of the appressorium. Consistent with these observations, the deletion of septin-related genes caused the failure of appressorium rupturing [14].

Though the actin cytoskeleton has been suggested to be closely related to infection of the pathogenic fungi, the actin-associated genes or proteins involved in this process remain largely unknown. The dynamics of actin depends mainly on the coordinated activities of highly conserved actin-associated proteins. Based on the proteomic analysis of *A. oligospora* in our lab, we noted that expression of the *arpc2* gene, which encodes one of seven subunits in the Arp2/3 protein complex, had increased 3.82-fold ($P < 0.05$) within 10 h of trap development [7]. However, construction of the *arpc2* knockout mutant in *A. oligospora* failed, probably because mutation of *arpc2* or the absence of the Arp2/3 complex could be lethal to the fungus (data not shown). Coronin is the other conserved component of the actin cytoskeleton found in all eukaryotes from yeast to mammals, and it functions as a regulator of the Arp2/3 complex [15−17]. At the molecular level, Crn1p has been shown to participate in both the assembly and disassembly of the actin cytoskeleton via interacting with the Arp2/3 complex [18,19] or ADF/cofilin [20], respectively. Furthermore, it has been reported that deletion of *crn1* led to the different phenotypes among the different organisms, such as in *Dictyostelium discoideum* and *Saccharomyces cerevisiae*. Thus, in our current study, our aim was to understand the potential roles of actin in nematode-trapping fungi, with a focus on trapping device production. Specifically, we observed changes in the actin cytoskeleton, trapping device formation and production of conidia, as well as their relationship when treated with LAT-B, an inhibitor of F-actin, or when the *crn1* gene was knocked out in *A. oligospora*.

2. Materials and methods

2.1. Fungal cultures

The wild strain of *A. oligospora* Fres ATCC24927 was cultured on plates of potato dextrose agar (PDA) and maintained on corn meal agar (CMA) medium (17 g corn meal, 10 g agar and 2 g K_2HPO_4 in 1 L of water, adjusted to pH 7 using 1 M NaOH) for trap formation and infection assay [21].

2.2. Fluorescence microscopy

To determine whether the actin cytoskeleton was involved in trap formation, FITC-phalloidin, a drug that selectively binds to F-actin, was employed to stain the trapping device and vegetative mycelia after the three-dimensional networks were induced by nematodes. To obtain fungal samples that could be more easily stained (e.g. FITC-labeled phalloidin) or observed under a microscope, the hyphae were first grown for 5 days on CMA media mounted on the glass slides in a petri dish. After being washed with 10 mM, pH 7.4 phosphate-buffered saline (PBS) buffer 3 times, the hyphae were treated with 0.5% Triton X-100 in PBS, followed by the same washes with PBS three additional times. Then the hyphae were stained for the actin cytoskeleton with 5 μM FITC-conjugated phalloidin (Sigma Aldrich, USA), following the protocol described previously [22].

The stained hyphae were observed with a Nikon E800 microscope using a 100-oil immersion objective lens and photographed using a Nikon DS-5Mc color camera. NIS-Elements F 2.30 software was utilized for image analysis (Instruments Europe B.V., Düsseldorf, Germany).

2.3. Trap formation and infection assay

Caenorhabditis elegans worms were used to induce trap formation of *A. oligospora* and monitor the infection process. The synchronized worms were grown to adulthood on the nematode growth medium (NGM, containing 50 mM NaCl, 20 g/L of agar, 2.5 g/L of peptone, 1.0 mM cholesterol,

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