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Original Contribution

Interaction among the vacuole, the mitochondria, and the oxidative stress response is governed by the transient receptor potential channel in *Candida albicans*

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ABSTRACT

Candida albicans is one of the most important opportunistic pathogens, causing both mucosal candidiasis and life-threatening systemic infections. To survive in the host immune defense system, this pathogen uses an elaborate signaling network to recognize and respond to oxidative stress, which is essential for its pathogenicity. However, the exact mechanisms that this fungus employs to integrate the oxidative stress response (OSR) with functions of various organelles remain uncharacterized. Our previous work implicated a connection between the calcium signaling system and the OSR. In this study, we find that the vacuolar transient receptor potential (TRP) channel Yvc1, one of the calcium signaling members, plays a critical role in cell tolerance to oxidative stress. We further provide evidence that this channel is required not only for activation of Cap1-related transcription of OSR genes but also for maintaining the stability of both the mitochondria and the vacuole in a potassium- and calcium-dependent manner. Element assays reveal that this TRP channel affects calcium influx and potassium transport from the vacuole to the mitochondria. Therefore, the TRP channel governs the novel interaction among the OSR, the vacuole, and the mitochondria by mediating ion transport in this pathogen under oxidative stress.

Introduction

Candida albicans is a well-known fungus colonizing on human beings, usually existing as a harmless part of the microbiome in healthy individuals [1,2]. However, this fungus may damage the epithelial barriers, invade deep mucosal tissues, and disseminate into the blood, causing both mucosal candidiasis and life-threatening systemic infections [3,4]. Especially, *C. albicans* related-systemic infections are on the rise in concert with the growing population of individuals suffering from HIV infection, organ transplantation, chemotherapy, indwelling catheter, total parenteral nutrition (TPN), and so on [5–8]. Several virulence-related properties, such as dimorphic switching and efficient responses to various environmental cues, are indispensible for its invasion and survival in the host [9,10]. Understanding how this fungus senses and responds to extracellular stimuli can provide significant information for designing antifungal strategies against *Candida*-related infections [5,11].

In the host, *C. albicans* must be frequently confronted with an immune defense system. The host response to this pathogen depends on fungal recognition by innate immune cells, including macrophages, neutrophils, and dendritic cells, followed by subsequent phagocytosis and killing of this pathogen [12–14]. The mechanism by which phagocytes kill *C. albicans* is thought to be associated with rapid production of reactive oxygen species (ROS, including superoxide anions, H_2O_2, hydroxyl radicals, and hypochlorous acid) initiated by activation of NADPH oxidase in phagocytes [12,15]. To escape the innate immune surveillance, *C. albicans* has a dedicated signaling network to recognize and respond to oxidative stress [16,17]. Cap1, a member of the basic region-leucine

Abbreviations: OSR, oxidative stress response; TRP, transient receptor potential; TPN, total parenteral nutrition; MMP, mitochondrial membrane potential; VMP, vacuolar membrane pemeabilization; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; MDA, malondialdehyde; TBA, thiobarbituric acid; ROS, reactive oxygen species; CAT, catalase; SOD, superoxide dismutase; ONPG, *O*-nitrophenyl-β-D-galactopyranoside; C-DCFDA, 5-(6)-carboxy-2′,7′-dichlorofluorescein diacetate; HACS, high-affinity calcium uptake system; LMP, lysosome membrane permeabilization

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zipper (bZip) transcription factor family, is a central oxidative stress response (OSR) regulator. Under oxidative stress, this factor is transported from the cytoplasm to the nucleus, mediating oxidant-dependent transcriptional activation of OSR genes, which are involved in redox controlling, ROS scavenging, energy metabolism, and the protein degradation pathway [18,19]. Several other regulators, such as Hog1 and Skn7, are also included in OSR [20–22]. In addition, we recently found that several members in the calcium signaling system, including Cch1 and Ecm7, also function in OSR, suggesting a connection between calcium signaling and OSR [23,24].

In fungal cells, both the vacuole and the mitochondrion are indispensible for physiological processes and involved in OSR. The fungal vacuole is an acidic organelle containing abundant hydrolytic enzymes and high-level ions, functioning in nutrient storage, maintenance of ion homeostasis, autophagy, adaptation to environmental stresses, and cellular differentiation [25–28]. For *C. albicans*, this organelle is also required for yeast-to-hypha switching and surviving in macrophage attacks [29], implying a role of the vacuole in OSR. On the other hand, the mitochondrion, termed the cellular energy factory, is recognized as the most important intracellular source of ROS. The generation of mitochondrial ROS is a determinant in cell functions, participating in abundant OSR-related signaling pathways [30]. Moreover, exposure to high-level ROS is a great risk of mitochondrial functions, leading to mitochondrial DNA mutations, lipid peroxidation, and opening of mitochondrial membrane channels. Especially, opening of these channels may result in decreased mitochondrial membrane potential (MMP) and release of mitochondrial components, causing mitophagy, apoptosis, or necrosis [31–33]. In mammalian cells, the mitochondria have a defense system that reduces the toxicity of ROS and inhibits ROS-caused apoptosis or necrosis through activation of mitochondrial antioxidant enzymes and mitochondrial ATP-dependent potassium channels (mitoK_{ATP}) [34,35]. However, the interaction between the vacuole and the mitochondria during OSR remains to be elucidated.

The transient receptor potential (TRP) channels comprise a superfamily of selective and nonselective cation-permeable ion channels. In recent years, more than 100 TRP members were identified in vertebrates, flies, worms, and yeasts [36,37]. These members have six predicted transmembrane domains (TMs) and a cation-permeable pore to mediate ion transport [36]. Most TRP channels function as cellular sensors, responding to touch, temperature, osmolarity, pheromones, taste, and other stimuli [36,38]. These channels predominately mediate calcium transport from the extracellular environment into the cell, or from intracellular calcium stores (the vacuole in fungal cells) to the cytoplasmic matrix, controlling the free cytoplasmic calcium levels [36–39]. Most recently, we characterized a TRP member in *C. albicans*, termed Yvc1 or TRPY3. This channel is localized on the vacuolar membrane, mediating vacuolar calcium release under hypertonic or alkaline stimulus. Moreover, it plays an important role in resistance to ethanol and the membrane-perturbing agent SDS, morphogenesis, and pathogenicity [40].

This study aimed to elucidate the role of the *C. albicans* TRP channel in OSR, and to reveal the relationship among the vacuole, the mitochondria, and the OSR linked by this channel. We first compared the suspectibility of the mutants of calcium signaling members to oxidative stress, and found that the TRP mutant is most sensitive to oxidative stress, indicating that the TRP channel is required for OSR. Detailed investigation further revealed that this channel governs OSR, mitochondrial ion homeostasis, and vacuolar membrane pemeabilization (VMP). Hence, this study uncovered a novel crosslink among the vacuole, the mitochondria, and the OSR governed by the TRP channel in *C. albicans*.

Materials and methods

Strains and culture conditions

All *C. albicans* strains used in this study are listed in Table S1. For deletion of *CMP1* encoding the catalytic subunit of calcineurin, the *cmp1::ARG4* and *cmp1::URA3* cassattes were amplified using the primers CMP1-5DR and CMP1-3DR (Table S2) from the plasmids pRS-ARG4Δ*Spe*I and pDDB57, respectively [41], and transformed into the wild-type strain BWP17, generating the *cmp1*Δ/Δ strain NKC75. The *CAT1*-disrupted strain *cat1*Δ/Δ (NKF139) and the autophagy-deficient strain *aut7*Δ/Δ (NKF140) were constructed with similar methods, using the primers CAT1-5DR and CAT1-3DR, the primers AUT7-5DR and AUT7-3DR (Table S2), respectively.

To construct the TRP mutant containing the OSR reporting plasmid p*PIPF7817-LacZ* [24], the TRP mutant *yvc1*Δ/Δ was transformed with the *Nru*I-digested plasmid, obtaining the reporter strain NKF132. To construct the strains containing the Cap1 reporting plasmid pCAP1-GFP [42], the strains NKF93 (*yvc1*Δ/Δ) and NKC73 (*cch1*Δ/Δ) were plated on SC agar (glucose 2%, yeast nitrogen base 0.67%, amino acid mixture 0.2%, agar 2%) containing 1% 5-fluoroorotic acid (5-FOA, BBI), generating the *URA3*-depleted strains NKF95 (*yvc1::ARG4/yvc1::dpl200*) and NKF97 (*cch1::ARG4/cch1::dpl200*). The strains BWP17, NKF95, and NKF97 were then transformed with the *Stu*I-digested plasmid pCAP1-GFP, obtaining the strains NKF135, NKF136 and NKF137, respectively.

To generate *CAT1*-overexpressed strains, the *CAT1* fragment was amplified from the wild-type genome using the primers CAT1-5C and CAT1-3C (Table S2), and cloned into the plasmid pAU34M [40], obtaining the *CAT1*-overexpressed plasmid *PACT1-CAT1*. The plasmid was then digested by *Bgl*II and transformed into the strains BWP17 (WT), NKF95 (*yvc1*Δ/Δ), and NKF139 (*cat1*Δ/Δ), obtaining the strains NKF141 (WT+*PACT1-CAT1*), NKF142 (*yvc1*Δ/Δ+*PACT1-CAT1*), and NKF143 (*cat1*Δ/Δ+*PACT1-CAT1*).

To construct the strains containing the GFP-tagged mitochondrial membrane protein Csp37, the GFP-URA3 fragment was amplified from the plasmid pGFP-URA3 using the primers CPS37-GFP-1 and CPS37-GFP-2 (Table S2), and transformed into the strains BWP17 (WT), NKF95 (*yvc1*Δ/Δ), and NKF140 (*aut7*Δ/Δ), obtaining the strains NKF144 (WT+Csp37-GFP), NKF145 (*yvc1*Δ/Δ+Csp37-GFP), and NKF146 (*aut7*Δ/Δ+Csp37-GFP).

Routinely, *C. albicans* strains were cultured in liquid YPD medium (yeast extract 1%, peptone 2%, glucose 2%) in a shaking incubator at 180 rpm and 30 °C. SD plates (glucose 2%, yeast nitrogen base 0.67%, amino acid dropout mixture 0.2%, agar 2%) were used to select *C. albicans* transformants.

Stress susceptibility

For testing the sensitivity of *C. albicans* strains to the oxidative stress agent H_2O_2, cells were overnight cultured in liquid medium, washed twice with PBS buffer (NaCl 0.8%, KCl 0.02%, Na_2HPO_4 0.142%, KH_2PO_4 0.027%, pH 7.4), and suspended in the same buffer with an initial OD_{600} of 0.5. Five microliters of 10-fold dilutions was spotted on the YPD plates containing 5 mM H_2O_2. YPD plates not containing H_2O_2 were used as the control. The plates were cultured at 30 °C for 2 days and then photograghed. To investigate the effect of potassium, sodium, and calcium on the sensitivity to H_2O_2, overnight cultured *C. albicans* cells were diluted with fresh liquid YPD medium to an OD_{600} of 0.1 and incubated with shaking at 30 °C for 4–5 h to the log phase. The cells were then harvested and diluted to an OD_{600} of 0.1 with fresh YPD medium containing 5 mM H_2O_2 plus KCl, NaCl, and $CaCl_2$ alone with different concentrations ranging from 0 to 200 mM, or containing 5 mM H_2O_2 plus 100 mM KCl, 100 mM NaCl, 100 mM $CaCl_2$, 50 mM KCl plus 50 mM NaCl, or