The Ubiquitin System and Morphogenesis of Fungal Pathogens

Daniel Kornitzer PhD

Department of Molecular Microbiology, Rappaport Faculty of Medicine, Technion and the Rappaport Institute for Research in the Medical Sciences, Haifa, Israel

Key words: *Candida albicans*, filamentation, ubiquitin, Cdc4

Abstract

Distinct fungal species exhibit different cellular morphologies, such as yeast and filamentous (hyphal and pseudohyphal) forms, that are reflected in the macroscopic colony morphology. Dimorphic and multimorphic fungi can switch between these different morphologies, enabling the utilization of different food supplies in the case of saprophytes, and contributing to pathogenesis in the case of parasites. Cellular morphogenesis is often regulated by signal transduction pathways, and is intimately linked to the cell cycle machinery. Here we describe the role of ubiquitin-mediated degradation of cell cycle regulators and transcription factors involved in fungal morphogenesis.

IMAJ 2006;8:243–245

Fungal dimorphism and pathogenicity

Many of the most common pathogenic fungi (e.g., the etiologic agents of blastomycosis, coccidioidomycosis, histoplasmosis and candidiasis) are dimorphic, i.e., they can adopt two or more distinct growth forms. The most frequently encountered fungal pathogen is *Candida albicans*, a commensal organism of the gastrointestinal tract and the main fungal agent of life-threatening, systemic infection among immunocompromised or debilitated patients [1]. The link between dimorphism and virulence has been best established with C. *albicans*, an organism that is able to switch between at least three distinct growth forms: an oblong yeast form, a hyphal or mold form (characterized by parallel cell walls without constrictions at the sites of septation), and a pseudohyphal form (characterized by unseparated chains of elongated cells) [2]. In a mouse model of systemic infection, C. *albicans* mutants unable to switch from the yeast form to the hyphal form showed strongly reduced virulence [3,4].

A variety of environmental conditions – the most potent of which are serum and 37°C – can promote the switch to hyphal growth in C. *albicans*. These stimuli act via partially identified signal transduction pathways to activate a number of transcription factors [reviewed in 5 and 6]. Hyphal growth requires maintenance of polarization within the cell in such a manner that growth occurs exclusively at the apex, in contrast to yeast growth, which is isotropic. Cell morphogenesis is closely associated with regulation of the cell cycle in many eukaryotes, but particularly in budding yeasts such as the well-studied baker's yeast (*Sacchraromyces cerevisiae*) [7,8]. In C. *albicans*, a number of cell cycle regulators were found to be involved in morphogenesis – e.g., the Polo-like kinase CaCdc5, a mitotic regulator [9], and Hgc1, a C. *albicans* cyclin related to the S. *cerevisiae* G1 cyclins Cln1 and Cln2 [4]. Recently, depletion of the C. *albicans* Cln3 homolog, an essential protein, was shown to induce hyphal or pseudohyphal growth, depending on the conditions [10,11]. Thus, in C. *albicans* as well, cell cycle regulators affect morphogenesis.

The SCF^{CDC4} ubiquitin ligase and morphogenesis

Ubiquitin-mediated protein degradation [12,13] plays a central role in cell cycle control. The substrate-recognition function of the ubiquitin system resides in the ubiquitin-ligase component. The multi-subunit SCF ubiquitin ligases (Skp1/Cullin/F-box) belong to the family of RING finger ubiquitin ligases [reviewed in 14]. The SCF core complex contains four subunits: Skp1, Cul1 (Cdc53 in S. *cerevisiae*), the RING finger protein Rbx1 (also Roc1 or Hrt1), and one of several F-box proteins [15]. Seventeen predicted F-box proteins were detected in the S. *cerevisiae* genome, including Cdc4 and Grr1 [16], and 17 at least could be detected in *Candida albicans* (our unpublished observations). SCF^{CDC4} – an SCF ubiquitin ligase complex that includes Cdc4 as substrate recognition component – is one of the ubiquitin ligases implicated in cell cycle entry and progression in yeast and mammalian cells. The mammalian homolog of Cdc4 is involved in degradation of cyclin E and of the proto-oncogene c-Myc, and was shown to function as a tumor suppressor gene. In S. *cerevisiae*, in contrast, CDC4 is essential for cell proliferation, and temperature-sensitive mutants arrest in G1 with elongated, multiple buds [17]. The critical substrate of yeast SCF^{CDC4} for the G1 to S transition is the cyclin-dependent kinase inhibitor Sic1 [18,19]. Other substrates of SCF^{CDC4} include another CDK inhibitor, Far1 [20], and a transcription factor, Gcn4 [21].

In the baker's yeast, S. *cerevisiae*, a nutrient-induced dimorphism exists that enables the cells to switch from the yeast morphology to the pseudohyphal morphology, a growth form consisting of chains of elongated yeast cells. Tec1 is a transcription factor that is central to this morphogenetic switch. Pseudohyphal growth is induced via a signal transduction pathway that includes the activation of the MAP kinase Kss1. Pheromone induces mating by activating the same MAP kinase cascade including, in addition to Kss1, the MAP kinase Fus3. The reason that pheromone, although inducing Kss1, does not cause pseudohyphal growth has only recently been uncovered: Fus3 specifically phosphorylates Tec1 [22], causing it to be targeted for degradation by an SCF complex [23],

SCF = Skp1/Cullin/F-box protein
CDK = cyclin-dependent kinase

probably SCF^{CDC4} [24]. Thus, in this case the ubiquitin system plays a dampening effect that enables the cell to distinguish between two different stimuli that use the same MAP kinase pathway to achieve two distinct developmental outcomes, mating vs. morphogenesis.

Given the established roles of Cdc4 in the regulation of the cell cycle and morphogenesis, we investigated its role in the dimorphic organism C. *albicans*. We found that deletion of CDC4 from the C. *albicans* genome is not lethal – unlike for example in S. *cerevisiae* – but results in a strain that is constitutively hyphal [25] [Figure 1]. Sol1, a protein related to the CDK inhibitor Sic1, was identified as a substrate of SCF^{CaCDC4} [25]. Sol1 clearly affects morphogenesis of C. *albicans*, presumably via its role in the inhibition of specific cyclin-CDK complexes, but it appears to have a distinct specificity from S. *cerevisiae* Sic1, based on the terminal arrest phenotype of cells overexpressing stabilized Sic1 vs. stabilized Sol1 [25]. On the other hand, Sol1 stabilization is not the sole reason for the distinct hyphal phenotype of the Ca*cdc*4-/- mutant, because the double Ca*cdc*4-/- *sol*1-/- mutant exhibits a constitutive hyphal morphology similar to that of the single Ca*cdc*4-/- mutant [25]. The additional SCF^{CaCDC4} substrate(s) involved in this phenotype have not yet been identified. One possible candidate was the homolog of the S. *cerevisiae* filamentation-inducing transcription factor Tec1, based on the fact that in S. *cerevisiae* Tec1 is degraded via the SCF^{CDC4} (see above); however, our preliminary results exclude CaTec1, as well as the hyphae-inducing transcription factors Efg1 and Cph1, and the hyphal phase-specific cyclin Hgc1 as SCF^{CaCDC4} substrates.

Regulation of SCF: the role of neddylation

SCF ubiquitin ligase activity can be regulated by different mechanisms, including the presence or absence of F-box proteins, e.g., SKP2 [26]; phosphorylation of the substrate, e.g., the SCF^{CDC4} substrates Sic1 [27,28] and Gcn4 [21,29]; and neddylation of the cullin subunit. Neddylation, or conjugation of the ubiquitin-like protein NEDD8/Rub1, at a single lysine residue in the C-terminal domain of Cul1, requires a mechanism similar to the ubiquitin system [30]. Neddylation is reversible: deneddylation of cullins is promoted by Csn5/Jab1, a subunit of another highly conserved multiprotein complex, the COP9 signalosome [31,32].

Genetic data suggest that the neddylation and deneddylation cycles of cullins might play a role in Ubc-ligase complex formation [33] and in cullin-dependent polyubiquitinylation of SCF target proteins [34]. The requirement for neddylation may involve CAND1, a protein that binds the unneddylated cullin and prevents Skp1/F-box protein binding [35,36]. Neddylation was initially found to dissociate CAND1 from the cullin [35,36]; however, results with a purified system indicate that neddylation by itself is unable to cause CAND1 dissociation [37], suggesting the involvement of additional factor(s). In the realm of fungal development, a Neurospora mutant defective in deneddylation activity was found to have a defect in the circadian clock controlling conidiation (spore formation) [38]. This could be attributed to a stabilization of the transcription factor FRQ, which is normally degraded by the SCF^{FWD-1} ubiquitin ligase. In

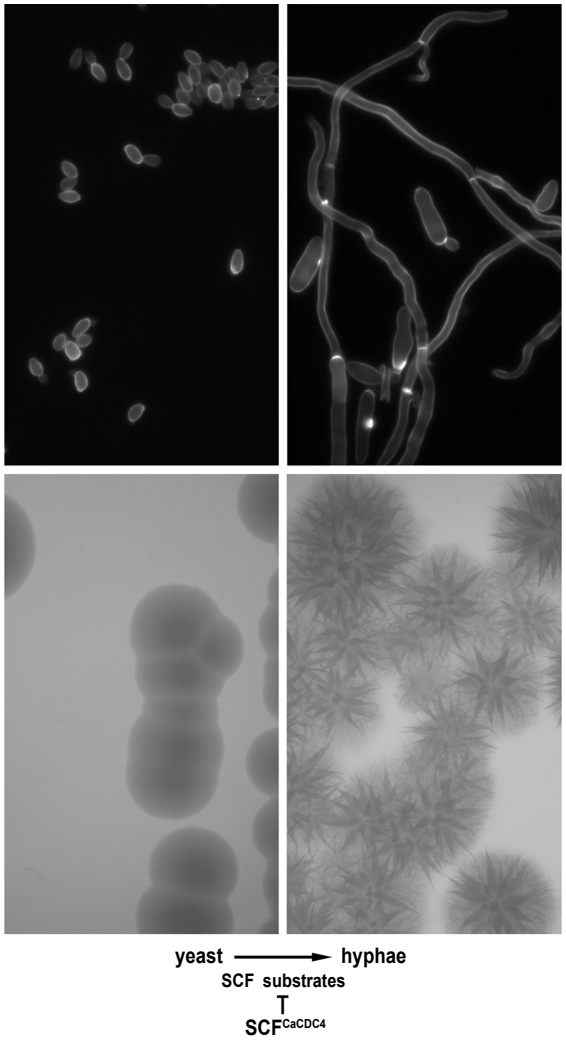

Figure 1. Cellular phenotype (upper panels) and colony phenotype (lower panels) of the C. *albicans cdc4-/-* mutant (right panels) vs. the wild-type strain (left panels) grown on YPD medium. The cell wall was stained with calcofluor and visualized with ultraviolet epifluorescence for the cellular phenotype (upper panels).

Aspergillus, the COP9 signalosome was found to be essential for sexual development [39]. Our preliminary results indicate that C. *albicans* contains a CaRub1/NEDD8 homolog. Three cullin homologs can be detected in the C. *albicans* genome sequence, of which one, orf19.1674, appears to be the Cdc53/CUL1 homolog. In preliminary experiments, we found that this protein is subject to neddylation. Jab1, the deneddylase subunit of the COP9/signalosome, was identified in the C. *albicans* genome and deleted. This deletion causes a hyperneddylation of CaCdc53, as expected. The Ca*rub*1-/- mutant favors the filamentous mode of growth, although not as strongly as the Ca*cdc*4-/- mutant, suggesting that modulation of SCF neddylation might constitute a mechanism for regulating the morphogenetic switch of C. *albicans*. Furthermore, the study of neddylation in C. *albicans* and other fungi may provide mechanistic insights into the role of this modification on SCF function.

Conclusions

Fungal cellular morphogenesis and virulence are connected. Cellular morphogenesis is affected by cell cycle regulators and via signal transduction cascades. Ubiquitin-mediated protein degradation affects morphogenesis by modulating the concentration of cell cycle regulators and transcription factors. Ubiquitination of these effectors is regulated by their phosphorylation. An additional potential layer of regulation involves modification of the ubiquitin ligases to increase or decrease their activity.

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Correspondence: Dr. D. Kornitzer, Dept. of Molecular Microbiology, Rappaport Faculty of Medicine, Technion, Haifa 31096, Israel.
Phone: (972-4) 829-5258
Fax: (972-4) 829-5254
email: danielk@techunix.technion.ac.il