Morphogenesis of a Human Fungal Pathogen Requires Septin Phosphorylation

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In this issue of Developmental Cell, Sinha et al. describe a posttranscriptional mechanism necessary for hyphal development of the human pathogen, Candida albicans. In this context, the kinase Gin4 phosphorylates the septin Cdc11 in uninduced yeast cells to prime them for fast action by the cyclin-dependent kinase Cdc28/Ccn1 at the time of hyphal induction. Joint phosphorylation of Cdc11 by these two kinases is essential for stable polarization of hyphal growth.

Septins are an evolutionarily conserved family of filament-forming proteins that play key roles in cell biology and development. In humans, septin genes have been implicated as oncogenes; mutations in Septin 9 cause the neurodegenerative disease hereditary neuralgic amyotrophy, and Septin 4 has been shown to be protective in a mouse model of Parkinson’s disease. In metazoan cells, septin scaffolds have multiple roles: they act in cell division, polarity determination, vesicle trafficking, and cytoskeletal dynamics. Septins were originally identified in the budding yeast, Saccharomyces cerevisiae, where the filaments form a collar at the bud neck that is composed of Cdc3, Cdc10, Cdc11, Cdc12, and Shs1. At cytokinesis, the collar splits in two, as it acts as a scaffold for the formation of the primary septum (Gladfelter et al., 2001). The septin filaments are attached to the inner surface of the plasma membrane where, in addition to their role in cytokinesis, they act as a barrier for the diffusion of proteins through the cell cortex (Dobbelaeere and Barral, 2004). They also regulate cell cycle progression through the operation of the morphogenesis checkpoint (Lew, 2003). Now, in this issue of Developmental Cell, Sinha et al. (2007) show that phosphorylation of the septin Cdc11 on adjacent residues by two kinases, Gin4 and Cdc28/Ccn1, plays a key role in the development of hyphal growth in the human fungal pathogen Candida albicans. The cyclin-dependent kinase Cdc28 has long been known to act as the master regulator of cell cycle progression and polarized growth in budding yeast. Yet our knowledge of its physiological targets remains surprisingly limited. The work by Sinha et al. provides a detailed molecular description of a Cdc28 target and in doing so brings an awareness of the action of the cell cycle engine and the developmental transition of an important pathogen to the growing field of septin biology.

Candida albicans is the most common human fungal pathogen. In addition to unpleasant but superficial mucosal infections such as vaginitis (thrush), it causes serious life-threatening bloodstream infections in vulnerable groups such as newborns and certain intensive care patients, especially those undergoing cancer chemotherapy, immunosuppressant therapy, or catetherization. A striking feature of C. albicans biology, which is thought to be essential for pathogenicity, is its capacity to grow in different morphological forms (Figure 1). These range from unicellular budding yeasts to true hyphae with parallel-sided walls. In between these two extremes, the fungus can exhibit a variety of growth forms that are filamentous but retain a constricting function between adjacent cellular compartments. These are collectively referred to as pseudohyphae. The organization of septin structures is different in yeast and pseudohyphae compared with hyphae (Sudbery, 2001). In yeast and pseudohyphae a septin collar forms at the bud neck (Figures 1A and 1B), which splits in two at cytokinesis as it organizes the formation of the primary septum, as it does in S. cerevisiae. When an unbudded yeast cell is induced to form a hyphal germ tube, a septin cap forms at the growing tip of the tube, while a band of septin bars forms at the base of the tube, next to the parental yeast cell (Figure 1C). As the germ tube elongates, a septin collar forms along its length; the basal band and apical cap disappear (Figure 1D). After mitosis, the collar splits into two clearly defined rings and organizes the formation of the primary septum (Figure 1E).

The Gin4 kinase was already known to be involved in septin organization. In S. cerevisiae gin4Δ mutants, septin collar organization is disturbed to produce a series of longitudinal bars reminiscent of the basal septin band in C. albicans (Longtine et al., 1998). In C. albicans, cells depleted of Gin4 due to conditional expression from the MET3 promoter form the basal septin bars, but not the septin collar (Wightman et al., 2004). Sinha et al. now show that Gin4 first acts to phosphorylate the septin Cdc11 at Ser395. This allows the cyclin-dependent kinase Cdc28 complexed to a particular cyclin, Ccn1, to phosphorylate the adjacent Ser394 residue. If phosphorylation of Ser394 is prevented, either by deletion of CCN1 or by a cdc11 S394A mutation, germ tubes evaginate normally and septins localize to a collar. However, after the septin collar forms, growth becomes isotropic distal to the collar, resulting in a swollen hyphal tip. Tip swelling in a ccn1Δ/Δ strain is rescued by CDC11 S394D S395D phosphomimetic mutations, showing that phosphorylation...
of Cdc11 Ser394 and Ser395 is the only Ccn1 function required for normal hyphal development.

Cdc11 phosphorylation by Gin4 is a cell-cycle-regulated event occurring during mitosis and cytokinesis phases of the previous cell cycle. Thus, Ser395 is already phosphorylated when un budded yeast cells are induced to form hyphae. After hyphal induction, Cdc28/Ccn1 associates with the septin complex within 5–10 min. This rapid response appears to be independent of transcription because it occurs in an efg1Δ/cph1Δ mutant lacking the transcription factors targeted by the signal transduction pathways that promote hyphal growth. This is an important observation, because a large amount of effort has been expended searching among the genes that are upregulated in hyphae for those that might control hyphal development. As Sinha et al. point out, their results should refocus attention on mechanisms that do not depend on hyphal-specific transcription.

Ccn1 is an interesting cyclin. Under the alias Cln1 it had previously been shown to be required for the maintenance, but not the establishment, of hyphal growth (Loeb et al., 1999), consistent with the conclusions of Sinha et al. Phylogenetic analysis shows that it represents the founding member of a novel cyclin class that is clearly distinct from the G1 (Cln1–3) and G2 (Clb1–6) cyclin families in S. cerevisiae. Curiously, most fungal species contain a Ccn1 homolog except for S. cerevisiae and closely related species such as Ashbya gossypii and Kluyveromyces lactis.

Why does the phosphorylation of Cdc11 at early times after hyphal induction result in a defect in hyphal morphogenesis at a later time after the septin collar has formed? In S. cerevisiae, polarized growth has been shown to involve the directed flow of post-Golgi secretory vesicles to generate new cell wall and membrane upon fusion with the plasma membrane after docking with a multiprotein structure called the exocyst. It has been suggested that septins play a role in generating the characteristic constriction of S. cerevisiae yeast cells at the bud neck by targeting secretion to the bud base, with the resulting lateral growth producing the characteristic swelling on the daughter side of the neck (Glafelter et al., 2005). Although C. albicans yeast cells have a similar constriction, there are no constrictions at the site of the septin collar in hyphae; thus, the propensity of the septin collar to target polarized secretion must be suppressed. Perhaps phosphorylation of Cdc11 Ser394 by Cdc28/Ccn1 is the critical event responsible for this suppression. A possible mechanism is provided by another observation from the Wang laboratory that the exocyst landmark protein Sec3 coimmunoprecipitates with the septin Cdc11 in C. albicans (Li et al., 2007). Furthermore, sec3Δ mutants could initiate apparently normal germ tubes, but when the septin collar formed, growth became isotropic such that the tip became swollen—a phenotype reminiscent of that of the ccn1Δ and cdc11 S394A mutants. Thus Sec3 is required for polarized growth, but only after the septin collar forms. Perhaps Cdc11 in the septin collar competes with the hyphal tip for Sec3 localization. Phosphorylation by Gin4 and Cdc28/Ccn1 may weaken the affinity of Cdc11 for Sec3, ensuring that tip localization predominates.

REFERENCES


Figure 1. Septin Organization in C. albicans Yeast, Pseudo hyphae, and Hyphae

Septins (green) form a collar at the bud neck of yeast (A) and pseudohyphae (B) that splits into two rings during cytokinesis. Septin bars appear at the base of a hyphal germ tube and a septin cap at its tip (C). As the germ tube elongates, a septin collar appears along its length (D), which split into rings; the primary septum (blue) forms between the two rings (E). Septin is visualized as Cdc10-YFP; cells are counterstained with Calcofluor white. Scale bars: (A), (B), and (D), 5 μm; (C) and (E), 1 μm. All images are by P. Sudbery.