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Investigating Filamentous Growth and Biofilm/Mat Formation in Budding Yeast

Paul J. Cullen[1]

Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14260

Abstract

In response to nutrient limitation, budding yeast can undergo filamentous growth by differentiating into elongated chains of interconnected cells. Filamentous growth is regulated by signal transduction pathways that oversee the reorganization of cell polarity, changes to the cell cycle, and an increase in cell adhesion that occur in response to nutrient limitation. Each of these changes can be easily measured. Yeast can also grow colonially atop surfaces in a biofilm or mat of connected cells. Filamentous growth and biofilm/mat formation require cooperation among individuals; therefore, studying these responses can shed light on the origin and genetic basis of multicellular behaviors. The assays introduced here can be used to study analogous behaviors in fungal pathogens, which require filamentous growth and biofilm/mat formation for virulence.

INTRODUCTION

Microbial species use diverse strategies to compete for nutrients. Being nonmotile, fungal microorganisms have developed a unique behavior, called filamentous growth, in which cells change their shape and band together in chains or filaments to scavenge for nutrients. Many fungal species can also grow in interconnected mats of cells called biofilms. The budding yeast *Saccharomyces cerevisiae* shows these behaviors, providing a genetically tractable system to study the pathways that control nutrient-dependent foraging. Studies on filamentous growth have provided insights into how eukaryotic cells differentiate and cooperate with each other, and how genetic pathways control fungal pathogenesis. Fungal pathogens require filamentous growth and biofilm formation for virulence.

Filamentous Growth

In budding yeast, filamentous growth is triggered by nutrient limitation (Cullen and Sprague 2012). In particular, depletion of glucose or fixed nitrogen induces filamentous growth in both haploid and diploid cells (Cullen and Sprague 2002). The balance of the cell's nutrient levels is critical for commitment to the filamentous growth program: complete removal of nutrients triggers entry into stationary phase (G_0) in both haploids and diploids, and in diploids, depletion of both carbon and nitrogen sources induces sporulation (Neiman 2011). The decision of whether or not to undergo filamentous growth, and the coordination of the

[1]Correspondence: pjcullen@buffalo.edu.

response itself, is regulated by signal transduction pathways. Among the pathways that regulate filamentous growth are the RAS protein kinase A (PKA) pathway (Gimeno et al. 1992) and a mitogen-activated protein kinase (MAPK) pathway called the filamentous growth pathway (Roberts and Fink 1994). These pathways regulate changes in gene expression and alter the activity of target proteins, leading to the construction of a new cell type.

Although filamentous growth is thought to be a complex differentiation response, it includes three easily observable changes in cells (Fig. 1). First, cell polarity is altered. Cell polarity is determined by bud-site-selection proteins, which mark the different poles of the cell (Park and Bi 2007) and direct growth in different directions. Haploid cells bud in an axial pattern, and diploid cells in a bipolar pattern (Chant and Pringle 1995). During filamentous growth, both haploid and diploid cells switch to a distal-unipolar pattern [Fig. 1; (Gimeno et al. 1992; Roberts and Fink 1994; Cullen and Sprague 2002)] by using the distal-pole landmark Bud8 (Harkins et al. 2001). Second, an increase cell length is observed, resulting from a delay in the G_2 phase of the cell cycle [Fig. 1 (Kron et al. 1994)]. Finally, cells remain attached to each other (Fig. 1). Unlike yeast-form cells that fully separate after cytokinesis, filamentous cells retain connections between proteins and carbohydrates on the cell wall. One such protein, Flo11, is a mucin-like flocculin and the major cell adhesion molecule that regulates cell–cell adherence (Lambrechts et al. 1996; Lo and Dranginis 1998; Guo et al. 2000; Halme et al. 2004). Together, these changes clearly denote cells undergoing filamentous growth.

Biofilm/Mat Formation

Budding yeast can also undergo biofilm/mat formation (Reynolds and Fink 2001; Vachova et al. 2011), an ancient microbial response that involves regulating colonial growth at the level of cellular connectivity. In addition to its role in filamentous growth, Flo11 is also required for biofilm/mat formation. Flo11 specifically regulates the complex colony morphology of biofilm/mats (Granek and Magwene 2010), their rim-and-spoke pattern (Reynolds and Fink 2001), and the expansion of mats across surfaces (Reynolds and Fink 2001). Flo11 mediates cellular “sliding” in part because the protein is shed from cells, thereby attenuating cell adhesion and potentially conferring a cellular lubrication property (Karunanithi et al. 2010). Filamentous growth and biofilm formation have distinct regulatory features (Sarode et al. 2011; Ryan et al. 2012), yet are related in that both occur in response to nutrient limitation and require overlapping signaling pathways and target proteins. Under some conditions, filamentous growth and biofilm formation occur in concert, which indicates that these behaviors may represent aspects of a global foraging response (Karunanithi et al. 2012).

TECHNICAL APPROACHES

The associated protocols describe assays to measure and quantitate the changes that occur during filamentous growth and biofilm formation in yeast. The assays are designed to distinguish between phenotypes showed in high- and low-nutrient environments and between wild-type and mutants strains. A key feature of several of these assays is their simplicity. The plate washing assay (see Protocol: The Plate-Washing Assay: A Simple Test

for Filamentous Growth in Budding Yeast [Cullen 2015a]) and biofilm/mat assay (see Protocol: Biofilm/Mat Assays for Budding Yeast [Cullen 2015b]) require minimal reagents and measure changes in colony patterns that are visible to the naked eye and interpretable without specialized equipment. In the single-cell invasive growth assay and pseudohyphal growth assay (both presented in Protocol: Evaluating Filamentous Yeast Growth at the Single-Cell Level [Cullen 2015c]), microscopic examination of cells allows quantitation of changes in budding pattern and cell length that occur during filamentous growth.

Three related assays measure the activity of the MAPK pathway that controls filamentous growth (all are presented in Protocol: Evaluating the Activity of the Filamentous Growth MAPK Pathway in Yeast [Cullen 2015d]). First, detecting phosphorylated MAPKs in yeast by western blotting using commercially available antibodies provides a direct measure of MAPK activity. Second, the pectinase assay measures the enzymatic activity of a secreted pectinase that is a target of the filamentous growth pathway. Finally, during filamentous growth, Flo11 (mentioned above) and other mucin-like proteins are shed from cells. Measuring Flo11 shedding provides information about protein levels and biofilm/mat patterning. Another mucin-like protein, Msb2, is the signaling glycoprotein that regulates the filamentous growth pathway (Cullen et al. 2004). Cleavage and release of the extracellular inhibitory domain of Msb2 is required for MAPK activity (Vadaie et al. 2008), which also corresponds to filamentous growth MAPK activity. Secretion profiling of yeast mucin-like proteins provides information about the role of MAPKs in the regulation of filamentous growth.

Finally, the *FLO11* gene is regulated by a large and complex promoter where multiple signals converge (Rupp et al. 1999). Measuring changes the expression of *FLO 11* (using techniques not described here) can provide a diagnostic readout of changes in the filamentous growth response.

Most yeast strains used in the laboratory do not show filamentous growth because they have acquired mutations as a result of genetic manipulation (Liu et al. 1996). The filamentous (Σ1278b) background is typically used to study filamentous growth (Gimeno et al. 1992). The genome sequence of the Σ1278b background is available (Dowell et al. 2010) as it is a collection of ordered deletion mutants (Ryan et al. 2012). These tools facilitate the genetic analysis of this growth response.

CONCLUSIONS

The current picture of filamentous growth is a complex one, in which multiple pathways and hundreds of targets coordinate a highly integrated response that we are only beginning to understand. Future studies of filamentous growth will aid in the understanding of the genetic basis of cell differentiation, development, and the regulation of multicellularity in eukaryotes. The assays described in the associated protocols are attractive in terms of their simplicity and potential use as teaching tools. Their versatility furthermore allows analysis of filamentous growth and biofilm formation in diverse fungal species including pathogens.

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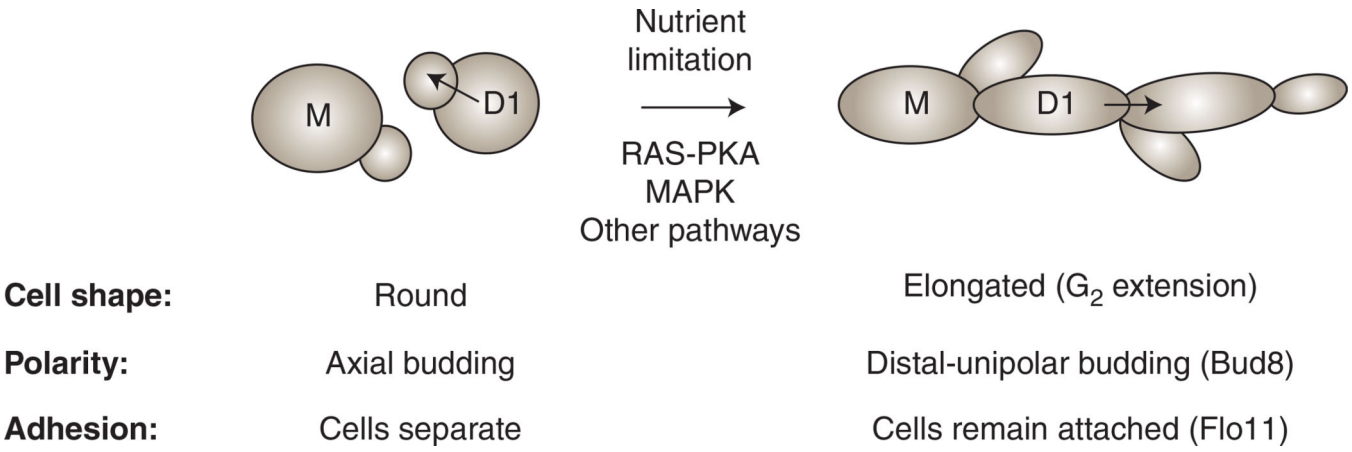

FIGURE 1.

Morphological changes that occur during filamentous growth in yeast. Under nutrient-rich conditions (*left*), yeast-form cells are round in shape and produce daughters that fully separate from their mothers. In haploid cells (shown), daughter cells (D1) bud back toward the mother cell (M) by axial budding (arrow toward left). Under nutrient-limiting conditions (*right*), cells become elongated and remain attached through Flo11. Daughter cells bud away from the mother cell (arrow toward right) by distal-unipolar budding by using the distal landmark Bud8. Signal transduction pathways (RAS-PKA and MAPK) regulate these changes.