**REVIEW
ARTICLE****Sensing, signalling and integrating physical processes during *Saccharomyces cerevisiae* invasive and filamentous growth**Sean P. Palecek,¹ Archita S. Parikh² and Stephen J. Kron^{2,3}

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Keywords: cell polarity, cell adhesion, pseudohyphal growth, MAPK, cAMP

Introduction

Simple fungi have evolved sophisticated mechanisms to sense and respond to environmental cues by activating developmental switches that result in coordinated changes in cell physiology, morphology and cell adherence. Critical depletion of nutrients often induces growth arrest to form spores capable of tolerating a wide range of environmental stresses. However, an alternative response is the dimorphic switch to filamentous growth, characterized by branching networks of chains of cells or hyphae to form a mycelium (Gancedo, 2001; Kron & Gow, 1995). Filamentous growth is considered an important adaptive response that functions analogously to cell motility in allowing a starving fungal colony to forage for nutrients (Lee & Elion, 1999). Branching filaments permit wider exploration of the environment at a lower biomass (energy) cost than nonfilamentous growth. Cell–cell adherence and highly polarized growth promote invasion of the substrate (Gancedo, 2001). Further, the high surface-to-volume ratio of filaments may facilitate transport of nutrients. The dimorphic switch is essentially modular and may be activated by a wide range of stimuli appropriate to the lifestyle of the fungus. Typically, the dimorphic switch in pathogenic fungi is tuned such that cells elongate or increase cell–cell adherence when exposed to their host (San-Blas *et al.*, 2000; Sanchez-Martinez & Perez-Martin, 2001). Such regulated dimorphism has been established as an important virulence factor, determining invasion and colonization by pathogenic organisms such as *Candida albicans*, *Magnaporthe grisea* (rice blast) and *Ustilago maydis* (corn smut) (Lengeler *et al.*, 2000; Sanchez-Martinez & Perez-Martin, 2001). Thus the dimorphic switch may prove to be an attractive target for chemical intervention in the prophylaxis and treatment of fungal infections in medicine and agriculture.

In turn, many industrial biochemical processes employ dimorphic yeasts and filamentous fungi to drive production of desired chemical compounds. Fermentation of carbohydrates in fruits, grains and other biomass to ethanol by *Saccharomyces cerevisiae* is the critical process for a wide range of products from fine wines to gasoline additives (Bothast *et al.*, 1999; Pretorius, 2000). *S. cerevisiae* and other simple fungi are also widely utilized in industrial production of small-molecule metabolites such as citrate and amino acids, bioremediation of toxic contaminants, fermentation of foodstuffs, production of complex organic compounds such as antibiotics, growth of starting material for protein purifications, and expression of heterologous proteins for vaccines, drugs and enzymes (Bennett, 1998; Chartrain *et al.*, 2000; Ostergaard *et al.*, 2000). The manner of organism proliferation, whether in a dispersed or aggregated form, is potentially a limiting factor in production of the desired metabolite(s), utilization of the growth medium and/or separation of cell mass from the soluble product. The classic example of self-clearing of beers at the end of the fermentation by the flocculation and settling of ale yeast underlines that control over cell morphology and/or cell–cell adhesion is a highly desirable characteristic of an industrial micro-organism (Hammond, 1995; Straver *et al.*, 1993). A detailed understanding of how metabolite sensing and signalling leads to changes in physical properties of cells will be critical for cellular engineering approaches to control dimorphism. For example, maintaining cell dispersion is desired during a fermentation to maximize mass transport and reaction rates but inducing flocculation can facilitate separation of the cells from fermentation products. In turn, it should be kept in mind that adhesion and morphology are not the only characteristics that change during the dimorphic transition. Many fungi also switch their patterns of metabolic conversions, gene expression and protein secretion (Viard & Kuriyama,

Fig. 1. Diploid *S. cerevisiae* grows as mounds of independent yeast-form cells on the surface of rich media (a). When starved for nitrogen, these cells switch to pseudohyphal growth, which is characterized by branching chains of cells that spread over and into the agar (b). In comparison, the dimorphic switch induced by nitrogen starvation in haploid *S. cerevisiae* leads to limited changes in cell morphology and the formation of short filaments (c). However, mutants lacking *SIR4* and expressing both mating loci display the nearly full expression of the filamentous phenotype in haploid strains (d).

1997; Wartmann & Kunze, 2000). Yeast form and filamentous cells also differ in their expression and accumulation of undesirable cell constituents and the ease with which cells can be disrupted or fractionated (Gibbs *et al.*, 2000; Khale & Deshpande, 1992; San-Blas, 1993).

Clearly, active control of dimorphic growth can take advantage of these circuits to increase uptake of reactants or secretion of products in industrial applications or to potentially provide new strategies to combat colonization or invasion by dimorphic fungi. As a first approach to molecular dissection of fungal dimorphism, we and others have turned to laboratory strains of the budding yeast *S. cerevisiae* and have examined the molecular and genetic determinants of pseudohyphal growth. In its starvation-induced filamentous differentiation response, *S. cerevisiae* switches from growth as mounds of independent yeast-form cells on the surface of agar media (Fig. 1a) to branching chains of cells that spread over and into the agar (Fig. 1b). Yeast-form growth of diploid *S. cerevisiae* is characterized by ovoid-shaped cells, a G_1 cell cycle delay, asymmetric cell division, bipolar bud site selection and cell separation following mitosis. Filamentous or pseudohyphal growth in diploid *S. cerevisiae* cells is characterized by cell elongation, a mitotic delay, symmetric cell division, unipolar budding and persistent physical attachment of mother and daughter cells (Gimeno *et al.*, 1992; Kron *et al.*, 1994).

In comparison to diploid cells, dimorphic switching in haploid *S. cerevisiae* is more subtle, leading to increased cell–cell adhesion but only limited changes in cell morphology (Fig. 1c). However, mutations in genes regulating mating type permit the nearly full expression of the filamentous phenotype in haploid strains (Fig. 1d). Since the discovery of the *S. cerevisiae* pseudohyphal differentiation response to nitrogen starvation a decade ago (Gimeno *et al.*, 1992), this phenomenon has been characterized extensively (Gancedo, 2001; Lengeler *et al.*, 2000; Pan *et al.*, 2000). Other signals such as poorly utilized carbon sources also induce filamentous growth. Much of the signal transduction mechanism has been mapped, from the nutrient receptors to transcription factors. Finally, molecular effectors of the physical components of pseudohyphal growth have been uncovered. This review details the current state of knowledge of environmental sensing, signal transduction and the physical mechanisms of filamentous growth in *S. cerevisiae*.

Environmental sensing mechanisms

Nitrogen starvation

Laboratory culture conditions for *S. cerevisiae*, whether in liquid media or on solid agar, have been developed to optimize cell growth. Typically, these conditions initially provide an excess of all necessary nutrients. Cells respond to such rich media containing abundant nitrogen sources and a fermentable carbon source by adopting a dispersed yeast form and growing rapidly, with the fermentation mixed actively by evolved carbon dioxide and/or mechanical agitation. A similar pattern is observed during the early stages of fermentation of beer worts and wine musts (Kleyn & Hough, 1971). However, once depletion of the media results in sufficiently low nutrient concentrations to limit cell growth, yeast undergo divergent responses that reflect the nutrients available in the media and the cell's micro-environment. At the diauxic shift, when fermentable carbon sources are exhausted, cells transiently arrest and then reprogram their metabolic patterns to metabolize ethanol and continue slow, vegetative growth (Russell *et al.*, 1993). Coincident with this switch, cells may become flocculent or change morphology. Later in the fermentation, when confronted by oxygen depletion, critical deprivation of a complex nutrient or complete carbon or nitrogen source starvation, haploid and diploid cells arrest in stationary phase, a G_0 exit from the cell cycle. During conditions of moderate nitrogen starvation and when nonfermentable carbon sources and oxygen are available, diploid cells can enter meiosis and perform sporulation before arresting. However, during moderate nitrogen limitation in the presence of adequate fermentable carbon source, a situation that wild yeasts may experience when colonizing ripened fruits, yeast continue slow vegetative growth and perform a dimorphic switch (Gimeno *et al.*, 1992). Diploid cells switch from yeast form to invasive and/or filamentous growth while haploid cells adopt an invasive

growth phenotype (Gimeno *et al.*, 1992), forming a film that spreads over and into the solid substrate.

The high-affinity ammonium permease Mep2 is one receptor that cells use to detect nitrogen compounds. Strains lacking Mep2 can acquire ammonium and grow at normal rates but do not form filaments in response to ammonium limitation (Lorenz & Heitman, 1998b). These results suggest that Mep2 senses ammonium starvation and produces a signal leading to filamentous differentiation. Mep1, a low-affinity permease, may also play a role in nitrogen sensing since deletion of *MEP1* allows downstream activation of filament formation in a strain lacking Mep2 (Lorenz & Heitman, 1998a). Perhaps Mep1 and Mep2 signals are integrated to provide a more accurate measure of nitrogen availability.

Amino acid concentrations also affect filamentous growth, either by providing a nitrogen source or via an independent signalling mechanism. For example, mutations in the *SSY1* amino acid permease or the *PTR3* peptide permease regulator induce elongation and enhance invasive growth in haploid strains (Klasson *et al.*, 1999). Mutations in *SHR3*, which is involved in processing amino acid permeases, also enhance filamentous growth (Gimeno *et al.*, 1992). The amino acid sensor may be intracellular. The 'charge' of the glutamine tRNA isoform tRNA-CUG is a candidate as a mutation in this tRNA results in pseudohyphal growth and sporulation at high nitrogen concentrations (Murray *et al.*, 1998). Similarly, components of nitrogen catabolism also play a role in filamentous growth and may be involved directly or indirectly in sensing nitrogen concentrations and transmitting filamentous growth signals. In the nitrogen regulation pathway, the regulator *MKS1* acts upstream of the transcription factors *URE2* and *GLN3* to promote transcription of *DAL5*, an allantoinase and ureidosuccinate permease. Overproduction or depletion of *Mks1* reduces filamentation on low nitrogen media (Edsikes *et al.*, 1999). Deletion of *URE2* and *GLN3* also results in the loss of filament formation in response to low nitrogen (Lorenz & Heitman, 1998b). A key issue in understanding nitrogen regulation of filamentous growth will involve differentiating nitrogen uptake and assimilation versus nitrogen sensing and signalling. Separate pathways might exist, or regulation of each might be integrated. A more in-depth study of this process with new genetic tools will be required to answer this question.

Carbon source regulation

Another receptor necessary for pseudohyphal differentiation is Gpr1, a cell surface G-protein-coupled receptor that binds to the heterotrimeric GTP-binding protein alpha subunit Gpa2 (Xue *et al.*, 1998; Yun *et al.*, 1998). Strains lacking Gpr1 are defective in both diploid filament formation and haploid invasive growth (Lorenz *et al.*, 2000b; Tamaki *et al.*, 2000). Nitrogen starvation induces *GPR1* transcription (Xue *et al.*, 1998) but the

primary function of Gpr1 appears to be glucose sensing. Gpr1 binds glucose with a very low affinity and activates cAMP signalling in a Gpa2-dependent manner (Lorenz *et al.*, 2000b; Rolland *et al.*, 2000; Yun *et al.*, 1998). Gpr1 may monitor glucose levels necessary for filamentous growth, or may integrate glucose and nitrogen signals in the pseudohyphal pathway.

A role for glucose transporters during filamentous growth has not been identified. However, mutations in mannose utilization and protein glycosylation pathways activate a mitogen activated protein kinase (MAPK) signalling pathway used in invasive and filamentous growth (Cullen *et al.*, 2000). Also, metabolic enzymes may directly or indirectly regulate filamentous phenotypes. For example, a mutation in the NADP-linked glutamate dehydrogenase *GDH3* induces elongation and polarized budding in haploid cells (Wilkinson *et al.*, 1996). In addition, mutations in genes encoding phosphoglucose isomerase (*PGI1*), 6-phosphogluconate dehydrogenase (*GND1*) and alcohol dehydrogenase (*ADH1*) constitutively activate invasive growth in haploid strains and enhance pseudohyphal development in nitrogen-deprived diploids (Palecek *et al.*, 2000).

One aspect of carbon source regulation of haploid invasive growth requires Snf1-mediated gene repression (Carlson, 1999; Gancedo, 1998). In the absence of glucose, cells elongate, the budding pattern polarizes and cells invade agar (Cullen & Sprague, 2000). Snf1 is required for expression of glucose-repressed genes responsible for invasive growth in the absence of glucose, perhaps via the transcription factor Sip4 (Cullen & Sprague, 2000). It remains to be determined which genes are regulated by Snf1 and which of these may contribute to cell invasion.

Starch degradation also appears to be linked to pseudohyphal development. Transcription of the glycoamylase-encoding genes *STA1–3* is repressed by *STA10*, but restored by overexpression of *MSN1* (Lambrechts *et al.*, 1996). Interestingly, *MSN1* overexpression also induces filamentous and invasive growth. Therefore, *STA1–3* may be coregulated with effectors of filamentous growth (Gagiano *et al.*, 1999), possibly suggesting an evolutionary development of invasive growth based on carbon metabolism. These recent studies demonstrate that carbon source metabolism is as important as nitrogen concentrations in determining cell growth states. In fact, the two signals likely act in concert to determine cell morphology.

Taken together, studies on how nitrogen and carbon starvation induce invasive and filamentous growth suggest multiple regulatory points in each pathway, and crosstalk between the pathways. Changing any single component may rearrange metabolic fluxes in a manner that is difficult to predict. New approaches to metabolic systems modelling and design (Christensen & Nielsen, 2000; Schilling *et al.*, 1999) will likely contribute to identifying the particular components that signal invasion and filamentation, and offer predictions for how to regulate intracellular activities of those components.

Fig. 2. cAMP-PKA pathway regulates filamentous growth in *S. cerevisiae*. Glucose and nitrogen sensors stimulate cAMP production and activate PKA signalling. PKA activates transcription of genes regulated by filamentous response elements (FREs).

Signal transduction during filamentous growth

The signalling pathways responsible for regulating filamentous growth are quite complex, involving several parallel pathways encompassing crosstalk and feedback. Yet, considerably more is known about the signal transduction pathways than the sensors and effectors of pseudohyphal differentiation. The two best characterized pathways regulating filament formation are the cAMP-dependent protein kinase (Protein Kinase A, PKA) and STE MAPK cascades (reviewed by Borges-Walmsley & Walmsley, 2000; D'Souza & Heitman, 2001; Lengeler *et al.*, 2000; Pan *et al.*, 2000; Posas *et al.*, 1998).

PKA-stimulated filamentous growth

In yeast vegetative growth, activation of Ras2 stimulates the adenylate cyclase Cyr1 and elevates intracellular cAMP concentrations (see Fig. 2). cAMP activates the yeast PKAs, each of which consists of one of three catalytic subunits, Tpk1–3, that form a complex with a single cAMP-binding regulatory subunit, Bcy1. Ras2 is required for pseudohyphal growth and a dominant active *RAS2*^{Val19} allele induces filamentous growth even in the absence of nitrogen starvation (Gimeno *et al.*,

1992). The role of PKA during filamentous growth is more complex. Mutations in *BCY1* activate PKA and enhance filamentous growth at both low and high nitrogen concentrations (Pan & Heitman, 1999). Tpk2 is required for filamentous growth but Tpk1 and Tpk3 are not (Robertson & Fink, 1998; Robertson *et al.*, 2000). In fact, mutations in *TPK2* repress filamentation while mutations in *TPK1* and *TPK3* enhance filamentous growth (Pan & Heitman, 1999). Furthermore, mutations in *TPK2* are epistatic to mutations in *TPK1* and *TPK3*, suggesting that Tpk1 and Tpk3 exert negative feedback on cAMP production, preventing activation of Tpk2. Interestingly, overexpression of the Yak1 kinase, an antagonist of the cAMP-PKA pathway, enhances pseudohyphal growth in response to nitrogen starvation (Zhang *et al.*, 2001). In the absence of glucose, Yak1 localizes to the nucleus where it phosphorylates Pop2, a transcriptional regulator of glucose-repressed genes (Moriya *et al.*, 2001). Thus Yak1 may be a key link between glucose sensing and invasive or filamentous growth.

The Gpr1 receptor and Gpa2 G-protein also activate adenylate cyclase and PKA but do not appear to function upstream of Ras2. Incubation with cAMP or deletion of the *PDE2* phosphodiesterase each restores filamentous growth to a *gpa2Δ/gpa2Δ* strain (Kubler *et al.*, 1997; Lorenz & Heitman, 1997). *BCY1* mutations also enhance filamentous growth in *GPA2* and *GPR1* mutant strains (Pan & Heitman, 1999). A surprising role for the Plc1 phospholipase C may be to regulate activation of Gpa2 by Gpr1. *PLC1* deletion, which prevents Gpr1 from associating with Gpa2, also inhibits filament formation. Plc1 activity may have a role in coupling carbon and nitrogen signalling at the sensor level (Ansari *et al.*, 1999).

MAPK-stimulated filamentous growth

In addition to stimulating PKA activity, Ras2 also affects filamentous growth via a MAPK signalling cascade (Mosch *et al.*, 1999) (Fig. 3). The filamentous MAPK cascade contains the protein kinases Ste20 (MAPKKKK), Ste11 (MAPKKK), Ste7 (MAPKK), Kss1 (MAPK) and the transcription factor Ste12 (Posas *et al.*, 1998).

Kss1 can both activate and inhibit filamentous growth. Unphosphorylated Kss1 binds to and prevents Ste12 from activating transcription of genes contributing to pseudohyphal growth. Conversely, Ste7 phosphorylation of Kss1 relieves its inhibition of Ste12 in addition to activating Kss1 (Bardwell *et al.*, 1998; Cook *et al.*, 1997), which further increases transcription through Ste12. Two additional proteins, Dig1 and Dig2, are required for Kss1-mediated repression of Ste12. A *dig1Δ dig2Δ* double mutant strain invades constitutively (Cook *et al.*, 1997). Interestingly, *KSS1* deletion does not affect invasive growth in this mutant, perhaps since the repression and activation functions of Kss1 are both abrogated. This mutation does provide a useful means for shielding transcriptional events from signalling

Fig. 3. MAPK pathway regulates filamentous growth in *S. cerevisiae*. External signals activate Ras2 and the MAPK signalling cascade to induce transcription of genes regulated by filamentous response elements (FREs).

through the MAPK filamentous growth pathway since the mechanisms required for filamentous growth are still in place, but elements downstream of Kss1 are isolated from any upstream activation or repression. The activation–repression system of Kss1 may provide a mechanism for cells to isolate invasive and pseudohyphal growth signals through the MAPK cascade from pheromone and osmotic shock signals through the same pathway. Mating pheromone activates Kss1, but Fus3 blocks invasive growth initiation via this activation (Sabbagh *et al.*, 2001). Interestingly, transcription and activity of the Ty1 retrotransposon is also regulated reciprocally by the invasive growth and mating pathways. Kss1 activates invasive growth and Ty1 transposition through Ste12 and Tec1 (Morillon *et al.*, 2000) while the Fus3 MAPK inhibits both invasive growth and Ty1 transposition (Conte & Curcio, 2000).

The PAK kinase Ste20 and the MEK kinase Ste11 appear to integrate multiple signals to modulate activation of the filamentous MAPK pathway. Dominant active alleles of the Rac/Rho GTPase *CDC42* induce constitutive pseudohyphal differentiation while dominant negative alleles of *CDC42* block filamentation in re-

sponse to the activated *RAS2^{Val19}* allele (Mosch *et al.*, 1996). When bound to GTP, Cdc42 binds and activates Ste20, and requires Ste20 to induce filamentous growth (Peter *et al.*, 1996). Several *CDC42* mutations block elongation in response to low nitrogen but do not affect cell morphology in rich media, placing *CDC42* in the nitrogen signalling pathway (Mosch *et al.*, 2001). Two-hybrid analysis of *cdc42* mutants suggests that Gic1, Gic2 and the PAK-like protein kinase Skm1, in addition to Ste20, may function as effectors of Cdc42 in the pseudohyphal and invasive pathways. The yeast 14-3-3 proteins Bmh1 and Bmh2 also associate with Ste20 and are essential for pseudohyphal growth in strains carrying activated alleles of *CDC42* or *RAS2* (Roberts *et al.*, 1997). Ste50 binds to Ste11 and is required for activation during filamentous signalling and other responses (Jansen *et al.*, 2001).

Cell cycle signals may also impinge on activation of the MAPK pathway at the level of Ste20. Overexpression of the *G₁* cyclins *CLN1* and *CLN2* enhances filamentous and invasive growth, represses pheromone signalling, and results in phosphorylation of Ste20 (Fujita *et al.*, 1999; Madhani *et al.*, 1999; Oehlen & Cross, 1998). However, another study demonstrated that *Cln1* and *Cln2* act in parallel to the STE MAPK pathway during filamentous growth (Loeb *et al.*, 1999). Hsl7, a negative regulator of the Swe1 protein kinase, apparently also binds to Ste20 and Cdc42 (Fujita *et al.*, 1999). *HSL7* deletion induces cell elongation and filament formation in rich medium, but this phenotype is diminished by a *STE20* deletion.

Transcriptional pathways connecting signalling to gene expression in filamentous growth

The transcription factor Tec1 is required for filamentous growth stimulated by the MAPK cascade but not by activated PKA (Gavrias *et al.*, 1996; Pan & Heitman, 1999). Both Ste12 and Tec1 bind to filamentous and invasion response elements (FREs), including an FRE in the *TEC1* promoter (Madhani *et al.*, 1997). Therefore, positive feedback appears to be present in filamentous signalling at the transcriptional level and may serve to amplify the filamentous growth signal. The transcription factor Flo8 is also required for pseudohyphal and invasive growth (Liu *et al.*, 1996) by regulating expression of the flocculins *FLO1*, 9 and 11 (Kobayashi *et al.*, 1999) as well as *STA1–3* (Gagiano *et al.*, 1999). Flo8 appears to regulate invasive and filamentous growth in a pathway independent from Ste12 but acts downstream of the cAMP-PKA pathway (Rupp *et al.*, 1999).

Phd1 is a transcription factor that induces filamentous differentiation when overexpressed (Gimeno & Fink, 1994). *PHD1* transcription is induced by mating pheromones and nitrogen starvation, demonstrating another link between the mating and filamentous growth pathways (Erdman *et al.*, 1998). In combination with a *STE12* mutation, deletion of *PHD1* completely suppresses diploid filamentous growth, suggesting that it functions in a parallel pathway (Lo *et al.*, 1997). The

forkhead transcription factors Fkh1 and Fkh2 also regulate filament formation and invasive growth. Deletion of both *FKH1* and *FKH2* induces filamentous growth while a single deletion of either forkhead gene has no effect (Hollenhorst *et al.*, 2000). Fkh1 and Fkh2 regulate transcription of *CLB2* and other genes involved in mitosis that antagonize filamentous growth, further demonstrating the link between the cell cycle and pseudohyphal growth (Zhu *et al.*, 2000). The forkhead factors are required for proper expression of the transcription factors Swi5 and Ace2, antagonists of filamentous growth that direct expression of proteins involved in cell cycle exit and cytokinesis. The transcription factor Ash1 also functions late in the cell cycle, but its activity is instead necessary for pseudohyphal growth, perhaps to regulate interactions between mother and daughter cells (Chandarlapaty & Errede, 1998).

Transcriptional repression also regulates the activity of genes influencing pseudohyphal growth. For example, the transcriptional repressor Sfl1 interacts with the Tpk2 subunit of PKA, but not the Tpk1 or Tpk3 subunits (Robertson & Fink, 1998). Sfl1 also acts with the Ssn6–Tup1 general corepressor complex to regulate transcription of genes including *FLO11*, suggesting that Sfl1 mediates relief of transcriptional repression by cAMP signalling (Conlan & Tzamarias, 2001). Inactivation of Sok2 enhances invasion and filament formation, and Sok2 overexpression represses these processes. Sok2 appears to act downstream of the PKA subunits (Ward *et al.*, 1995) but also in a separate, PKA-independent pathway (Pan & Heitman, 2000). Genome array analysis of a *sok2Δ/sok2Δ* strain reveals that the transcription factors *PHD1*, *ASH1* and *SWI5* are normally repressed by Sok2 (Pan & Heitman, 2000). However, transcription of these genes is not affected by *TPK2* deletion.

The RNA polymerase II complex also regulates invasive and filamentous growth, perhaps by altering expression of effectors and repressors of these processes. Overexpression of the RNA polymerase subunit *RPB7* causes cell elongation and filament formation (Khazak *et al.*, 1995). Also, mutations in other components of the RNA polymerase II complex – Ssn3, Ssn8, Srb8, Cse2 and Med1 – constitutively activate invasive growth in haploids (Palecek *et al.*, 2000).

Other pathways involved in filamentous differentiation

A recent observation has been that stress-induced signalling has an important role in filamentous growth. In addition to nitrogen and carbon starvation, oxygen limitation may also affect dimorphic switching in both haploids and diploids (Wright *et al.*, 1993). Osmotic shock transduced via the Sho1 receptor may also enhance filamentous growth (O'Rourke & Herskowitz, 1998). Additionally, various alcohols also generate morphological changes in cells. Ethanol enhances diploid strain filamentation, and other alcohols including 1-

butanol, isoamyl alcohol and n-amyl alcohol stimulate filamentous growth in haploids growing in glucose-containing liquid medium (Lorenz *et al.*, 2000a). Perhaps a shared metabolic intermediate is responsible for generating a filamentous growth signal or the induction of filamentous growth may reflect that the alcohols serve as cellular 'irritants'. Skn7, a transcription factor that mediates oxidative stress responses downstream of PKA, is required for filament formation in response to nitrogen starvation (Lorenz *et al.*, 2000b). Mutations in *SKN7* can also induce constitutive invasive growth in haploids (Palecek *et al.*, 2000). In addition, the stress-responsive transcriptional repressor Xbp1 represses *CLB2* expression to induce cell elongation and filament formation in response to nitrogen starvation (Miled *et al.*, 2001). Ras2 activity also decreases the activity of the stress-responsive transcription factors Msn2 and Msn4. This suppressed stress response has been suggested to be responsible for inducing invasive growth (Stanhill *et al.*, 1999).

The unfolded protein response pathway transcription factor Hac1 plays an interesting role in pseudohyphal differentiation. Hac1 activates transcription of endoplasmic-reticulum-resident chaperones and is also stimulated by the unfolded protein response, a signal generated by unfolded proteins in the ER lumen. Deletion of *HAC1* derepresses filamentous growth while overexpression of *HAC1* represses pseudohyphae formation (Schroder *et al.*, 2000). *HAC1* mRNA is cleaved by Ire1 and spliced by tRNA ligase. High nitrogen concentrations stimulate this splicing, which ceases during nitrogen starvation. Therefore, nitrogen starvation may at least partially stimulate filamentous growth by slowing protein translation rates, inactivating the unfolded protein response, and inhibiting *HAC1* splicing (Schroder *et al.*, 2000).

Other yeast developmental responses, such as entering the sporulation pathway, also respond to environmental stresses. In general, sporulation occurs under conditions of nitrogen and carbon starvation while pseudohyphal differentiation is induced in nitrogen-poor but carbon-rich environments. This switch may be mediated by Gpa2, which, when overexpressed, reduces sporulation but enhances filamentous growth (Donzeau & Bandlow, 1999; Lorenz & Heitman, 1997). Gpa2 appears to direct cells toward pseudohyphal growth rather than sporulation by directly interacting with the Ime2 kinase (Donzeau & Bandlow, 1999). In turn, overexpression of Ime2 inhibits pseudohyphal growth but enhances sporulation in the presence of glucose and nitrogen while *RIM1*, 8, 9 and 13 mutations reduce *IME2* activity and switch cells from meiotic division to filamentous growth (Li & Mitchell, 1997). Mutations in *BIR1*, a gene homologous to inhibitor of apoptosis (IAP) proteins in vertebrates, also enhance pseudohyphal growth but block sporulation during carbon source deprivation (Uren *et al.*, 1999).

Not surprisingly, genes linking nutrient regulation to cell growth and division also affect filament formation. The abundance and activity of the G₁ cyclin Cln3 has

Fig. 4. Regulation of physical responses during filamentous growth. Cell adhesion, elongation and polarized bud site selection contribute to filamentous growth (a). Swe1 inhibits Clb2–Cdc28 activity to regulate apical growth and cell shape (b). Several different pathways affect invasive growth via bud site selection (c).

multiple connections to cell-size control and nutrient sensing during vegetative growth – Cln3 accumulates when cells have sufficient nutrients to continue growth. Deletion of *CLN3* confers enhanced pseudohyphal growth (Loeb *et al.*, 1999), suggesting that Cln3 abundance may carry part of the nitrogen starvation signal. Overexpression of *WHI2*, a gene required for cell cycle

arrest upon carbon source depletion, stimulates filamentous growth (Radcliffe *et al.*, 1997). However, *WHI2* is not required for pseudohyphal growth and is partially blocked by mutations in the STE MAPK pathway. Deletion of the *Whi3* RNA-binding protein abrogates filamentous growth, leading to small round cells on nitrogen starvation media (Mosch & Fink, 1997).

Table 1. Cell–cell adhesion

Gene name	Protein function	Role in invasion (mutant phenotype)
<i>ACE2</i>	Transcription factor; DNA-binding protein	Regulates <i>CTS1</i> transcription; involved in regulation of G_1 genes including <i>ASH1</i> , <i>CDC6</i> , <i>EGT2</i> , <i>PCL2</i> , <i>PCL9</i> , <i>RME1</i> and <i>SIC1</i> ; mutation enhances invasive and filamentous growth
<i>ASH1</i>	Transcription factor; repressor	Required for transcription of <i>FLO11</i> ; mutant defective in filamentous growth
<i>CTS1</i>	Endochitinase	Required for separation of mother and daughter cells following cytokinesis; diploid mutant displays enhanced filamentous growth
<i>EGT2</i>	Endoglucanase	Involved in separation of mother and daughter cells following cytokinesis; mutant diploid displays enhanced filamentous growth
<i>FLO8</i>	Transcription factor	Regulates <i>FLO11</i> transcription downstream of the PKA pathway; mutants defective in invasive and filamentous growth
<i>FLO11</i>	Cell-surface flocculin	Promotes agar invasion and cell adhesion; mutants defective in invasive and filamentous growth
<i>STE12</i>	Transcription factor	Regulates <i>FLO11</i> transcription downstream of the MAPK pathway; mutants defective in invasive and filamentous growth
<i>SWI5</i>	Transcription factor; DNA-binding protein	Involved in regulation of G_1 genes including <i>ASH1</i> , <i>CDC6</i> , <i>EGT2</i> , <i>PCL2</i> , <i>PCL9</i> , <i>RME1</i> and <i>SIC1</i> ; mutant diploid displays enhanced filamentous growth
<i>TEC1</i>	Transcription factor; DNA-binding protein	Regulates <i>FLO11</i> transcription downstream of the MAPK pathway; mutant diploid defective in filamentous growth

Invasive growth is inversely proportional to ploidy for haploid to tetraploid cells (Galitski *et al.*, 1999), suggesting a gene-dose-dependent regulation of invasive growth or a connection to cell size, which increases proportionally to ploidy. Similarly, deletion of *SIR2* or *SIR4* results in a loss of silencing of silent mating type cassettes, and expression of diploid specific genes in haploid cells. Consequently, *sir2* and *sir4* haploid mutants undergo polarized bud site selection and haploid invasive growth (Palecek *et al.*, 2000). These mutants also exhibit moderate pseudohyphal growth (Fig. 1), suggesting that mating type, in addition to ploidy, plays a role in filamentous growth.

Physical events during invasive and filamentous growth

The signalling pathways governing formation of filamentous networks of yeast cells ultimately affect physical properties of individual cells, such as adhesion, budding polarity and shape. How each of the pathways described above regulates each physical process remains somewhat of a mystery, but we are beginning to understand how these processes contribute to filamentous and invasive growth (see Fig. 4).

Adhesion and cytokinesis

Cell attachment is critical during both invasive and filamentous growth. Without sufficient attachment, filaments may not possess the mechanical integrity to penetrate their surrounding matrix. Attachment can be regulated in at least two different ways: persistence of adhesion following cell separation or incomplete mother–daughter separation. Genes regulating each of

these mechanisms are listed in Table 1. In *S. cerevisiae*, cell–cell adhesion that can be detected as formation of multicellular aggregates is mediated in part by flocculins, a family of highly glycosylated glycosylphosphatidylinositol-anchored proteins. Flo11 appears to be the most important flocculin during invasive and filamentous growth. Deletion of *FLO11* eliminates agar invasion as well as filament formation and cells grow in disorganized piles on the agar surface (Lo & Dranginis, 1998). In turn, the extent of filament formation and invasive growth increases as *FLO11* expression increases (Palecek *et al.*, 2000). Increasing *FLO11* expression polarizes cell shape, suggesting that Flo11 normally participates in determining polarity and morphology in addition to its role in adhesion and flocculation (Palecek *et al.*, 2000). Perhaps Flo11 serves a signalling function as well, feeding back to enhance cell elongation. Fig2, an adhesin required for mating, can partially substitute for Flo11-mediated adhesion when overexpressed in a *FLO11* mutant strain (Guo *et al.*, 2000), suggesting some redundancy among flocculins. *FLO11* is highly regulated, containing several repression and activation sequences in its promoter (Rupp *et al.*, 1999). Ste12 and Tec1 regulate transcription from the MAPK cascade downstream of Kss1 while Flo8 regulates Flo11 transcription downstream of PKA (Madhani *et al.*, 1999).

A lack of mother–daughter separation following cytokinesis may provide an alternative to cell–cell adhesion during filament formation. In pseudohyphal filaments, a complete septum is formed between mother and daughter cells at cytokinesis but it persists rather than being cleaved, maintaining a stable connection (Kron *et al.*, 1994). In the vegetative cell cycle, the Ace2 and Swi5 transcription factors induce the endochitinase Cts1 and

Table 2. Bud site selection

Gene name	Protein function	Role in invasion (mutant phenotype)
<i>ACT1</i>	Structural protein	Involved in cell polarity
<i>AXL1</i>	Protease; hydrolase	Required for axial budding pattern; required for cell fusion during mating; mutant haploids invade
<i>BEM1</i>	Unknown – localizes to bud tip	Required for polarized apical growth
<i>BNI1</i>	Unknown – localizes to bud tip and bud neck	Required for polarized apical growth and bipolar budding pattern in diploids
<i>BUD1/RSR1</i>	GTP-binding protein/GTPase	Required for bud site selection in both haploids and diploids; haploid and diploid mutants display random bud site selection; diploid mutants defective for pseudohyphal growth
<i>BUD2</i>	GAP	Required for bud site selection in both haploids and diploids; haploid and diploid mutants display random bud site selection
<i>BUD3</i>	Bud site marker	Required for bud site selection in haploids; mutants bud at distal site and invade agar
<i>BUD4</i>	Bud site marker	Required for bud site selection in haploids; mutants bud at distal site and invade agar
<i>BUD5</i>	GEF	Required for bud site selection in both haploids and diploids; haploid and diploid mutants display random bud site selection
<i>BUD6</i>	Unknown – localizes to bud neck	Required for bud site selection and polarized apical growth; diploid mutants display random bud site selection
<i>BUD8</i>	Bud site marker	Required for polar bud site selection in diploids; mutants bud at proximal pole and are noninvasive
<i>BUD9</i>	Bud site marker	Required for bipolar bud site selection in diploids; mutants bud at distal pole
<i>BUD10</i>	Unknown – localizes to bud site	Required for bud site selection in haploids
<i>CDC3</i>	Structural protein (septin)	Required for axial budding pattern in haploids; required for normal subcellular localization of Bni4p, Chs3p and Chs4p
<i>CDC10</i>	GTP-binding protein/GTPase; structural protein	Required for axial budding pattern in haploids; required for normal subcellular localization of Bni4p, Chs3p and Chs4p
<i>CDC11</i>	Structural protein (septin)	Required for axial budding pattern in haploids; required for normal subcellular localization of Bni4p, Chs3p and Chs4p
<i>CDC12</i>	Structural protein (septin)	Required for axial budding pattern in haploids; required for normal subcellular localization of Bni4p, Chs3p and Chs4p
<i>CDC24</i>	GEF	Required for budding and polarized apical growth

putative endoglucanase regulator Egt2 to cleave the septum in G₁ (Dohrmann *et al.*, 1996; Kovacech *et al.*, 1996). Mutating *CTS1* or *ACE2* induces invasive filamentous growth, even in a *flo8Δ* background (King & Butler, 1998). Similarly, mutations in *EGT2* enhance pseudohyphal growth in a *flo11Δ* strain (Pan & Heitman, 2000). Coupling septal maturation to cell adhesion, Swi5 regulates *FLO11* transcription as well, most likely via the transcription factor *ASH1* (Pan & Heitman, 2000).

Bud site selection

Haploid bud site selection is predominantly axial (proximal), with each new bud emerging adjacent to the birth scar that forms after cleavage of the septum at the mother–daughter neck. Table 2 lists genes that induce axial, polar or random bud site selection. Axial budding cells use a marker at the site of cytokinesis to direct assembly of budding components at the new bud site. These markers may include Bud3, Bud4, Bud10, Axl1

and the septins Cdc3, Cdc10, Cdc11 and Cdc12 (Madden & Snyder, 1998). Each of these proteins is necessary for proper axial bud site selection; deletion or mutations bias haploid cells toward distal pole budding but have no effect on bud site selection in diploids. In addition, cell wall synthesis proteins Kre9 and Hkr1 are involved in axial bud site selection (Brown & Bussey, 1993; Yabe *et al.*, 1996). Deletion of these proteins results in random budding. Pmt4, a protein mannosyl transferase that participates in O-linked glycosylation of secreted proteins, is also required for axial budding, probably via modification of Bud10 (Sanders *et al.*, 1999). Diploid cells normally undergo bipolar budding in which buds emerge somewhat stochastically either adjacent or opposite to the birth scar (reviewed by Chant, 1999; Madden & Snyder, 1998). A large group of proteins that are needed for bipolar budding has also been identified (e.g. Ni & Snyder, 2001). Cytoskeletal and cytoskeleton binding proteins are required for bipolar budding in diploids. Mutations in actin depolarize budding in mother cells but have no effect on daughter cell bud site

selection (Yang *et al.*, 1997). Similar budding defects occur when actin-binding proteins Bni1, Rvs167, Sac6 (fimbrin), Sla2, Srv2 and Tpm1 (tropomyosin) or actin-organizing proteins Sla1 and Rvs161 are mutated (Madden & Snyder, 1998; Mosch & Fink, 1997; Yang *et al.*, 1997). Another class of proteins required for both axial and distal bud site selection constitute a GTPase module. Rsr1 is the GTPase, Bud2 the GTPase-activating protein (GAP) and Bud5 the guanine nucleotide exchange factor (GEF) and together the complex directs bud assembly and growth at the proper location. Mutation in any component of this module causes random budding in both haploids and diploids and moderately inhibits invasive and filamentous growth (Madden & Snyder, 1998; Taheri *et al.*, 2000). In addition, Rsr1 interacts with the Cdc42 Rac/Rho-type GTPase, its GEF Cdc24 and Bem1, and is thought to direct polarized growth to the tags marking the appropriate bud site.

In diploid pseudohyphal growth in response to nitrogen starvation, bud site selection becomes skewed to a unipolar distal pattern where each new bud emerges from the pole opposite the birth scar (Gimeno *et al.*, 1992; Kron *et al.*, 1994). Mutations in genes required for bipolar budding in diploids such as *BUD1*, *BUD5* or *BUD8* prevent filament formation (Gimeno *et al.*, 1992; Lo *et al.*, 1997; Mosch & Fink, 1997). *BUD8* mutations switch diploid cells from bipolar bud site selection to proximal pole budding while mutations in *BUD9* favour distal bud site selection (Zahner *et al.*, 1996). Both Bud8 and Bud9 localize to the distal pole, suggesting that Bud8 serves as an initiator for distal bud site assembly while Bud9 is an inhibitor (Taheri *et al.*, 2000). Some mutations in *ACT1* also repress cell elongation and filamentous differentiation in response to low nitrogen (Cali *et al.*, 1998). Also, mutations in the actin-binding proteins Tpm1, Sac6, Srv2 and Bni1 prevent filament formation (Mosch & Fink, 1997). Tpm1 and Bni1 mutants can still invade agar, suggesting that the actin cytoskeleton controls physical processes regulating filamentation but does not affect invasive growth. Deletion of *MYO3* and *MYO5*, genes encoding myosin I proteins, also prevent cell elongation and polarized bud site selection (Goodson *et al.*, 1996). Other genes important in distal pole bud site selection include Spa2, Pea2 and Bud6/Aip3, which form a complex that localizes to budding and growth sites. Spa2 interacts with Ste11 and Ste7 while Bud6 binds Ste11 and actin, suggesting that the Spa2–Pea2–Bud6 complex may link filamentous growth signalling through the MAPK cascade to bud site selection. Deleting *SPA2*, *PEA2* or *BUD6* increases random bud site selection in mother cells, depolarizes cell surface expansion and reduces cell elongation (Sheu *et al.*, 2000; Zahner *et al.*, 1996). Cdc42 and its effector Gic2 are required for localization of Spa2 and Bud6 to bud sites (Jaquenoud & Peter, 2000). Spa2 and Sph1 may also be involved in localizing MAPK signalling during pseudohyphal growth. *SPH1* and *SPA2* double deletion cells cannot invade or form filaments (Roemer *et al.*, 1998). Sph1

interacts with Ste7 and Ste11, suggesting that the polarization effects of these proteins may be due to localized MAPK signalling; however, it is unclear whether *sph1 spa2* mutant phenotypes are due to signalling or physical defects, or both.

Polarized budding can promote haploid invasive growth provided a sufficient level of cell–cell adhesion exists. Mutations in *BUD3*, *BUD4*, *BUD10* and *AXL1* switch haploids to a polar budding pattern and turn on invasive growth independent of induction of Flo11, but they have no effect on filamentous growth in diploids (Palecek *et al.*, 2000).

Cell shape and polarity

Although cell attachment and bud site selection are sufficient for growth in chains, the full pseudohyphal phenotype also encompasses an elongation of the usual ovoid cell morphology. This regulation of cell shape is coupled to cell cycle progression. Buds emerge late during the G_1 phase of the cell cycle. Initially, buds grow apically with new material added at the bud tip. Eventually, growth becomes isotropic with material added throughout the bud. This switch between apical and isotropic growth affects cell shape and filament formation (reviewed by Pruyne & Bretscher, 2000). Disproportionate apical growth causes elongated cells while extended isotropic growth results in round cells. Molecular regulation of the cell cycle controls the switch between apical and isotropic growth, enhancing pseudohyphal differentiation via cell morphology. Table 3 summarizes genes involved in morphology regulation via the cell cycle. In general, Cln cyclins are polarizing while Clb cyclins are depolarizing. Overexpression of Cln1 or Cln2 promotes filamentous growth while deletion of G_1 cyclins Cln1 and Cln2 causes a severe defect in elongation and filamentation (Loeb *et al.*, 1999; Madhani *et al.*, 1999). Mutations in *GRR1* or *CDC53* may enhance apical growth, cell elongation and invasive growth in part by stabilizing G_1 cyclins. In contrast, deletion of the G_2 cyclin Clb2 enhances polarized growth and bud site selection while overexpression of *CLB2* suppresses elongation due to the ectopically activated MAPK pathway by reducing the G_2 /M delay in the cell cycle (Ahn *et al.*, 1999; Edgington *et al.*, 1999).

Septin mutations also enhance apical growth and bud elongation due to a G_2 delay (Longtine *et al.*, 2000). Improper septin assembly in the bud neck appears to activate the Swe1 kinase by inactivating Elm1, Hsl1 and Hsl7. Swe1 then phosphorylates Cdc28, inactivating the Clb–Cdc28 complex and delaying cell cycle progression. Analogous to the bud morphogenesis checkpoint, which limits mitosis unless a bud has formed, this pathway may represent a feedback circuit that restricts mitosis until a proper cytokinetic apparatus can be assembled. Mutations in *ELM1*, *HSL1* or *HSL7*, overexpression of *SWE1*, or deletion of the antagonistic phosphatase

Table 3. Cell morphology

Gene name	Protein function	Role in invasion (mutant phenotype)
<i>CDC53</i>	Structural protein	Inhibits apical growth; targets degradation of proteins, including G ₁ cyclins; haploid mutants elongated, bud at proximal site and invade agar
<i>CLB2</i>	G ₂ cyclin	Depolarizes actin; inhibits polarized growth and elongation; mutants display increased invasive and filamentous growth
<i>CLN1</i>	G ₁ cyclin	Required for initiation of DNA replication and for bud emergence; enhances apical growth and cell elongation; major G ₁ cyclin involved in filamentous growth; mutants defective in invasive and filamentous growth
<i>CLN2</i>	G ₁ cyclin	Required for initiation of DNA replication and for bud emergence; enhances apical growth and cell elongation; mutants defective in invasive and filamentous growth
<i>ELM1</i>	Protein kinase	Regulates Swe1; controls bud growth during mitosis; regulates Clb2–Cdc28; mutants invade agar and display enhanced filamentous growth
<i>GRR1</i>	Complex assembly protein	Inhibits apical growth; targets degradation of proteins, including G ₁ cyclins; haploid mutants elongated, bud at proximal site and invade agar
<i>HSL1</i>	Protein kinase	Inhibits Swe1; involved in transmitting nutritional information to Cdc28; involved in switching from polarized to isotropic bud growth; inhibits cell elongation and filamentous growth; mutants invade agar and display enhanced filamentous growth
<i>HSL7</i>	Repressor	Inhibits Swe1; inhibits cell elongation, polarized bud emergence and filamentous growth; mutants invade agar and display enhanced filamentous growth
<i>SWE1</i>	Protein kinase	Required for apical growth; inhibits Cdc28 and mitosis; mutants defective for filamentous growth

MIH1 all enhance elongation, polar bud site selection, filament formation and invasive growth via Swe1-dependent tyrosine phosphorylation and inactivation of Cdc28 (Ahn *et al.*, 1999; Bouquin *et al.*, 2000; Edgington *et al.*, 1999; Palecek *et al.*, 2000).

Other physical effects

Yeast–matrix interactions other than adhesion may also enable invasive or filamentous growth. The MAPK Kss1 regulates expression of *PGU1*, an enzyme that degrades polygalacturonic acid (Madhani *et al.*, 1999). Perhaps other matrix-degrading enzymes are also regulated by filamentous growth signalling pathways. Furthermore, cell wall composition may affect yeast–matrix interactions. Mutations in *DFG16* decrease invasive growth but do not affect cell morphology or polarity (Mosch & Fink, 1997). These *dfg16* strains exhibit an increased ratio of mannose to glucose in their cell wall and a decreased sensitivity to the cell-surface-perturbing chemical calcofluor white.

Conclusions and perspectives

Pseudohyphal development in *S. cerevisiae* appears to be more complex than a simple differentiation switch. The process integrates signals from several nutrients and metabolic byproducts to construct a physical response that alters the adhesion, budding polarity, morphology and cell cycle control of individual cells in order to organize them into a structured network. Signals travel

through two major pathways, cAMP-PKA and MAPK cascades, and elicit several distinct yet coregulated transcriptional responses that eventually affect how cells interact with each other and their environment.

Studies of filamentous and invasive growth of *S. cerevisiae* have also provided insight into the dimorphism of other fungal organisms, including pathogenic fungi and organisms used in biochemical processes (reviewed by Borges-Walmsley & Walmsley, 2000; Brown & Gow, 1999; Lengeler *et al.*, 2000). While the stimuli inducing filamentation in the different organisms are likely to be quite distinct, the signalling pathways and physical responses seem to be well conserved. In addition, the transition from yeast form to hyphal or pseudohyphal growth is linked to virulence in a number of pathogenic fungi. For example, in the animal pathogen *C. albicans*, a serum factor initiates hyphal growth in a Ras1-dependent manner (Feng *et al.*, 1999). The MAPK and PKA pathways also transmit filamentation signals in *C. albicans*. Deletion of the transcription factors Cph1 and Efg1, effectors of the MAPK- and PKA-signalling pathways, respectively, partly eliminates filamentation and reduces virulence in mice (Brown & Gow, 1999; Lo *et al.*, 1997).

Filament formation and virulence in the plant pathogen *U. maydis* also require the cAMP-PKA and MAPK pathways. However, in *U. maydis*, cAMP switches filamentous cells to a yeast-form phenotype, opposite of the effect seen in *S. cerevisiae* (Kronstad *et al.*, 1998). Likewise, activation of PKA suppresses filament forma-

tion and virulence (Durrenberger *et al.*, 1998). Disruption of the MAPK pathway by mutating the MAPKKK *UBC4*, MAPKK *UBC5* or MAPK *UBC3* disrupts filament formation and pathogenicity, suggesting that the MAPK pathway stimulates filamentous growth in *U. maydis* (Andrews *et al.*, 2000).

S. cerevisiae provides a genetically suitable organism for studying dimorphism of fungal organisms. Pathways and processes identified and characterized in *S. cerevisiae* will no doubt advance our understanding of analogous regulation of morphogenesis and pathogenicity in other organisms. In addition, this information may greatly facilitate the design of strains optimized for biochemical processing. Major challenges still remain in determining how cells sense filamentous growth signals from their environment, how various signalling cascades process and integrate filamentous growth signals, and how these signals manifest in physical processes leading to filament formation. Specifically, crucial challenges in the coming years from the sensory standpoint are to identify what metabolites cells sense and the receptors for these metabolites. At the signalling level, new strategies to identify nonlinear complexities in signal transduction, such as crosstalk and feedback loops, must be developed. Finally, an integrated model of how cell attachment, shape and budding polarity are regulated by these signalling pathways will lead to a better understanding of filamentous and invasive growth.

Acknowledgements

The authors thank their colleagues in the Kron and Palecek laboratories for helpful input and careful reading of the manuscript. S.P.P. was an Amgen Fellow of the Life Sciences Research Foundation and S.J.K. was a James S. McDonnell Foundation Scholar. Our studies have been supported by National Science Foundation grant MCB-9875976 to S.J.K.

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