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Control of pseudohyphae formation in *Saccharomyces cerevisiae*

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Abstract

Pseudohyphal growth in both haploid and diploid strains of *Saccharomyces cerevisiae* reflects concerted changes in different cellular processes: budding pattern, cell elongation and cell adhesion. These changes are triggered by environmental signals and are controlled by several pathways which act in parallel. Nitrogen deprivation, and possibly other stresses, activate a MAP kinase cascade which has the transcription factor Ste12 as its final target. A cAMP-dependent pathway, in which the protein kinase Tpk2 plays a specific role, is also required for the morphogenetic switch. Both pathways contribute to modulate the expression of the *MUC1/FLO11* gene which encodes a cell-surface flocculin required for pseudohyphal and invasive growth. The MAP kinase cascade could also control the activity of the cyclin/Cdc28 complexes which affect both the budding pattern of yeast and cell elongation. A further protein which stimulates filamentous growth in *S. cerevisiae* is Phd1; although its mode of action is unknown, it may be regulated by a cAMP-dependent protein kinase, as occurs with the homologous protein Efg1 from *Candida albicans*, which is required for the formation of true hyphae. Morphogenesis in different yeast genera share common elements, but there are also important differences. Although a complete picture cannot yet be drawn, partial models may be proposed for the interaction of the regulatory pathways, both in the case of *S. cerevisiae* and in that of *C. albicans*.

Keywords: Dimorphism; Pseudohypha; Invasive growth; Yeast; *Candida albicans*; *Saccharomyces cerevisiae*

Contents

1. Introduction

The well known baker's or brewer's yeast, *Saccharomyces cerevisiae*, grows usually as single budding cells; Pas-

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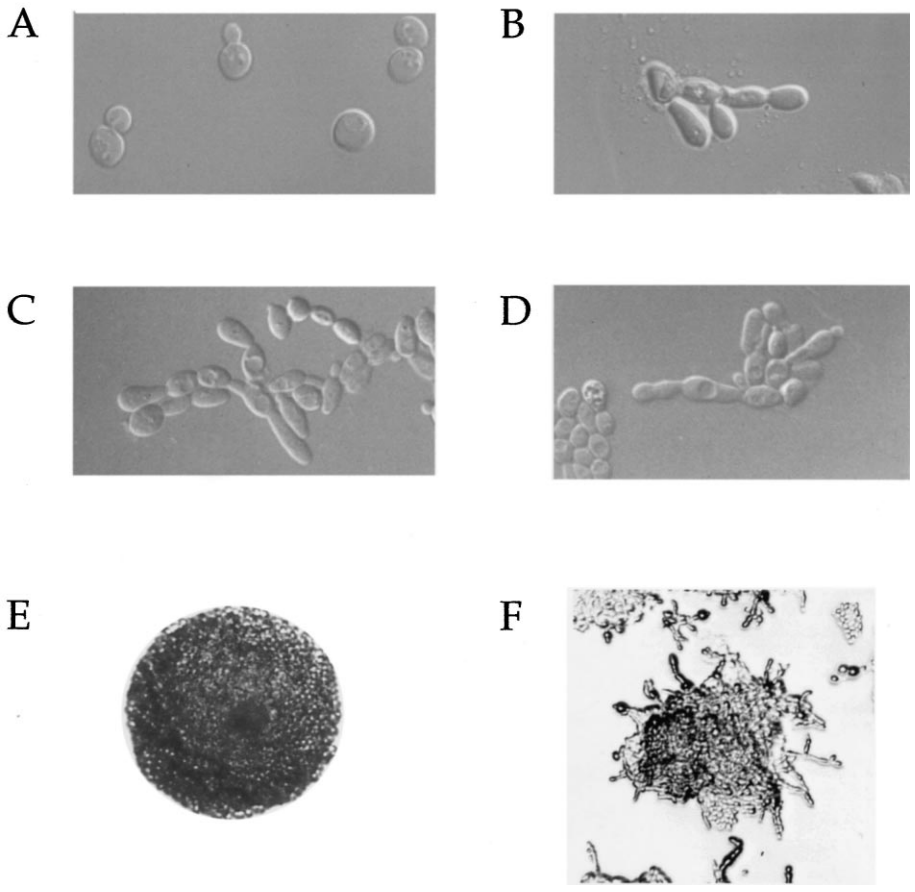

Fig. 1. Cell and colony morphologies of *S. cerevisiae*. Individual cells growing as yeast (A) or pseudohyphae (B–D). Colonies from yeast cells (E) or pseudohyphae (F).

teur referred to them as 'globules'. Although scattered observations on the ability of *S. cerevisiae* to grow in a filamentous form were made long ago [1], the fact that most laboratory strains grow exclusively in the unicellular form [2] meant that dimorphism in this yeast did not receive significant attention until the last decade. In 1992 Gimeno et al. [3] reported that, in a particular diploid strain of *S. cerevisiae*, nitrogen starvation caused formation of pseudohyphae and led to filamentous growth. During pseudohyphal growth (PH growth) the cells become elongated, budding occurs synchronously in unipolar fashion and the buds do not separate, producing the chains of cells which are called pseudohyphae (Fig. 1). Some *S. cerevisiae* haploid strains may also show a change in morphology, for instance, after extended growth on YPD plates [4]. This has been called invasive growth because the filaments penetrate within the agar, below the colony. Although I will use in this review the now well established terms of PH growth for diploids and invasive growth for haploids, this does not mean that diploids cannot invade or that haploids are unable to form pseudohyphae. Both diploids and haploids form pseudohyphae, although in diploids these filaments extend on the surface of the agar away from the colony, while for haploids on YPD the filaments do not extend significantly. In addition, diploids invade agar even more strongly than haploids: their filaments resist vigorous washing of cells from agar surfaces, whereas haploids do not support such a harsh treatment but are not removed by a gentle washing [5–7].

The ability to form pseudohyphae upon nutrient deprivation has been lost in most laboratory strains which were selected for their tendency to form cells which separate easily [2]. In the wild this ability could provide a selective advantage, as it would facilitate foraging for scarce nutrients. In addition, the capacity for developing pseudohyphae in response to stress [8] may allow escape from a harmful environment.

Although dimorphism is less prominent in *S. cerevisiae* than in other yeasts, the wide range of tools available for the study of *S. cerevisiae* has made this yeast the organism of choice to study the factors involved in the switch from unicellular to PH growth. Information on these factors is rapidly increasing and many important features of the process have just been uncovered. Therefore recent reviews on the subject [2,9,10] are already becoming outdated. In this review I have tried to present, in a systematic way, what is already established about this process and what are the obscure points which remain to be investigated.

2. Two signaling pathways which act coordinately

The morphogenetic switch in *S. cerevisiae* requires the cooperation of two different signaling pathways, a MAP kinase cascade and a cAMP-dependent pathway, whose elements are described in detail below. A central element in the switch appears to be the GTP-binding protein Ras2. When this protein is constitutively activated by a $RAS2^{Val19}$ mutation, which causes decreased GTPase activity, it can trigger enhanced PH growth in diploids [3,11] and invasive growth in haploids [12]. It should be noted, however, that while in a *ras2* haploid invasive growth is prevented [12], in a *ras2*/*ras2* diploid starved for nitrogen PH growth still occurs, although the pseudohyphae are composed of cells that are round instead of being elongated [13].

2.1. The MAP kinase cascade

The MAP kinase pathway involved in PH and invasive growth includes a large number of elements. The available evidence supports the scheme shown in Fig. 2, which operates as follows. Upon receipt of an adequate signal (see below) Ras2 is activated and activates the guanine nucleotide exchange factor Cdc24. Activated Cdc24, in turn, activates Cdc42 by facilitating the formation of its GTP-bound form [14]. Activated Cdc42 interacts with the protein kinase Ste20 [14,15], and displaces the negative regulator Hsl7 [16]. In this way Ste20 can activate the MAP kinase cascade formed by Ste11, Ste7 and Kss1 [17–19]. An important function of the interaction of Cdc42 with Ste20 appears to be the targeting of Ste20 towards the site of growth, a process required for a pseudohyphal growth

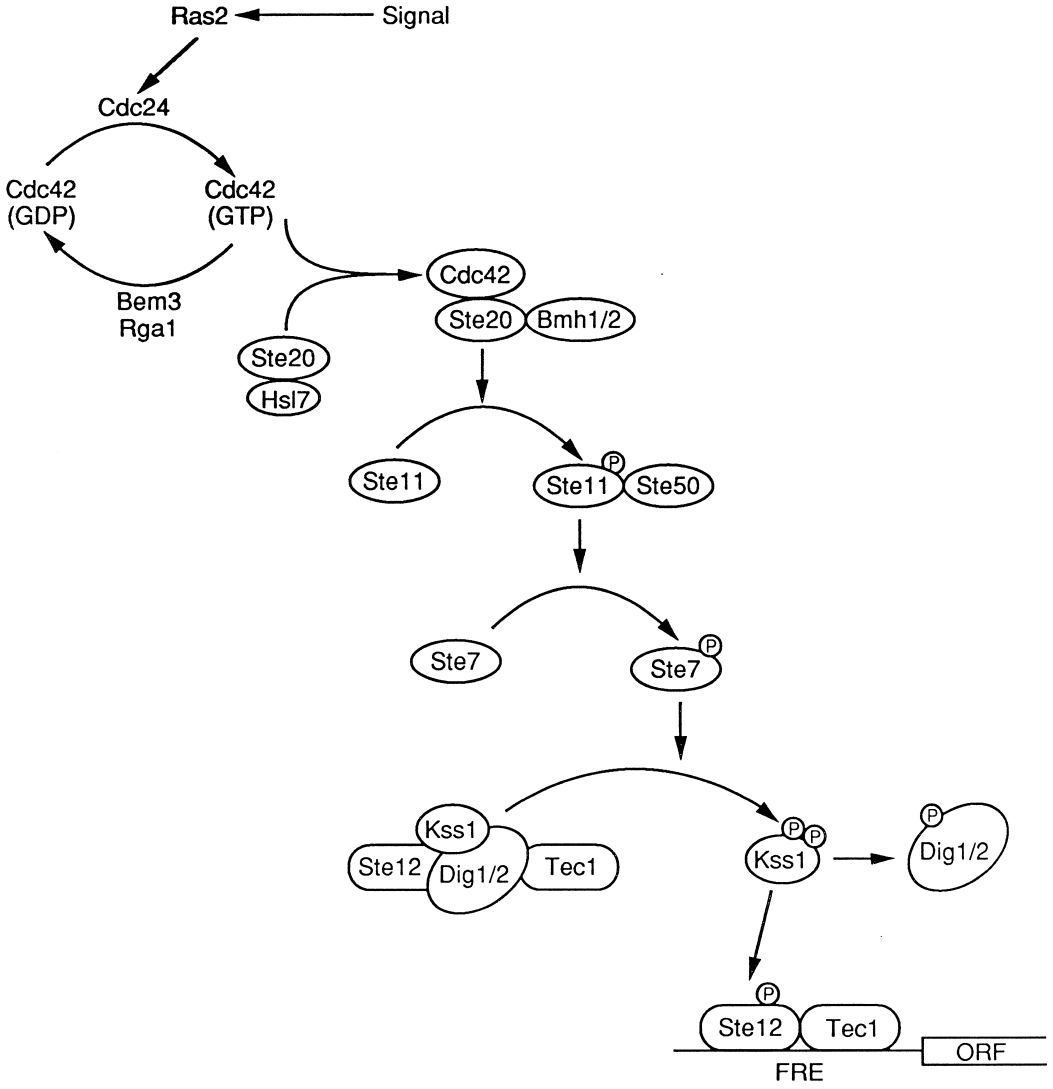

Fig. 2. Triggering of the MAP kinase cascade leading to pseudohyphal and invasive growth in *S. cerevisiae*. An external signal activates Ras2 which, in turn, through a series of intermediate steps, causes the phosphorylation of the MAPKKK Ste11, the MAPKK Ste7 and the MAPK Kss1. The final targets are genes with a FRE element in their promoter. Arrows indicate activation. For details, see text.

pattern [14,15]. The MAP kinase Kss1 in its unphosphorylated form is able to interact with the transcription factor Ste12 and with the negative regulators Dig1(Rst1) and Dig2(Rst2), thereby potentiating the Dig-mediated block of Ste12 activity [20,21]. When Kss1 is phosphorylated by Ste7, both on a threonine and on a tyrosine residue, it is able to phosphorylate Ste12 and Dig1/2; the Dig proteins dissociate and this allows derepression of the target genes to which Ste12 may bind [21]. Elements called FRE (for Filamentation and invasive Response Element) have been identified in the promoters of several genes [22]. These are composite elements with two adjacent binding sites for the transcription factors Ste12 and Tec1 which bind cooperatively to the consensus sequences TGAAACA and CATTCT/C respectively. Dig1 and Dig2 interact with Tec1 in a two-hybrid assay [21] but there is no evidence that Kss1 phosphorylates Tec1. On the other hand, there is a FRE element in the *TEC1* promoter itself, which is required for PH growth [22]. The MAP kinase cascade appears therefore to regulate both *TEC1* expression through the FRE element and Ste12 activity through its phosphorylation.

Further proteins which participate in the modulation of this MAP kinase cascade are Bmh1, Bmh2 and Ste50. The proteins Bmh1 and Bmh2 appear to play a redundant role, they have been found to associate with Ste20 and are critical for the expression of a reporter gene under the control of a FRE element [23] These proteins are not involved in invasive growth but are required for cell elongation. Since *bmh1* mutants have been isolated which cause a reduced expression of a FRE-*lacZ* reporter gene but allow normal elongation, Bmh proteins may play a role in elongation, independent of the MAP kinase cascade [23]. Deletion of *STE50* leads to defects in PH growth and since Ste50 associates with Ste11 it has been suggested that Ste50 is necessary for Ste11 activity [24].

The GTPase activating proteins Bem3 and Rga1 appear to stimulate the hydrolysis of GTP on Cdc42 [25,26] and a *rga1bem3* double mutant has an aberrant, elongated cell morphology [26]. It is not known, however, whether pseudohyphal or invasive growth are stimulated in this double mutant. There is also no information on possible mechanisms of control for the proteins Bem3 and Rga1 which may operate when morphogenesis is induced.

Some of the elements of this MAP kinase pathway are shared with other regulatory pathways: pheromones activate the Ste20/Ste11/Ste7 cascade and a high osmolarity causes activation of Ste11 (reviewed in [9]). It is, therefore, important to prevent inappropriate cross-talk between the pathways. This is achieved through the specificity of the MAP kinases themselves, Kss1 involved in PH growth and invasiveness, Hog1 responding to osmotic stress, and Fus3 being responsible for the pheromone response. In the absence of the Fus3 protein, however, Kss1 can be recruited in response to pheromones and then activate both the mating response and invasive growth [22]. Similarly, in a

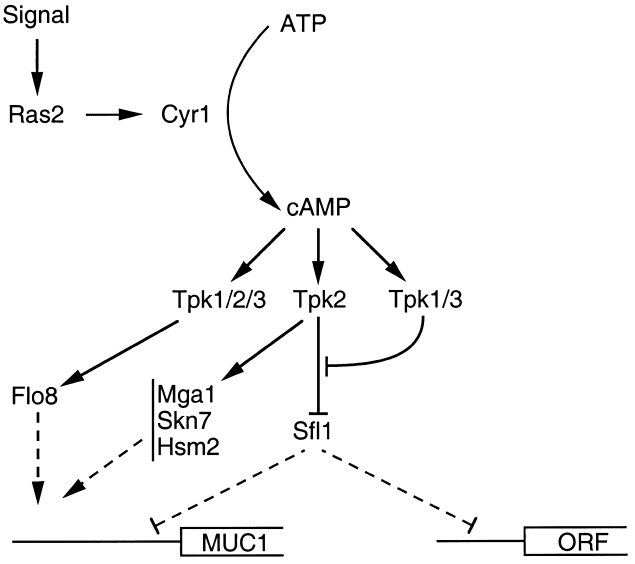

Fig. 3. cAMP-dependent pathway leading to pseudohyphal and invasive growth in *S. cerevisiae*. Activation of Ras2 stimulates adenylate cyclase, Cyr1, and the resulting increase in cAMP level causes an activation of the Tpks. These specifically activate or inhibit a number of proteins which act on different target genes. Arrows indicate activation, lines with bars indicate inhibition, broken lines are used for tentative, not yet proven, interactions. For details, see text.

pbs2-3 strain, defective in the HOG pathway, Kss1 is phosphorylated in response to hyperosmotic shock and this leads to the production of long projections by the yeast [27]. It can be noted that Hog1 has some inhibitory effect on the *STE* signaling pathway and that *hog1* strains are consistently more filamentous than wild-type strains [22].

2.2. The cAMP-dependent pathway

Activated Ras2 can interact with Cyr1, adenylate cyclase [28]; this results in an increase in the intracellular concentration of cAMP which in turn activates the cAMP-dependent protein kinases (Fig. 3). Although any of the three cAMP-dependent protein kinases from *S. cerevisiae* is sufficient for cell viability, they play different roles in PH differentiation [29,30]. Deletion of *TPK2* blocks PH growth, while deletion of *TPK3* stimulates it. For *TPK1* there is a lack of agreement between different groups, while Robertson and Fink [29] did not observe any effect when the gene was interrupted, Pan and Heitman [30] reported that Tpk1 had a repressing role on PH growth. In two-hybrid assays only Tpk2 was shown to interact with Sfl1 and with Mga1 [29]. The proteins Sfl1 and Mga1 contain a type of helix-turn-helix DNA binding-motif first described in the heat-shock transcription factor Hsf1 [31] and consensus Tpk phosphorylation sites (5 in Sfl1 and 2 in Mga1). Sfl1 acts as a repressor for the *SUC2* gene [32] and its deletion enhances PH growth [29]. The deletion of *MGA1* did not interfere with the formation of elongated cells during nitrogen starvation but

caused a large increase in the proportion of cells budding randomly [33]; the defect in pseudohyphal growth was reported as strong [33] or 'not dramatic' [29].

Two other proteins with the same helix-turn-helix motif, Skn7 and Hsm2, could also be involved in PH growth since a deletion of either of the corresponding genes causes a moderate defect in this type of growth [33]. This points to Sfl1 and other proteins of the family as possible substrates for Tpk2, which would play opposing roles: Sfl1 would act as a repressor and Mga1, Skn7 and Hsm2 as (redundant?) activators. The downstream target(s) of these proteins in the regulation of pseudohyphal growth have not been yet identified but the gene *MUC1/FLO11* (see below) could be a good candidate, since *MUC1/FLO11* mRNA levels are increased in a *sfl1* mutant and *sfl1muc1/flo11* homozygous diploids are defective in filamentation. The fact that filamentation is not completely abolished in the double mutant, as it is in a *muc1/flo11* mutant, suggests that Sfl1 is able to repress other genes involved in pseudohyphal development [29]. In a *bcy1* mutant where the Tpks are constitutively activated, there is an increase in *MUC1/FLO11* expression which is strictly dependent on *FLO8*, a gene involved in flocculation [34] and required for PH growth [6] (see below); this could indicate that *FLO8* is also a target of the cAMP-dependent protein kinases [30]. The fact that a double mutant *sfl1tpk2* shows no defect in filamentation [29], suggests that the activation of *FLO8* is not strictly dependent on Tpk2 and can be performed also by Tpk1 and/or Tpk3.

A further role of the Ras2/cAMP pathway in stimulating invasive growth may be the suppression of the activity of the stress-responsive transcription factors Msn2 and Msn4. It has been shown that invasive growth does not take place in an haploid *ras2*Δ mutant, but occurs in *ras2*Δ*msn2*Δ*msn4*Δ or *ras2*Δ*yap1*Δ mutants [35]. This suggests that the product of some gene(s) activated by the transcription factors Msn2/4 and yAP-1 has a negative effect on invasiveness.

The importance of the cAMP-dependent pathway for morphogenesis is highlighted by the fact that external cAMP stimulates PH growth [13,36] while this form of growth is blocked by overexpression of the phosphodiesterase Pde2 [37].

2.3. Interaction between the pathways

The two pathways activated by Ras2 converge to regulate the expression of the same gene, *MUC1/FLO11*, which encodes a cell-surface flocculin required for PH growth of diploid cells and invasive growth of haploids [38,39]. The *MUC1/FLO11* promoter is much larger than most yeast promoters, *cis*-acting elements being present at least at 2.8 kb from the first coding triplet [40]. It is also extremely complex, as it appears to contain at least four upstream activating sequences and nine upstream repressing sequences. *MUC1/FLO11* expression requires Tec1, Ste12 and Flo8, but the effect of *ste12* and *flo8* mutations can be suppressed by overexpression of *FLO8* and *STE12*, respectively [40]. However, Flo8 is specifically required for the cAMP-mediated increase in *MUC1/FLO11* transcription [40]. While it has been found that the upstream region of the *MUC1/FLO11* promoter contains a FRE element with a Ste12-binding site and a Tec1-binding site in close proximity [39], it is not yet established whether the nuclear protein Flo8 binds to the *MUC1/FLO11* promoter or plays a more indirect role. It has also been suggested that Ste12 may act at some other site of the *MUC1/FLO11* promoter, independently of Tec1, interacting perhaps with a different activator [40].

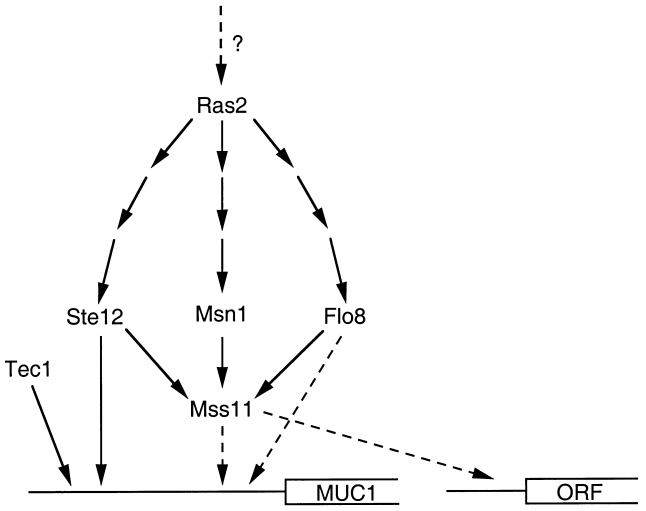

Fig. 4. Suggested model for the relationship between different elements which control the expression of *MUC1/FLO11*. Broken lines are used for tentative, not yet proven, interactions. For details see text.

Although Flo8 may also facilitate an increased transcription of *FLO1*, a gene encoding a cell wall protein involved in flocculation [34], neither Flo1 nor the related protein Flo5 appear required for PH growth [30].

Further elements involved in the transcriptional activation of *MUC1/FLO11* have been characterized recently. Msn1, a nuclear protein with capacity for transcriptional activation [41], and Mss11 are required for invasive growth; they may act upstream of *MUC1/FLO11* since their absence may be partially compensated by expression of *MUC1/FLO11* under the control of a strong constitutive promoter [42]. On the other hand, MUC1/FLO11 cannot be the only target for Mss1, since overexpression of MSS11, but not of MSN1, allows weak invasive growth in a *muc1/flo11* strain. Whereas Mss11 appears to act downstream of Ste12 and of Msn1, Msn1 and Ste12 would act independently in parallel pathways, which are both Ras2-dependent [42]. There is also evidence that Mss11 acts downstream of Flo8 to trigger invasive growth and that Flo8 and Msn1 act independently of each other [43]. A tentative model is shown in Fig. 4.

Another mechanism which would allow the two Ras2-dependent pathways to interact is the operation of the

Tpks on the Ste12 and/or Tec1 transcription factors. This is suggested by the observation that in *ras2* haploid cells, overexpression of *TPK1, TPK2* or *TPK3* stimulates the expression of a FRE-*lacZ* reporter gene [12].

The observation that Σ1278b strains are more competent than most laboratory strains for filamentous growth has been related with the fact that the usual laboratory strains have a defect in the *FLO8* gene [2]. It has also been reported that the Ras2/cAMP pathway is overactive in the Σ1278 background [35]. It may be concluded therefore that the unique behavior of Σ1278b strains is due to a combination of several peculiarities of its genetic background.

2.4. Upstream signals

A variety of conditions promote PH growth, among them nitrogen limitation [3], a poor carbon source in the medium [8,38], or some possible stress conditions [8,44].

How these situations trigger the operation of the MAP kinase cascade and of the cAMP-dependent protein kinase pathway is not well understood. There are, however, some elements involved in signaling which have already been identified and which will be discussed in this section (cf. Fig. 5).

Surprisingly, although Ras2 has been placed upstream of both the MAPK and the cAMP-dependent pathways, there is at present no information on the mechanisms by which Ras2 may be activated in conditions leading to PH and invasive growth.

In SLAD medium, a medium with only 50 μM ammonium [3], Sho1, a putative membrane-spanning protein [45], is required for PH growth of a diploid [46]. It has been therefore proposed that Sho1 may respond not only to high osmolarity [45] but also to a 'low nitrogen' signal and relay it to Ste20 and the MAPK cascade which activates Ste12 and Tec1 [46].

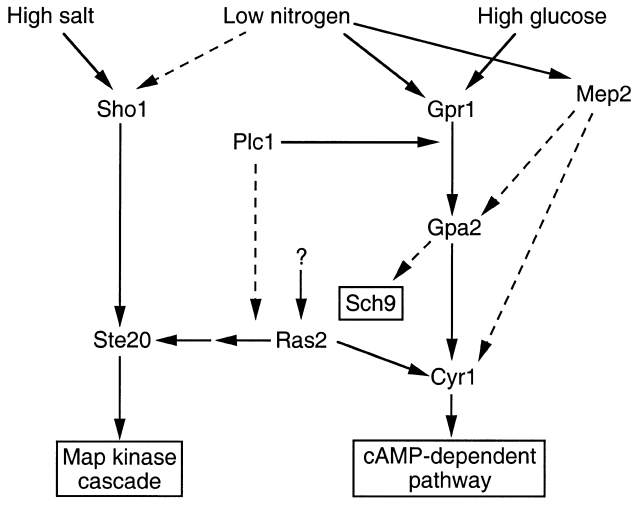

Fig. 5. Multiple signals activate the MAP kinase cascade and the cAMP-dependent pathway. Broken lines are used for tentative, not yet proven, interactions. For details, see text.

PH growth requires also the G protein Gpa2 [13,36] and its coupled receptor Gpr1 [47–49]. It has been suggested that Gpr1 plays a role in nitrogen detection and, in fact, *GPR1* mRNA was strongly induced in response to starvation for nitrogen but not for a carbon source [50]. However, there is also recent evidence that Gpr1 can monitor glucose [49,51,52] and it has been proposed that both low nitrogen and a high level of glucose or a related sugar are required to trigger PH growth [49].

There is, in addition, a high affinity ammonium permease, Mep2, also needed for PH growth of a diploid in SLAD medium [53] which suggests that Mep2 could act as a nitrogen sensor. There is no evidence, however, for a direct interaction between Mep2 and Gpa2. It is noteworthy that another ammonium permease, Mep1, may inhibit pseudohyphal differentiation under certain conditions [53].

The elements Gpr1, Gpa2 or Mep2 appear to work through the cAMP pathway, since the defects in filamentation of a *gpr1*, a *gpa2* or a *mep2* mutant are suppressed by a $RAS2^{Val19}$ mutation or by external cAMP [13,36,48,49,53]; the defect in filamentation in a *gpr1* mutant is also suppressed by a *bcy1* mutation [30].

The phosphatidylinositol-specific phospholipase C, Plc1, is required for the interaction between Gpr1 and Gpa2. Two-hybrid assays and coimmune precipitation of epitope-tagged proteins demonstrated that Plc1 can bind both Gpr1 and Gpa2, while the association between Gpr1 and Gpa2 required the presence of Pcl1 [47]. This explains why is Plc1 necessary for PH growth upon nitrogen starvation [47]. On the other hand the fact that the mutation $RAS2^{Val19}$ suppresses the filamentation defects of *gpr1* and *gpa2* strains, but not those of a *plc1* strain, suggests that Plc1 has a further function downstream of Ras2, perhaps controlling the MAP kinase pathway via Cdc42 [47]. Although it had been proposed that Gpa2 could act in a cAMP-independent pathway that activates the protein kinase Sch9 [50], it was later shown that Sch9 is not absolutely required for PH growth and could even play a negative role on invasive growth and on *MUC1/FLO11* expression in haploid cells [49].

A further element which may play a role in signaling the availability of nitrogen is the glutamine $tRNA_{CUG}$ molecule. A mutation which affects this tRNA allows PH growth in a nitrogen-rich solid complex medium where wild-type cells grow exclusively in the yeast form [54]. It has also been found that the MAP kinase pathway and the Ste12 transcription factor are dispensable for the PH growth of the $tRNA_{CUG}$ mutants [54], an observation which suggests that $tRNA_{CUG}$ operates through the cAMP-dependent signaling pathway. However, the putative regulatory protein interacting with $tRNA_{CUG}$ has not been yet identified.

It has been reported recently that in a Σ1278b diploid strain a mild thermal stress or the presence of aliphatic

alcohols (ethanol, propanol or butanol) induces invasive growth on YPglucose; the alcohols trigger also the formation of pseudohyphae [8] In *pde2* diploid cells, which do not share the Σ1278b genetic background, alcohols or a high salt concentration induce PH growth, but only in the presence of cAMP in the medium [8]. Since Sho1 activates Ste11 in response to osmotic stress [55], Sho1 is likely to act as the sensor for high salt which triggers morphogenesis. On the other hand, information is lacking on the target(s) for thermal stress and aliphatic alcohols, although they may act by modifying the structure of the plasma membrane.

3. Control of specific morphological changes

The switch between growth in the yeast form and PH growth in *S. cerevisiae* involves important changes in the pattern of progression through the cell cycle. For cells in the yeast form, the bud grows first predominantly at its apex; later on growth becomes more uniform over its whole surface, and finally the daughter cell separates from the mother before reaching the size of the latter. As a consequence, the smaller new cell has to spend some time growing in G_1 before reaching the critical size for progression to S phase at Start. In contrast, during PH growth there is an extended G_2/M period and when the septum separating mother and daughter is formed the daughter and the mother cells are the same size, so that both cells can start a new cycle synchronously [56].

Cell cycle progression is mediated by the association of the protein kinase Cdc28 with distinct groups of cyclins, mainly Cln1, Cln2 or Cln3 during the G1 phase and Clb1 or Clb2 before mitosis [57]. This suggests that Cdc28 and/or the Cln and Clb cyclins may be targets for the signal transduction pathways leading to filamentation and there is recent evidence for a lengthening of the G_2/M growth period induced by an activation of the MAP kinase pathway which inhibits mitotic cyclin/Cdc28 complexes [58].

Filamentous growth involves changes in the budding pattern, in elongation and in invasiveness. Although these cellular processes are interconnected, they are not always controlled by the same elements [5]. For instance, while both Ste12 and Tpk2 are required for agar invasion, Ste12 has little influence in the pattern of budding and Tpk2 does not affect cell elongation [30]. Therefore the different processes leading to PH growth will be considered separately.

3.1. Regulation of the budding pattern

Pseudofilamentation is absolutely dependent on a polar budding pattern, the normal pattern of growth in diploid cells. The mode of budding is controlled by a large number of genes which are listed in Table 1 and which will be discussed below.

Mutations in *BUD1, BUD2* or *BUD5* which cause a random distribution in the localization of new buds do not allow the formation of pseudohyphae in diploids [3,59] or haploids [4]. Similarly a *bud8*/*bud8* diploid mutant which buds with high frequency at the birth end of the cell is also defective in PH growth [5]. Interestingly neither Bud1 nor Bud8 are required for cell elongation or agar invasion [4,5].

Other mutations have been described which facilitate filamentation by changing the budding pattern. A *bud4*

Table 1
S. cerevisiae genes implicated in the control of the budding pattern and in elongation

Gene	Effects	Reference
BUD1/2/5	Deletion causes random distribution of buds	[3,4,59]
BUD8	Deletion causes budding at the birth end	[5]
BUD4	Deletion causes polar budding in haploids	[60,61]
WHI2	Overexpression causes polar budding in haploids	[62]
CDC28	The *cdc28-127* mutation causes polar budding in haploids	[63]
SEM1	Interferes with the shift to unipolar budding	[64]
ACT1	Required for unipolar bud selection and for elongation	[66]
CLN1/2	Products interact with Cdc28. At least one of them required for PH growth	[67–69]
CLB1/2	Products interact with Cdc28. Overexpression blocks PH growth and suppresses *cdc28-127* phenotype. Deletion causes elongation and bypasses the requirement of Cln1 for elongation	[58,63,67]
CLN3	Deletion bypasses the requirement of Cln1 for elongation	[67]
GRR1	Product participates in the degradation of Cln1 and Cln2. Deletion promotes PH growth	[67,70,71]
SWE1	Swe1 inhibits Cdc28. Deletion impairs PH growth	[63]
HSL1	Hsl1 inhibits Swe1. Deletion promotes PH growth	[63]
MIH1	Mih1 antagonizes Swe1. Deletion promotes PH growth	[58]
ELM1	Encodes a protein kinase which may activate Hsl1. Deletion promotes elongation and PH growth	[70,72]
CDC12	Encodes a septin essential for cytokinesis. Mutation causes an elongated morphology	[70]
CDC55	Encodes a regulatory subunit of PPase 2A; related with elongation	[5,72]
BUD6, BNI1, SPA2, PEA2	The products of these four genes function together in a complex to promote polarized cell growth. Mutants are defective in cell polarity and in cell elongation	[5,74]
TPM1	Encodes major form of tropomyosin; could interact with actin	[5]
ASH1	Encodes a repressor which accumulates in daughter cells. Deletion impairs PH growth	[75]

mutation, which causes polar budding in haploid cells allows a moderate increase in filamentation [60,61]. Similarly, overexpression of the *WHI2* gene triggers polar budding in haploids, hinders the completion of cytokinesis, and causes hyperpolarized growth which results in the production of cells with an elongated shape; nevertheless, these cells do not invade the agar [62].

The mutation *cdc28-127* in the cyclin-dependent kinase Cdc28 replaces also the axial budding pattern of an haploid strain by an unipolar or bipolar budding which causes PH growth, even during exponential growth in liquid cultures of YPD [63]. It should be noted that *cdc28-127* does not cause delayed cell separation, a further indication that the different processes contributing to pseudofilamentation may be regulated by different elements.

It has been reported that deletion of *SEM1* allows pseudohyphae formation upon nitrogen starvation in a *flo8* strain and it was suggested that Sem1 interferes with the shift from bipolar to unipolar distal budding which is required for PH growth [64]. Although the mode of action of Sem1 is not known, this small acidic protein appears to play a negative regulatory role in exocyst function, and this would support the idea that there is a link between exocytosis and polarization of growth, involving the actin cytoskeleton [65]. Unipolar bud selection in pseudohyphal cells is also dependent on actin function [66].

3.2. Regulation of elongation

Mutations in a large number of genes affect cell elongation (see Table 1). It is important to note that in some cases the cells may become elongated due to a delay in mitosis and a defect in the switch from apical to isotropic growth. Nevertheless, it is still debated whether this is the mechanism underlying the change in cell shape observed in pseudohyphae. Among the genes related with elongation, those encoding cyclins, or proteins able to regulate cyclins or the cyclin-dependent kinase Cdc28 itself, are prominent. At least one of the G_1 cyclins, Cln1 or Cln2, is required for PH growth and haploid invasive growth [67–69], while overexpression of the cyclins Clb1 or Clb2 blocks PH growth triggered by low nitrogen, even in the presence of the dominant active mutations $RAS2^{Val19}$ or *STE11-4* [58], and suppresses the elongation phenotype of a *cdc28-127* mutant [63]. Since deletion of *CLB2* produces an elongation phenotype, even in rich medium [63,67], it appears that the complexes Cdc28-Clb and Cdc28-Cln play opposite roles in the morphogenic process [57]. The negative role of Clb2 would be dependent on the Cln3 cyclin, since both a *cln3* and a *clb2* mutation bypass the requirement of Cln1 for elongation [67]. This indicates that the MAP kinase cascade activates at least two parallel pathways with some degree of redundancy. It is interesting to note that, although expression of *CLN1* is under the control of Tec1 and Ste12 [68], overexpression of *STE20* or the dominant mutation *STE11-4* can partially suppress defects in PH growth of a *cln1/cln1 cln2/cln2* mutant [67]. It has been suggested that the complex Cdc28-Cln1/2 would promote vegetative morphogenic functions of Ste20 while inhibiting functions related with pheromone signaling [69]. The observation that a *grr1* mutation promotes PH growth [70] may in turn be related with the fact that Grr1 participates in the degradation of Cln1 and Cln2 [71]. Grr1, however, should control at least another protein involved in elongation, since a *grr1/grr1 cln1/cln1 cln2/cln2* mutant forms elongated cells even in a rich medium [67]. The crucial role of Cdc28 in controlling *S. cerevisiae* morphogenesis is also shown by the fact that PH growth is facilitated by inhibition of Cdc28 by Swe1 (which phosphorylates Tyr-19) and impaired by Hsl1, a protein kinase which inhibits Swe1 [63] or by Mih1, a protein phosphatase which acts as antagonist of Swe1 [58].

A variety of mutants which present an elongated morphology have been called *elm* [70,72]; other mutants such as *bni1*, *spa2*, *tpm1*, *pea2/dfg9*, *dfg5* and *dfg10* are defective in both cell polarity and cell elongation, but still invade the agar [5]. *ELM1* encodes a serin–threonine protein kinase [72,73] which appears located mainly at the bud neck [74]. It may activate the protein kinase Hsl1 [63] to allow cytokinesis and mitosis to proceed. The *elm1* mutation would then cause a delay in cytokinesis which results in elongation of the cells and eventually PH growth. Another *elm* mutation has been characterized as *cdc12* [70] and its phenotype can be related to the fact that *CDC12* encodes one of the septin proteins essential for cytokinesis. The role played by Cdc55, a regulatory subunit of yeast PPase 2A, is less clear. Whereas a diploid with a single functional copy of the *CDC55* gene shows an elongated morphology [72], a diploid with the homozygous *cdc55-100* mutation is impaired in cell elongation [5].

While Dfg5 and Dfg10 have not been characterized further, Spa2, Pea2, Bni1 and another protein, Bud6, appear to function together in a complex to promote polarized cell growth [75]. These proteins, and also Tpm1, a major form of tropomyosin, could interact with actin and thereby direct the actin cytoskeleton to specified growth sites in the yeast cells. It should be noted that Spa2 is also able to interact with Ste11 and Ste7, two elements of the MAP kinase pathway which controls PH growth [75]. Some mutations in actin interfere with cell elongation suggesting that the polarization of actin patches at the tip of the cell is important for elongation [66].

It has been reported that strains with a deletion of the *ASH1* gene are impaired in PH growth [76]. At the end of mitosis there is an asymmetric accumulation of the repressor Ash1 in daughter cells due to an asymmetric distribution of *ASH1* mRNA which is dependent on proteins such as Act1, Tpm1 or Bni1 which regulate the actin cytoskeleton [77]. It would be interesting to check whether the defect in pseudofilamentation observed in a *ash1*Δ mutant is actually due to the lack of the Ash1 protein or may be a secondary effect of the lack of *ASH1* mRNA which could

distort the structure of the actin cytoskeleton required for polarized growth. Although this mechanism may seem rather unusual, it has recently been reported that the association between the complex She3–Myo4 and the She2 protein is dependent on the presence of Ash1 mRNA [78].

3.3. Regulation of agar invasion

In a general screen to isolate genes involved in filamentous growth, in which different classes of mutants were identified, only one mutant, *dfg16*, was obtained which was defective in invasion but not affected in cell polarity or filament formation [5]. *DFG16* encodes a protein predicted to contain six membrane-spanning domains, which could play a role in cell wall synthesis [79], but has not been further characterized. A *cdc 55-100* mutant was impaired both in cell elongation and in invasiveness [5].

In addition, some actin alleles cause a marked defect in agar invasiveness; a possible reason for this would be the participation of actin in the secretion of putative hydrolases able to facilitate growth through the agar [66].

It should be noted that not every gene which could have been identified was revealed in the screening described in [5]. The *MUC1/FLO11* gene discussed in Section 2.2 was not found, for example, although it has been reported to be required for agar invasion and cell–cell adhesion but not for mediating cell elongation or the budding pattern switch [30,67].

4. Other genes involved in PH growth and invasive growth

Overexpression of *PHD1* induces vigorous PH growth of diploid cells on rich medium [60] and *PHD1* expression is strongly induced in a medium with low nitrogen [80]. Nevertheless, deletion of *PHD1* does not interfere with the filamentation response to nitrogen starvation, and overexpression of *PHD1* does not induce PH growth in wild-type haploid strains, although it does in a *bud4* mutant strain, which has the polar budding pattern [60]. Phd1 has been shown to be localized in the nucleus and to contain a region similar to one present in different fungal transcriptional regulators [60]. This region includes a positively charged stretch followed by a helix-turn-helix motif potentially able to bind DNA; there is no information, however, on the possible target(s) of Phd1. In any case the enhancement of filamentous growth by Phd1 is independent of Tpk2 [30], Tec1 [81], Ste12 or Ash1 [76]. Although this enhancement is prevented in a *gpa2*Δ mutant [36], it takes place in the diploid double mutants *gpa2mep1* or *mep1-mep2* [53]. A protein with significant homology to Phd1 in its putative DNA-binding region, Sok2, has been found to play a negative role on PH growth since *sok2/sok2* diploid strains form pseudohyphae at an accelerated rate [37]. Here again the target(s) for Sok2 are not known but it should be noted that the positive effect of a *SOK2* deletion on filamentation requires the presence of *PHD1*, although *sok2phd1* diploids still exhibit PH growth when starved for nitrogen.

Pseudohyphae formation may require an enhanced production of chitin, since pseudohyphae have been shown to accumulate chitin on their whole surface and not only at the septa [8]. This is consistent with the observation that Σ1278b-derived strains which form pseudohyphae easily [2] have a 30-fold lower chitinase activity and a level of chitin at least 70% higher than strains unable to undergo PH growth [82]. It can be noted also that in yeasts with an impairment in PH growth caused by a non-functional *FLO8* allele, disruption of *ACE2*, which encodes a positive regulator of the chitinase gene *CTS1*, results in the production of pseudohyphae. Since disruption of the *CTS1* gene itself in a *flo8* background also allows PH growth, but at a reduced level [82], Ace2 may control another gene involved in PH growth.

So many genes have been described as involved in the control of PH growth that it becomes difficult to distinguish between direct and indirect effects. For instance, the impairment of *dom34* mutants in forming pseudohyphae appears related to a decrease in bulk protein translation, which, in turn, alters the cell cycle [83]. The observation that *shr3/shr3* diploids show enhanced filamentous growth can be connected with a role of Shr3 in the secretory pathway specifically required for the localization of amino acid permeases in the plasma membrane [84]. In the absence of a functional Shr3, the yeast exhibits starvation responses, even when amino acids are present in the medium. *MSN5* is one of the genes identified in different screenings, for which no role has been suggested. Overexpression of the gene restores filamentation in a *mep1-mep2* mutant, and its deletion in a wild-type strain causes a moderate defect in PH growth [33]. Since Msn5 is involved in the nuclear export of phosphorylated proteins [85] it could facilitate the removal from the nucleus of some regulatory protein which represses PH growth.

A form of the RNA polymerase II holoenzyme includes a negative regulator formed by Srb10, a cyclin-dependent kinase, and several associated proteins. This Srb–CDK complex, which would function through phosphorylation of the RNA polymerase carboxy-terminal domain, appears to down-regulate a small fraction of *S. cerevisiae* genes, many of them derepressed during nutrient deprivation [86]. It has been found that diploid cells lacking Srb10 exhibit increased PH growth and this could be related with the observation that in a *srb10*Δ mutant growing in YPD, *MUC1/FLO11* is induced 15-fold and *FLO1* up to 100-fold [86]. Since the lack of Srb10 did not affect significantly the mRNA levels of either *STE12, TEC1, FLO8* or *SFL1*, Srb10 could control directly the transcription of the *FLO1* and *MUC1/FLO11* genes. As cells enter the diauxic shift Srb10 levels decrease strongly and other control mechanisms should become operative.

Recently, targets for the MAP kinase Kss1 have been

looked for by performing transcript profiling experiments [68,87]. Although dozens of genes regulated by the Kss1 signaling pathway have been identified, most of them are not required for invasive growth in haploid cells [68]. This could indicate that many of these genes play a redundant role, but also that some of them participate in a process related to filamentous growth but not required for morphogenesis. A clear-cut example is the *PGU1* gene, which encodes a secreted enzyme that hydrolyzes polygalacturonic acid, a component of the plant polysaccharide pectin. This enzyme is not required for invasiveness but would facilitate the access of *S. cerevisiae* to substrates such as rotting fruits [68]. Profiling experiments have also confirmed that there is some functional overlap between the filamentous growth and mating responses [87].

5. Differences in the behavior of haploids and diploids

PH growth of diploid cells and invasiveness of haploids present very similar requirements. Both depend on the MAPK cascade, starting with activation of Cdc42, including the Ste20, Ste11, Ste7 and Kss1 proteins and their downstream targets, the transcription factors Ste12 and Tec1 [4,11,12,17]. Flo8 [6] and Muc1/Flo11 [39] are also required for the morphogenetic change of both haploids and diploids. Finally the PKA pathway plays a role in haploids [12] as it does in diploids.

However, the regulation of these pathways is different in the two types of cells. Whereas, in an haploid strain, *MUC1*/*FLO11* mRNA levels increased steadily during exponential growth in YPD and decreased to very low levels at later phases of growth, in diploid cells growing in YPD *MUC1*/*FLO11* mRNA levels always remained low [39]. In SLAD medium, on the other hand, while *MUC1*/*FLO11* mRNA levels increased steadily with growth in diploids, in haploids there was some increase in the first hour of growth and then a subsequent decrease [39]. Expression of a reporter gene depending on the transcription factors Ste12 and Tec1 was 10-fold higher in haploids than in diploids in a complete mineral medium (SC). The rate of transcription of the reporter gene was increased in SLAD medium, which has a low level of ammonium, the effect being more marked in the case of diploid cells (nine-fold vs. three-fold for haploids) [11].

It has been stated [12] that while in haploids an increase in protein kinase A activity stimulated the expression of a reporter gene controlled by Ste12 and Tec1, in diploids a very high protein kinase A activity inhibited the transcription of this reporter gene. In an haploid strain in SLAD medium external cAMP repressed the expression of the reporter gene up to 50-fold, while in SC medium cAMP had no effect [36]. In the same SC medium the overexpression of *TPK1, TPK2* or *TPK3* increased the expression of the reporter gene in a *ras2* haploid three to five-fold [12]. For diploid strains growing on SLAD, overexpression of *TPK1* increased the expression of the reporter gene about two-fold [11]. Looking at the effects of an activation of the PKA pathway on the expression of the reporter gene controlled by Ste12 and Tec1, no clear difference emerges between haploids and diploids. On the other hand, the effects are strongly dependent on the metabolic conditions of the cell.

As discussed in Section 2.1, regulation of cyclin activity plays a role in the control of morphogenesis. There are, however, some differences in the behavior of haploid and diploid cells. Whereas single knockouts of *CLN1* or *CLN2* do not block invasive growth in haploids [69], a *cln1*/*cln1* diploid strain, but not a *cln2*/*cln2* strain, is unable to induce pseudohyphae upon stimulation of the MAP kinase pathway [67,68].

The most striking difference between the two types of cells appeared to be that haploids are able to invade agar in the presence of a good nitrogen source, while diploids are not [4]. However, in a diploid growing on a rich medium (YP), filamentation and invasiveness may be induced by cAMP together with a stressful condition such as a poor carbon source or the presence of aliphatic alcohols [8].

Although *TEC1* transcription is higher in haploid than in diploid cells, there is little correlation between *TEC1* transcription and haploid invasive growth, an observation which suggests that a low level of *TEC1* transcription is sufficient for invasive growth [88]. It has been shown that invasive growth in haploids does not take place in *rim* mutants, while in homozygous diploid *rim* mutants there is no defect in PH growth [89]. The exact role played by the Rim proteins is not yet known, but from the characteristics of these proteins (yeast protein databases) several points emerge. Rim101 (earlier called Rim1) is a Zn finger protein with similarity to PacC from *Aspergillus*. It is activated by limited proteolysis which, in turn, requires the genes *RIM8, RIM9* and *RIM13* [89]. *RIM8* has not been cloned, Rim9 may acetylate Rim13 and Rim13, similar to PalB, a calpain-like protein from *Aspergillus nidulans*, may be the protease which cleaves Rim101.

6. Regulation of dimorphism in other fungi

Although dimorphism has been observed in a large number of fungi, most studies on this process have been carried out in two main species, *S. cerevisiae* and the pathogenic fungus *Candida albicans* (see [90] for a recent review). *C. albicans* can grow as a budding yeast or as pseudohyphae, and may also develop germ tubes and true hyphae, in which growth takes place by apical extension and division occurs without leaving constrictions at the septa. Pseudohyphae formation is induced by growth on a variety of solid media (Spider, milk-Tween, etc.) while true hyphae are formed in liquid media containing serum or in Lee's medium at pH 6.7.

The role of the MAP kinase pathway in the transition from yeast to pseudohypha in *C. albicans* has been well established. Functional homologues of the *S. cerevisiae STE20, STE7* and *KSS1* genes have been identified; these are *CST20* [91,92] *HST7* [91–93] and *CEK1* [94] respectively, and interruption of these genes causes lack of pseudofilamentation in solid media but no defect in the formation of hyphae in response to serum. In contrast another Ste20-like protein, Cla4, appears to be absolutely required for hyphal growth; homozygous *cla4* mutants show an aberrant morphology which may indicate a role for Cla4 in cytokinesis [95]. In this respect it can be noted that there is evidence that Cla4 is important for the formation of the septin ring [96], although a requirement for Cla4 for pseudohyphal growth in *S. cerevisiae* has not been directly tested. The downstream target of the MAP kinase pathway in *C. albicans* is Cph1, the homologue of *S. cerevisiae* Ste12, and homozygous *cph1* mutants show also a defect in pseudohyphae formation on a solid medium, such as Spider medium, but are able to form hyphae in liquid Lee's medium and to induce germ tubes in response to serum [97]. Although an homologue of the *S. cerevisiae TEC1* gene has been identified by DNA sequencing, its role on morphogenesis has not been yet studied. On the other hand, while PH growth is constitutive in *C. albicans cpp1* mutants, lacking a protein phosphatase which acts on phosphorylated Cek1 [98], there is no information on the effects on PH growth of a *MSG5* deletion in *S. cerevisiae*, although recent evidence suggests that the protein phosphatase Msg5 acts on Kss1 [27].

Targets for Cph1 have not been identified but *INT1,* a gene encoding a surface protein with a cytoplasmic tail, may be a candidate. Disruption of *INT1* suppressed hyphal growth in Spider or milk-Tween solid media but not on Lee's medium or in the presence of serum [99]. Besides, overexpression of *INT1* led to the production of germ tubes in a haploid *S. cerevisiae* strain and even in the corresponding *ste12* mutant [100].

Morphogenesis in *C. albicans* has been shown to respond also to Efg1, a homologue of *S. cerevisiae* Phd1. Reduced expression or deletion of *EFG1* caused an inability to form germ tubes and true hyphae in the presence of serum, but elongated pseudohyphal cells could be observed in certain solid media [101,102]. While overexpression of *EFG1*-enhanced pseudohyphal growth [101], a double mutant *cph1*/*cph1 efg1*/*efg1* was completely defective in filamentous growth [102]. It has been postulated that Efg1 lies in a Ras-activated, cAMP-dependent pathway [90], and recent results indicate that mutation of the single possible PKA phosphorylation site in Efg1 to alanine does not allow filamentation, whereas a change to glutamate results in hyperfilamentation (D. Bockmühl and J.F. Ernst, personal communication). Evidence is also accumulating that a cAMP-dependent protein kinase is involved in the dimorphic transition in *C. albicans*. Dibutyryl-cAMP enhanced germ-tube formation in exponentially growing cells [103], inhibitors of protein kinase A blocked the germ-tube formation induced by *N*-acetyl glucosamine, although not that induced by serum [104], and *Catpk2* mutants are impaired in hyphal development unless *EFG1* is overexpressed [105]. One of the targets of the cAMP-activated protein kinase may be the glucosamine-6-phosphate synthase required to provide the substrate for chitin synthesis, which is more abundant in mycelium than in yeast cells [106].

Very recently the *RAS1* gene from *C. albicans* has been isolated. No other close homologue of *RAS* seems to be present in this species and it has been shown that a *ras1*Δ/*ras1*Δ homozygous strain could no longer form germ tubes and hyphae, although it still formed some pseudohyphae when exposed to serum [107]. A *C. albicans* chaperonin, Cct8, appears to play a role in morphogenesis. Filamentation is defective both in a *cct8*/*CCT8* strain and in a strain overexpressing *CCT8* [108]. In *S. cerevisiae,* Cct8 from *C. albicans* can block pseudohyphal growth induced by $RAS2^{Val19}$, an observation which suggests that an excess of chaperonin subunits interferes with the Ras signal transduction pathway [108].

No homologue for Flo8, the target for the cAMP pathway in *S. cerevisiae*, has been reported in *C. albicans* and this is also the case for the *MUC1*/*FLO11* gene, which plays a central role in *S. cerevisiae*.

Hyphal development in *C. albicans* is under both positive and negative control. Deletion of the *TUP1* [109] or *RBF1* [110] genes stimulates filamentous growth. Homozygous *tup1* mutants display filamentation in a great variety of media but no germ tube formation progressing to true hyphae in the presence of serum. A double mutant *tup1cph1* behaves as a *tup1* mutant, and it has been suggested that repression by Tup1 is relieved via Cph1 [109]. However, as discussed below, Tup1 and Cph1 are probably acting in different pathways. It can be noted that in *S. cerevisiae* the lack of Tup1 has a different effect: it reduces PH growth in diploids and invasiveness in haploids [109] and it induces flocculation [111].

A further gene involved in *C. albicans* morphogenesis is *HWP1*, a hypha-specific gene, which encodes a cell surface protein involved in host attachment but of unknown function in hyphal development. In *hwp1* mutants the ability to form hyphae on solid medium is lost and in the presence of serum there are reduced levels of peripheral hyphae [112]. The ability to invade the agar beneath the colony is maintained as well as the capacity to form germ tubes in liquid media. Expression of *HWP1* is dependent on *EFG1* but is not dependent upon *CPH1*. *HWP1* is expressed at a reduced level in a strain with a deletion in *RBF1* and expression can take place in non-inducing conditions in a *tup1* null mutant [112]. Since the expression of *HWP1* is repressed by Tup1 but does not require Cph1, the suggestion that Cph1 acts by counteracting the action of Tup1 remains unproven. Although *HWP1* can be situated downstream of *EFG1*, the inability of constitutively ex-

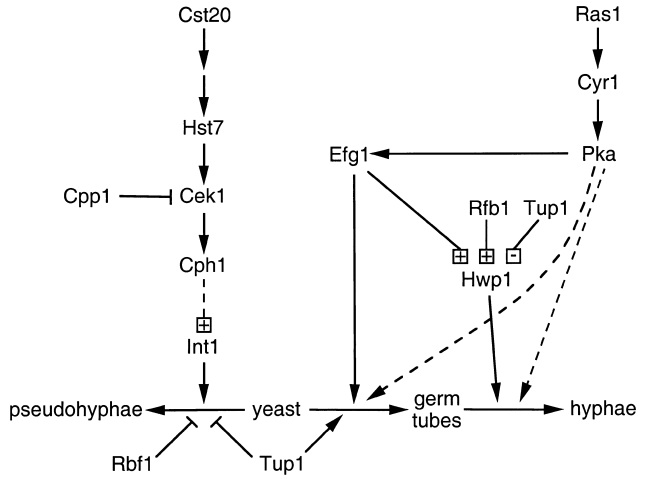

Fig. 6. Signaling pathways triggering hyphal and pseudohyphal growth in *C. albicans*. Arrows indicate activation, lines with bars indicate inhibition. + indicates activation of transcription and − repression. Broken lines are used for tentative, not yet proven, interactions. For details see text.

pressed *HWP1* to suppress an *efg1* null mutation indicates that Efg1 regulates additional genes required for hyphal development [112]. Recent evidence also suggests that Cph1, Efg1 and Tup1 control different pathways which make additive contributions to filamentous growth and that a fourth pathway may still operate when the others are blocked [113]. A tentative model integrating the data available is shown in Fig. 6. It should also be noted that, as reported for *S. cerevisiae*, G_1 cyclins are involved in morphogenesis. In a *Cacln1/Cacln1* mutant in liquid Lee's medium the *HWP1* gene is not induced and hyphal growth does not take place [114]. In a serum-containing medium, on the other hand, the *cln1* mutation has only a slight effect on the induction of *HWP1* and does not interfere with germ tube formation or hyphal growth [114].

Finally, the interruption in *C. albicans* of *HRM101*, the homologue of *S. cerevisiae RIM101*, blocks filamentation on Spider plates [115]. This is in contrast with the situation in *S. cerevisiae*, where PH growth is not impaired in homozygous diploids, although *rim101* haploids are defective in invasive growth [89].

As happens with *S. cerevisiae*, other genes which have been implicated in morphogenesis may affect differentiation in an indirect way. For instance, disruption of the *C. albicans TPS1* gene encoding trehalose-6-phosphate synthase impaired the formation of hyphae during incubation with serum at 37°C, in the presence of glucose, or during growth on galactose or glycerol at 42°C [116]. In the first case the impairment could be due to alterations in the metabolism of glucose, whereas in the second it may reflect a need for trehalose for the maintenance of the hyphae at a high temperature.

In *Yarrowia lipolytica* the protein Mhy1, which is able to bind to a STRE sequence, is required for the yeast-to-hypha transition [117]. This is in contrast with the situation in *S. cerevisiae*, where the absence of either or both the transcription factors Msn2 and Msn4, which bind to the STRE sequences has no effect in pseudohyphae formation [30], and can even facilitate the morphogenetic switch in some genetic backgrounds [35]. During the yeast-to-hypha transition the transcription of *MHY1* is strongly increased and Mhy1 is concentrated in the nuclei of actively growing cells found at the hyphal tip. Mhy1 transcription is unaffected by thermal stress and decreases upon carbon source starvation and osmotic or oxidative shock [117].

It is interesting to note that while *C. albicans* hyphal development is activated by a variety of stresses such as nitrogen starvation or an increased temperature or pH, nitrogen starvation inhibits hyphae formation in liquid cultures of *Y. lipolytica* [118]. A further peculiarity of *Y. lipolytica* is that the *YlSTE7* gene, an homologue of the *STE7* genes from *C. albicans* and *S. cerevisiae*, is not required for hyphal development (A. Domínguez, personal communication). On the other hand, deletion of *YlTUP1* stimulates hyphal growth as it does in *C. albicans* (A. Domínguez, personal communication).

XPR2 which encodes an alkaline extracellular proteinase from *Y. lipolytica* appears to be involved in regulation of dimorphism [118]; the expression of *XPR2*, in turn, is controlled by Rim101, homologous to *S.c.* Rim101 [119,120], while the processing of the native Xpr2 is carried out by a dibasic endoprotease Xpr6 [121]. This regulatory pathway would be equivalent to the pathway controlled by the *RIM* genes in *S. cerevisiae*.

The product of the homeogene *HOY1*, which shows some regions of homology with the *S. cerevisiae* transcriptional activator Pho2, is required for hyphal formation in *Y. lipolytica* [122]. The expression of *HOY1* increases when the yeast cells are induced to form hyphae but there is not yet information on the genes which could be controlled by Hoy1.

Even the fission yeast *Schizosaccharomyces pombe* may undergo pseudohyphal growth in certain stress conditions. Although very little is known on the mechanisms underlying the morphological transition, there have been reports that a defect in a MAP kinase, Pmk1/Spm1 [123,124] or in a MEK kinase, Mkh1 [125] interferes with the completion of cytokinesis and results in growth of filaments with multiple septa and nuclei. The absence of a coiled-coil protein, Alm1, with similarity to myosin and other fibrous proteins, results in the production of a high proportion of elongated mononucleate cells [126]. Since Alm1 is associated with the medial region of the cells during nuclei separation at anaphase, it has been suggested that Alm1 plays a role in initiating cell division.

The fungal pathogens *Cryptococcus neoformans* and *Ustilago maydis* show some characteristics which set them apart from the fungi we have considered until now. Under suitable environmental conditions haploid, budding cells mate and generate a dikaryotic filamentous cell type

[9,127]. However, a true dimorphic transition from a haploid yeast phase to a hyphal phase has also been observed for both species [128,129]. In the case of *C. neoformans* this transition, called haploid fruiting, is stimulated in the presence of a dominant RAS1 mutant protein [130] and requires a STE12 homologue [130,131]. STE12 in turn is under the control of the G-protein β subunit GBP1, which has been proposed to activate a MAP kinase pathway [132]. Although cAMP has been shown to suppress the mating defect of a *gpa1* mutant lacking a G-protein α subunit homologue, there is no evidence that cAMP affects haploid fruiting [133].

For *U. maydis,* a disruption of the genes encoding adenylate cyclase (*UAC1*) or the major catalytic subunit of cAMP-dependent protein kinase (*ADR1*) resulted in a constitutive filamentous phenotype [129,134]. Filamentous growth in a *uac1* mutant was suppressed by the lack of the MAP kinase kinase, Fuz7, or of the MAP kinase, Ubc3 [135]. These observations support the idea that morphogenesis in *U. maydis* is controlled by both cAMP and MAP kinase signal transduction pathways. However, while in the well studied cases of *S. cerevisiae* and *C. albicans*, cAMP stimulates filamentation, in the case of *U. maydis* cAMP is required for budding growth. It may be noted, too, that for *Mucor rouxii*, cAMP levels were found to decrease before the yeast-to-hypha transition induced by exposure to air and that this transition was blocked by addition of dibutyryl-cAMP [136].

7. Conclusions and perspectives

The role played in morphogenesis by a MAP kinase cascade and by a cAMP-dependent pathway has been clearly established in *S. cerevisiae*. The MAP kinase cascade is required for cell elongation while the cAMP-dependent protein kinase Tpk2 controls the switch to unipolar budding [30]. For invasiveness both pathways appear redundant to some extent since a defect in one of the pathways may be compensated by an increased activity in the other [40].

However, large gaps in knowledge remain, which concern different aspects of the morphogenetic process. Without being exhaustive, the following points can be listed:

1. How do external signals trigger the operation of the regulatory pathways?
   The receptor Gpr1 and its coupled G protein, Gpa2, appear to be important in the response to the nutritional situation, and a role has also been suggested for the membrane protein Sho1. However, due to the fact that Ras2 is situated upstream of the two well-known regulatory pathways, it should be expected that some signal is transmitted to the Ras2 protein itself and this point has not yet been addressed.
2. What are the final targets for the different regulatory pathways?
   Actin appears to play a very important role in determining the budding pattern and the morphology of the cells (round vs. elongated), but it is not yet clear how Tpk2 and the MAP kinase cascade influence the structure of the actin cytoskeleton.
   It is also clear that the formation of chains of cells as well as invasiveness would require specific cell-surface proteins. The only such protein identified up to now is Muc1/Flo11 but other proteins may be involved and it could be worth looking more closely at the putative membrane protein Dfg16 required for agar invasion.
3. What are the roles for a number of elements involved in PH growth but which have not been yet connected with the main regulatory pathways?
   One of these elements is the cyclin-dependent kinase Cdc28. It has been suggested that the MAP kinase cascade which leads to the activation of Ste12 could modulate the cyclin/Cdc28 complexes either by promoting hyperphosphorylation of Cdc28 [63] or by inhibiting the mitotic Clb/Cdc28 complexes [58]. However, the evidence for this is still rather indirect and further research will be necessary to test these suggestions.
   Another protein which stimulates PH growth, but for which no mechanism of action has been described is Phd1. It has been shown that Phd1 acts independently of Tpk2 [30], but Phd1 could be a substrate for Tpk1 or Tpk3. In this context, it can be noted that the Phd1 homologue in *C. albicans* (Efg1) is likely to be controlled through phosphorylation by a cAMP-dependent protein kinase (D. Bockmühl and J.F. Ernst, personal communication).

An intriguing point which has not received much attention is the fact that invasiveness of haploid strains grown in liquid medium and spotted in test plates is strongly dependent on the growth phase at which the cells were sampled from the liquid cultures. Late-exponential phase yeast cells showed a stronger invasive phenotype. It has been suggested that the phase of growth could even affect the results of epistasis analysis [42]. Differences due to the growth state of the cells used in different laboratories may explain some of the contradictions between reports from different groups.

When the factors which regulate morphogenesis in different yeasts are compared, it is plain that no unified picture emerges. Nitrogen starvation stimulates filamentous growth in both *S. cerevisiae* and *C. albicans*, but inhibits hyphae formation in cultures of *Y. lipolytica*. With respect to cAMP, it has a positive effect on filamentation in *S. cerevisiae* and *C. albicans*, but a negative one in *M. rouxii* or *U. maydis*. There are also marked differences between *S. cerevisiae* and *C. albicans*: disruption of *TUP1* stimulates hyphal growth in *Candida* and reduces PH growth in *Saccharomyces* diploids, whereas disruption of *RIM101*

has no effect in a *Saccharomyces* diploid, but disruption of its homologue, *HRM101*, impairs filamentation in *Candida*. On the other hand some signaling pathways, such as the MAP kinase cascade leading to phosphorylation of Ste12, the cAMP-dependent pathway, or the pathway involving Phd1 in *S. cerevisiae* and its homologue Efg1 in *C. albicans* are found in the two yeasts although they could differ in the regulatory details. Therefore, even in the absence of extensive studies in species other than *S. cerevisiae* and *C. albicans*, it could be concluded that the fungal signal transduction pathways have been only partially conserved during evolution. Depending on the ecological niche where each particular species has evolved, diversity has been generated, through selection of the mechanisms which allowed a better adaptation to the features of each particular environment.

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