SIGNALLING TOWARDS CELL WALL SYNTHESIS IN BUDDING YEAST

Vladislav Raclavský

Centre of Molecular Biology and Medicine, Faculty of Medicine, Palacký University, CZ-775 15 Olomouc, Czech Republic

Received February 10, 1998

Key words: Saccharomyces cerevisiae / Budding yeast / Cell wall / Signalling / PKC1

The budding yeast *Saccharomyces cerevisiae* has long proved to be a very useful model in cell biology. Its cell morphology is established and maintained at least in part by the cell wall, a rigid but dynamic structure that affords mechanical protection. Although fungal cell walls represent an unique phenomenon, recent progress in research has shown striking parallels between yeast and mammalian cells in the area of cell morphogenesis and proliferation. Further studies promise to shed common light on the processes of cell morphogenesis including the intersections with proliferation control. This review focuses on the recent progress in this promising area in the yeast *Saccharomyces cerevisiae*.

The process of cell wall synthesis in *Saccharomyces cerevisiae* was reviewed by several authors recently[1,2,3]. Briefly, the cell wall represents a complex structure of cross-linked chitin, β-(1,6)-d-glucan, β-(1,3)-D-glucan and mannoproteins. Chitin and β-(1,3)-D-glucan are synthesized by enzymatic complexes at the cell membrane and extruded into the periplasmic space, mannoproteins are synthesized along the yeast secretory pathway, and the site of β-(1,6)-D-glucan synthesis is still unknown. The principal motif which interconnects individual cell wall constituents was recently identified by Kollár[4] et al. The mechanisms of cross-linking of the polymers in the wall remain unknown, however. Recently, nevertheless, substantial progress has been achieved in understanding the signalling pathways which target the cell wall construction.

DISSECTING THE PKC1-PATHWAY

Levin[5] et al. first described a *Saccharomyces cerevisiae* homolog of rat protein kinase C (PKC) gene, deletion of which resulted in a cell cycle arrest between DNA replication and mitosis, and was represented by cells with small buds. The same gene was later detected to complement mutants showing a cell lysis defect in hypotonic environment[6] and mutants hypersensitive to the PKC-inhibitor staurosporine[7]. The cell lysis defect was compensated in an osmotically stabilized medium and the cell walls of pkc1 mutants were darker than wild-type cell walls, which was supposed to reflect changes in cell wall composition[6]. Further, loss of PKC1 function was shown to result in cessation of protein synthesis[8]. When the first dominant extragenic supressor, BCK1, was isolated due to its ability to bypass the requirement for functional PKC1 gene[9], the hunting of kinases functioning in a presumed kinase cascade was started. Bck1p was shown to be a protein kinase, which functions most probably downstream of PKC1 in a common pathway, as bck1 mutants (i) showed a pattern of cell lysis similar to that of pkc1 mutants, and (ii) BCK1 supressor mutations bypassed the requirement for PKC1[9]. As PKC1 is essential at all temperatures and BCK1 is only essential at 37 °C, Lee and Levin[9] suppose a bifurcated pathway, where BCK1 functions in one of the pathway branches downstream of PKC1. In this model, loss of either branch of the pathway should not be as severe as the defect associated with the loss of PKC1.

A MAP kinase homolog MPK1 was identified as a further component of this pathway by Lee[10] et al. This gene was identified as dosage supressor of a bck1 deletion defect and mpk1 mutants showed a cell lysis defect indistinguishable from the defect associated with deletion of bck1. As neither expression of an activated allele of BCK1 nor overexpression of wild-type BCK1 supressed the mpk1 defect, it was concluded that MPK1 functions downstream of BCK1[10]. Later Mazzoni[11] et al. detected the SLT2 gene which was found to be identical with MPK1. Mutation of this gene was detected as an enhancer of the division defect of cells expressing a partially inactivated cdc28. The results suggested that the Mpk1p/Slt2p is either a downstream activation target of the Cdc28 kinase or that Slt2p and Cdc28p function in parallel in promoting bud emergence and subsequent growth[11]. The slt2 mutant

cells were also shown to exhibit a number of phenotypes that recall those of yeast cytoskeletal mutants including morphological defects, delocalization of both chitin deposition and actin cortical spots, and accumulation of secretory vesicles. This clearly demonstrates that Mpk1p/Slt2p is involved in polarized growth, possibly through phosphorylation of one or more cytoskeletal components. A pair of redundant protein kinase genes MKK1/MKK2 was further isolated by Irie[12] et al. as supressors of the pkc1 cell lysis defect. The mkk1/mkk2 double mutant showed a temperature-sensitive cell lysis defect similar to that showed by bck1. Overexpression of MKK1 suppressed the growth defect of bck1 and overexpression of MPK1 suppressed the defect of the mkk1/mkk2 double mutant, whereas an activated allele of BCK1 did not suppress the defect of the mkk1/mkk2 double disruption. Thus, it can be supposed that the pair of Mkk1p/Mkk2p kinases functions downstream of Bck1p and upstream of Mpk1p in the pathway[12].

Finally, Levin[13] et al. summarized these findings in a model of the PKC1-pathway and described the cell wall ultrastructure in Pkc1p-depleted cells. These cells showed cell walls reduced in thickness to approximately 60 % of wild-type cells. Either marked thinnings of the cell wall or even single breaches releasing plums of membranes and cytoplasm were often observed at the bud tips. Based on the above mentioned findings the PKC1-pathway has also been termed as the cell integrity and proliferation pathway. Based on (i) the epistatic experiments, (ii) on the fact that Pkc1p phosphorylates Bck1p in vitro, and (iii) on the much lower levels of Mpk1p protein kinase activity in bck1 mutants, it is generally accepted that this pathway represents a three-gene module characteristic of the extracellulary regulated kinase pathways. In this pathway, the signal should be transfered from Pkc1p to the MAPKKK (mitogen-activated protein kinase kinase kinase) Bck1p, next through the pair of MAPKKs (MAP kinase kinases) Mkk1p/Mkk2p, finally to the MAPK (MAP kinase) Mpk1p. A two-hybrid screen for protein-protein interactions confirmed some of the interactions which could be expected from epistatic experiments, however other were not confirmed and some new unexpected interactions were detected[14]. Bck1p-Mkk1p, Bck1p-Mkk2p and Mkk2p-Mpk1p interactions were confirmed, but not the Pkc1p-Bck1p interaction. Capacity for possible Bck1p-Bck1p dimerization was newly found as well as a Pkc1p-Mkk1p interaction, which may represent a feedback loop as an attenuation mechanism. These results suggest that the members of the pathway form a complex in vivo.

IDENTIFYING THE TARGETS OF PKC1-PATHWAY

Although it was clear that the PKC1-pathway functions in cell wall integrity, cell polarity, and proliferation control, some of the targets were identified only recently and others may be still unknown. First, two nonhistone chromosomal proteins Nhp6A and Nhp6B described earlier[15,16] as homologous to bovine high mobility group protein 1 (HMG1) were identified to be multicopy suppressors of the synthetic lethality of bck1 and spa2[17]. The NHP6A/NHP6B genes were shown to be functionally redundant. Strains from which both NHP6 genes were deleted shared many phenotypes with pkc1, bck1 and mpk1 mutants. They showed an osmotically remediable temperature-sensitive growth defect and also exhibited a variety of morphological and cytoskeletal defects[17]. Nhp6Ap/Bp were found to be required for the induction of a subset of genes, however none of them involved in cell wall synthesis, integrity or polarity. Namely, activation of the CUP1 (copper-binding metallothionein), CYC1 (cytochrome c), GAL1 (galactokinase), and DDR2 (for DNA damage response) genes was decreased or abolished completely in the nhp6A/B double mutant[18].

In another screen, Watanabe[19] et al detected a transcription factor RLM1 to interact genetically with the PKC1-pathway by suppressing the growth-inhibitory effect of MKK1 overexpression. Although rlm1 mutants were able to grow, they displayed a caffeine-sensitive phenotype similar to that of bck1, mkk1/mkk2 or mpk1 mutants and a gene fusion providing Rlm1 transcriptional activation suppressed defects associated with bck1 and mpk1 disruptions[19]. The supposed role of Rlm1p as a transcription factor which functions downstream of Mpk1p was confirmed by Dodou and Treisman[20], who showed the C-terminal sequences of RLM1 to function as Mpk1p-dependent transcriptional activators and described the Rlm1p DNA-binding specificity as the consensus sequence CTA(T/A)$_4$TAG. Rlm1p was demonstrated to activate transcription in a manner regulated by Mpk1p which was shown to directly phosphorylate it[21]. Finally, RLM1 is also able to suppress the calcineurin-requirement for viability of an fks1 mutant (see also the subchapter „Cell wall and ion stress“) indicating that Rlm1p regulates expression of FKS2 gene coding for the catalytic subunit of glucan synthase 2[22]. However Rlm1p is not required for the heat stress activation of FKS2 through the PKC1-pathway[22], thus an alternative branch should exist as well. Further, in a screen for hypo-osmolarity-sensitive mutants Shimidzu[23] et al. identified a pkc1 mutant which overproduced a β-glucanase, encoded by BGL2. Disruption of BGL2 partially rescued the growth rate defect, suggesting that the PKC1 kinase cascade regulates BGL2 expression negatively. However, evidence for the mediators of this negative regulation is still lacking.

Substantial progress in identifying PKC1-pathway targets were done by Igual[24] et al. who studied mpk1 disruptants and detected a strong reduction in expression of FKS1 (β-(1,3)-D-glucan synthase 1 catalytic subunit), MNN1 (α-1,3-mannosyltransferase), CHS3 (chitin synthase 3 catalytic subunit), and significant reduction in the expression of GAS1 (GPI-anchored protein involved in β-(1,3)-D-glucan synthesis), and KRE6 (engaged in synthesis of β-(1,6)- and β-(1,3)-D-glucan). They further detected synthetic lethality of pkc1/swi4 double mutants. Swi4p forms together with Swi6p the heterodimeric transcription factor SBF which regulates gene expression in late G_1 by its binding to a specific promoter sequence called the SCB element. This element was found in the promoters of many genes involved in the synthesis of cell

wall components and clear cell cycle regulation peaking near the G_1/S boundary was observed in transcription of FKS1, MNN1, CHS3, GAS1, KRE6 and VAN2 (may regulate Golgi function and glycosylation)[24]. Finally, phenotypic similarities between mpk1 and swi4 mutants and the fact that Swi4p and Swi6p are phosphoproteins led to an investigation of whether SBF might be activated by Mpk1p. Madden[25] et al. found SBF to exist in a complex with Mpk1p and detected the ability of Mpk1p to directly phosphorylate Swi4p and Swi6p in vitro. Although other results predict that SBF and Mpk1p may also have independent functions[24], it can be supposed that at least 5 genes directly involved in cell wall synthesis are transcriptionally activated by Mpk1-phosphorylated SBF. In addition, Mpk1p-activated SBF promotes expression of the PCL1 and PCL2 G_1 cyclins resulting probably in coordination of polarized growth events and entry into S phase[25]. However, other results question these conclusions and show that at least FKS1 should not be under the control of the PKC1-pathway[22].

ROLE OF RHO1P-GTPase

Small Rho-type GTPases have repeatedly been shown to play universal roles in signalling and morphogenesis. In the case of cell wall synthesis in yeast the Rho1p plays apparently a central role. It was first identified as a member of the Ras-superfamily (Rho for Ras-homologous)[26]. Later it was shown to be crucial for bud formation and was localized to the periphery of yeast cells including the site of bud emergence, the tip of the growing buds, and the mother-bud neck region of cells prior to cytokinesis[27]. The role of this protein in yeast was further elucidated by the finding that GTP-Rho1p but not GDP-Rho1p interacts with the region of Pkc1p containing the pseudosubstrate site and the C1 domain[28]. It was demonstrated that Rho1p regulates the activity of Pkc1p[29,30] and that there are at least two signalling pathways regulated by Rho1p[28]. Moreover, the Rho1p was further identified as a regulatory subunit of β-1,3-D-glucan synthase[31,32]. There are two genes coding for β-(1,3)-D-glucan synthase catalytic activity, FKS1 and FKS2, which are differentially expressed[33], however it is not certain, that both gene products are regulated by Rho1p in vivo. Co-purification and co-immunoprecipitation of Rho1p and Fks1p, but not Fks2p were described[31,32], however glucan synthase activities in both fks1 and fks2 membrane preparations were described to be equally sensitive to inactivation by Rho1p-specific ribosylation, suggesting that Rho1p is the required, or at least predominant, regulatory component of glucan synthase containing either Fksp isoform[32].

As Rho proteins function in the organization of the cytoskeleton in animal cells, an analogous role integrating cell wall synthesis and polarity controls could be expected for Rho1p. In a two-hybrid screen BNI1, known to be implicated in cytokinesis or establishment of cell polarity, was identified[34]. BNI1 is a member of the formin family which forms complexes with actin-associated proteins, profilin and Bud6p[35]. Thus, Rho1p regulates not only the glucan synthase activity directly and the expression of several genes engaged in cell wall synthesis through the PKC1-pathway, but it regulates the cytoskeleton assembly as well, probably at least through Bni1p and through Mpk1p. As Rho1p functions upstream of Pkc1p, its regulation will be reviewed in the next section.

WHAT ARE THE SIGNALS THAT ACTIVATE THE PKC1-PATHWAY?

Members of the family of mammalian protein kinase C kinases generally respond to extracellular signals that act through receptor-mediated hydrolysis of phosphatidylinositol-4,5-bisphosphate to diacylglycerol and inositol-1,4,5-trisphosphate[36]. Diacylglycerol serves as a second messenger to activate PKC[37,38], and inositol-1,4,5-trisphosphate functions to mobilize Ca^{2+} from intracellular stores[39]. Calcium ions serve as cofactors for Pkc1p activation. However, it remains unclear if the yeast Pkc1p may be activated in an analogous way, as the in vitro Pkc1p activity is calcium independent[40]. Kamada[41] et al. showed that the Mpk1p is activated in response to mild heat shock, rapid reduction in extracellular osmolarity, and treatment with chlorpromazine. All of these stimuli are supposed to have one characteristic feature in common: plasma membrane stretch. The PKC1-pathway response to mild heat shock is not responsible for heat shock-induced expression of genes under STRE or HSE control. As there is a considerable delay in Mpk1p response to the mild heat shock, Kamada[41] et al. suppose the activation of Pkc1p can be attributable to increased membrane fluidity and/or to development of weakness in the cell wall which result in plasma membrane stretch. Both of the other stimuli observed to activate Mpk1p, namely rapid reduction of extracellular osmolarity and insertion of the amphipathic drug chlorpromazine into the plasma membrane lipid bilayer, should cause a plasma membrane stretch as well. It can be only speculated about how the plasma membrane stretch signal could be tranformed and transmitted to Pkc1p. Yeast cells possess mechanosensitive ion channels which pass both cations and anions[42] and a calcium pulse was described in response to hypotonic shock in yeast[43]. Calcium ions can thus be candidates for Pkc1p activation or co-activation, as the thermosensitivity of pkc1 mutants can be supressed by growth at high levels of calcium[44]. However, the recent finding that FKS2 gene is regulated in response to ion stress via the Ca^{2+}-dependent phosphatase calcineurin offers alternative explanation for the impact of increase in Ca^{2+} as well (see also the next section). Anyway, the role of PKC1-pathway in hypotonic shock signalling is clear and unquestioned[45]. Recently it was demonstrated that an increase in FKS2 transcription induced by mild heat shock is mediated independently both by calcineurin signalling in early response and by PKC1-pathway signalling in late response[22].

Another possible candidate for the upstream regulator of Pkc1p activity was isolated in a screen for supersensitivity to the protein kinase C inhibitor staurosporine. The STT4 gene codes for a phosphatidylinositole-4-kinase,

which functions probably in the same pathway upstream of PKC1[46]. Finally, more putative upstream regulators of Pkc1p were isolated recently. A HCS77 gene coding for a putative integral membrane protein shows dose-dependent suppression of swi4 mutants. hcs77 mutants exhibit phenotypes like mpk1 mutants, are defective in heat shock induction of Mpk1p activity and can be partially suppressed by overexpression of PKC1[47]. A family of genes WSC1, WSC2 and WSC3 coding for putative integral membrane proteins was further described to function upstream of PKC1 in mild heat shock signalling as well[48]. Thus, there are lot of new findings concerning stress signalling through PKC1-pathway. Although our knowledge is not complex enough, better understanding of the modes of Pkc1p-stress activation can be expected soon.

However, PKC1-signalling does not function in stress-signalling solely. In *S. cerevisiae*, entry into the mitotic cell cycle is regulated at a point late in the G_1 interval, operationally defined as START. Cyclin Cln3 was shown to activate the central Cdc28 protein kinase, which in turn transcriptionally activates the CLN1 and CLN2 genes[49]. Sudden accumulation of cyclins Cln1 and Cln2 occurs during late G_1, which is required for the execution of most START-related events. Cln1 together with Cln2 were demonstrated to further activate the Cdc28 protein kinase to regulate the other START-regulated events. Bud emergence is one of these events and this requires activation of cell wall synthesis. The possible role of PKC1-pathway in this process was supported by the isolation of a mpk1 mutant as an enhancer of the division defect associated with cdc28 mutation[50]. It was proposed that Mpk1p may function downstream or in parallel with Cdc28p in stimulating polarized growth. Further, activation of Mpk1p during periods of polarized growth was found. In a screen for mutations that decrease the effectiveness of signaling by the Cdc28 kinase, the PKC1 gene was identified, and a cell cycle dependent hydrolysis of phosphatidylcholine to diacylglycerol was demonstrated[51]. These results implied that an activation of Pkc1p may be mediated via Cdc28p-dependent stimulation of phosphatidylcholine-specific phospholipase C (PC-PLC). Based on experiments with suppression of the growth defect associated with swi4 mutation it was concluded that PKC1-pathway promotes bud emergence in a Cdc28-Cln1– and Cdc28-Cln2-dependent process[47].

Another element which regulates the PKC1-pathway is the GTPase Rho1p, as already mentioned above. Like other small GTPases, Rho1p switches between an inactive GDP-bound form and an active GTP-bound form. The switch from inactive to active form is mediated by the GEF (GDP/GTP exchange factor) Rom2p[52], the switch from active to inactive form is mediated by the GAP (GTPase activating protein) Bem2p[53], and the inactive GDP-bound state may be stabilized by the rho-GDI (GDP dissociation inhibitor) described to function in yeast rho-proteins[54]. As another protein which interacts with Rho1p and may function in regulating the binding of Rho1p to its targets or regulatory proteins the Bem4p was isolated[55]. The GEF Rom2p was further shown to be activated by the phosphatidylinositol kinase Tor2p[56], which is involved in nutrient sensing independent from the RAS/cAMP pathway[57]. If nutrients are available, Tor2p in concert with Tor1p activates translation initiation and Tor2p controls organization of the actin cytoskeleton[58]. Both processes mediate progression through early G_1 if nutrients are available, or eventually stop it in response to starvation. As the regulation of Pkc1p by Rho1p was clearly established, it can be supposed that cell wall synthesis is regulated in response to nutrient availability through the Tor2p-Rom2p-Rho1p-Pkc1p pathway as well. Indirect evidence supporting this suggestion was demonstrated by the finding that bem2 mutants (Bem2p functions as GAP for Rho1p) display not only disturbed polarization, but defects in cell wall thickness, in resistance to sonication and to the action of hydrolytic enzymes as well[59].

CELL WALL AND ION STRESS

As already mentioned above, the PKC1-pathway and thus changes in cell wall synthesis play crucial role in response to hypotonic shock or adaptation to hypotonic environment[45]. Another well studied signalling pathway, namely the HOG-pathway (high osmolarity-glycerol), plays a crucial role in the response to hypertonic stress. The main target of this pathway is the glycerol accumulation which compensates for the loss of water in hypertonic environment. This pathway was not reported to influence cell wall synthesis yet, thus the most recent review[60] should be consulted for detailed information. However, cell wall synthesis is undoubtedly regulated in response to ion stress. In yeast, Ca^{2+}-dependent Ser/Thr protein phosphatase 2B (calcineurin) was shown to play a crucial role in the regulation of salt homeostasis in response to ion stress[61]. This is done through the Tcn1p transcription factor, which was shown to regulate expression of ENA1 gene (plasma membrane Na^{+}-pump)[61], PMR1 gene (Ca^{2+}-pump)[62], and PMC1 gene (vacuolar Ca^{2+}-ATPase)[62]. In our context it is important that mutants deficient in the glucan synthase catalytic subunit FKS1 show a calcineurin-dependent growth[63]. Also, it was shown that overexpression of MPK1 suppresses the mutation of the gene CNB1 which encodes the calcineurin regulatory subunit, demonstrating partial functional redundancy of the PKC1- and calcineurin-pathways[64]. Calcineurin was further shown to affect the budding pattern and cell morphology[65] and the upstream element calmodulin concentrates at regions of active cell growth[66]. All these findings indicate calcineurin-signaling to be involved in cell polarity and cell wall synthesis. The engagement in cell wall synthesis was finally confirmed by the recent finding that calcineurin activates FKS2 transcription through the Tcn1p transcription factor[62,67]. As already mentioned above, the FKS2 transcription is under control of the PKC1-pathway as well[22].

In an attempt to identify novel phosphatases in yeast the protein phosphatases PPZ1 and PPZ2 were isolated[68,69] and it was shown that they are involved in the maintainance of yeast osmotic stability, possibly in a way related to the function of the PKC1-pathway[70,71]. This

was further supported by the fact that these protein phosphatases bypass the requirement for functional PKC1 and MPK1 genes in a dosage-dependent fashion[72]. Later it was shown that these phosphatases are important determinants in salt tolerance and play a functional role opposite to calcineurin, most probably through repression of the ENA1 gene[73]. Two further proteins were shown to possibly function in a common pathway branch with PPZ1/PPZ2: Bck2p, a serine/threonine-rich protein[72], and Bro1p, required for regulation of cell proliferation in response to nutrient limitation[74]. However, it remains unclear what the cell wall synthesis targets of the PPZ1/PPZ2-pathway are. There are two models proposed for the apparent partial redundancy of the PKC1-pathway and the PPZ1/PPZ2-pathway. In one model, Pkc1p regulates the Ppz1p/Ppz2p branch of the pathway parallel to the branch represented by Bck1p-Mkk1p/Mkk2p-Mpk1p, in a second model, Ppz1p/Ppz2p are not regulated by Pkc1p, but they contribute to the pathway at a point below or at the same level as Mpk1p[72]. The first model could be favored by the finding that recombinant Ppz1p was detected to be phosphorylated in vitro by protein kinase C[75].

MATING PROJECTION FORMATION

During mating, *Saccharomyces cerevisiae* cells form mating projections, also called shmoos, which growth towards the source of sexual pheromone from the mating partner. A cell fusion occurs then at the contact site of the mating partners. Both of these processes require regulation of cell wall lysis and synthesis. The pheromone signalling pathway was the first and best studied signalling pathway in yeast. It was also the first time when a gene involved in cell wall synthesis, namely the CHS1 (chitin synthase) gene, was identified to be regulated in response to a signal in yeast[76]. Deposition of chitin at the tips of shmoos was described[77], Chs1p is, however, not responsible for this synthesis and the β-subunit of protein geranylgeranyltransferase type-I encoded by CDC43/CAL1 was shown to be required for this synthesis[78]. The engagement of this gene in cell wall synthesis was shown earlier when it was described to be required for chitin synthase 3 activity[79], although its role in cell wall synthesis and regulation is far more complex[80]. However, in the case of mating projection formation no changes in CDC43/CAL1 transcription were detected[78], thus its requirement for pheromone induced chitin synthesis indicates only that Chs3 is probably responsible for the chitin deposition at the tip of the mating projection. The CHS1 transcription is activated probably due to the fact that Chs1 acts as a repair enzyme counteracting the lytic processes needed during mating projection formation and cell fusion.

The yeast mating pathway was described to consist of a receptor, which activates a heterotrimeric G-protein by dissociation of an inhibitory Gα subunit from stimulatory Gβγ subunit. This activates then a kinase cascade consisting of Ste20p-Ste11p-Ste7p-Fus3p,Kss1p, where Ste5p functions as a protein scaffold for the interaction[81]. Which way is this pathway coupled with control of cell wall synthesizing enzymes? One piece of evidence was demonstrated by the finding that Mpk1p phosphorylation is induced if cells are treated with sexual pheromone[82,50]. Zarzov[50] et al. showed that this activation of the PKC1-pathway does not require transcriptional activation by the Ste12p transcription factor, which is regulated by the pheromone signalling kinase cascade, but it does require functional STE20 and BCK1 genes. Buehrer and Errede[83] demonstrated that the Mpk1p activation is only partially impaired in cells lacking Bck1p and propose that activation of transcription by Ste12p serve as a messenger of the pheromone action in PKC1-pathway activation as well. Further, they described that Spa2p and Bni1p contribute in a nonadditive fashion not only to the timing and normal morphology of mating projections but also to the timing of Mpk1p activation[83].

In addition to activation of the PKC1-pathway, Ste20p was shown to bind to the essential Rho-type GTPase Cdc42p[84], which plays a key role in inducing polarization of the actin cytoskeleton and in promoting the localized deposition of secretory vesicles at the projection site[85]. Cdc42p was also shown to interact with the formin Bni1p mentioned earlier, thus possibly linking the pheromone response to regulation of the actin cytoskeleton[35]. In this context it should be further mentioned, that conserved domain in yeast myosins Myo3p and Myo5p was identified as a unique phosphorylation site for Ste20p[86]. The signalling complex of Ste20p may be stabilized by binding to cytoskeleton through its interaction with Bem1p, which interacts with both actin and Ste20p. The role of Bem1p is very important as well, as it interacts further with Cdc24p (GEF for Cdc42p), Ste5p and Far1p, which is a Fus3p substrate required for G_1 arrest and proper polarized growth during mating[87,53,88].

Steps towards understanding another link between mating and cell wall synthesis were started when Ca^{2+}-uptake was detected in response to mating pheromone[89] and found to be essential for survival of pheromone-induced growth arrest[90]. Later, calcineurin activated by Ca^{2+}-calmodulin was shown to be the mediator of this signal[91,92], but it was shown that the essential function of calcineurin in this response may be distinct from its role in modulation of intracellular Ca^{2+}-levels[93]. The link to cell wall synthesis was finally established when FKS2 was detected as one of the targets of calcineurin signalling, as already mentioned above[22]. Although two genes were isolated which code for components of the channel that should mediate Ca^{2+} uptake in response to mating pheromone and salt stress[94–96], the mechanism of its activation remains unclear.

PSEUDOHYPHAL GROWTH

Saccharomyces cerevisiae cells were described to undergo a dimorphic transition that results in invasive filamentous growth of pseudohyphae in response to starvation for nitrogen[97]. This unique process that allows yeasts to forage for nutrients requires a complex regulati-

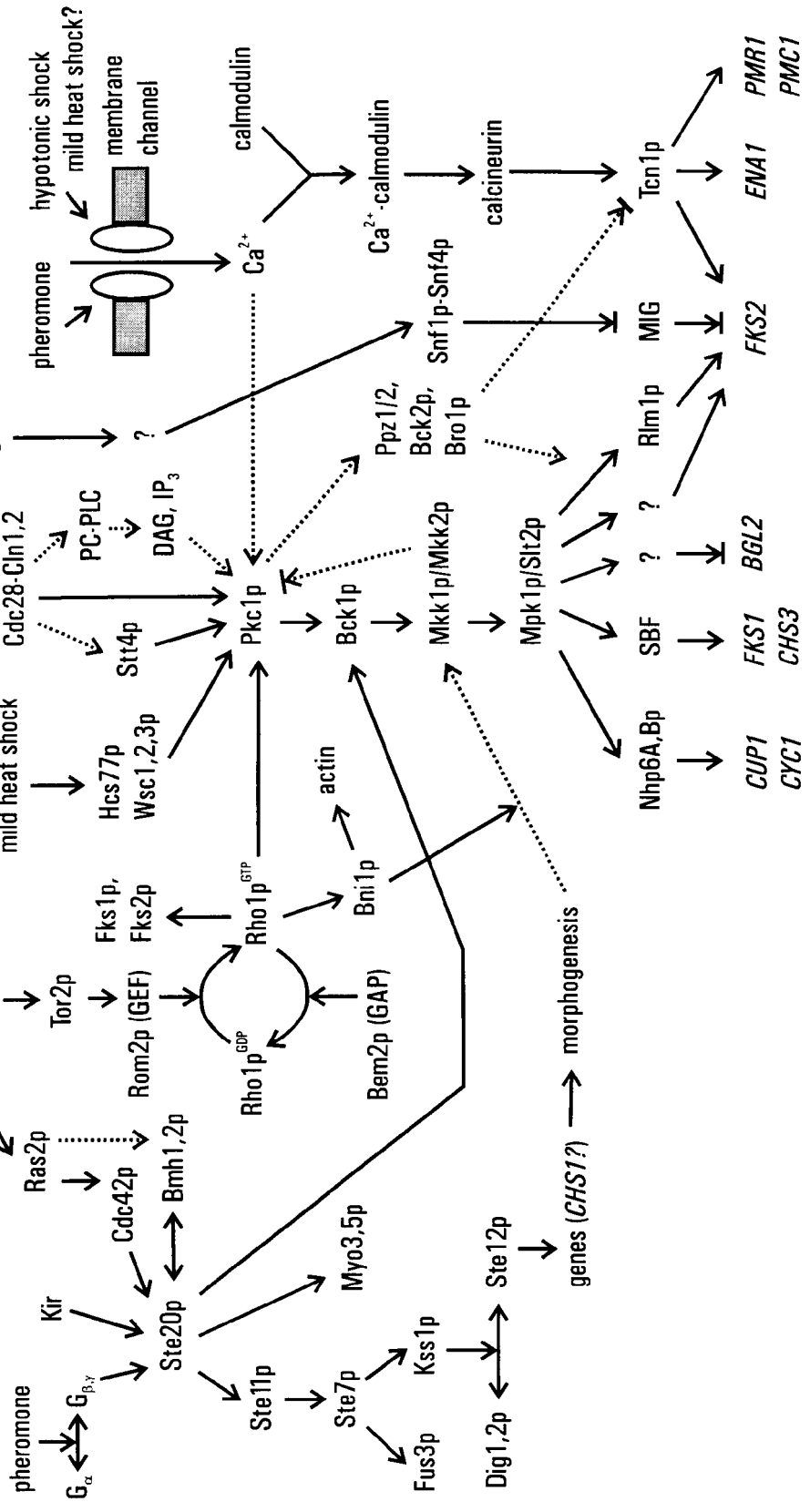

Fig. 1. Scheme of potential signalling pathways towards cell wall synthesis in *Saccharomyces cerevisiae*. Symbols: ⟶ known stimulation, ⇢ speculative stimulation, ⟞ known inhibition, ⇢⟞ speculative inhibition, ⟷ association-dissociation, ⇄ turnover.

on of several cellular functions including pattern of cell division, cell polarity determination, and cell wall synthesis as well. The mechanisms of this complex regulation are still rather unknown, although several components involved in the processes of dimorphic switch were identified. A model integrating the known relationships into a general scheme was proposed recently[98]. We will focus only on those facts which can be supposed to play a role in cell wall synthesis. First, Ras2p was shown to be involved in filamentous growth regulation[97]. Then, the constituents of the pheromone response pathway Ste20p, Ste11p, Ste7p kinases and Ste12p transcription factor were shown to be involved in filamentous growth signalling[99]. Later, a stimulated allele of RAS2 was shown to signal towards Ste20p via the Rho-type GTPase Cdc42p[100]. As another upstream activator of Ste20p, a Ras-family G-protein Kir was described as well[101]. Further, homologs of 14–3–3 proteins Bmh1p and Bmh2p were shown to be essential for pseudohyphal development signalling but not for mating signalling[102]. As they associate with Ste20p in vivo, they are candidates for the discrimination between mating signalling and pseudohyphal growth signalling at the intersection of the pathways represented by Ste20p. Further, it was shown that although the pseudohyphal growth signalling pathway shares Ste20p, Ste11p, Ste7p, Kss1p[103] and Ste12p with the mating pathway, the response is discriminated at the level of Ste12p transcription factor[100]. The primary function of this pathway is to repress the invasive growth in haploids and the pseudohyphal growth in diploids. This repression is mediated by Kss1p in its inactive state and Ste7p acts to relieve this repressive action[103]. The Ste12p transcription factor is probably blocked in its action by the Dig1p and/or Dig2p. If these are phosphorylated by activated Kss1p, Ste12p is permitted to promote gene expression[104,105].

Although the relationship of the above described signalling to cell wall synthesis was not targeted yet, it can be supposed, that analogies or even the same links can be supposed like those which function in the case of pheromone signalling. Thus, the Ste20p-Bck1p link or the relationship between morphogenetic events due to transcription activation through Ste20p and PKC1-pathway activation are the candidates. Nevertheless, any other links including the RAS/cAMP or Elm1 pathways cannot be excluded. One such link was indicated by the finding that FKS2 transcription is, among others, under control of the SNF1 protein kinase and the MIG1 transcriptional repressor[22], which are responsible for „general repression“ in presence of glucose. In context of the cell wall modification in invasive growth a Muc1 protein should be further mentioned at least[106]. Muc1p is a putative integral membrane-bound protein, similar to mammalian mucin-like proteins that have been implicated to play a role in the ability of cancer cells to invade other tissues. The protein backbone of these proteins is very rigid and long, thus Muc1p is supposed to span the cell wall and to help yeast cells to anchor themselves on the surface of the substrate during invasive growth[106].

FINAL COMMENTS

We were certainly not able to include all of the current available knowledge on signals and signalling pathways which function towards cell wall synthesis. In addition, new progress can be observed continuously in this fast developing research area. The complete genome information and well working methodology available in *Saccharomyces cerevisiae* are the best prerequisites to get an even more integrative view of cell morphogenesis soon. Understanding how this is achieved in simple cells will provide us with important clues to interpret how higher cellular systems adapt their shape to their environment.

ACKNOWLEDGEMENTS

V. Raclavský was supported by the Ministry of Education, Youth and Sport (VS 96154).

REFERENCES

1. Cid, V. J., Durán, A., del Rey, F., Snyder, M. P., Nombela, C., Sánchez, M. (1995) Molecular basis of cell integrity and morphogenesis in *Saccharomyces cerevisiae*. Microbiol. Rev., *59*, 345–386.
2. Cabib, E., Drgoň, T., Drgoňová, J., Ford, R. A., Kollár, R. (1997) The yeast cell wall, a dynamic structure engaged in growth and morphogenesis. Biochem. Soc. Trans., *25*, 200–204.
3. Klis, F. M., Caro, L. H., Vossen, J. H., Kapteyn, J. C., Ram, A. F., Montijn, R. C., Van Berkel, M. A., Van den Ende, H. (1997) Identification and characterization of a major building block in the cell wall of *Saccharomyces cerevisiae*. Biochem. Soc. Trans., *25*, 856–860.
4. Kollár, R., Reinhold, B. B., Petráková, R., Yeh, H. J., Ashwell, G., Drgoňová, J., Kapteyn, J. C., Klis, F. M., Cabib, E. (1997) Architecture of the yeast cell wall. β-(1,6)-glucan interconnects mannoprotein, β-(1,3)-glucan, and chitin. J. Biol. Chem., *272*, 17762–17775.
5. Levin, D. E., Fields, F. O., Kunisawa, R., Bishop, J. M., Thorner, J. (1990) A candidate protein kinase C gene, PKC1, is required for the *Saccharomyces cerevisiae* cell cycle. Cell, *62*, 213–224.
6. Paravicini, G., Cooper, M., Friedli, L., Smith, D. J., Carpentier, J.–L., Klig, L. S., Payton, M. A. (1992) The osmotic integrity of the yeast cells requires a functional PKC1 gene product. Mol. Cell. Biol., *12*, 4896–4905.
7. Yoshida, S., Ikeda, E., Uno, I., Mitsuzawa, H. (1992) Characterization of a staurosporine- and temperature-sensitive mutant, stt1, of *Saccharomyces cerevisiae*: STT1 is allelic to PKC1. Mol. Gen. Genet., *231*, 337–344.
8. Levin, D. E., Barlet-Heubusch, E. (1992): Mutants in the *Saccharomyces cerevisiae* PKC1 gene display a cell cycle specific osmotic stability defect. J. Cell Biol., *116*, 1221–1229.
9. Lee, K. S., Levin, D. E. (1992) Dominant mutations in a gene encoding a putative protein kinase (BCK1) bypass the requirement for a *Saccharomyces cerevisiae* protein kinase C homolog. Mol. Cell. Biol., *12*, 172–182.
10. Lee, K. S., Irie, K., Gotoh, Y., Watanabe, Y., Araki, H., Nishida, E., Matsumoto, K., Levin, D. E. (1993) A yeast mitogen-activated protein kinase homolog (Mpk1p) mediates signalling by protein kinase C. Mol. Cell. Biol., *13*, 3067–3075.
11. Mazzoni, C., Zarzov, P., Rambourg, A., Mann, C. (1993) The SLT2(MPK1) MAP kinase homolog is involved in polarized cell growth in *Saccharomyces cerevisiae*. J. Cell Biol., *123*, 1821–1833.

12. Irie, K., Takase, M., Lee, K. S., Levin, D. E., Araki, H., Matsumoto, K., Oshima, Y. (1993) MKK1 and MKK2, which encode Saccharomyces cerevisiae mitogen-activated protein kinase-kinase homologs, function in the pathway mediated by protein kinase C. Mol. Cell. Biol., *13*, 3076–3083.
13. Levin, D. E., Bowers, B., Chen, Ch.–Y., Kamada, Y., Watanabe, M. (1994) Dissecting the protein kinase C/MAP kinase signalling pathway of *Saccharomyces cerevisiae*. Cell. Mol. Biol. Res., *40*, 229–239.
14. Paravicini, G., Friedli, L. (1996) Protein-protein interactions in the yeast PKC1 pathway: Pkc1p interacts with a component of the MAP kinase cascade. Mol. Gen. Genet., *251*, 682–691.
15. Kolodrubetz, D., Haggren, W., Burgum, A. (1988) Amino-terminal sequence of a *Saccharomyces cerevisiae* nuclear protein, NHP6, shows significant identity to bovine HMG1. FEBS Lett., *26*, 175–179.
16. Kolodrubetz, D., Burgum, A. (1990) Duplicated NHP6 genes of *Saccharomyces cerevisiae* encode proteins homologous to bovine high mobility group protein 1. J. Biol. Chem., *25*, 3234–3239.
17. Costigan, C., Kolodrubetz, D., Snyder, M. (1994) NHP6A and NHP6B, which encode HMG1-like proteins, are candidates for downstream components of the yeast SLT2 mitogen-activated protein kinase pathway. Mol. Cell. Biol., *14*, 2391–2403.
18. Paull, T. T., Carey, M., Johnson, R. C. (1996) Yeast HMG proteins NHP6A/B potentiate promoter-specific transcriptional activation in vivo and assembly of preinitiation complexes in vitro. Genes Dev., *10*, 2769–2781.
19. Watanabe, Y., Irie, K., Matsumoto, K. (1995) Yeast RLM1 encodes a serum response factor-like protein that may function downstream of the Mpk1 (Slt2) mitogen-activated protein kinase pathway. Mol. Cell. Biol., *15*, 5740–5749
20. Dodou, E., Treisman, R. (1997) The Saccharomyces cerevisiae MADS-box transcription factor Rlm1 is a target for the Mpk1 mitogen-activated protein kinase pathway. Mol. Cell. Biol., *17*, 1848–1859.
21. Watanabe, Y., Takaesu, G., Hagiwara, M., Irie, K., Matsumoto, K. (1997) Characterization of a serum response factor-like protein in Saccharomyces cerevisiae, Rlm1, which has transcriptional activity regulated by the Mpk1 (Slt2) mitogen-activated protein kinase pathway. Mol. Cell. Biol., *17*, 2615–2623.
22. Zhao, C., Jung, U. S., Garrett-Engele, P., Roe, T., Cyert, M. S., Levin, D. E. (1998) Temperature-induced expression of yeast FKS2 is under the dual control of protein kinase C and calcineurin. Mol. Cell. Biol., *18*, 1013–1022.
23. Shimizu, J., Yoda, K., Yamasaki, M. (1994) The hypo-osmolarity-sensitive phenotype of the *Saccharomyces cerevisiae* hpo2 mutant is due to a mutation in PKC1, which regulates expression of β-glucanase. Mol. Gen. Genet., *242*, 641–648.
24. Igual, J. C., Johnson, A. L., Johnston, L. H. (1996) Coordinated regulation of gene expression by the cell cycle transcription factor SWI4 and the protein kinase C MAP kinase pathway for yeast cell integrity. EMBO J., *15*, 5001–5013.
25. Madden, K., Sheu, Y.-J., Baetz, K., Andrews, B., Snyder, M. (1997) SBF cell cycle regulator as a target of the yeast PKC-MAP kinase pathway. Science, *275*, 1781–1784.
26. Madaule, P., Axel, R., Myers, A.M. (1987) Characterization of two members of the rho gene family from the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA, *84*, 779–783.
27. Yamochi, W., Tanaka, K., Nonaka, H., Maeda, A., Musha, T., Takai, Y. (1994) Growth site localization of Rho1 small GTP-binding protein and its involvement in bud formation in *Saccharomyces cerevisiae*. J. Cell. Biol., *125*, 1077–1093.
28. Nonaka, H., Tanaka, K., Hirano, H., Fujiwara, T., Kohno, H., Umikawa, M., Mino, A., Takai, Y. (1995) A downstream target of RHO1 small GTP-binding protein is PKC1, a homolog of protein kinase C, which leads to activation of the MAP kinase cascade in *Saccharomyces cerevisiae*. EMBO J., *14*, 5931–5938.
29. Drgoňová, J., Drgoň, T., Tanaka, K., Kollár, R., Chen, G.-Ch., Ford, R. A., Chan, C. S. M., Takai, Y., Cabib, E. (1996) Rho1p, a yeast protein at the interface between cell polarization and morphogenesis. Science, *272*, 277–279.
30. Kamada, Y., Quadota, H., Python, Ch., Anraku, Y., Ohya, Y., Levin, D. E. (1996) Activation of yeast protein kinase C by Rho1 GTPase. J. Biol. Chem., *271*, 9193–9196.
31. Quadota, H., Python, Ch. P., Inoue, S. B., Arisawa, M., Anraku, Y., Zheng, Y., Watanabe, T., Levin, D. E., Ohya, Y. (1996) Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-β-glucan synthase. Science, *272*, 279–281.
32. Mazur, P., Baginski, W. (1996) In vitro activity of 1,3-β-D-glucan synthase requires the GTP-binding protein Rho1. J. Biol. Chem., *271*, 14604–14609.
33. Mazur, P., Morin, N., Baginsky, W., El-Sherbeini, M., Clemas, J. A., Nielsen, J. B., Foor, F. (1995) Differential expression and function of two homologous subunits of yeast 1,3-β-D-glucan synthase. Mol. Cell. Biol., *15*, 5671–5681.
34. Kohno, H., Tanaka, K., Mino, A., Umikawa, M., Imamura, H., Fujiwara, T., Fujita, Y., Hotta, K., Qadota, H., Watanabe, T., Ohya, Y., Takai, Y. (1996) Bni1p implicated in cytoskeletal control is a putative target of Rho1p small GTP binding protein in *Saccharomyces cerevisiae*. EMBO J., *15*, 6060–6068.
35. Evangelista, M., Blundell, K., Longtine, M. S., Chow, C. J., Adames, N., Pringle, J. R., Peter, M., Boone, C. (1997) Bni1p, a yeast formin linking cdc42p and the actin cytoskeleton during polarized morphogenesis. Science, *276*, 118–122.
36. Hokin, L. E. (1985) Receptors and phosphoinositol-generated second messengers. Annu. Rev. Biochem., *54*, 205–235.
37. Takai, Y., Kishimoto, A., Kikkawa, U., Mori, T., Nishizuka, Y. (1979) Unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system. Biochem. Biophys. Res. Commun., *91*, 1218–1224.
38. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U., Nishizuka, Y. (1980) Activation of calcium and phospholipid dependent protein kinase by diacylglycerol: Its possible relation to phosphatidyl inositol turnover. J. Biol. Chem., *255*, 2273–2276.
39. Berridge, M. J., Irvine, R. F. (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature, *312*, 315–321.
40. Watanabe, M., Chen, C.-Y., Levin, D. E. (1994) *Saccharomyces cerevisiae* PKC1 encodes a protein kinase C (PKC) homolog with a substrate specificity similar to that of mammalian PKC. J. Biol. Chem., *269*, 16829–16836.
41. Kamada, Y., Jung, U. S., Piotrowski, J., Levin, D. E. (1995) The protein kinase C-activated MAP kinase pathway of *Saccharomyces cerevisiae* mediates a novel aspect of the heat shock response. Genes Dev., *9*, 1559–1571.
42. Gustin, M. C., Zhou, X.-L., Martinac, B., Kung, Ch. (1988) A mechanosensitive ion channel in the yeast plasma membrane. Science, *242*, 762–765.
43. Batiza, A. F., Schulz, T., Masson, P. H. (1996) Yeast respond to hypotonic with a calcium pulse. J. Biol. Chem., *38*, 23357–23362.
44. Levin, D. E., Barlett-Heubusch, E. (1992) Mutants in the *Saccharomyces cerevisiae* PKC1 gene display a cell cycle-specific osmotic stability defect. J. Cell Biol., *116*, 1221–1229.
45. Davenport, K. R., Sohaskey, M., Kamada, Y., Levin, D. E., Gustin, M. C. (1995) A second osmosensing signal transduction pathway in yeast. J. Biol. Chem., *270*, 30175–30161.
46. Yoshida, S., Ohya, Y., Goebl, M., Nakano, A., Anraku, A. (1994) A novel gene, STT4, encodes a phospatidylinositol 4-kinase in the PKC1 protein kinase pathway of *Saccharomyces cerevisiae*. J. Biol. Chem., *269*, 1166–1171.
47. Gray, J. V., Ogas, J. P., Kamada, Y., Stone, M., Levin, D. E., Herskowitz, I. (1997) A role for the Pkc1 MAP kinase pathway of *Saccharomyces cerevisiae* in bud emergence and identification of a putative upstream regulator. EMBO J., *16*, 4924–4937.
48. Verna, J., Lodder, A., Lee, K., Vagts, A., Ballester, R. (1997) A family of genes required for maintenance of cell wall integrity and for stress response in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA, *94*, 13804–13809.

49. Dirick, L., Boehm, T., Nasmyth, K. (1995) Roles and regulation of Cln-Cdc28 kinases at the start of the cell cycle of *Saccharomyces cerevisiae*. EMBO J., *14*, 4803–4813.
50. Zarzov, P., Mazzoni, C., Mann, C. (1996) The SLT2(MPK1) MAP kinase is activated during periods of polarized cell growth in yeast. EMBO J., *15*, 83–91.
51. Marini, N. J., Meldrum, E., Buehrer, B., Hubberstey, A. V., Stone, D. E., Traynor-Kaplan, A., Reed, S. I. (1996) A pathway in the yeast cell division cycle linking protein kinase C (Pkc1) to activation of Cdc28 at START. EMBO J., *15*, 3040–3052.
52. Manning, B. D., Padmanabha, R., Snyder, M. (1997) The Rho-GEF Rom2p localizes to sites of polarized cell growth and participates in cytoskeletal functions in *Saccharomyces cerevisiae*. Mol. Biol. Cell, *8*, 1929–1844.
53. Peterson, J., Zheng, Y., Bender, L., Myers, A., Cerione, R., Bender, A. (1994) Interactions between the bud emergence proteins Bem1p and Bem2p and Rho-type GTPases in yeast. J. Cell Biol., *127*, 1395–1406.
54. Masuda, T., Tanaka, K., Nonaka, H., Yamochi, W., Maeda, A., Takai, Y. (1994) Molecular cloning and characterization of yeast rho GDP dissociation inhibitor. J. Biol. Chem., *269*, 19713–19718.
55. Mack, D., Nishimura, K., Dennehey, B. K., Arbogast, T., Parkinson, J., Toh-E, A., Pringle, J. R., Bender, A., Matsui, Y. (1996) Identification of the bud emergence gene BEM4 and its interaction with Rho-type GTPases in *Saccharomyces cerevisiae*. Mol. Cell. Biol., *16*, 4387–4395.
56. Schmidt, A., Bickle, M., Beck, T., Hall, M. N. (1997) The yeast phosphatidylinositol kinase homolog TOR2 activates RHO1 and RHO2 via the exchange factor ROM2. Cell, *88*, 531–542.
57. Barbet, N. C., Schneider, U., Helliwell, S. B., Stansfield, I., Tuite, M. F., Hall, M. N. (1996) TOR controls translation initiation and early G1 progression in yeast. Mol. Biol. Cell, *7*, 25–42.
58. Schmidt, A., Kunz, J., Hall, M. N. (1996) TOR2 is required for organization of the actin cytoskeleton in yeast. Proc. Natl Acad. Sci. USA, *93*, 13780–13785.
59. Cid, V. J., Cenamor, R., Sánchez, M., Nombela, C. (1998) A mutation in the Rho1-GAP-encoding gene BEM2 of *Saccharomyces cerevisiae* affects morphogenesis and cell wall functionality. Microbiology, *144*, 25–36.
60. Varela, J. C. S., Mager, W. H. (1996) Response of *Saccharomyces cerevisiae* to changes in external osmolarity. Microbiology, *142*, 721–731.
61. Mendoza, I., Rubio, F., Rodriguez–Navarro, A., Pardo, J. M. (1994) The protein phosphatase calcineurin is essential for NaCl tolerance of *Saccharomyces cerevisiae*. J. Biol. Chem., *269*, 8792–8796.
62. Matheos, D. P., Kingsbury, T. J., Ahsan, U. S., Cunningham, K. W. (1997) Tcn1p/Crz1p, a calcineurin-dependent transcription factor that differentially regulates gene expression in *Saccharomyces cerevisiae*. Genes Dev., *15*, 3445–3458.
63. Eng, W.-K., Faucette, L., McLaughlin, M. M., Cafferkey, R., Koltin, Y., Morris, R. A., Young, P. R., Johnson, R. K., Livi, G. P. (1994) The yeast FKS1 gene encodes a novel membrane protein, mutations in which confer FK506 and cyclosporin A hypersensitivity and calcineurin-dependent growth. Gene, *151*, 61–71.
64. Nakamura, T., Ohmoto, T., Hirata, D., Tsuchyia, E., Miyakawa, T. (1996) Genetic evidence for the functional redundancy of the calcineurin- and Mpk1-mediated pathways in the regulation of cellular events important for growth in *Saccharomyces cerevisiae*. Mol. Gen. Genet., *251*, 211–219.
65. Mendoza, I., Quintero, F. J., Bressan, R. A., Hasegawa, P. M., Pardo, J. M. (1996) Activated calcineurin confers high tolerance to ion stress and alters the budding pattern and cell morphology of yeast cells. J. Biol. Chem., *271*, 23061–23067.
66. Brockerhoff, S. E., Davis, T. N. (1992) Calmodulin concentrates at regions of cell growth in *Saccharomyces cerevisiae*. J. Cell Biol., *118*, 619–629.
67. Stathopoulos, A. M., Cyert, M. S. (1997) Calcineurin acts through the CRZ1/TCN1-encoded transcription factor to regulate gene expression in yeast. Genes Dev., *15*, 3432–3444.
68. Da Cruz e Silva, E. F., Hughes, V., McDonald, P., Stark, M. J., Cohen, P. T. (1991) Protein phosphatase 2B and protein phosphatase Z are *Saccharomyces cerevisiae* enzymes. Biochim. Biophys. Acta., *1089*, 269–272.
69. Posas, F., Casamayor, A., Morral, N., Ariño, J. (1992) Molecular cloning and analysis of a yeast protein phosphatase with an unusual amino-terminal region. J. Biol. Chem., *15*, 11734–11740.
70. Posas, F., Casamayor, A., Ariño, J. (1993) The PPZ protein phosphatases are involved in the maintainance of osmotic stability of yeast cells. FEBS Lett., *318*, 282–286.
71. Hughes, V., Muller, A., Stark, M. J., Cohen, P. T. (1993) Both isoforms of protein phosphatase Z are essential for the maintenance of cell size and integrity in Saccharomyces cerevisiae in response to osmotic stress. Eur. J. Biochem., *216*, 269–279.
72. Lee, K. S., Hines, L. K., Levin, D. E. (1993) A pair of functionally redundant yeast genes (PPZ1 and PPZ2) encoding type 1-related protein phosphatases function within the PKC1-mediated pathway. Mol. Cell. Biol., *13*, 5843–5853.
73. Posas, F., Camps, M., Ariño, J. (1995) The PPZ protein phosphatases are important determinants of salt tolerance in yeast cells. J. Biol. Chem., *270*, 13036–13041.
74. Nickas, M. E., Yaffe, M. P. (1996) BRO1, a novel gene that interacts with components of the Pkc1p-mitogen-activated protein kinase pathway in *Saccharomyces cerevisiae*. Mol. Cell. Biol., *16*, 2585–2593.
75. Posas, F., Bollen, M., Stalmans, W., Ariño, J. (1995) Biochemical characterization of recombinant yeast PPZ1, a protein phosphatase involved in salt tolerance. FEBS Lett., *368*, 39–44.
76. Appeltauer, U., Achstetter, T. (1989) Hormone-induced expression of the CHS1 gene from *Saccharomyces cerevisiae*. Eur. J. Biochem., *181*, 243–247.
77. Scheckman, R., Brawley, Y. (1979) Localized deposition of chitin on the yeast cell surface in response to mating pheromone. Proc. Natl. Acad. Sci. USA, *76*, 645–649.
78. Choi, W.–J., Santos, B., Durán, A., Cabib, E. (1994) Are yeast chitin synthases regulated at the transcriptional or posttranslational level? Mol. Cell. Biol., *14*, 7685–7694.
79. Valdivieso, M. H., Mol, P. C., Shaw, J. A., Cabib, E., Durán, A. (1991) CAL1, a gene required for activity of chitin synthase 3 in *Saccharomyces cerevisiae*. J. Cell. Biol., *114*, 101–109.
80. Omer, C. A., Gibbs, J. B. (1994) Protein prenylation in eukaryotic microorganisms: genetics, biology and biochemistry. Mol. Microbiol., *11*, 219–225.
81. Elion, E. A. (1995) Ste5: a meeting place for MAP kinases and their associates. Trends Cell. Biol., *5*, 322–327.
82. Errede, B., Cade, R. M., Yashar, B. M., Kamada, Y., Levin, D. E., Irie, K., Matsumoto, K. (1995) Dynamics and organization of MAP kinase signal pathways. Mol. Reprod. Dev., *42*, 477–485.
83. Buehrer, B. M., Errede, B. (1997) Coordination of the mating and cell integrity mitogen-activated protein kinase pathways in *Saccharomyces cerevisiae*. Mol. Cell. Biol., *17*, 6517–6525.
84. Leberer, E., Wu, C., Leeuw, T., Fourest–Lieuvin, A., Segall, J. E., Thomas, D. Y. (1997) Functional characterization of the Cdc42p binding domain of yeast Ste20p protein kinase. EMBO J., *16*, 83–97.
85. Li, R., Zheng, Y. Drubin, D. G. (1995) Regulation of cortical actin cytoskeleton assembly during polarized growth in budding yeast. J. Cell. Biol., *128*, 599–615.
86. Wu, C., Lytvyn, V., Thomas, D. Y., Leberer, E. (1997) The phosphorylation site for Ste20p-like protein kinases is essential for the function of myosin-I in yeast. J. Biol. Chem., *272*, 30623–30626.
87. Leeuw, T., Fourest-Lieuvin, A., Wu, C., Chenevert, J., Clark, K., Whiteway, M., Thomas, D. Y., Leberer, E. (1995) Pheromone response in yeast: Association of Bem1p with proteins of the MAP kinase cascade and actin. Science, *270*, 1210–1213.
88. Lyons, D. M., Mahanty, S. K., Choi, K.–Y., Manadhar, M., Elion, E. A. (1996) The SH3-domain protein Bem1 coordinates mitogen

activated protein kinase cascade activation with cell cycle control in *Saccharomyces cerevisiae*. Mol. Cell. Biol., *16*, 4095–4106.

89. Ohsumi, Y., Anraku, Y. (1985) Specific induction of Ca2+ transport activity in MATa cells of *Saccharomyces cerevisiae* by a mating pheromone, alpha factor. J. Biol. Chem., *260*, 10482–10486.
90. Iida, H., Yagawa, Y., Anraku, Y. (1990) Essential role for induced Ca2+ influx followed by $[Ca2+]_i$ rise in maintaining viability of yeast cells late in the mating pheromone response pathway. A study of $[Ca2+]_i$ in single Saccharomyces cerevisiae cells with imaging of fura-2. J. Biol. Chem., *265*, 13391–13399.
91. Cyert, M. S., Thorner, J. (1992) Regulatory subunit (CNB1 gene product) of yeast Ca2+/calmodulin-dependent phosphoprotein phosphatases is required for adaptation to pheromone. Mol. Cell. Biol., *12*, 3460–3469.
92. Moser, M. J., Geiser, J. R., Davis, T. N. (1996) Ca^{2+}–camodulin promotes survival of pheromone-induced growth arrest by activation of calcineurin and Ca^{2+}-calmodulin-dependent protein kinase. Mol. Cell. Biol., *16*, 4824–4831.
93. Withee, J. L., Mulholland, J., Jeng, R., Cyert, M. S. (1997) An essential role of the yeast pheromone-induced Ca2+ signal is to activate calcineurin. Mol. Biol. Cell, *8*, 263–277.
94. Iida, H., Nakamura, H., Ono, T., Okumura, M. S., Anraku, Y. (1994) MID1, a novel Saccharomyces cerevisiae gene encoding a plasma membrane protein, is required for Ca2+ influx and mating. Mol. Cell. Biol. *14*, 8259–8271.
95. Paidhungat, M., Garrett, S. (1997) A homolog of mammalian, voltage-gated calcium channels mediates yeast pheromone-stimulated Ca^{2+} uptake and exacerbates the cdc1(Ts) growth defect. Mol. Cell. Biol., *17*, 6339–6347.
96. Fischer, M., Schnell, N., Chattaway, J., Davies, P., Dixon, G., Sanders, D. (1997) The Saccharomyces cerevisiae CCH1 gene is involved in calcium influx and mating. FEBS Lett., *419*, 259–262.
97. Gimeno, C. J., Ljungdahl, P. O., Styles, C. A., Fink, G. R. (1992) Unipolar cell divisions in the yeast *S. cerevisiae* lead to filamentous growth: Regulation by starvation and RAS. Cell, *68*, 1077–1090.
98. Garret, J. M. (1997) The control of morphogenesis in *Saccharomyces cerevisiae* by Elm1p kinase is responsive to RAS/cAMP pathway activity and tryptophan availability. Mol. Microbiol., *26*, 809–820.
99. Liu, H., Styles, C. A., Fink, G. R. (1993) Elements of the yeast pheromone response pathway required for filamentous growth of diploids. Science, *262*, 1741–1744.
100. Moesch, H.–U., Roberts, R. L., Fink, G. R. (1996) Ras2 signals via the Cdc42/Ste20/mitogen-activated protein kinase module to induce filamentous growth in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA, *93*, 5352–5356.
101. Dorin, D., Cohen, L., Del Villar, K., Poullet, P., Mohr, R., Whiteway, M., Witte, O., Tamanoi, F. (1995) Kir, a novel Ras-family G-protein, induces invasive pseudohyphal growth in *Saccharomyces cerevisiae*. Oncogene, *11*, 2267–2271.
102. Roberts, R. L., Mosch, H. U., Fink, G. R. (1997) 14–3–3 proteins are essential for RAS/MAPK cascade signaling during pseudohyphal development in *S. cerevisiae*. Cell, *89*, 1055–1065.
103. Cook, J. G., Bardwell, L., Thorner, J. (1997) Inhibitory and activating functions for MAPK Kss1 in the *S. cerevisiae* filamentous-growth signalling pathway. Science, *390*, 85–88.
104. Cook, J. G., Bardwell, L., Kron, S. J., Thorner, J. (1996) Two novel targets of the MAP kinase Kss1 are negative regulators of invasive growth in the yeast *Saccharomyces cerevisiae*. Gen. Dev., *10*, 2831–2848.
105. Tedford, K., Kim, S., Sa, D., Stevens, K., Tyers, M. (1997) Regulation of the mating pheromone and invasive growth responses in yeast by two MAP kinase substrates. Curr. Biol., *7*, 228–238.
106. Lambrechts, M. G., Bauer, F. F., Marmur, J. Pretorius, I. S. (1996) Muc1, a mucin-like protein that is regulated by Mss10, is critical for pseudohyphal differentiation in yeast. Proc. Natl. Acad. Sci. USA, *93*, 8419–8424.