Zinc Finger Protein Prz1 Regulates Ca$^{2+}$ but Not Cl$^{-}$ Homeostasis in Fission Yeast

IDENTIFICATION OF DISTINCT BRANCHES OF CALCINEURIN SIGNALING PATHWAY IN FISSION YEAST*

Received for publication, December 18, 2002, and in revised form, January 31, 2003
Published, JBC Papers in Press, March 13, 2003, DOI 10.1074/jbc.M212900200

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Calcineurin is an important mediator that connects the Ca$^{2+}$-dependent signaling to various cellular responses in a wide variety of cell types and organisms. In budding yeast, activated calcineurin exerts its function mainly by regulating the Crz1p/Tcn1 transcription factor. Here, we cloned the fission yeast prz1+ gene, which encodes a zinc finger transcription factor highly homologous to Crz1/Tcn1. Similar to the results in budding yeast, calcineurin dephosphorylated Prz1 and resulted in the trans-location of Prz1 from the cytoplasm to the nucleus. Prz1 expression was stimulated by high extracellular Ca$^{2+}$ in a calcineurin-dependent fashion. However, unlike in budding yeast, the prz1-null cells did not show any phenotype similar to those previously reported in calcineurin deletion such as aberrant cell morphology, mating defect, or hypersensitivity to Cl$^{-}$. Instead, the prz1-null cells showed hypersensitivity to Ca$^{2+}$, consistent with a dramatic decrease in transcription of Pmc1 Ca$^{2+}$ pump. Interestingly, overexpression of Prz1 did not suppress the Cl$^{-}$ hypersensitivity of calcineurin deletion, and overexpression of Pmp1 MAPK phosphatase suppressed the Cl$^{-}$ hypersensitivity of calcineurin deletion but not the Ca$^{2+}$ hypersensitivity of prz1 deletion. In addition, mutations in the its2/1/cps1+, its8+, and its10/1/edc7+ genes that showed synthetic lethal genetic interaction with calcineurin deletion did not exhibit synthetic lethality with the prz1 deletion. Our results suggest that calcineurin activates at least two distinct signaling branches, i.e., the Prz1-dependent transcriptional regulation and an unknown mechanism, which functions antagonistically with the Pmk1 MAPK pathway.

Calcineurin is a Ca$^{2+}$/calmodulin-dependent serine/threonine protein phosphatase consisting of a catalytic subunit and a regulatory subunit (1). In mammalian cells, calcineurin plays an important role in various Ca$^{2+}$-mediated processes including T-cell activation (2, 3), cardiac hypertrophy (4), neutrophil chemotaxis (5), apoptosis (6), angiogenesis (7), and memory development (8). For many of these cellular events, calcineurin exerts its function by regulating the NF-AT family members. Calcineurin directly dephosphorylates NF-AT transcription factors, causing their activation and trans-location from the cytoplasm to the nucleus (9). Furthermore, calcineurin is specifically inhibited by the immunosuppressants cyclosporin A and FK506 (10), and these drugs have been a powerful tool for identifying many of the roles of calcineurin.

In the budding yeast Saccharomyces cerevisiae, calcineurin-deficient strains exhibit normal growth under standard conditions (11, 12). However, calcineurin function is required for cell viability under some specific growth conditions. Calcineurin mutants deficient for either the catalytic subunits (CNA1/CNA2) (11, 12) or the regulatory subunit (CNB1) (13, 14) die in the presence of high concentrations of different ions including manganese, sodium, lithium, and hydroxyl ions (15–18). Some of these ion sensitivities are because of a defect in the calcineurin-dependent regulation of several ion transporter genes including PMR1, PMR2, and PMC1 (16, 19) whose expressions are regulated through the Crz1/Tcn1 transcription factor (20, 21). The expression of FKS2, which encodes a β,1,3-glucan synthase, is also regulated by Crz1 through a calcineurin-dependent mechanism (20). When calcineurin is activated, it dephosphorylates Crz1, causing its rapid trans-location from the cytoplasm to the nucleus (22), suggesting similar modes of regulation by calcineurin for its downstream transcription factors in budding yeast and mammals. A disruption of CRZ1 gene caused similar phenotypes as those of calcineurin mutants, and in calcineurin mutants, these phenotypes are suppressed by CRZ1 overexpression (20). These results suggest that Crz1 functions downstream of calcineurin to effect most of the calcineurin-dependent cellular responses in budding yeast. Furthermore, recent genome-wide analysis of gene expression regulated by the calcineurin/Crz1 signaling pathway confirm that Crz1 is the major and possibly the only effector of calcineurin-regulated gene expression in budding yeast (22).

We have been studying the calcineurin signaling pathway in fission yeast Schizosaccharomyces pombe because this system is amenable to genetic analysis and has many advantages in...
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Experimental Procedures

Strains, Media, and Miscellaneous Procedures—S. pombe strains used in this study are listed in Table I. The complete medium, YPD (1% yeast extract, 2% polypeptide, 2% glucose), and the minimal medium, Edinburgh minimal medium (35), have been described previously (34). SPA mating and sporulation medium contained 10 g/liter glucose, 1 g/liter KH2PO4, 1 g/liter Na2HPO4, 1 ml/liter 1000× vitamin stock solution (same as those used for EMM), and 30 g/liter agar. Standard methods for S. pombe genetics were followed according to Moreno et al. (35). FKS06 was provided by Fujisawa Pharmaceutical Co. (Osaka, Japan). Calcineurin and calmodulin were prepared from bovine brain as described previously (36).

Gene disruptions are denoted by lowercase letters representing the disrupted gene followed by two colons and the wild-type gene marker followed by two colons and the wild-type gene marker (for example, przl::ura4+). Also, gene disruptions are denoted by an abbreviation of the gene preceded by a (for example, Δprzl). Proteins are denoted by Roman letters, and only the first letter is capitalized (for example, Przl). Data base searches were performed using the National Center for Biotechnology Information BLAST network service (www.ncbi.nlm.nih.gov) and the Sanger Center S. pombe data base search service (www.sanger.ac.uk).

Cloning and Tagging of the przl Gene—The przl gene was amplified by PCR with the genomic DNA of S. pombe as a template. The sense primer used for PCR was 5'-CG GGA TCC ATG GAG GCTCAA AGG TCA GAA GAA GCC AT-3' (BamHI site and start codon are underlined), and the antisense primer was 5'-CG GGA TCC TCA TTG TTG TGT GCT TGA GGC GGC-3' (BamHI site and stop codon are underlined). The amplified product was digested with BamHI, and the resulting fragment was subcloned into Bluescript SK(+)..

For ectopic expression of proteins, we used the thiamine-repressible nmt1 promoter at various levels of expression (37). Expression was repressed by the addition of 4 µg/ml thiamine to EMM and was induced by washing and incubating the cells in EMM lacking thiamine. To express GFP-Przl1, the complete open reading frame of przl was ligated to the C terminus of the GFP carrying the S65T mutation (38). GFP-Przl1 fully complements the growth defects of a przl-null strain (data not shown). The GFP-fused gene was subcloned into pREP1, pREP41, or pREP81 vectors to express the gene at various levels. Maximum expression of the fused gene was obtained using pREP1, whereas pREP81 contained the most attenuated version of the nmt1 promoter (37). To obtain the chromosome-born GFP-Przl1 instead of the plasmid-born GFP-Przl1, the fused genes with the nmt1 promoter at various levels were subcloned into the vector containing the ura4 marker and were integrated into the chromosome at the ura4 gene locus of KP1245 (h- leu1-32 ura4-294) (39).

Deletion of przl Gene—A one-step gene disruption by homologous recombination (40) was performed. The przl::ura4- disruption was constructed as follows. Cloned open reading frame of the przl gene in the Bluescript vector was digested with BamHI and EcoRI, and the resulting fragment containing ~80% przl gene was subcloned into the BamHI/EcoRI site of pUC119. A SmaI fragment containing the ura4 gene then was inserted into the EcoRI site of the previous construct, causing the interruption of the open reading frame. A fragment containing disrupted przl gene was transformed into diploid cells. Stable integrants were selected on medium lacking uracil, and disruption of the gene was checked by genomic Southern hybridization (data not shown).

Cell Extract Preparation and Immunoblot Analysis—For the analysis of electrophoretic mobility shift of Przl1, whole-cell extracts were prepared from cultures of wild-type or calcineurin-null cells expressing GFP-Przl1 grown at 30 °C to mid-log phase. Cells were resuspended in 450 µl of ice-cold homogenizing buffer, 50 µl Tris-HCl, pH 7.8, containing 2 mM EDTA, 1 mM dithiothreitol, and a mixture of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 0.1 mM sodium metabisulfite, 0.01 µg/ml chymostatin, 2 µg/ml aprotonin, 1 µg/ml pepstatin A, 1 µg/ml phosphoramidon, and 0.5 µg/ml leupeptin). Glass beads (0.2 g) were then added, and cells were broken mechanically by vortexing for 30 s, after which the tubes were placed on ice for 30 s. Vortexing and cooling were repeated five times, after which the glass beads and cellular debris were removed by centrifugation at 15,000 × g for 5 min. Protein extracts (10–20 µg/µl) were subjected to SDS-PAGE and immunoblotted with anti-GFP antibody.

Northern Blot Analysis—Total RNA was isolated by the method of Kohler and Domdey (41). 20 µg of total RNA/lane was subjected to electrophoresis on denaturing formaldehyde 1% agarose gels and transferred to nylon membranes. Hybridization was performed using DIG-labeled antisense cRNA probes coding for Przl1 and Pmc1 (SPBC1A4.10c). The DIG-labeled hybrids were detected by an enzyme-linked immunoassay using an anti-DIG-alkaline-phosphatase antibody conjugate. The hybrids were visualized by chemiluminescence detection on a light-sensitive film according to the manufacturer’s instructions (Roche Applied Science).

### Table I

<table>
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<td>Our stock</td>
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<tr>
<td>HM228</td>
<td>h- his2</td>
<td>Our stock</td>
</tr>
<tr>
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<td>h- leu1-32 ura4-D18 pph6::ura4+</td>
<td>This study</td>
</tr>
<tr>
<td>KP162</td>
<td>h- leu1-32 yrp5-15</td>
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<tr>
<td>KP165</td>
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**Terms of relevance to higher systems**—**S. pombe** has a single gene encoding the catalytic subunit of calcineurin, pph1+ (26). We have developed a genetic screen for mutants that depend on calcineurin for growth using the immunosuppressant FK506 and have given the designation its mutants. The analyses of the its mutants revealed that calcineurin is implicated in cytokinesis, septation initiation network, and exocytic pathway in fission yeast (27–31). We have shown that fission yeast calcineurin plays an essential role in maintaining chloride ion homeostasis and acts antagonistically with the Pmk1 pathway.

**Calcineurin and PRZ1 in S. Pombe**—S. pombe has a single gene encoding the catalytic subunit of calcineurin, pph1+ (26). We have developed a genetic screen for mutants that depend on calcineurin for growth using the immunosuppressant FK506 and have given the designation its mutants. The analyses of the its mutants revealed that calcineurin is implicated in cytokinesis, septation initiation network, and exocytic pathway in fission yeast (27–31). We have shown that fission yeast calcineurin plays an essential role in maintaining chloride ion homeostasis and acts antagonistically with the Pmk1 pathway.
**Calcineurin and PRZ1 in S. Pombe**

**Fig. 1.** Prz1 is a C2H2-type zinc finger protein and is a calcineurin substrate. A, schematic structure of Prz1 protein. Denoted are the SSR and three putative zinc fingers (ZnFs). B, sequence alignment of the three zinc finger motifs from Prz1 and Crz1 from budding yeast (21). Residues conserved in two sequences are boxed and highlighted. Residues that coordinate zinc ions are indicated by asterisks. C, the calcineurin-specific inhibitor FK506 induces a Prz1 protein mobility shift. Cells expressing GFP-Prz1 were incubated in EMM medium containing 4 μg/ml thiamine at 30 °C with CaCl2 (50 mM) or FK506 (0.5 μM) for 30 min as noted. The electrophoretic mobility of the GFP-Prz1 protein was investigated by immunoblotting using a GFP-specific antiserum. D, the mobility shift of Prz1 caused by calcineurin treatment in vitro is calmodulin-dependent. GFP-Prz1 was purified by immunoprecipitation from Δppb1 cells expressing GFP-Prz1, incubated with 1 mM CaCl2 and calcineurin (lane 1) or with 1 mM CaCl2, calmodulin, and calcineurin (lane 2) for 15 min at 37 °C, and analyzed by immunoblotting.

**RESULTS**

**Microscopic Analysis**—Cells were grown to exponential phase in YPD or EMM medium and shifted to various conditions as indicated in the figure legends. In some cases, cells were washed with phosphate-buffered saline, pH 7.0, and then stained with Hoechst 33,342 or Calcofluor to visualize the DNA or septum, respectively, before microscopic observation. Cells were microscopically examined under an Axioskop microscope (Carl Zeiss Inc.). Photographs were taken with a SPOT2 digital camera (Diagnostic Instruments Inc.). Images were processed with the CorelDRAW software (Corel Corporation Inc.).

**Identification of the S. pombe prz1** Gene—A BLAST program search using the peptide sequence of Crz1, the calcineurin-responsive zinc finger transcription factor of S. cerevisiae (20, 21) against the S. pombe protein data base at the Sanger Center revealed an open reading frame, SPAC4G8.13c, exhibiting significant similarity to Crz1 (score = 293, p = 6.3e−25, identities = 58/125 [46%], positives = 75/125 [60%]). We named the gene prz1 (for Ppb1-responsive zinc finger protein). As shown in Fig. 1, A and B, the prz1 gene encodes a protein of 681 amino acids that contains three C2H2-type zinc finger motifs at its carboxy terminus highly homologous to those of Crz1. However, unlike Crz1, Prz1 does not have a polyglutamine tract, which acts as a transcriptional activation domain in many cases (42). Outside of the zinc finger domain, Prz1 also contains a serine-rich region (SRR, residues 57–219, N score = 8.748 (Prosite)), but it shows low homology with that of Crz1.

**Prz1 is Dephosphorylated by Calcineurin**—We examined whether Prz1 phosphorylation is modulated by calcineurin. GFP-Prz1 protein showed a significant alteration in its mobility when analyzed by immunoblot. GFP-Prz1 protein isolated from cells treated with 50 mM CaCl2 migrated on SDS-PAGE gels with a significantly larger apparent molecular mass than that from non-treated wild-type cells, whereas GFP-Prz1 isolated from cells treated with 50 mM CaCl2 migrated with a slightly smaller apparent molecular mass (Fig. 1C). The change in the ratio of the faster and slower migrating GFP-Prz1 species suggested that there is a calcineurin-dependent change in the phosphorylation state of Prz1. To establish that the mobility change of GFP-Prz1 was indeed the result of variable degrees in phosphorylation, protein extracts from calcineurin-null cells were treated with purified calcineurin in vitro. Treatment of the larger form of GFP-Prz1 with calcineurin converted it to the smaller form (Fig. 1D). This change in apparent molecular mass was dependent on Ca2+ ion and calmodulin (Fig. 1D) and was blocked by the addition of FK506 (data not shown). These findings confirmed that Prz1 is hyper-
The indicates a dividing cell whose nucleus shows intense GFP fluorescence. GFP-Prz1 to the nucleus is induced by Ca²⁺ shift to growth at 42°C in wild-type but not in calcineurin-null cells grown at 27°C after a 30°C—31°C temperature upshift on the steady-state levels of Prz1 mRNA. Fig. 2B showed the level of Prz1 mRNA accumulation was induced rapidly in wild-type cells expressing the constitutively active calcineurin (Ppb1ΔC) increased the steady-state levels of Prz1 mRNA (Fig. 2C).

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Involved in the regulation of Ca$^{2+}$/H$_9$004, or (ppb1 analysis of Pmc1 mRNA expression. Wild-type, calcineurin-null thus, the Ca$^{2+}$A). Interestingly, both of the Prz1-null cells were hypersensitive to Ca$^{2+}$null cells could not grow in the presence of 0.15M CaCl$_2$ or 0.15M Ca(NO$_3$)$_2$. Northern blot analysis of Pmc1 mRNA expression. Wild-type, calcineurin-null (ppb1) cells were incubated in YPD medium at 30°C with 100 mM CaCl$_2$ for 15 min. Total RNA (20 μg) were subjected to Northern analysis using a DIG-labeled Pmc1 cRNA.

Serine-rich region of Prz1 and that of Crz1 or the NF-ATc with the exception of the richness in serine residue, although significant sequence similarity between the serine-rich region of Crz1 and NF-ATc transcription factor has been reported previously (22).

**Prz1-null Cells Are Hypersensitive to Ca$^{2+}$ but Not to Cl$^-$**—To further investigate the relationship between Prz1 and calcineurin, we analyzed prz1-null cells for phenotypes exhibited by calcineurin-null mutants. Calcineurin-null mutants were hypersensitive to Cl$^-$ and failed to grow in the presence of 0.15M MgCl$_2$ or 0.3M KCl (32). Contrary to our expectation, prz1-null cells grew normally in the YPD plate containing 0.2M MgCl$_2$ where calcineurin-null cells did not grow (Fig. 5A). Interestingly, both of the prz1-null cells and calcineurin-null cells could not grow in the presence of 0.15M CaCl$_2$ or 0.15M Ca(NO$_3$)$_2$ (Fig. 5A). These results suggest that Prz1 is involved in the regulation of Ca$^{2+}$ ion homeostasis but not that of Cl$^-$ ion homeostasis. In budding yeast, Crz1, the homolog of Prz1, is required for calcineurin-dependent transcriptional regulation of *PMC1*, which encodes a Ca$^{2+}$ pump playing a key role in Ca$^{2+}$ tolerance (43). Consistently, Northern blot analysis revealed that calcineurin (ppb1) deletion and prz1 deletion resulted in a marked reduction in Pmc1 mRNA levels (Fig. 5B). In fission yeast, the disruption of *pmc1* gene (SPBC1A4.10c) resulted in severe hypersensitivity to Ca$^{2+}$ (data not shown). Thus, the Ca$^{2+}$ hypersensitivity of prz1-null cells can be explained, at least in part, by lowered level of Pmc1.

As noted above, calcineurin-null cells of *S. pombe* are sensitive to Ca$^{2+}$. In contrast to *S. pombe*, calcineurin-null cells of *S. cerevisiae* are resistant to CaCl$_2$ and show increased growth on medium containing high levels of Ca$^{2+}$, whereas the crz1-null cells similar to the prz1-null cells are highly Ca$^{2+}$-sensitive (20). Thus, the roles of calcineurin in Ca$^{2+}$ homeostasis in these two yeasts are suggested to be quite different.

**prz1-null and Calcineurin-null Mutants Have Distinct Phenotypes in Cell Morphology and Mating**—In addition to ion homeostasis, prz1-null cells showed phenotypes distinct from those of calcineurin-null cells in cell morphology and mating (Fig. 6). As shown in Fig. 6A, calcineurin-null cells were enlarged, multiseptated and branched, consistent with the previous study by Yoshida et al. (26), which suggests the involvement of calcineurin in the regulation of the cell polarity and cytokinesis. On the other hand, prz1-null cells were indistinguishable from the wild-type cells in morphology. Furthermore, unlike calcineurin-null cells, which were sterile as reported by Yoshida et al. (26), prz1-null cells were fertile and their mating efficiency and spore morphology were indistinguishable from wild-type cells (Fig. 6B).

**Some of the Mutants That Show Synthetic Lethality with Calcineurin Deletion Do Not Show Synthetic Lethality with prz1 Deletion**—As described above, we have isolated mutants that depend on calcineurin for growth using the immunosuppressant FK506 and have given the designation its mutants (27–31). These mutants showed synthetic lethal genetic interaction with calcineurin. Therefore, their genes encode the functional proteins that may share essential function with calcineurin. To examine the relationship between these genes and the prz1 gene, tetrad analysis of a diploid derived from a cross between the *its* mutants with prz1-null cells was performed. As shown in Table II, we found that prz1-null mutation was synthetically lethal with *its3* and *its5byp3-15* mutants that encode phosphatidylinositol 4-phosphate 5 (PI(4)P5)-kinase and a
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TABLE II
Genetic interactions between prz1+ or ppb1+ and its (immunosuppressant- and temperature-sensitive) mutants

<table>
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<th>its3</th>
<th>its5/eye3–4s</th>
<th>ita8</th>
<th>its10/cdc7–10</th>
<th>SYNTHESIS</th>
<th>SIN pathway</th>
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</table>

small GTPase of the Rab/Ypt family, respectively (27, 31). On the other hand, mutations in its2/kps1–, its8–, and its10/cdc7– genes (encoding β-glucan synthase, glycosylphosphatidylinositol anchor synthetic enzyme, and protein kinase implicated in septation initiation, respectively) (28, 30, 44) did not show synthetic lethality with prz1-null mutation and double mutants were obtained (Table II). The Δprz1 its8 double mutant grew slower and was more temperature-sensitive than the its8 single mutant (data not shown), indicating a functional overlapping between Prz1 and the glycosylphosphatidylinositol anchor synthetic pathway.

Prz1 Is Not Involved in Chloride Ion Homeostasis That Is Antagonistically Regulated by Calcineurin and the Pmk1 MAPK Pathways—Our previous study showed that calcineurin acts antagonistically with the Pmk1 MAPK pathway in Cl– ion homeostasis and inhibition of Pmk1 MAPK pathway suppressed the Cl– hypersensitivity of calcineurin-null cells (32, 33). Consistent with our previous results, overexpression of pmp11+ gene encoding a MAPK phosphatase for Pmk1 suppressed the Cl– hypersensitivity of calcineurin-null cells. On the other hand, overexpression of pmp11+ did not affect the Ca2+ hypersensitivity of prz1-null cells (Fig. 7A). Furthermore, overexpression of prz11+ did not suppress the MgCl2 hypersensitivity of calcineurin-null cells. In addition, overexpression of constitutively active calcineurin did not suppress the CaCl2 hypersensitivity of prz1-null cells (Fig. 7A), whereas overexpression of prz11+ partially suppressed the CaCl2 hypersensitivity of calcineurin-null cells (data not shown). These results suggest that Prz1 acts downstream of calcineurin and regulates Ca2+ homeostasis. These results also suggest that Prz1 is not involved in Cl– homeostasis that is antagonistically regulated by calcineurin and the Pmk1 MAPK pathways (32, 33). A model consistent with these data is presented in Fig. 7B.

DISCUSSION

We report here the identification and characterization of Prz1, the S. pombe homolog of Crz1, which is a C2H2-type zinc finger protein that binds to the calcineurin-dependent response element and that regulates transcription of various target genes in budding yeast. Similar to its budding yeast homolog, the S. pombe Prz1 is dephosphorylated by calcineurin and its trans-location from the cytoplasm to the nucleus is caused by the activation of calcineurin. However, unlike in budding yeast, prz1-null phenotypes are quite different from those of calcineurin-null cells as shown in the present study. In budding yeast, crz1-null cells showed similar phenotypes as those of calcineurin-null cells, such as hypersensitivity to Mn2+ or Li+, and survival defect when incubated with α-factor (20). Furthermore, a recent genome-wide analysis of gene expression regulated by the calcineurin/Crz1 signaling pathway confirms that Crz1 is the major and possibly the only effector of calcineurin-regulated gene expression in budding yeast (23). On the other hand, with the exception of its hypersensitivity to Ca2+, prz1-null cells showed no typical phenotypes as those observed in calcineurin-null cells, such as aberrant cell morphology, mating defect, or hypersensitivity to Cl–. In addition, our preliminary genome-wide analysis using S. pombe DNA microarray suggests that the gene expression pattern of prz1-null cells is considerably different from that of calcineurin-null cells in fission yeast.2 These results strongly suggest that there are at least two branches of calcineurin signaling pathway. Related to this issue is the observation that in S. cerevisiae, crz1-null cells and calcineurin-null cells show opposing phenotypes in the condition of high Ca2+, i.e. crz1-null cells are highly Ca2+-sensitive similar to prz1-null cells, whereas cnb1-null cells are resistant to this ion and show increased growth on medium containing high levels of Ca2+ (20), showing that there is also branching in calcineurin signaling in S. cerevisiae.

Obviously, one branch is the Prz1-dependent branch that regulates the expression of Pmc1 Ca2+-pump. The Ca2+ sensitivity of prz1-null cells is consistent with the markedly reduced level of Pmc1 mRNA, suggesting a similar regulatory mechanism of Ca2+ homeostasis in these two distantly related yeasts (20, 21). To further analyze the heterogeneous nature of the calcineurin signaling pathway, we examined the genetic interaction between prz1 deletion and its5 mutants that are synthetically lethal with calcineurin deletion. As shown in Table II, prz1 deletion is synthetically lethal with mutations in the ita3– and its5–ptyp3– genes encoding a PI(4)P5 kinase and a Rab

family protein (27, 31), respectively, suggesting that these gene products and Prz1 play an overlapping essential function.

In the previous study (32), we showed that overexpression of Pmp1 MAPK phosphatase could suppress the aberrant cell morphology and Cl⁻ hypersensitivity of the calcineurin deletion. Thus, the second branch of the calcineurin signaling pathway seems to act antagonistically with the Pmk1 MAPK pathway and regulate various cellular events, such as morphology and Cl⁻ homeostasis. Furthermore, prz1 deletion is not synthetically lethal with mutations in the tis2/\textit{cps1}⁻, tis8⁺, and \textit{its10/\textit{cdc7}⁺} genes (encoding β-glucan synthase, glycosylphosphatidylinositol biosynthetic enzyme, and a protein kinase in the SIN pathway, respectively) (28, 30, 44) (Table II). Thus, it is suggested that these three genes seem to have some genetic interactions with the second branch of the calcineurin signaling pathway. As these genes encode proteins that seem to be involved in the cell wall synthesis, these genetic data are in good agreement with the hypothesis that the Pmk1 MAPK pathway is involved in the regulation of cell wall integrity (34).

In our preliminary studies in which we have been searching for downstream targets of the Pmk1 MAPK, we identified several candidates including certain novel putative transcription factors. Studies are in progress to determine whether these factors play some roles in the Cl⁻ homeostasis and are functionally related to the calcineurin signaling pathway.

Acknowledgments—We thank Mitsuhiro Yanagida (Kyoto University, Kyoto, Japan), Takashi Toda and Paul Nurse (Cancer Research UK London Institute, London, United Kingdom) for their generous gift of strains and plasmids, and Susie O. Sio for critical reading of the paper.

REFERENCES


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doi: 10.1074/jbc.M212900200 originally published online March 13, 2003

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