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The function and regulation of protein phosphatase Z in *Candida albicans*

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1. Introduction

Candida albicans is an opportunistic pathogen which occurs in the healthy human body under physiological conditions, but it can cause mycosis in immuncompromised patients. *Candida albicans* is the most common fungal pathogen in clinics. The infectiveness of this fungus is largely affected by its morphological changes. Several studies showed that the hyphae and pseudohyphae forms are important in mycosis. Commonly, fluconazole and/or amphotericin B are used in the medical treatment of systemic candidiasis, but the therapy often fails because *Candida* species are becoming increasingly resistant to antifungals. Thus, it is important to search for new potential antifungal targets. In the treatment of candidiasis, complete elimination of *C. albicans,* that constitutes an integral part of normal human flora, is not an absolute requirement, because it may result in overproduction of other fungi or bacteria. Therefore, it is reasonable to investigate *C. albicans* signaling pathways which can be targeted to influence cell growth and division, or morphological changes.

Protein phosphorylation and dephosphorylation is known to be a reversible posttranslation modification that regulates important signal transduction pathways in eukaryotic cells. Protein phosphorylation is catalyzed by protein kinases, and dephosphorylation is carried out by protein phosphatases. Protein phosphatase Z (PPZ) is a fungus specific Ser/Thr specific protein phosphatase that was first characterized in *Saccharomyces cerevisiae* and subsequently it was described in several fungus species, including *C. albicans*. Since protein PPZ plays important physiological roles and is restricted to the fungi, it can be a potential target of signal transduction therapy.

Candida albicans has a single protein phosphatase Z gene, *CaPPZ1*. This gene and its product, the CaPpz1 protein has been first reported and characterized by our research group. Previously we demonstrated that CaPpz1 is involved the in cation homeostasis, regulation of membrane potential, cell wall integrity, and the oxidative stress response. Importantly the deletion of the *CaPPZ1* gene reduced virulence. Since the infectiveness of *C. albicans* is related to the yeast-to-hyphae, we investigated if the elimination of CaPpz1 affects fungal morphology.

All of the known PPZ enzymes contain well conserved globular C-terminal catalytic domain, and a variable, disordered N-terminal domain. With our collaborating partners we used homology based modeling and x-ray diffraction analysis to determine the structure of the C-terminal catalytic domain of CaPpz1. We verified that the structure of the CaPpz1 catalytic domain is very similar to that of the well-known protein phosphatase 1 catalytic subunit

(PP1c), but it also contains unique elements that are specific to the CaPpz1 phosphatase. Our biochemical experiments revealed that typical PP1c regulatory proteins do not affect the phosphatase activity of CaPpz1. The distinct regulation of CaPpz1 may be explained by the lack of conservation of critical amino acid residues in the inhibitor-binding site and/or by the presence of CaPpz1-specific structural elements, including the intrinsically disordered N-terminal domain that may block the binding of typical PP1c interacting proteins. Together, our structural and biochemical studies excluded the possibility that PP1c regulators would play a significant role in the controlling of CaPpz1 activity. This is why searching for CaPpz1-specific regulators in *C. albicans* is an important research goal.

In earlier publications it was reported that the ScHal3 protein was a specific regulator of ScPpz1 in *S. cerevisiae*. ScHal3 and one of its paralogs, ScVhs3 could inhibit the phosphatase activity of ScPpz1. Subsequently, ScHal3 and its two paralogs, ScVhs3 and ScCab3 were found to form a heterotrimer that has Phospho-Pantothenoyl-Cysteine DeCarboxylase (PPCDC) activity and catalyzes an essential step of CoA biosynthesis in the budding yeast. Consequently, ScHal3 is a moonlighting protein that besides its essential role in CoA biosynthesis has a second function as a phosphatase regulator. By the bioinformatic analysis of databases we identified two ScHal3 orthologs in *C. albicans* that we termed CaHal3 and CaCab3 on the bases of their amino acid sequence similarity to the ScHal3 and ScCab3 proteins. In our present study we investigated the function(s) of these two *Candida* ScHal3 orthologs.

2. Aims

Our first goal was to investigate the role of CaPpz1 in the morphological changes of *C. albicans.* For these experiments we used homozygous and heterozygous *cappz1* deletion mutant strains that were generated by Dr. Csaba Ádám some time ago in our laboratory. To start with, we planned some preliminary experiments by using classical microscopy methods to investigate how the partial or complete elimination of *CaPPZ1* gene affects the morphology and hyphal outgrowth of the fungus. If we find significant differences between the deletion mutant(s) and parental strain, we could extend these studies by the adaptation of video microscopic recording and image analyzing methods for a more detailed characterization of the morphological changes.

To uncover the potential mechanisms of CaPpz1 regulation, our goal was to determine the function(s) of the two ScHal3 orthologs, CaHal3 and CaCab3 that we have identified in *C. albicans*. The planned physiological investigations were facilitated by the availability of several *S. cerevisiae* mutant strains which had been generated by the research group of Prof. Joaquin Ariňo at the Autonomous University of Barcelona, Spain. These strains have been suitable for complementation analysis with the overexpressed *C. albicans* orthologs. First, we wanted to clarify the role of CaHal3 and CaCab3 in the essential PPCDC activity. In these experiments the complementation of the otherwise lethal *cab3* and *hal3/vhs3* mutants could be carried out with tetrad analysis.

Next we planned to examine the role of CaHal3 and CaCab3 in the regulation of protein phosphatase Z *in vivo* and *in vitro* experiments. To this end both of these proteins have to be expressed with appropriate tags in bacteria and in yeast cells for pull down experiments to investigate their interaction with different recombinant CaPpz1 enzyme forms. Based on previous publications we predicted that the ScHal3 orthologs would bind preferentially to the catalytic domain of the phosphatase CaPpz1-Cter, so we planned to test the interactions with the catalytic domain and with the full length phosphatase, too. If we can demonstrate meaningful protein-protein interactions between the potential regulators and the two CaPpz1 forms, we could test the effect of CaHal3 and CaCab3 on enzyme activity. Finally, we wanted to demonstrate the physiological significance of the protein-protein interactions. The expression of CaHal3 and CaCab3 in *hal3* mutant *S. cerevisiae* cells would reveal whether the expressed candida proteins can complement the phosphatase-associated mutant phenotypes under different stress conditions.

3. Methods

3.1 Microscopic investigations

The parental *C. albicans* SN87 strain, as well as the homozygous null mutant *cappz1* and heterozygous deletion mutant strains (*CaPPZ1-LEU2* and *CaPPZ1-HIS1*) were cultured and the cells were photographed under an Olympus BX40 microscope. Hyphal length was determined and analyzed with the Image J software.

3.2 Time lapse video microscopy

For time lapse video microscopy we used the homozygous *cappz1* deletion mutant that was compared to QMY23, a genetically matched control strain. Yeast cells were incubated for 5 hours under physiological conditions, in RMPI medium at 37 ^{o}C, in the presence of 5% CO_2. We monitored four parallel samples in the incubator with 4 Olympus microscopes. Pictures were taken every 5 seconds with 4 CCD cameras, and images were analyzed with the software of our collaborating partners.

3.3 *S. cerevisiae* drop test

HAL3 ortholog DNA sequences from *C. albicans* and *S. cerevisiae* were ligated into pWS93 plasmid, and these plasmids were transformed into wild type (BY4741) and *hal3* deletion mutant *S. cerevisiae* strains. The transformed cells were selected on SD-Ura medium. Cells were diluted to the same concentration and 3µl portions of the diluted cells were spotted on YPD agar as a control plate and on YPD agar containing different concentrations of stress agents. Pictures were taken after 48 h and 72 h incubation at 28^{o}C.

3.4 Tetrad analysis

S. cerevisiae strains were transformed with expression plasmids carrying Hal3 orthologs. One colony of each transformant was cultured in SD-Ura medium for overnight. Cells were transferred to INE medium and were grown for several days. Tetrads were isolated with zymolyase and were dissected using the MSM 300 Yeast Dissection Microscope (Singer Instruments). The genotype of each spore was determined using selective agar plates.

3.4 DNA cloning

CaHAL3 and *CaCAB3* ORFs were amplified with PCR from *C. albicans* genomic DNA. We used Phusion and Phire polymerases in an Eppendorf Mastercycler® PCR cycler. The restriction digestion of vector and inserts was performed with Promega endonucleases enzymes. Insert and vector DNA were treated with phosphatase, ligated and transformed into *E. coli* or *S. cerevisiae* cells. *E. coli* competent cells were prepared with $CaCl_2$. *S. cerevisiae* competent cells were prepared with LiAc, and were transformed in the presence of PEG. Vector constructs were purified from the transformants that grew on selective agar plates, and all of the constructs were verified by DNA sequencing.

3.5 Expression of recombinant proteins

Recombinant proteins were expressed in *E. coli* BL21 DE3 RIL cells. Bacterial cells transformed with pET28a-based plasmids were grown in the presence of kanamycin and chloramphenicol while the cells harboring pGEX-6p-1-based plasmids were cultivated in the presence of ampicillin and chloramphenicol. CaPpz1 and its catalytic domain (CaPpz1-Cter) were expressed in the presence of $MnCl_2$. We optimized all protein expression procedures, and all recombinant proteins were expressed with different IPTG concentrations, temperatures, and expression times.

3.6 Purification of recombinant proteins with affinity chromatography

Bacteria containing the expressed proteins were lysed and spun to separate cell debris from soluble proteins. GST-tagged proteins were purified on Glutathione Sepharose 4B resin with batch technique. GST was removed from resin bound proteins with PreScission protease. CaPpz1 was expressed from pET28a to produce 6xHis-CaPpz1 that was purified on Ni-NTA agarose. Protein concentrations were determined according to Bradford. The protein preparations were characterized with SDS-PAGE analysis according to Laemmli.

3.7 Detection of protein-protein interactions with pull down experiments

The GST tagged CaPpz1 catalytic domain (CaPpz1-Cter) was bound to Glutathione Sepharose 4B matrix and was incubated with purified recombinant CaHal3, CaCab3, and ScHal3 proteins at 25°C for 40 min. The matrix bound interacting proteins were eluted, separated with SDS-PAGE and were detected with Coomassie Brilliant Blue staining. This insensitive method allowed the detection of the strongest interactions only.

In the reverse pull down experiment, the GST-tagged potential inhibitors were bound to Glutathione Sepharose 4B and were incubated with 6xHis-CaPpz1. The matrix bound interacting proteins were eluted and after separation with SDS-PAGE were transferred onto Hybond ECL membrane. The presence of the phosphatase was detected with Western blotting with anti-6xHis antibody. This method was suitable for the detection of weaker interactions as well.

To verify protein-protein interactions we performed pull down assays in which GST-CaPpz1 was bound to Glutathione Sepharose 4B that was incubated with HA-tagged interacting proteins expressed in *S. cerevisiae*. The HA-tagged proteins had not been purified; rather protein extracts of the appropriate transformed yeast cells were used in these experiments. The matrix bound proteins were eluted from the resin and were separated with SDS-PAGE. The detection of interacting proteins was carried with Western blotting with anti-HA antibody.

3.10 Protein phosphatase activity assay

The phosphatase activities of the different protein phosphatase Z enzyme forms were measured with ^{32}P-labeled myosin light chain (^{32}P-MLC) substrate. In this novel, sensitive radioactive assays, the phosphatase activities of the CaPpz1, CaPpz1-Cter, ScPpz1, and ScPpz1-Cter recombinant enzymes were measured in the presence of 2 mM $MnCl_2$.

4. Results and Discussion

4.1 The role of CaPpz1 in fungal morphology

The role of the *Candida albicans CaPPZ1* gene product CaPpz1 in fungal morphology was examined in two steps. In preliminary experiments, hyphal elongation of the *cappz1* deletion mutant was compared to that of the parental SN87 strain in the absence and in the presence FBS (an inducer of the hyphal outgrowth) by classical microscopy. The homozygous *cappz1* mutant exhibited significantly reduced hyphal growth rate under both experimental conditions. The deletion of a single *CaPPZ1* allele had only a negligible effect indicating that the marker genes (*HIS1, LEU2*) incorporated into the *Candida* genome during gene disruption have no effect on hyphal extension. Consequently, the reduced hyphal growth rate is most likely related to the complete loss of the CaPpz1 phosphatase.

We confirmed and extended the preliminary result by the comparison of the *cappz1* deletion mutant with the QMY23 control strain that has a completely identical genetic background. For the continuous, long time monitoring of morphological changes under nearly physiological conditions we adopted a time-lapse videomicroscopy method, which has been previously used to analyze human cell lines. By the application of the genetically matching control strain, and a more advanced microscopic setup we were able to confirm our preliminary results concerning the reduced rate of hypha formation in the absence of the CaPpz1 phosphatase. We also noted that the growth rate followed a normal distribution in the QMY23 control strain and become more irregular in the *cappz1* mutant suggesting that the CaPpz1 phosphatase is important in the regulation of the morphology changes in *C. albicans*. Furthermore the use of time-lapse videomicroscopy allowed us to investigate the adhesion of the fungal cells to the wall of the culturing flask. We found that the adhesion of *cappz1* cells required more time than that of the QMY23 cells, and consequently the onset of the hyphae outgrowing and hyphae elongation was delayed in the deletion mutant.

In summary, the adaptation of the videomicroscopy method was an important step forwards the better understanding of the morphological transitions in *C. albicans*. The long time continuous observation of the fungal cells confirmed earlier observations and revealed novel features of the process. The method proved to be suitable to investigate the effects of different mutations, and to test the effect of small courum molecules on the morphological changes of the pathogenic fungus.

Our results may have some important consequences as the morphological changes of *C. albicans* are related to the infectiveness of the opportunistic pathogen. Since Cappz1 is

fungus specific one van envisage a phosphatase inhibition based signal transduction therapy that would have minimal side effects and would prevent *Candia* infection without completely killing of the fungal cells. Our results suggest CaPpz1 be a novel target for antifungal therapy.

4.2 The regulation of CaPpz1

It has been already known that the ScHal3 was a natural inhibitor protein of ScPpz1 in *S. cerevisiae*. By bioinformatic analysis of DNA and protein databases we identified two ScHal3 orthologues in *C. albicans*: the gene products of the orf19.7378 and orf19.3260 up till now uncharacterized genes. We found by amino acid sequence comparisons that the protein product of orf19.7378 is highly similar to ScHal3, so we termed it CaHal3. The CaHal3 protein contains the conserved His amino acid that is essential for PPCDC activity in ScHal3. Our phylogenetic analyses confirmed that CaHal3 belongs to the family of Hal-like proteins. Based on this structural analysis our working hypothesis that CaHal3 is a functional PPCDC subunit that is required for CoA biosynthesis, and at the same time it has a moonlighting function, it can act as a CaPpz1 inhibitor. On the other hand the gene product of orf19.3260 the CaCab3.protein was much more related to ScCab3 according to its primary structure...in CaCab3 we found the Asn motif and a Cys amino acid residue that are essential for PPCDC activity. In addition, our phylogenetic analysis proved that CaCab3 belongs to the Cab3-like protein family. Thus we predicted that CaCab3 is a PPCDC subunit that has an important physiological role in CoA biosynthesis, but it has not got any moonlighting function. However, we noted that ScCab3 contains an about 30 amino acid long non-homologues sequence that is similar to ScHal3, so were not able to exclude the possibility it may be involved in CaPpz1 regulation.

To check the validity of our working hypothesis we investigated the functions of the two ScHal3 orthologs by biochemical and molecular genetics methods. First we tested the essential PPCDC related functions of the *Candida* ScHal3 orthologues. For this purposes we utilized heterozygous *cab3* and *hal3/vhs3* mutants *S. cerevisiae* strains. We found with the aid of with tetrad analysis that the expression of CaCab3 rescued the lethality of the *cab3* mutant, thus it functions as a PPCDC subunit. Likewise, the expression of CaHal3 rescued the lethality of the *hal3/vhs3* mutant, indicating that CaHal3 was another subunit of PPCDC. Taken together we proved that the essential functions of CaHal3 and CaCab3 in CoA biosynthesis were well conserved in *C. albicans.*

Next we investigated the phosphatase inhibitory capacity i. e. the moonlighting functions ScHal3 orthologues. We performed with pull down experiments, to test the putative protein-protein interactions and demonstrated that the recombinant CaHal3 and CaCab3 proteins formed a complex with either the catalytic domain or with the full length CaPpz1 enzyme. These results are in correlation with previously publications telling that in *S. cerevisiae* both ScHal3 and ScCab3 can interact with ScPpz1, but only ScHal3 can inhibit the ScPpz1 phosphatase activity.

To check our second hypothesis we investigated the effect of the ScHal3 orthologs on the protein phosphatase activity. For the phosphatase activity measurements we used a new assay that utilizes radiolabeled myosin light chain substrate (^{32}P-MLC20). This method proved to be more sensitive than the traditional pNPP based photometric assay, and was especially suitable to test the effects of protein interactions on enzyme activity. We found that both forms of CaPpz1, i.e. the catalytic domain and the full length protein effectively dephosphorylated the myosin light chain substrate. However, the specific activity of the full length CaPpz1 was much lower than that of its catalytic domain, indicating that the N-terminal domain of the enzyme has a negative regulatory role as it hinders the binding of the protein substrate to the catalytic center. The phosphatase activity of both enzyme forms was inhibited by CaHal3 and CaCab3 in a concentration dependent manner. These results partially contradict previous reports telling that in *S. cerevisiae* only ScHal3 is an effective phosphatase inhibitor but ScCab3 does not influence the enzyme activity of ScPpz1. To clarify the situation we repeated our experiments with full length ScPpz1 and its catalytic domain. The *in vitro* phosphatase assays fully confirmed our results. Both of the ScPpz1 enzyme forms effectively dephosphorylated the radiolabeled myosin light chain substrate. The specific activity of the catalytic domain was much higher than that of the full length enzyme. The effect of the two ScHal3 orthologues was identical, namely both CaHal3 and CaCab3 inhibited phosphatase activity of the two ScPpz1 phosphatase species in a concentration depend manner. Thus, the disordered N-terminal domains of the S. cerevisiae ScPpz1 and C. albicans CaPpz1 have a similar negative effect on phosphatase activity despite the fact that their amino acid sequences are quite divergent. Not only the specific activity was similar, but also our data clearly show that, CaHal3 and CaCab3 are both specific phosphatase regulators under *in vitro* conditions.

Finally, we scrutinized if the two *C. albicans* ScHal3 orthologues behaved as effective phosphatase regulators under *in vivo* conditions. To answer this question, were expressed the ScHal3 orthologues in *hal3* deletion mutant *S. cerevisiae* strain. The efficiency of the

expression was verified with Western blot. It has been known, that the *hal3* mutant cells were sensitive to salt stress, and to toxic cations. According to previous publications the overexpression of ScHal3 rescued the sensitive phenotype and made the mutant cell tolerant to these stress conditions. On the other hand, the overexpression of ScCab3 protein was not effective; in fact it caused a slight anti-Hal3 effect in similar experiments. When we expressed CaHal3 protein in *hal3* mutant *S. cerevisiae* cells, in contrary to our expectation, did not observe any protective effect against salt stress and toxic cations. Even more unexpectedly the expression of CaCab3 made the cells tolerant against LiCl and Hyromycin B, thus CaCab3 behaved like ScHal3 *in vivo*. To verify the reliability of this unexpected observation we carried out several control tests. We excluded the accidental mixing of the samples by insert specific colony PCR. We also tested growth rate of the same transformed cell under limited potassium ion concentrations, and got the same results as before. So we had to conclude that, there was no experimental error, and indeed CaCab3 acts as a phosphatase regulator under *in vivo* conditions.

It is known from the literature, that ScPpz1 exhibits an indirect effect in the *slt2* MAP kinase mutated *S. cerevisiae* cells. The deletion of the Slt2 kinase results in a lytic phenotype that is aggravated by the genetic elimination of ScPpz1 or by the overexpression of ScHal3. This lytic phenotype can be easily detected as the *stl2* cells are sensitive for cell wall damage agents like caffeine. We utilized this testing system to determine the effects of our ScHal3 orthologues in *slt2* and *slt/hal3* mutant *S. cerevisiae* cells. We found that the expression of CaHal3 had a small anti-Hal3 effect, just like the ScCab3, while the expression of the CaCab3 protein reduced caffeine tolerance similarly to CaHal3 in *slt2* mutants. The results obtained with *slt2/schal3* double mutant cells were very much similar to that of our previous *in vivo* tests, namely CaHal3 had no effect or exhibited a weak anti-Hal3 effect like ScCab3, while CaCab3 behaved like ScHal3 did, as it aggravated the lytic phenotype. Thus, in a different testing system and in another mutant with more complex genetic background, we found again that only CaCab3 acts as a physiological phosphatase regulator.

Our investigations of the essential functions of the ScHal3 orthologues proved that the structure based identification of the two, up till now unidentified, ScHal3 orthologues in *C. albicans* was correct. CaHal3 is similar to ScHal3 and CaCab3 corresponds to ScCab3, as their essential role in CoA biosynthesis has been well conserved during evolution; the two proteins are functional subunits of the PPDCD enzyme.

As expected, CaHal3 and CaCab3 can bind to the full length CaPpz1 enzyme and to its C-terminal catalytic domain, too. The ScHal3 orthologues bind the catalytic domain stronger

than full length protein indicating that these proteins preferentially bind the C-terminal domain, while the N-terminal domain blocks the formation of this protein-protein complex.

According to our *in vitro* phosphatase assays both ScHal3 orthologues can inhibit the phosphatase activity of CaPpz1 and ScPpz1 enzyme and their catalytic domains in a concentration depend manner Thus, under *in vitro* conditions both CaHal3 and CaCab3 behave as potential phosphatase regulators. In contrast, under physiological conditions in our *in vitro* experiments we found unexpectedly that CaHal3 was ineffective, or it had anti-Hal3 effect like ScCab3. On the other hand CaCab3 proved to be similar to ScHal3 in *hal3 S. cerevisiae* deletion mutant cells under salt stress, and presence of toxic cation, as well as in the *slt2/hal3* double mutant *S. cerevisiae* cells in the presence cell wall damaging caffeine.

These results indicate that while the essential function of ScHal orthologs is conserved during evolutionarily, the moonlighting function phosphatase regulatory functions of the same proteins evolved independently in *C. albicans*. It is possible that a unique 30 amino acid long non-homologues protein segment that is present in both ScHal3 and CaCab3 proteins may have a significant role in the inhibition of the phosphatase activity.

The exact function of the disordered N-terminal domain in Ppz enzymes has not been revealed yet. Our results suggest that N-terminal domain has a negative, inhibitory role in the regulation of the protein phosphatase activity and in the protein-protein interactions; however more experiments are needed to verify this hypothesis.

5. Summary

Protein phosphatase Z is a so called “novel” type Ser/Thr phosphatase that is restricted to the fungal kingdom. In the opportunistic pathogen *Candida albicans* there is only one gene (*CaPPZ1*) that codes for the CaPpz1 protein. The latter, like its better known *Saccharomyces cerevisiae* counterpart ScPpz1, is involved in the determination of cell wall integrity, preservation of osmotic stability, and cation homeostasis as well as in the mechanism of oxidative stress response. In the present study we investigated its additional roles and the ways of its regulation.

The function of CaPpz1 in the hypha formation of the filamentous fungus was analyzed by classical microscopy and by long term videomicroscopy. Both of the methods revealed a significant delay in the rate of hyphal outgrowth in the *cappz1* deletion mutant relative to the control strain. In addition the knockout mutant cell exhibited slower adhesion to solid surfaces. Thus we identified some novel morphological functions of CaPpz1 that may be related to the virulence of *C. albicans*.

The ScHal3 protein was originally described in *S. cerevisiae* as a specific ScPpz1 inhibitor. Recently, it has been reported that ScHal3 together with its two paralogs (ScVhs3 and ScCab3) acts as an essential subunit of the heterotrimeric phosphopanthotenoyl cysteine decarboxylase (PPCDC) enzyme that is required for CoA biosynthesis. In a database search we found two Hal3 orthologs in *C. albicans* that we termed CaHal3 and CaCab3 based on their amino acid sequence similarities to the *S. cerevisiae* counterparts. In the course of molecular genetics functional analysis we found that both of the *Candida* proteins preserved their essential function as building blocks of the PPCDC enzyme. In addition we demonstrated by *in vitro* experiments with recombinant proteins that both CaHal3 and CaCab3 bind to the CaPpz1 enzyme and are able to inhibit its protein phosphatase activity. The complementation of *S. cerevisiae* deletion mutants revealed that overexpression of CaCab3 was able to replace ScHal3 in functional tests that are related to phosphatase inhibition. In contrast, the CaHal3 protein proved to be ineffective under the same conditions. Our *in vivo* experiments suggest that only CaCab3 can act as a physiological regulator of CaPpz1. According to our results the essential functions of the CaHal3 and CaCab3 proteins as PPCDC subunits have been well conserved but the moonlighting function of CaCab3 as a phosphatase inhibitor evolved separately during evolution.

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List of publications related to the dissertation

1. **Petrényi, K.**, Molero, C., Kónya, Z., Erdődi, F., Ariño, J., Dombrádi, V.: Analysis of Two Putative Candida albicans Phosphopantothenoylcysteine Decarboxylase / Protein Phosphatase Z Regulatory Subunits Reveals an Unexpected Distribution of Functional Roles.
PLoS One. 11 (8), e0160965, 2016.
DOI: http://dx.doi.org/10.1371/journal.pone.0160965
IF: 3.057 (2015)

2. Szemán-Nagy, G., Hennig, G. W., **Petrényi, K.**, Kovács, L., Pócsi, I., Dombrádi, V., Bánfalvi, G.: Time-lapse video microscopy and image analysis of adherence and growth patterns of Candida albicans strains.
Appl. Microbiol. Biotechnol. 98 (11), 5185-5194, 2014.
DOI: http://dx.doi.org/10.1007/s00253-014-5696-5
IF: 3.337

3. Ádám, C., Erdei, É., Casado, C., Kovács, L., Gonzalez, A., Majoros, L., **Petrényi, K.**, Bagossi, P., Farkas, I., Molnár, M., Pócsi, I., Ariño, J., Dombrádi, V.: Protein phosphatase CaPpz1 is involved in cation homeostasis, cell wall integrity and virulence of Candida albicans.
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DOI: http://dx.doi.org/10.1099/mic.0.057075-0
IF: 2.852

List of other publications

4. Chen, E., Choy, M. S., **Petrényi, K.**, Kónya, Z., Erdődi, F., Dombrádi, V., Peti, W., Page, R.: Molecular Insights into the Fungus-Specific Serine/Threonine Protein Phosphatase Z1 in Candida albicans.
mBio. 7 (4), e00872-16, 2016.
DOI: http://dx.doi.org/10.1128/mBio.00872-16

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5. Molero, C., **Petrényi, K.**, Gonzalez, A., Carmona, M., Gelis, S., Abrie, J. A., Strauss, E., Ramos, J., Dombrádi, V., Hidalgo, E., Ariño, J.: The Schizosaccharomyces pombe fusion gene hal3 encodes three distinct activities.
Mol. Microbiol. 90 (2), 367-382, 2013.
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