Communicative & Integrative Biology 4:2, 186-187; March/April 2011; ©2011 Landes Bioscience

Calcium signaling components in the human pathogen *Cryptococcus neoformans*

Livia Kmetzsch,[1] Charley Christian Staats,[1,2] Marcio L. Rodrigues,[3] Augusto Schrank[1,2] and Marilene Henning Vainstein[1,2,*]

[1]Centro de Biotecnologia; Universidade Federal do Rio Grande do Sul; Porto Alegre, RS, Brazil; [2]Departamento de Biologia Molecular e Biotecnologia; Universidade Federal do Rio Grande do Sul; Porto Alegre, RS, Brazil; [3]Laboratório de Estudos Integrados em Bioquímica Microbiana; Instituto de Microbiologia Professor Paulo de Góes; Universidade Federal do Rio de Janeiro; Rio de Janeiro, RJ, Brazil

Calcium signaling through calmodulin and the phosphatase calcineurin are required for key events of the biology of the human pathogen *Cryptococcus neoformans*, including mating, morphogenesis, growth at 37°C and virulence. In a recent work we described the functional characterization of a new component of this calcium signaling network: the vacuolar calcium exchanger Vcx1. This transporter is involved in calcium tolerance and virulence in *C. neoformans*. Two other uncharacterized calcium transporters which are putative orthologs of *Saccharomyces cerevisiae PMC1* (a vacuolar calcium ATPase) and *PMR1* (a Golgi calcium ATPase) are also functional in *C. neoformans*. No ortholog of *CRZ1,* the target of calcineurin in other fungi, has been identified in *C. neoformans*, indicating a high complexity in cryptococcal calcium-related pathways. Future studies are necessary for the complete understanding of calcium signaling regulation in *C. neoformans*.

Key words: *Cryptococcus neoformans*, calcium, calcineurin, virulence, calcium transport

Submitted: 11/22/10

Accepted: 11/22/10

DOI: 10.4161/cib.4.2.14271

*Correspondence to: Marilene Henning Vainstein; Email: mhv@cbiot.ufrgs.br

Addendum to: Kmetzsch L, Staats CC, Simon E, Fonseca FL, de Oliveira DL, Sobrino L, et al. The vacuolar Ca^{2+} exchanger Vcx1 is involved in calcineurin-dependent Ca^{2+} tolerance and virulence in *Cryptococcus neoformans*. Eukaryot Cell 9:1798–805. PMID: 20889719; DOI: 10.1128/EC.00114-10.

The calcium-calcineurin signaling pathway in *Cryptococcus neoformans* is fundamental for sensing and adaptation to the human host milieu.[1] In our recent work, a new component of the *C. neoformans* Ca^{2+} signaling network was characterized: the vacuolar calcium exchanger Vcx1. Mutant cells lacking Vcx1 expression had altered calcineurin-dependent Ca^{2+} tolerance and a reduced ability to kill mice. The loss of *VCX1* gene activity did not influence cell wall integrity or capsule size, but resulted in decreased secretion of the major capsular polysaccharide glucuronoxylomannan (GXM) to culture supernatants. The Vcx1 knockout strain was also more susceptible to killing by murine macrophages.[2] The cellular and molecular connections that could explain the relationship of calcium transport with polysaccharide secretion remain to be elucidated.

The phosphatase calcineurin, the major component of the calcium signaling pathway, is required for several crucial events of the *C. neoformans* biology, such as mating, morphogenesis, growth at 37°C and virulence.[3-7] *C. neoformans* calmodulin, which senses cytosolic calcium and activates calcineurin, is essential for viability and acts in response to high temperature.[1] Besides Vcx1, two important calcium transporters, Cch1 and EcaI, were described in reference 8 and 9. The plasma membrane calcium channel Cch1 mediates calcium entry in *C. neoformans* cells and is required for calcium uptake in low-calcium environments.[8] The sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase EcaI participates in stress tolerance.[9] All these components, Vcx1, Cch1 and EcaI, are involved in *C. neoformans* virulence, making clear the importance of calcium transport in fungal virulence.[2,8,9] In fact, calcium-related pathways have been associated to key pathogenic steps in important fungal pathogens, including *Candida albicans* and *Aspergillus fumigatus*.[10,11] Searches in the *C. neoformans* genome database (www.broadinstitute.org/annotation/genome/cryptococcus_neoformans/MultiHome.html) revealed the presence of two putative orthologs of

well-characterized calcium transporters of *Saccharomyces cerevisiae*, *PMC1* (a vacuolar calcium ATPase) and *PMR1* (a Golgi calcium ATPase).[12,13] In *C. neoformans*, we demonstrated that the *PMC1* ortholog is upregulated in the Vcx1 knockout strain, probably due to a compensatory effect. We speculate that this observation is related to the fact that *PMC1* also transports calcium into vacuoles, generating functional redundancy. This finding indicates the existence of a complex system that regulates calcium transport, including the participation of uncharacterized *C. neoformans* calcium transporters.

The transcriptional regulation of the calcium-calcineurin signaling pathway has been extensively studied in filamentous fungi and yeasts.[14-20] The zinc finger transcription factor Crz1 is the molecular target of calcineurin, that mediates nuclear translocation of Crz1 after dephosphorylation.[19] Orthologs of *S. cerevisiae* Crz1 are involved in virulence and pathogenesis in several plant and human fungal pathogens, such as *Magnaporthe oryzae*, *Botrytis cinerea*, *A. fumigatus* and *Candida glabrata*.[17,18,21-23] Up to now, no ortholog of the *CRZ1* gene has been identified in *C. neoformans*. Probably, *C. neoformans* contains diverse or more than one transcription factor responsive to calcineurin.[1] This scenario suggests that the characterization of potential Crz1 orthologs and/or novel calcium transporters is necessary to a broader understanding of the complex regulation of calcium signaling in the human pathogen *C. neoformans*.

References

1. Kozubowski L, Lee SC, Heitman J. Signaling pathways in the pathogenesis of Cryptococcus. Cell Microbiol 2009; 11:370-80.
2. Kmetzsch L, Staats CC, Simon E, Fonseca FL, de Oliveira DL, Sobrino L, et al. The vacuolar Ca^{2+} exchanger Vcx1 is involved in calcineurin-dependent Ca^{2+} tolerance and virulence in *Cryptococcus neoformans*. Eukaryot Cell 9:1798-805.
3. Cruz MC, Fox DS, Heitman J. Calcineurin is required for hyphal elongation during mating and haploid fruiting in *Cryptococcus neoformans*. EMBO J 2001; 20:1020-32.
4. Fox DS, Cruz MC, Sia RA, Ke H, Cox GM, Cardenas ME, et al. Calcineurin regulatory subunit is essential for virulence and mediates interactions with FKBP12-FK506 in *Cryptococcus neoformans*. Mol Microbiol 2001; 39:835-49.
5. Odom A, Muir S, Lim E, Toffaletti DL, Perfect J, Heitman J. Calcineurin is required for virulence of *Cryptococcus neoformans*. EMBO J 1997; 16:2576-89.
6. Kraus PR, Fox DS, Cox GM, Heitman J. The *Cryptococcus neoformans* MAP kinase Mpk1 regulates cell integrity in response to antifungal drugs and loss of calcineurin function. Mol Microbiol 2003; 48:1377-87.
7. Kraus PR, Heitman J. Coping with stress: calmodulin and calcineurin in model and pathogenic fungi. Biochem Biophys Res Commun 2003; 311:1151-7.
8. Liu M, Du P, Heinrich G, Cox GM, Gelli A. Cch1 mediates calcium entry in *Cryptococcus neoformans* and is essential in low-calcium environments. Eukaryot Cell 2006; 5:1788-96.
9. Fan W, Idnurm A, Breger J, Mylonakis E, Heitman J. Eca1, a sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, is involved in stress tolerance and virulence in *Cryptococcus neoformans*. Infect Immun 2007; 75:3394-405.
10. Reedy JL, Filler SG, Heitman J. Elucidating the *Candida albicans* calcineurin signaling cascade controlling stress response and virulence. Fungal Genet Biol 47:107-16.
11. Steinbach WJ, Cramer RA Jr, Perfect BZ, Asfaw YG, Sauer TC, Najvar LK, et al. Calcineurin controls growth, morphology and pathogenicity in *Aspergillus fumigatus*. Eukaryot Cell 2006; 5:1091-103.
12. Cunningham KW, Fink GR. Calcineurin-dependent growth control in *Saccharomyces cerevisiae* mutants lacking PMC1, a homolog of plasma membrane Ca^{2+} ATPases. J Cell Biol 1994; 124:351-63.
13. Marchi V, Sorin A, Wei Y, Rao R. Induction of vacuolar Ca^{2+}-ATPase and H^+/Ca^{2+} exchange activity in yeast mutants lacking Pmr1, the Golgi Ca^{2+}-ATPase. FEBS Lett 1999; 454:181-6.
14. Hagiwara D, Kondo A, Fujioka T, Abe K. Functional analysis of C_2H_2 zinc finger transcription factor CrzA involved in calcium signaling in *Aspergillus nidulans*. Curr Genet 2008; 54:325-38.
15. Soriani FM, Malavazi I, da Silva Ferreira ME, Savoldi M, Von Zeska Kress MR, de Souza Goldman MH, et al. Functional characterization of the *Aspergillus fumigatus* CRZ1 homologue, CrzA. Mol Microbiol 2008; 67:1274-91.
16. Soriani FM, Malavazi I, Savoldi M, Espeso E, Dinamarco TM, Bernardes LA, et al. Identification of possible targets of the *Aspergillus fumigatus* CRZ1 homologue, CrzA. BMC Microbiol 10:12.
17. Cramer RA Jr, Perfect BZ, Pinchai N, Park S, Perlin DS, Asfaw YG, et al. Calcineurin target CrzA regulates conidial germination, hyphal growth and pathogenesis of *Aspergillus fumigatus*. Eukaryot Cell 2008; 7:1085-97.
18. Choi J, Kim Y, Kim S, Park J, Lee YH. MoCRZ1, a gene encoding a calcineurin-responsive transcription factor, regulates fungal growth and pathogenicity of *Magnaporthe oryzae*. Fungal Genet Biol 2009; 46:243-54.
19. Cyert MS. Calcineurin signaling in *Saccharomyces cerevisiae*: how yeast go crazy in response to stress. Biochem Biophys Res Commun 2003; 311:1143-50.
20. Karababa M, Valentino E, Pardini G, Coste AT, Bille J, Sanglard D. CRZ1, a target of the calcineurin pathway in *Candida albicans*. Mol Microbiol 2006; 59:1429-51.
21. Miyazaki T, Yamauchi S, Inamine T, Nagayoshi Y, Saijo T, Izumikawa KS, et al. Roles of calcineurin and Crz1 in antifungal susceptibility and virulence of *Candida glabrata*. Antimicrob Agents Chemother 54:1639-43.
22. Schumacher J, de Larrinoa IF, Tudzynski B. Calcineurin-responsive zinc finger transcription factor CRZ1 of *Botrytis cinerea* is required for growth, development and full virulence on bean plants. Eukaryot Cell 2008; 7:584-601.
23. Rispail N, Soanes DM, Ant C, Czajkowski R, Grunler A, Huguet R, et al. Comparative genomics of MAP kinase and calcium-calcineurin signaling components in plant and human pathogenic fungi. Fungal Genet Biol 2009; 46:287-98.