PLANT SIGNALING & BEHAVIOR
2020, VOL. 15, NO. 2, e1719312 (3 pages)
https://doi.org/10.1080/15592324.2020.1719312

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Stress-seventy subfamily A 4, A member of HSP70, confers yeast cadmium tolerance in the loss of mitochondria pyruvate carrier 1

Xin Li[a,b*], Han Zheng[a,b*], Lin Shi[a], Zhongliang Liu[c], Lilong He[a], and Jianwei Gao[a,b]

[a]Institute of Vegetables and Flowers, Shandong Key Laboratory of Greenhouse Vegetable Biology, Shandong Branch of National Vegetable Improvement Center, Huanghuai Region Vegetable Scientific Station of Ministry of Agriculture(Shandong), Shandong Academy of Agricultural Sciences, Jinan, China; [b]College of Life Science, Shandong Normal University, Jinan, China; [c]Taian Academy of Agricultural Sciences, Taian, China

ABSTRACT

Mitochondrial pyruvate carrier (MPC), which transports pyruvate into mitochondria, is a key regulatory element in the material metabolism and energy metabolism. Since MPC was firstly identified in yeast in 2012, many groups have investigated the function of MPC. As MPC is a classic material transporter, the focus of previous studies has been placed on its role in pyruvate transport. In this study, we discovered a novel Cd resistant gene, stress-seventy subfamily A 4 (SSA4), which can recover the Cd sensitive phenotype in the yeast *MPC1* mutant strain. It is suggested that, except for adjusting metabolism, MPC can regulate stress tolerance by regulating downstream genes in yeast. Previously, we discovered a Cd related gene, AGP30, which is associated with MPC1 in *Arabidopsis*. These results indicate that MPC can regulate Cd tolerance through downstream genes in both *Arabidopsis* and yeast. This study will pave the way for further exploring the bypass pathways of MPC at the molecular level, and the interaction between MPC and the downstream genes in biology.

ARTICLE HISTORY
Received 5 December 2019
Revised 13 January 2020
Accepted 14 January 2020

KEYWORDS
Yeast; mitochondrial pyruvate carrier; Cd stress; transcript profile

Mitochondrial pyruvate carriers (MPCs), which are the critical regulation elements for both substance and energy metabolisms, were firstly identified in Drosophila and yeast.[1,2] Previously, we found that MPC1 is required for Cd tolerance in *Arabidopsis* and yeast. In *Arabidopsis*, MPC prevents Cd toxicity by sustaining TCA cycle and ATP, and alleviates the pressure of GSH synthesis.[3] Recently, AGP30, a Cd tolerance related gene, was also proven to be involved in Cd tolerance associated with AtMPC1 in *Arabidopsis*. When treated with Cd, the transcript abundance of AGP30 decreased by half; without *AtMPC1*, the abundance dropped over 50 fold.[4] These results indicate that the function of MPC in Cd tolerance regulation is complex, and there exist downstream candidate genes which associate Cd stress in *Arabidopsis*. In yeast, the mutant strain *Δmpc1* also shows a sensitive phenotype compared with the wild-type strain JRY472 when treated with 50 μM Cd. Although MPC shares similar metabolic and Cd resistance mechanisms in *Arabidopsis* and yeast, it is unclear whether other molecular bypass pathways exist in regulating Cd toxicity in yeast under the control of MPC. To verify this, RNA-seq analysis was conducted on Cd-treated yeast cells comparing *Δmpc1* against the wild-type strain (JRY472) (Figure 1(a)). Differentially expressed genes (DEGs) were identified with good repeatability from three biologically independent repeats (adjusted *P* value < .05), and log2 ratio >0.7 or <-0.7 were selected for the following analysis (Supplementary Table 1). Out of the DEGs, 1023 genes were up-regulated and 1003 genes were down-regulated.

To understand the molecular regulatory mechanism, Gene Ontology (GO) enrichment analysis of the DEGs was primarily carried out by the GOseq.[5] As shown in Figure 1(c) and Supplementary Table 2, 20 pathways were enriched, including pathways in biological process, cellular component and molecular function. Out of the enriched pathways, most are related to metabolism or material synthesis, and there is no term describing heavy metal stress or oxidative stress. Considering there are many terms related to heavy metal or oxidative stress in the GO database, our result suggested that the Cd tolerance function of MPC could be not due to the classical heavy metal or oxidative stress tolerance. We also conducted Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. As shown in Figure 1(d), most of the pathways were involved in material metabolisms. These results indicated that the Cd sensitive phenotype caused by the loss of MPC1 in yeast is due to the interruption of some metabolic pathways or material pathways.

To test whether downstream candidates were involved in the Cd stress tolerance mechanism regulated by MPC1, four most significantly down-regulated DEGs (*SPG1, MEP2, SSA4*, and YGR035C) were selected (Table 1). The *SPG1* is required for high temperature survival during stationary phase, and detected

CONTACT Jianwei Gao jianweigao3@qq.com Institute of Vegetables and Flowers, Shandong Key Laboratory of Greenhouse Vegetable Biology, Shandong Branch of National Vegetable Improvement Center, Huanghuai Region Vegetable Scientific Station of Ministry of Agriculture (Shandong), Shandong Academy of Agricultural Sciences, Jinan 250100, China; Lilong He hllong1984@163.com Institute of Vegetables and Flowers, Shandong Key Laboratory of Greenhouse Vegetable Biology, Shandong Branch of National Vegetable Improvement Center, Huanghuai Region Vegetable Scientific Station of Ministry of Agriculture (Shandong), Shandong Academy of Agricultural Sciences, Jinan 250100, China
*These authors contributed equally to this work.
Supplemental data for this article can be accessed on the publisher's website.

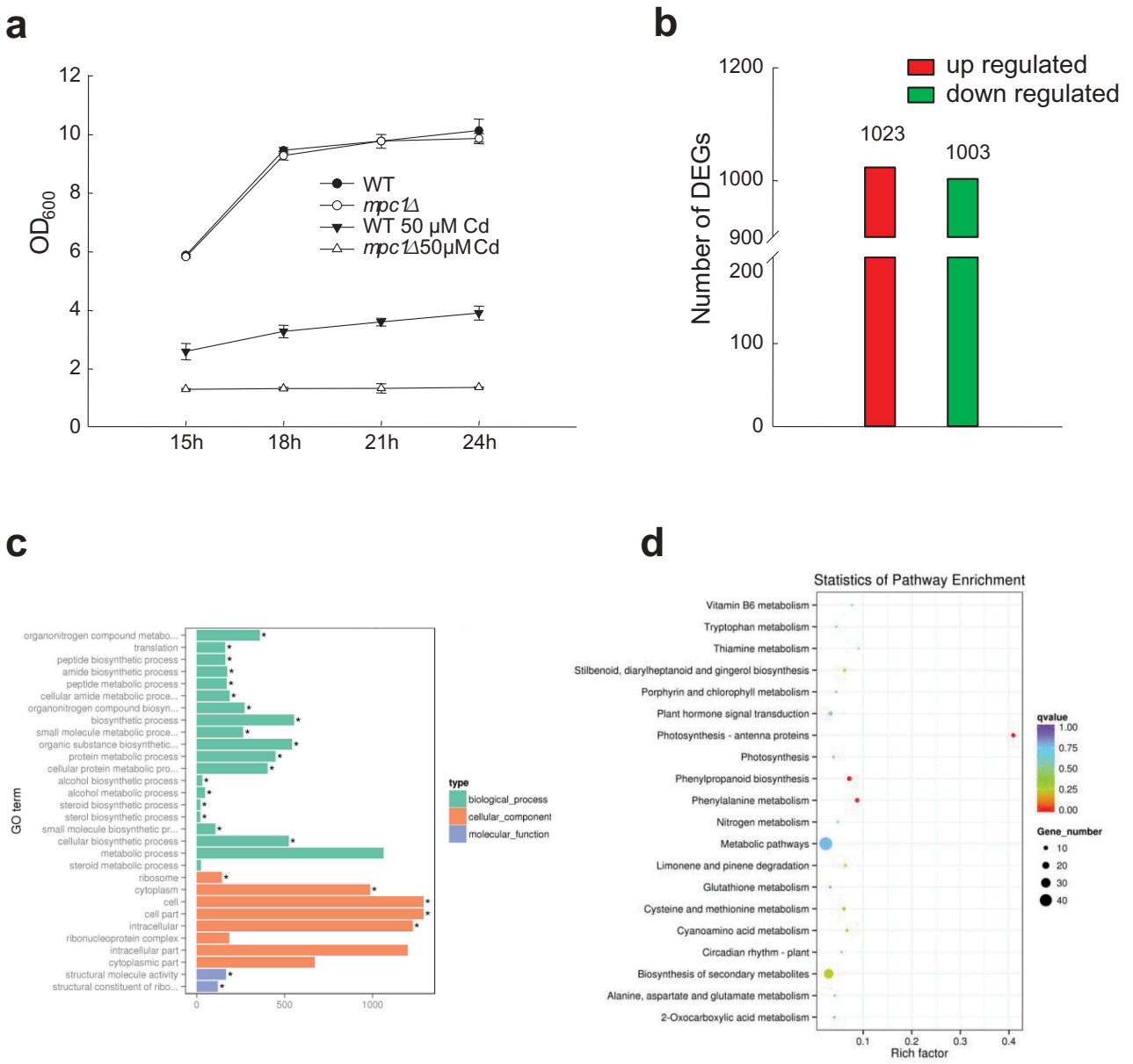

Figure 1. RNA-seq analysis. (a) Yeast wild type strain and *mpc1Δ* cells were grown at 30°C in YPAD liquid media and exposed to 50 μM $CdCl_2$ at the concentration of 0.3 OD_{600}. Cell density was monitored with the absorbance at 600 nm at 15, 18, 21, 24 h after treatment. Error bars indicate ± SD from three independent experiments. After centrifuging 30s at 12000g, the yeast cells in tubes were instantly freezed in liguid nitrogen, and then stored in −80°C. (b) Numbers of DEGs in the comparison group (*Δmpc1-1* VS Wt). (c) The most 30 enriched GO terms in the comparison group (*Δmpc1-1* VS Wt). * represent the corrected *P* value of the term < 0.05 in GO term enrichment analysis. (d) The most enriched KEGG pathways in the comparison group (*Δmpc1-1* VS Wt). Rich factor indicates the ratio which the DEG number compare to the background number in the enriched KEGG pathway. qvalue is *Pvalue* after multiple hypothesis test correction.

Table 1. The most significant down-regulated differentially expressed genes (DEGs) (Log2 (Fold Change) < −4) in Δ*mpc1* VS WT comparison group discovered by RNA-seq.

Gene_ID	Read Count (Δ*mpc1*)	Read Count (WT)	Log2 (Fold Change)	P value	Corrected P Valve	Associated Gene Name
YGR236C	68.05330935	2347.520994	−5.1083	3.78E-06	5.37E-05	*SPG1*
YNL142W	719.9926712	16594.1596	−4.5265	1.69E-35	3.76E-32	*MEP2*
YER103W	1935.029646	41848.86568	−4.4348	6.84E-41	2.28E-37	*SSA4*
YGR035C	353.1023624	5883.860286	−4.0586	9.08E-35	1.51E-31	-//-

in highly purified mitochondria in high-throughput studies.[6,7] As an ammonium transport protein, *MEP2* regulates pseudohyphal differentiation and is controlled by phospho-silencing.[8,9] SSA4 is a member of Heat Shock Protein 70 (HSP70) gene family.[10–12] Although HSP70 members can be induced and associate with heavy metal stress, [13–15] there is no evidence indicating that SSA4 confer Cd tolerance in any species. YGR035C whose transcription is activated by paralogous transcription factors Yrm1p and Yrr1p along with genes involved in multidrug resistance is potential Cdc28p substrate and uncharacterized.[16,17] After the four genes were cloned into the yeast expressing recombination vectors (pGPD::DEGs) and yeast transformation, a serial dilution assay

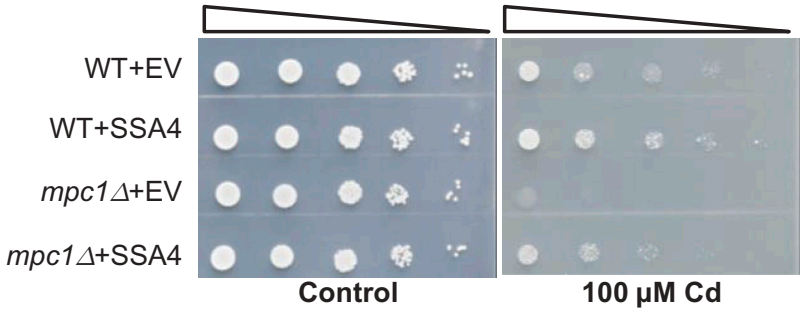

Figure 2. Yeast dilution bioassay with wild-type strain, *mpc1Δ* transformed with pRS416 and pRS416 expressing *ScMPC1* in SC medium. Triangles represent serial 10-fold dilutions (starting concentration of 0.3 OD_{600}). Representative test from three reproducible experiments was shown.

was conducted to test Cd tolerance. As shown in Figure 2, Stress-seventy subfamily A 4 (SSA4) can partially recover the Cd sensitive phenotype of *▵mpc1*. Our results indicate that, except for metabolic regulation, MPC1 also regulated Cd tolerance through SSA4, which is a downstream gene in the *MPC1* controlled Cd resistant pathway. Previously, we reported that MPC prevents Cd toxicity by sustaining the TCA cycle and glutathione synthesis. We also discovered a resistant gene, AGP30, which is related to Cd tolerance and associated with MPC1. It is suggested that MPC1 plays a role in Cd tolerance through regulating metabolism and downstream gene pathways in *Arabidopsis*. In this study, we found MPC1 was also required for Cd tolerance through regulating metabolism and downstream Cd tolerance genes in yeast. Although MPC is a classic key material transporter, it could not affect downstream genes by direct interaction, however, under Cd stress, MPC can confer the tolerance associating with some specific gene. The function of MPC in molecular regulatory pathways needs to be further explored.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This study was supported by National Key Research and Development Program of China [grant no. 2017YFD0101801], Modern Agricultural Industrial Technology System Funding of Shandong Province, China [grant no. SDAIT-05-04], Shandong Upgraded Project of "Bohai Granary" Science and Technology Demonstration Engineering in 2019, the Special Foundation for Postdoctoral Innovation Projects under Grant 201603036, Taishan Scholars Program of Shandong Province (No. tsqn201909167).

References

1. Bricker DK, Taylor EB, Schell JC, Orsak T, Boutron A, Chen YC, Cox JE, Cardon CM, Van Vranken JG, Dephoure N, et al. 2012. A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. Science. 337:96–100. doi:10.1126/science.1218099.
2. Herzig S, Raemy E, Montessuit S, Veuthey JL, Zamboni N, Westermann B, Kunji ER, Martinou JC. 2012. Identification and functional expression of the mitochondrial pyruvate carrier. Science. 337:93–96. doi:10.1126/science.1218530.
3. He L, Jing Y, Shen J, Li X, Liu H, Geng Z, Wang M, Li Y, Chen D, Gao J, et al. 2019. Mitochondrial pyruvate carriers prevent cadmium toxicity by sustaining the TCA cycle and glutathione synthesis. Plant Physiol. 180:198–211. doi:10.1104/pp.18.01610.
4. Jing Y, Shi L, Li X, Zheng H, He L. 2019. AGP30: Cd tolerance related gene associate with mitochondrial pyruvate carrier 1. Plant Signal Behav. 14:1629269. doi:10.1080/15592324.2019.1629269.
5. Young MD, Wakefield MJ, Smyth GK, Oshlack A. 2010. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biol. 11:R14. doi:10.1186/gb-2010-11-2-r14.
6. Martinez MJ, Roy S, Archuletta AB, Wentzell PD, Anna-Arriola SS, Rodriguez AL, Aragon AD, Quinones GA, Allen C, Werner-Washburne M. 2004. Genomic analysis of stationary-phase and exit in saccharomyces cerevisiae: gene expression and identification of novel essential genes. Mol Biol Cell. 15:5295–5305. doi:10.1091/mbc.e03-11-0856.
7. Reinders J, Zahedi RP, Pfanner N, Meisinger C, Sickmann A. 2006. Toward the complete yeast mitochondrial proteome: multidimensional separation techniques for mitochondrial proteomics. J Proteome Res. 5:1543–1554. doi:10.1021/pr050477f.
8. Boeckstaens M, Llinares E, Van Vooren P, Marini AM. The TORC1 effector kinase Npr1 fine tunes the inherent activity of the Mep2 ammonium transport protein. Nat Commun. 2014;5.
9. Lorenz MC, Heitman J. 1998. The MEP2 ammonium permease regulates pseudohyphal differentiation in saccharomyces cerevisiae. Embo Journal. 17:1236–1247. doi:10.1093/emboj/17.5.1236.
10. Chughtai ZS, Rassadi R, Matusiewicz N, Stochaj U. 2001. Starvation promotes nuclear accumulation of the hsp70 Ssa4p in yeast cells. J Biol Chem. 276:20261–20266. doi:10.1074/jbc.M100364200.
11. Gokhale KC, Newnam GP, Sherman MY, Chernoff YO. 2005. Modulation of prion-dependent polyglutamine aggregation and toxicity by chaperone proteins in the yeast model. J Biol Chem. 280:22809–22818. doi:10.1074/jbc.M500390200.
12. Werner-Washburne M, Stone DE, Craig EA. 1987. Complex interactions among members of an essential subfamily of hsp70 genes in Saccharomyces cerevisiae. Mol Cell Biol. 7:2568–2577. doi:10.1128/MCB.7.7.2568.
13. Begum N, Hu Z, Cai Q, Lou L. Influence of PGPB inoculation on HSP70 and HMA3 gene expression in switchgrass under cadmium stress. Plants (Basel). 2019;8.
14. Hui F, Liu J, Gao Q, Lou B. 2015. Piriformospora indica confers cadmium tolerance in nicotiana tabacum. J Environ Sci (China). 37:184–191. doi:10.1016/j.jes.2015.06.005.
15. Wang W, Vinocur B, Shoseyov O, Altman A. 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci. 9:244–252. doi:10.1016/j.tplants.2004.03.006.
16. Lucau-Danila A, Delaveau T, Lelandais G, Devaux F, Jacq C. 2003. Competitive promoter occupancy by two yeast paralogous transcription factors controlling the multidrug resistance phenomenon. Journal of Biological Chemistry. 278:52641–52650. doi:10.1074/jbc.M309580200.
17. Ubersax JA, Woodbury EL, Quang PN, Paraz M, Blethrow JD, Shah K, Shokat KM, Morgan DO. 2003. Targets of the cyclin-dependent kinase Cdk1. Nature. 425:859–864. doi:10.1038/nature02062.