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SHORT COMMUNICATION

AGP30: Cd tolerance related gene associate with mitochondrial pyruvate carrier 1

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ABSTRACT

Heavy metal ions which are not essential elements for basic metabolism severely threaten human health through food chain. As the most water-soluble and absorbed heavy metal ion, Cadmium (Cd) is easily accumulated and contaminates plants. Previously, mitochondrial pyruvate carrier 1 (MPC1) was proved to be required for Cd tolerance and Cd^{2+} exclusion. In this study, we carried out following mRNA expression profile analysis on Cd-treated *mpc1-1* and wild-type plants. After further selection of differential expressed genes and Cd tolerance tests in yeast, we have discovered a novel Cd tolerance related gene: *AGP30*, which specifically expresses in root and is significantly regulated by MPC under Cd stress. This protein mainly localize in the cell wall of cells in root meristem region, which was consistent with our former Cd^{2+} flux measurement. In conclusion, our work discovered a new Cd resistant gene for utilizing in transgenic crops for preventing Cd^{2+} influx.

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Mitochondrial pyruvate carriers (MPCs), which transport pyruvate into mitochondria for generating Acetyl-CoA, and finally drive TCA activity, are the key elements in the cellular basic metabolism.[1,2] Recently, we found that MPC1 prevent Cd stress and Cd^{2+} influx by sustaining TCA cycle and ATP level in *Arabidopsis*.[3] In plant, MPC members were identified to be involved in kinds of stress regulation.[4–6] Moreover, MPC1 had proved to be interacted with its homologous proteins: MPC2 (MPC2L), NRGA1 and MPC3, and form kinds of MPC complexes.[3,6] However, the details of MPC in the regulation of downstream genes in plants are unclear. Although MPC is a kind of transporter, which should have no direct responsive or associated proteins or genes, some putative indirect candidates are still interesting to be further analyzed for Cd tolerance. To examine whether the gene expression pattern has changed, an RNA-seq analysis was conducted on Cd-treated plants comparing *mpc1-1* against the wild-type control (Figure 1(a)). Differential expressed genes (DEGs) with statistically significant change in the comparison, which DEG comparison log2 ratio >2 or <-2 were collected for clustering analysis, and there are 33 up-regulated and 8 down-regulated including MPC1 itself (Figure 1(b,c)). Loss of *AtMPC1* may lead to the decreased expression of the putative indirect regulatory downstream genes under Cd stress. In order to find out the putative candidate in Cd tolerance, we focus on the seven significant down-regulated DEGs (Table 1), and selected the most significant three down-regulated DEGs (*AGP30, CLE5* and *JAL9*) for further analysis. As an easy and fast tolerance test system, yeast (*Saccharomyces cerevisiae*) can be used to test some special tolerance function of the genes. Previously, *saccharomyces cerevisiae* JRY472 showed Cd sensitive phenotype when lack MPC1.[3] So, we cloned the genes into the yeast expressing recombination vector pRS416, which is under control of the pGDP, and then transformed them in JRY472 and *mpc1Δ*. Serial dilution assays were conducted to test Cd tolerance in yeast. As shown in Figure 2, *AGP30* could recover the Cd sensitivity of *mpc1Δ*. This result indicated that AGP30 could be a novel Cd tolerance related gene.

AGP30 (Arabinogalactan protein 30), which specifically expresses in the cell walls of the primary root, plays a role in root regeneration and seed germination.[7] However, there is no evidence showing that AGP30 related to the tolerance of heavy metal stress. For plants, root is the main channel of heavy metal ion influx,[8–10] and root cell wall should be the first line of defense to resist heavy metal ion invasion.[11–13] Thus, there should existing Cd resistant proteins localized in root cell walls. Interestingly, the transcripts of *AGP30* significantly accumulate in the cell walls of root meristem region.[14,15] Previously, we had measured the Cd ion flux in *Arabidopsis* root in meristematic zone.[3] The detective point of *mpc1-1* showed enhanced Cd influx compared to wild-type plant. Thus, we speculate that the deletion of AGP30 should also be one reason of Cd invasion except the decrease of ATP level. Additionally, to confirm the relationship between AGP30, MPC1 and Cd stress, we conducted RT-qPCR in *mpc1-1* and wild-type plants without or with Cd stress. As shown in Figure 3, without stress, the AGP30 transcript abundance did not delete in *mpc1-1* compared to wild-type plants, however, when treated with 50 μM $CdCl_2$, the

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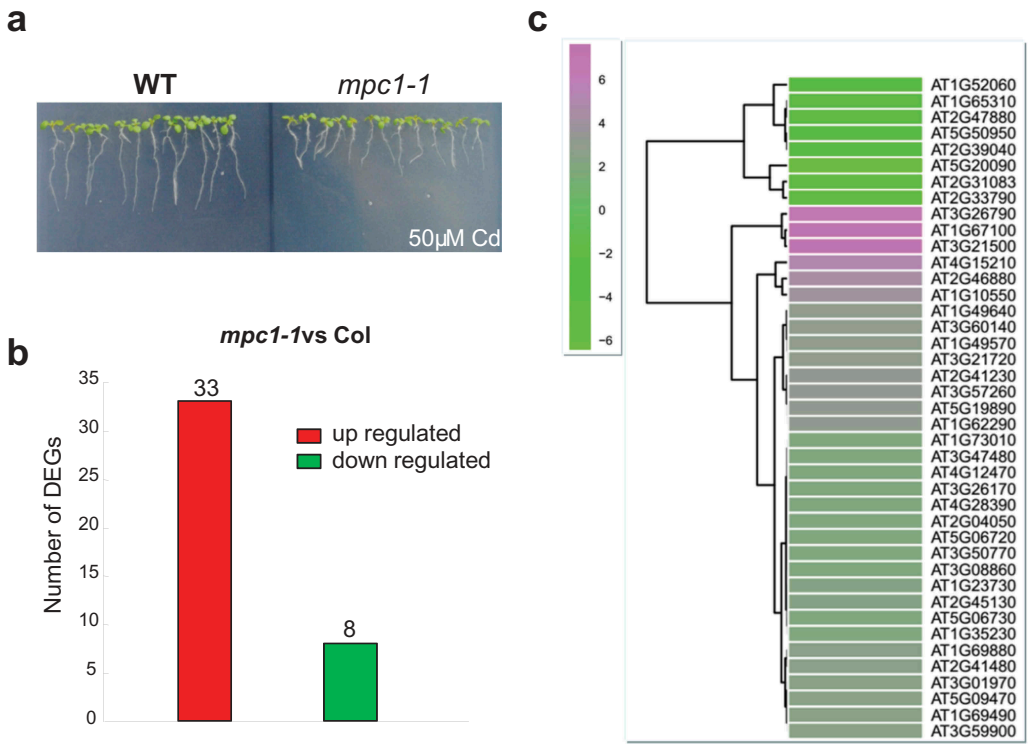

Figure 1. RNA-seq analysis. (a) Arabidopsis plants grown on 0.5X MS plates vertically for 3 d were transfered to plates with 50 μM CdCl2 for culturing another 7 d. whole plantlets were instantly freezed in liguid nitrogen, and than stored in −80°C. (b) Numbers of DEGs in *oxs2-1* VS Wt group. (c) DEGs which comparison log2 ratio >2 or <-2 were collected for clustering analysis. Expression ratios shown as log2 values. Magenta represents increased expression; green represents decreased expression compared to Col. Vertical axis shows fold enrichment of relative transcript levels between *mpc1-1* and wild-type plants.

Table 1. Summary of significant down-regulated differentially expressed genes in *mpc1-1* VS WT comparison group discovered by RNA-seq (Figure 1(c)).

Gene_id	Readcount _*mpc1*	Readcount _Col	log2FoldChange	*mpc1-1* vs Col	Col vs *mpc1-1*	padj	Associated Gene Name
AT5G20090	8.418565681	723.1528101	−6.4246	0.011641475	85.89976458	1.34E-81	MPC1
AT2G33790	5.935108725	204.1548384	−5.1042	0.029071605	34.3978262	1.30E-11	AGP30
AT2G31083	1.282069928	31.16551444	−4.6034	0.041137454	24.30874772	0.00036245	CLE5
AT1G52060	11.62816875	128.724492	−3.4686	0.090333771	11.0700571	8.82E-13	JAL9
AT2G39040	9.676693472	48.65136367	−2.3299	0.19889871	5.027684695	0.0050019	PER24
AT5G50950	83.7015594	392.6306867	−2.2298	0.2131814	4.690840762	0.00078333	FUM2
AT2G47880	14.51504021	63.16384212	−2.1216	0.22979983	4.351613306	0.029287	GRXC13
AT1G65310	26.22389186	107.3363649	−2.0332	0.244315073	4.093075333	0.01831	XTH17

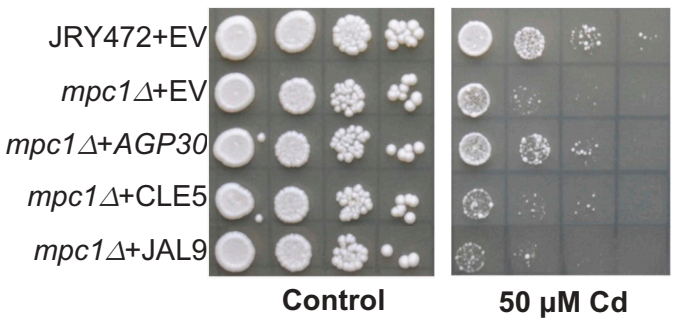

Figure 2. Cd tolerance test in yeast. Yeast dilution bioassay with wild-type strain, *mpc1Δ* transformed with pRS416 and pRS416 expressing *AGP30* in SC medium. Triangles represent serial 10-fold dilutions (starting concentration of 0.3 OD600). Representative test from three reproducible experiments is shown.

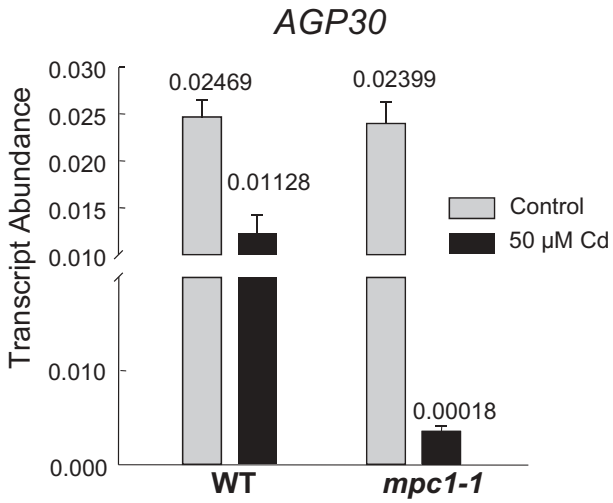

Figure 3. Expression of AGP30 (relative to ACT2 control) in 10-d-old Arabidopsis seedlings exposed to 0 or 50 mM CdCl2. Error bars indicate ± SD from three independent experiments. *P* value of Student's t test: *mpc1-1* compared with the wild type. ***P < .001.

abundance level decreased about 62 fold. Moreover, in wild-type plants, when treated with Cd, the transcript abundance of AGP30 also decreased to the half level of the normal condition. It is suggested that the AGP30 response to Cd, and is

dramatically regulated by MPC1 with Cd stress. Considering the expression of *AGP30* decreased in plants with Cd, and AGP30 can restore the Cd sensitive phenotype in *mpc1Δ*, *AGP30* may someday be engineered for heavy metal stress tolerance in crops.

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