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Short Communication

Intracellular localization of Na^+/H^+ antiporter from *Malus zumi* (MzNHX1)

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In this study, we examined the intracellular localization of the product of Na^+/H^+ antiporter gene (*MzNHX1*) cloned from *Malus zumi*. Analysis using yeast cells expressing a fusion protein of MzNHX1 and green fluorescent protein confirmed the localization of MzNHX1 on the tonoplast.

Key words: Intracellular localization, eGFP, *Malus zumi*, Na^+/H^+ antiporter, yeast.

INTRODUCTION

Na^+/H^+ antiporters are widespread membrane proteins found in bacteria, yeasts, animals and plants (Mennen et al.,1990; Lv et al., 2003), regulating the internal pH, Na^+ level and cell volume in the cytoplasm. Na^+/H^+ antiporters are located on either the plasma membrane or the tonoplast (Apse et al., 1999). Lots of experiment results show that the salt tolerance of plants and yeasts is highly related to the level of Na^+/H^+ antiporting activity (Yokoi et al., 2002). Under high saline conditions, Na^+/H^+ antiporters on plasma membrane can eliminate Na^+ from the cells and vacuolar Na^+/H^+ antiporters can compartmentalize Na^+ into the vacuoles. Thus the Na^+ concentration and Na^+/K^+ ratio in plants and yeasts cytoplasm maintain a moderate level to adapt the saline conditions (Shi et al., 2000). The Na^+/H^+ antiporters have been payed more and more attention to its role in plant salt tolerance. To this day, Na^+/H^+ antiporter genes have been isolated from many different plants, including *OsNHX1* from rice (Fukuda et al., 1999), *VvNHX1* from rape (Wang et al., 2003), *TtNHX1* from New Zealand spinach (Lv et al., 2004), and *InNHX2* from Japanese morning glory (Ohnishi et al., 2005). We have also cloned a Na^+/H^+ antiporter gene named *MzNHX1* from the *Malus zumi* with moderate salt tolerance. The result of sequence analysis showed that it was highly homologous to the vacuolar Na^+/H^+ antiporter gene.

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The purpose of this study was to confirm the intracellular localization of MzNHX1 protein by experimental measure.

MATERIALS AND METHODS

Chemicals, enzymes, vectors and strains

Restriction endonucleases, *Taq* DNA polymerase and other ordinary chemicals were obtained from Tiangen Biotech Company (Beijing, China); *Saccharomyces cerevisiae* DY1457, *Escherichia coli* DH-5α and vectors pEZS-NL, pYES2 were kept by the Key Laboratory of Beijing Municipality of Stress Physiology and Molecular Biology for Fruit Tree (Beijing, China).

Vector construction

A pair of primers was used to amplify the full open reading frame of *MzNHX1*. The primers sequence were 5'-ATAGGTACCCAATGGCGGTTCCACATTTG-3' (KpnI cutting site added) and 5'-ACCGGATCCccTTGCCACTGAACATTGTTG-3' (termination codon deleted, BamHI cutting site added). The PCR product and pEZS-NL plasmid were digested with KpnI and BamHI, then the two digested products were linked to be pEZS-NL + *MzNHX1* (Figure 1A) by T4 DNA ligase. The recombinant plasmid was transformed into DH-5α for amplifying, then recombinant plasmid DNA was extracted. The plasmids pEZS-NL + *MzNHX1* and pEZS-NL were digested with KpnI and XbaI to obtain *MzNHX1* + *eGFP* and *eGFP* DNA fragments. The two fragments were linked to the product of pYES2 plasmid digested by KpnI and XbaI respectively. Thus the recombinant yeast expression vectors pYES2 + *MzNHX1* + *eGFP* and pYES2 + *eGFP* (as control) were ready (Figure 1B).

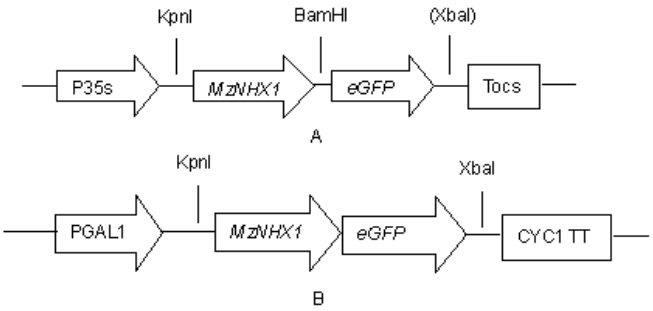

Figure 1. Schematic diagram of expression vector structure. A. pEZS-NL+*MzNHX1.* B. pYES2+*MzNHX1+eGFP*.

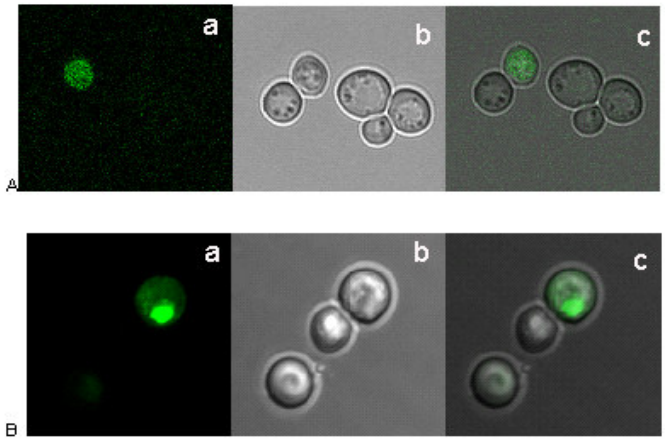

Figure 2. Localization of MzNHX1::eGFP fusion protein expressed in yeast cells. A: control; B: luminescence of MzNHX1::eGFP fusion protein in yeast cells; a. eGFP b. bright field c. a and b are overlaid in c.

Transformation of expression vector to yeast

Preparation of medium for yeast growth (YPD) or selection (SD-Ura) and transformation of yeast by lithium acetate referred to the method of Gietz and Woods (2002).

Observation of transformed yeasts under microscope

Picking single colony from SD-Ura$^-$ plate, shaking cultivated at 30℃, 200 rpm in liquid SD-Ura$^-$ medium (Galactose as carbon source to induce GAL promoter expression) for 48 h. Taking 900 µl culture mixed with 100 µl glycerol, dropping 200 µl mixture on the slide and overlaid with cover slip, kept at 4℃. Glycerol is not essential for instant observation.

The yeast cells were observed under common florescence microscope first, then observed the luminescence for more precise subcellular localization of MzNHX1 under laser scanning confocal microscope.

RESULTS AND DISCUSSION

Observed under the laser scanning confocal microscope, it showed uniform and very weak green fluorescence in the entire control yeast cells (Figure 2 A-a). This was because only eGFP without signal peptide was expressed in control yeast as heterologous protein, eGFP stayed in cell sap after synthesis on the ribosome. The signal peptide of MzNHX1 protein guided the fusion protein of MzNHX1::eGFP to its purpose location. So the vacuole of the yeast cells expressed fusion protein of MzNHX1::eGFP showed strong fluorescence (Figure 2 B-a). This result showed that MzNHX1 protein localized on tonoplast and might play a role in compartmentalizing Na^+ into vacuoles.

The yeasts have been used to express exogenous protein since 1980s. The exogenous protein can be located on different membrane in yeast cells, which is impossible in bacteria. There are vacuoles in yeast cells but smaller than that of plant cells, the volume is only 1/3-1/2 of whole yeast cell, and they usually appear during the mid to late period of cell development. Regardless of the fusion protein locates on the tonoplast or the plasma membrane, it can be observed very clearly.

The enhanced GFP can catalyze itself to form chromophoric groups and send out green fluorescence under the blue light emission, therefore it can be fused with the goal protein as fluorescence tagged molecule used in intracellular localization of protein specifically (Sullivan et al., 1998; belson et al., 1999). The eGFP is widely used because of its other advantage that can be used *in vivo* determination (Quaedvlieg et al., 1998).

Regarding the intracellular localization of Na^+/H^+ antiporter protein which locates on the plasma membrane or the tonoplast, it is wise to use eGFP as fluorescence mark in yeast cells, because that the technology of this method is mature, the operation is simple, the experimental period is short, but the effect is obvious.

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