Functional Studies of Thioredoxin in *Arabidopsis thaliana*

Catharine Lie, Ling Meng, Bob B. Buchanan and Peggy G. Lemaux
Plant & Microbial Biology, University of California, Berkeley 94720

Abstract

Trx knockout lines were screened and Trx over-expressing lines of *A. thaliana* were generated. The knockout lines were germinated on media with kanamycin for selection for the T-DNA insert. The T-DNA insertions in putative knockout lines were screened for using PCR; TRX expression will be studied using RT-PCR. Three out of seven knockout lines were confirmed to have a T-DNA insertion; the remainder of the lines is still being screened. The confirmed lines need to be examined further using RT-PCR. Plants from confirmed knockout lines will be subjected to different stress treatments for further observation of phenotype. The data produced will be used to speculate on the function of Trxs.

Introduction

Thioredoxins (Trxs) are proteins involved in cellular redox regulation and appear to be particularly important in plants compared to other organisms. Plants contain a large number of Trx genes compared to mammalian organisms; in *Arabidopsis thaliana* 19 different Trx genes have been identified. This research focuses on three different Trxs in *A. thaliana*: AtTRXh9, AtTRXputative, and AtTRX X. AtTRXh9 has been shown to localize in the cell wall/plasma membrane and appears to be able to move from cell to cell, based on observations using a GFP fusion to AtTRXh9; and AtTrx putative has been shown to localize in the chloroplast, again using the same technique of GFP fusions.

Comparative analysis will be performed on *Arabidopsis* plants that have gained or lost expression of specific Trxs that will be grown in normal and stress conditions in order to observe differences in the phenotypes of plants and determine function. Prior to the stress experiments, Trx knockout mutant lines and Trx over-expressing lines were first screened for the presence of T-DNA insertion and gene expression, respectively.

Materials and Methods

Screening of Trx knockout mutant lines

Trx knockout mutant lines were obtained from the Arabidopsis Biological Resource Center (ABRC). These lines were screened for the presence of T-DNA insertions by PCR with primers specific for given Trxs. Plants homozygous for the T-DNA insertion will be further analyzed for RNA expression using RT-PCR to screen for null knockout plants.

Plants were grown in kanamycin-containing media for selection of those containing T-DNA insertions and were moved to soil after 2 weeks. DNA was extracted from the floral meristem. Extracted DNA was amplified with PCR using specific primers; annealing temperature used was 54˚C. PCR products were checked with gel electrophoresis, run for 45 min at 70mV. Selected DNA fragments, based on migration distances relative to molecular weight markers, were isolated and used for sequencing to confirm the T-DNA insertion.

Screening of Trx over-expressing lines

Trx overexpressing lines were generated by *Agrobacterium*-based stable transformation of *Arabidopsis* (ecotypes Columbia and Landsberg). The full-length sequence of Trx, fused in

frame with eGFP (enhanced Green Fluorescence Protein) at the N-terminus, was driven by the 35 S promoter, and cloned into the binary vector pART27, and transformed into *Arabidopsis* by the flower dip method.

Transformed plants were selected on MS media containing kanamycin and analyzed for GFP expression by observing green fluorescence under the fluorescence microscope. Plants with the strongest GFP signals, indicating the highest levels of Trx expression, were selected and given various stress treatments.

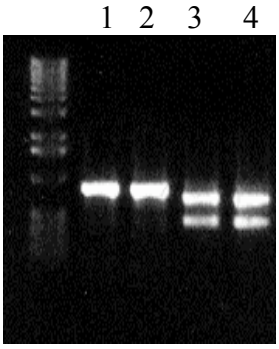
Stress treatments for overexpressing lines

1. Salt stress treatment: NaCl [100mM], [200mM], [300mM]
2. Oxidative stress treatment: hydrogen peroxide [10mM], [5mM], [1mM]

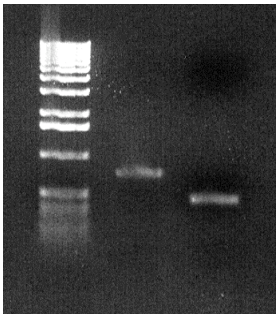
Seeds from overexpressed lines for stress treatments are divided into:

1. Control (no stress treatment)
2. Stress treatment after 2-3 days
3. Stress treatment given immediately after cold treatment

Results



Column1: amplified from wild type *Arabidopsis*
Columns 2, 3, 4 from putative knockout line;
Columns 3 and 4 contain extra band.



Column 1: Column 2:
Two bands from gel above were separately isolated and used for sequencing, to confirm the insertion of the T-DNA.

Three out of seven different knockout lines have been confirmed to have a T-DNA insertion.

At trxh9 081049	Still in screening process	
At trxh9 086660	T-DNA insertion is confirmed	Inserted in Exon
At trxput 020019	Still in screening process	
At trxput 028162	T-DNA insertion is confirmed	Inserted in Intron
At trxput 059334	Still in screening process	
At trxX 128914	Still in screening process	
At trxX 125897	T-DNA insertion is confirmed	Inserted in Intron

From the overexpressed lines, Athn and Atput, plants, that show GFP expression and therefore are known to express the fused *trx* gene, were selected and are currently subjected to different stress treatments.

Discussion

The obtained putative knockout lines, that were screened and confirmed to have T-DNA insertions after sequencing, are heterozygous. No homozygous lines were obtained. possibly because the homozygous state is lethal. RT-PCR was used to check gene expression in the knockout lines.

In separate experiments using *in situ* hybridization, it was found that AtTRXh9 is located in the plasma membrane and appears able to move from cell to cell, as visualized using GFP fused to the Trx gene. (What about Trx put?)

The function of Trxs will be studied by observing phenotypes in plants grown under different stress treatments, either high salt or oxidative stress. Based on these observations, we will attempt to determine the functions of the three Trxs in plants.

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