Open Access

NAP-Related Protein 1 (Atnrp1) Overexpression Increases the Heat Tolerance of Arabidopsis Cells/Plantlets

Bíró J, Domoki M and Fehér A*

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Temesvári krt 62, H-6726 Szeged, Hungary

Abstract

Nucleosome assembly protein-related proteins, NRPs, were overexpressed in transgenic Arabidopsis plants. This resulted in increased survival rate of seedlings and cells exposed to 45ºC for one hour. Heat shock resulted in the accumulation of the proteins, detected in the cytosol of untreated plants, in the nuclear protein fraction. DNA repair and genotoxic stress related-gene expression were unaltered in the transgenic plants as compared to controls.

Keywords: Nucleosome assembly protein-related protein; Elevated temperature; Survival; Transgenic; Cellular localization

Introduction

Nucleosome-assembly protein-related proteins (NRPs) are multifunctional proteins having histone chaperone [1] and histone phosphatase inhibitor [2] properties and being implicated in root meristem maintenance [1], mitotic regulation [1], chromatin-mediated gene silencing [1], genotoxic sensitivity [1] and homologous recombination [3]. The drosophila homologue of plant NRPs, the SET protein, has been shown to accumulate at heat-shock gene loci in response to increased temperature where it co-localized with Ser10-phosphorylated histoneH3 [4]. It was therefore hypothesized that the drosophila SET protein prevents phosphatase2A (PP2A)-mediated histoneH3 dephosphorylation and contributes to chromatin modifications facilitating heat shock protein gene transcription [4].

Based on the structural and functional homology of SET and NRP proteins [2], we investigated whether the altered expression of the plant At NRP1 protein interferes with the heat sensitivity of Arabidopsis cells/seedlings.

Experimental

Plant cultures and heat treatment

Experiments were carried out with the wild-type Columbia ecotype of Arabidopsis thaliana (L.) Heynh and it's At NRP1 overexpressor genetic transformant (NRP OX; kindly provided by Valerie Frankard, CropDesign N.V., Ghent, Belgium). In these plants, the Arabidopsis *nrp1-1* cDNA clone was cloned after the *Helianthus annuus* Gubb1 promoter for constitutive expression. T4 generation homozygous transgenic (OX) and wild type (WT) segregants of the same transformation event were used in the experiments.

Seeds of Arabidopsis thaliana were surface-sterilized by soaking in 70% ethanol (v/v) for 1 minute and in 30% bleach (v/v) for 10 minutes and then rinsed five times with sterile distilled water, and plated on solid Murashige and Skoog (MS) medium (Duchefa Biochemie, B.V., Haarlem, The Netherlands) containing 3% (w/v) sucrose, 0.6% (w/v) agar, with pH adjusted to 5.8. The seeds were germinated at 24ºC using an 8 hours light/16 hours dark light regime. Experiments were carried out at least in triplicates with 7-8-days-old seedlings grown in glass petri dishes on MS medium [2]. Heat shock treatment (45ºC for 45-60 minutes as indicated) was applied in a water bath (50 seedlings/dish; 4 dishes/line). Survival rates of plants (%) were visually determined one week after the treatment as the ratio of seedlings staying green and growing six weeks after the treatment. In the case of leaf protoplast-derived cells (for isolation and culture, [5]) dead cells were stained by Evans blue (0, 5% w/v). Altogether app. 1000 cells were investigated per line in three repetitions.

Western blotting

The antibodies used and the immunoblotting protocol is described elsewhere in details [2].

Gene expression analysis by real-time quantitative PCR (RT-QPCR)

RNA isolation, cDNA synthesis and RT-QPCR analysis by an ABI Prism 7700 sequence detection system have been described earlier [2]. DNA damage and repair-associated (PARP2, At4g02390; RAD51, At5g20850; AtMYB, At5g03780; RNR2, At3g27060) as well as the control unresponsive (UBC18, At5g42990) genes were selected based on the work of Chen et al. [6] (Table 1).

Results and Discussion

Using a specific antibody recognizing Arabidopsis NRP proteins

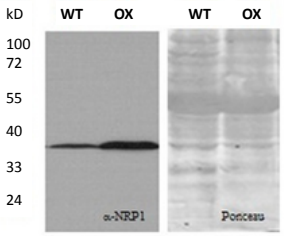

Figure 1: NRP protein level in wild type (WT) and overexpressing (OX) Arabidopsis plants as detected by a specific antibody (α-NRP1) Proteins were stained by Ponceau reagent as loading control.

***Corresponding author:** Fehér A, Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Temesvári krt. 62, H-6726 Szeged, Hungary, Tel: +36 62599701; E-mail: feher.attila@brc.mta.hu

Received April 26, 2013; **Accepted** July 10, 2013; **Published** July 17, 2013

Citation: Bíró J, Domoki M, Fehér A (2013) NAP-Related Protein 1 (Atnrp1) Overexpression Increases the Heat Tolerance of Arabidopsis Cells/Plantlets. J Plant Biochem Physiol 1: 115. doi:10.4172/2329-9029.1000115

[2] it was verified that NRP proteins accumulated in the AtNRP1 overproducing transgenic plants (Figure 1). 8-day-old seedlings of the wild type (WT) and NRP overexpressing (OX) plants were subjected to heat shock at 45ºC. In a series of experiments the NRP overexpressing seedlings exhibited increased survival rate one week after the treatment in comparison to controls (Figure 2A and 2B).

In order to test whether this difference in heat sensitivity of seedlings is also exhibited at the cellular level, leaf protoplasts have been isolated from WT and OX plants and were similarly subjected to a one hour 45ºC heat shock. Cell viability was determined using Evans blue staining dead cells. Similarly, as was observed in the case of seedlings, an app. 20% difference in cell viability could be detected in favor of NRP overexpressing cells (Figure 2C).

Basal thermo tolerance of plants depends on several factors, including hormonal regulation (ethylene, salicylic acid), production and scavenge of reactive oxygen species and the expression of various genes including heat shock transcription factors and heat shock proteins [7,8], several of which play also role during acquired thermotolerance. However, we have previously shown that NRPs are dispensable for the heat-induced expression of heat shock protein genes in Arabidopsis; despite the fact that they act as potent histoneH3 phosphatase inhibitors *in vitro* [2].

Strong heat causes DNA strand breaks what if not repaired can finally result in cell death. NRPs has recently been implicated in the maintenance of genome stability [3]. Therefore we investigated whether NRP overexpression may contribute to increased DNA repair and in this way can improve cellular survival. However, we could not detect any difference in the expression of selected DNA repair-associated (PARP2, RAD51) or genotoxic stress-upregulated (AtMYB and RNR2) genes (Figure 3). Therefore at present it is not known via what mechanism NRPs contribute to cellular heat tolerance in Arabidopsis. However, based on the presently known biochemical functions of plant NRPs,

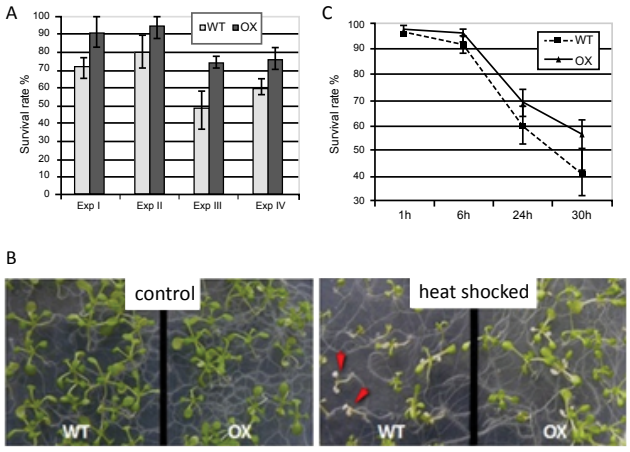

Figure 2: Increased survival of NRP overexpressing Arabidopsis plants (A,B) and cells (C) subjected to heat shock at 45ºC.

(A) Survival rate of one-week-old seedlings treated at 45ºC for 45 minutes (Exp I and II) or for 60 minutes (Exp III and IV) as determined 7-days after the treatment.

(B) Example of seedling growth one week after or without the treatment. Arrows indicate dead plants.

(C) Survival rate of leaf protoplast derived cells treated at 45ºC for 60 minutes and scored for survival by the help of Evans blue staining at the indicated time points. WT-wild type; OX-overexpressor.

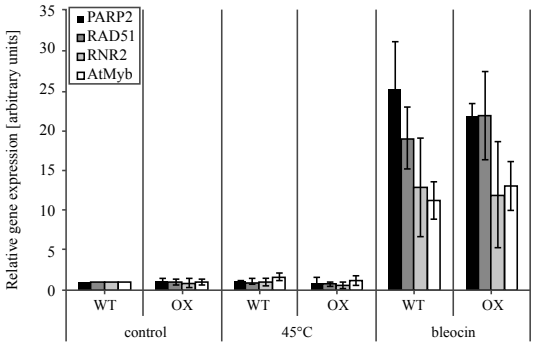

Figure 3: Results of a gene expression analysis by real-time quantitative PCR. 8-days-old seedlings of non-transgenic (WT) and NRP1-overexpressing transgenic (OX) Arabidopsis lines were heat shocked (45ºC for 1 hour) or were grown in the presence of 10 µg/ml bleomycin. Relative expression (treated/control) is shown for the DNA repair-associated (PARP2, RAD51) and genotoxic stress-upregulated (AtMYB and RNR2) genes [6]. Values were normalized to the genotoxic stress un-responsive UBC18 gene expression [6].

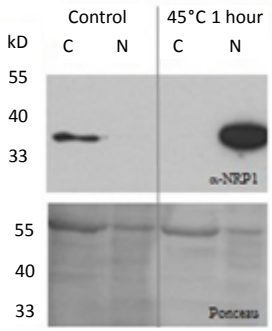

Figure 4: The NRP proteins are detected in the cytoplasmic fraction in seedlings grown at normal culture conditions (24ºC) but in the nuclear fraction of heat shocked (45ºC for 1 hour) cells as detected by a specific antibody (α -NRP1). Proteins were stained by Ponceau reagent as a loading control.

it might be related to their capability to influence chromatin structure and/or phosphatase activity [1,2].

Several functions, such as gene silencing, mitosis regulation and homologous recombination [1,3] of NRPs are associated with nuclear functions, and especially with the organization of the chromatin. Interestingly, when NRP proteins were detected by Western blotting in control cells and in cells heat shocked for one hour at 45ºC, it was observed that while in untreated cells the proteins were in the cytosolic, in heat shocked cells they were in the nuclear protein fractions (Figure 4). Therefore one can suppose that these proteins shuttle between the cytosol and the nucleus in response to heat shock. However, the detection of NRP proteins in the cytosol of WT plants is contradictory to a previous report describing the nuclear localization of green fluorescent protein-NRP fusions [1].

The animal homologues of NRPs are also nuclear proteins having, however, cytoplasmic functions as well [9]. Therefore, further studies are required to determine the dynamic cellular location of plant NRPs and its role in the high temperature response of plant cells.

Acknowledgements

The presented work was supported by the NKTH-OTKA grant K68051.

Primer name	UPL	Sequence (5'-3')
PARP2 FW	68	GCGAACTATTGCTATGCCAAC
PARP2 REV		ATGTCTCCCAAAGCAACCTC
RAD51 FW	134	GATCACGGGAGCTCGATAAA
RAD51 REV		GCGGAACTCACCATATAACTCTG
UBC18 FW	67	ACAGCAATGGACATATTTGTTTAGA
UBC18 REV		TGATGCAGACTGAACTCACTGTC
RNR2 FW	143	GAATCATCGCTTTCGCTTG
RNR2 REV		TTCAGCCAGAAGATTGAACAAA
AtMyb FW	134	TGTGGCTAAGTCAAAGACAGTGA
AtMyb REV		TCCACTCCCACCTTTAGCAT

(UPL = Roche "Universal probe library" number of TaqMan probes used together with the primers to ensure specificity.)

Table 1: Sequence of primers used in the study.

References

1. Zhu Y, Dong A, Meyer D, Pichon O, Renou JP (2006) *Arabidopsis NRP1* and *NRP2* Encode Histone Chaperones and Are Required for Maintaining Postembryonic Root Growth. Plant Cell 18: 2879-2892.
2. Bíró J, Farkas I, Domoki M, Otvös K, Bottka S (2012) The histone phosphatase inhibitory property of plant nucleosome assembly protein-related proteins (NRPs). Plant Physiol Biochem 52: 162-168.
3. Gao J, Zhu Y, Zhou W, Molinier J, Dong A, et al. (2012) NAP1 family histone chaperones are required for somatic homologous recombination in Arabidopsis. Plant Cell 24: 1437-1447.
4. Nowak SJ, Pai CY, Corces VG (2003) Protein phosphatase 2A activity affects histone H3 phosphorylation and transcription in Drosophila melanogaster. Mol Cell Biol 23: 6129-6138.
5. Abel S, Theologis A (1994) Transient transformation of Arabidopsis leaf protoplasts: a versatile experimental system to study gene expression. Plant J 5: 421-427.
6. Chen IP, Haehnel U, Altschmied L, Schubert I, Puchta H (2003) The transcriptional response of Arabidopsis to genotoxic stress - a high-density colony array study (HDCA). Plant J 35: 771-786.
7. Larkindale J, Knight MR Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. Plant Physiol 128: 682-695.
8. Larkindale J, Hall JD, Knight MR, Vierling E (2005) Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol 138: 882-897.
9. Park YJ, Luger K (2006) Structure and function of nucleosome assembly proteins. Biochem Cell Biol 84: 549-558.

Citation: Bíró J, Domoki M, Fehér A (2013) NAP-Related Protein 1 (Atnrp1) Overexpression Increases the Heat Tolerance of Arabidopsis Cells/Plantlets. J Plant Biochem Physiol 1: 115. doi:10.4172/2329-9029.1000115