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

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**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 45°C 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 SET protein, has been shown to accumulate at heat-shock gene loci. 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 SET protein, has been shown to accumulate at heat-shock gene loci.

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.

**Results and Discussion**

Using a specific antibody recognizing Arabidopsis NRP proteins 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).

**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 NRPI overexpressor genetic transformant (NRP OX; kindly provided by Valerie Frankard, CropDesign N.V., Ghent, Belgium). In these plants, the Arabidopsis npr1-1 cDNA clone was cloned after the

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 24°C using an 8 hours light/16 hours dark 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 (45°C 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
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 45°C. 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 45°C 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, 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 45°C, 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
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References


Table 1: Sequence of primers used in the study.

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<th>Primer name</th>
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(UPL = Roche “Universal probe library” number of TaqMan probes used together with the primers to ensure specificity.)