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 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

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

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[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 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).

Baseline theromo 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 thermostolerance. 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 histone H3 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-regulated (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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(UPL = Roche “Universal probe library” number of TaqMan probes used together with the primers to ensure specificity.)

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