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Arabidopsis NAP-related proteins (NRPs) contribute to the coordination of plant growth, developmental rate, and age-related pathogen resistance under short days

Balázs Barna[c,1], Katalin Gémes[a,e,1], Mónika Domoki[a], Dóra Bernula[a], Györgyi Ferenc[a], Balázs Bálint[d], István Nagy[b,d], Attila Fehér[a,e,*]

[a] Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62, H-6726, Hungary
[b] Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62, H-6726, Hungary
[c] Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman Ottó út 15, 1022 Budapest, Hungary
[d] SeqOmics Biotechnology Ltd, Vállalkozók útja 7, 6782 Mórahalom, Hungary
[e] Department of Plant Biology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary

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ABSTRACT

Plant nucleosome assembly protein-related proteins (NRPs) are histone chaperons involved in nucleosome turnover. Despite this basic cellular function, the *Arabidopsis nrp1-1 nrp2-1* knock out mutant has been reported to exhibit only mild seedling root phenotypes and to significantly affect the expression of only few hundred genes Zhu et al. (2006). Here we report that NRP loss-of-function as well as the ectopic overexpression of At NRP1 significantly affected the growth, development, and the pathogen response of *Arabidopsis* plants under short day conditions. The *nrp1-1 nrp2-1* mutant grew faster and flowered weeks earlier than the wild type and the overexpressor. The latter developed slower and flowered at a lower number of leaves than the mutant and the wild type. Moreover, the mutant was more sensitive, the overexpressor was more tolerant to pathogen-induced necrosis correlating with their more adult and juvenile character, respectively. Transcriptomic comparison of mature non-bolting plants agreed with the phenotypes. The presented and other published data indicate that although NRPs might not be absolutely required for normal plant growth and development, their level needs to be controlled to allow the epigenetic coordination of metabolic, growth, defence and developmental processes during the acclimation to unfavourable growth conditions such as short days.

1. Introduction

The nuclear genome of eukaryotes is structured by the help of specific proteins, the histones, resulting in the formation of the chromatin. The basic unit of this DNA-protein complex is the nucleosome, where 147 base pair of DNA is wrapped around a histone octamer consisting of two-two copies of H2A, H2B, H3, and H4, respectively [2]. The inter-nucleosomal DNA region is associated with H1 histones having role in the higher-order organization of the chromatin. In addition, all histones have variants increasing the structural and functional variability of nucleosomes. The genomic functions, including replication, repair, and transcription, largely depend on the accessibility of the DNA by various enzyme complexes. Therefore, the histone variants are in a continuous turnover that allows temporal and spatial reorganization of the overall nucleosome structure. This dynamic is, however, tightly controlled. Several covalent modifications of histones influence DNA-histone and histone-histone interactions and consequently the architecture and dynamics of the chromatin [3].

Histone chaperones control histone turnover. These acidic proteins binding to the positively charged histones facilitate their transport and have role in nucleosome assembly/disassembly determining the specificity of the DNA-histone interaction [4]. One of the families of histone chaperones includes the nucleosome assembly proteins (NAPs; [5]) sharing a structurally conserved fold, the NAP domain. These proteins have been shown to have many functions in addition to serve as histone chaperones [5]. The family consists of the NAP1 protein and its close relatives as well as the structurally more distinct NAP-related SET (S.E. Translocation) protein having various other names due to its many functions (see e.g. in [6].

The NAP family was evolutionary conserved in all eukaryotes.

* Corresponding author at: Department of Plant Biology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary.
E-mail address: feher.attila@brc.mta.hu (A. Fehér).
[1] These two authors contributed equally to the results.

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Arabidopsis as well as rice have four NAP1 (NAP1; 1–4 also called NAPL1-4) and two NAP1-related proteins (NRP1 and 2 also called NAPL6 and 5) [7,8]. Plant NAP1 as well as NRP proteins were shown to bind histones H2A and H2B [1,9,10] and are considered as histone H2A and H2B chaperones [8]. In accordance with their general function, the expression of the NAP1 as well as the NRP genes is rather ubiquitous in the *Arabidopsis* plant except NAP1; 4 that has a tissue specific expression in root segments and in the pollen grain [11]. The various proteins were found to have diverse intracellular distribution patterns depending on the investigated species, tissue, or physiological state as well as the used detection method [9–12]. While some members seem to shuttle between the cytoplasm and the nucleus, others seem to be mostly cytoplasmic or nuclear. Functional studies indicated that plant NAP1 proteins may be involved in the regulation of microtubule dynamics and the cell cycle [10], DNA repair [11], somatic homologous recombination [13,14], and the regulation of transcription [11] (for review see [8]). The NRP1 and 2 proteins have been implicated in post-embryogenic root growth, mitotic regulation, DNA repair, and transcription [1,15,16] as well as somatic homologous recombination [14]. However, their role in these processes as well as the influenced genes seem to be distinct from those of the NAP1;1-4 proteins (e.g. [8,11,14,16]).

The plant NRP1 and NRP2 proteins fall into a phylogenetically distinct NAP1 subfamily also containing the animal SET and the yeast Vps75 proteins [7,8]. Both SET and Vps75 have several functions in association with various protein complexes (see [6,17], and the references therein). It is unknown how many of these interactions/functions are conserved in plants. In this regard, we have recently demonstrated that the *Arabidopsis* and Medicago NRP1 proteins have protein phosphatase 2A inhibitory activities similarly to their animal homologue, the SET protein [6]. Furthermore, González-Arzola and co-workers demonstrated that both the *Arabidopsis* NRP1 and the human SET (also called TAF-Iβ) proteins can interact with cytochrome c and control DNA repair in a similar manner [16].

Interestingly, despite of their basic cellular functions and the hundreds of affected genes [1,11], the loss of NAP1 and/or NRP functions were reported to exhibit mild or no phenotypes in *Arabidopsis* [1,11]. Although the *nap1;1 nap1;2 nap1;3* triple mutant exhibited increased sensitivity to various stresses, its growth and development were unaffected under normal conditions [11,13]. The *nrp1-1 nrp 2-1* double mutant seedlings have short highly branched roots, but the mature plants have no any remarkable phenotype [1]. These plants exhibited, however, increased sensitivity against genotoxic stresses [1,13]. Even the sextuple mutant where all the six NAP1 family genes (including genes coding for the NRPs) had been deleted exhibited undisturbed growth and development under normal conditions [14].

Investigating the gene expression pattern of *nrp1-1 nrp2-1* mutant seedlings, Zhu and coworkers [1] determined 102 genes that were differentially regulated in the double mutant in comparison to the wild type. *In silico* analysis of this gene set indicated that many of them might also be regulated by heat and/or pathogen infection [6]. Therefore, we decided to test the potential role of these proteins in heat shock as well as pathogen responses. In previous publications we have reported that although the overexpression of At NRP1 somewhat increased the heat tolerance [18], the NRPs were dispensable for heat-shock-induced gene expression in *Arabidopsis* [6]. To get a deeper insight into the reactions of the mutant and overexpressor plants to pathogens, we report here their response to artificial infections using the biotroph powdery mildew *Golovinomyces orontii*, the hemibiotroph *Pseudomonas syringae* bacteria, and the necrotroph *Sclerotinia sclerotiorum* fungus. In the pathological experiments, from practical points of view, plants grown at short day condition (11 h light/13 h dark) were used. It was observed that under this circumstance the NRP protein level strongly affected the growth, development, and senescence of *Arabidopsis* plants, as was indicated by plant size, flowering time, and photosynthetic pigment content. The effect of NRP protein absence and At NRP1 overexpression, respectively, on the growth, development, senescence, and pathogen sensitivity of short-day-grown (8 h light 16 h dark) *Arabidopsis* is reported in this paper. Transcriptomic data obtained with mature, senescing plants grown at short days were used to strengthen the phenotypic observations including the pathogen responses. The potential role of NRPs as histone chaperones in the coordination of short-day responses of *Arabidopsis* plants is discussed.

2. Materials and methods

2.1. Plants and pathogens

Experiments were carried out with the wild-type Columbia ecotype of A. thaliana (L.) Heynh, its double mutant *nrp1-1 nrp2-1* and overexpressing *nrp1ox* lines. The *nrp1-1 nrp2-1* double mutant *Arabidopsis* plants are deficient in At NRP1 and At NRP2 expression and protein [1,6]. The seeds were kindly provided for us by Dr. Yan Zhu (State Key Laboratory of Genetic Engineering, Institute of Plant Biology, School of Sciences, Fudan University, Shanghai, China). The seeds of the At NRP1 genetic transformant (*nrp1ox*) approximately 2-fold overexpressing the At NRP1 protein under the control of the sunflower *GUbB1* ubiquitin promoter was kindly provided by Dr. Valerie Frankard (CropDesign N.V., Ghent, Belgium).

The immunological detection of NRP protein levels in these and wild type plants is shown in the Supplementary material (Supplementary Fig. 1).

Seeds of *Arabidopsis* plants were sown into pots and kept 3 days at 5 °C in dark, then put into a growth chamber (for the pathological experiments and pigment determination 11 h light/13 h dark, 140 μmol/m2/s, for the gene expression experiments 8 h light/16 h dark, 190 μmol/m2/s) with the temperature of 20 °C and the plants were grown for the next 40–100 days depending on the type of the experiment.

The biotrophic powdery mildew (*Golovinomyces orontii*) strain MPIZ (kindly provided by Dr. Paul Schulze-Lefert, Max Planck Institute for Plant Breeding Research, Cologne, Germany) was used in the experiments. Strain MPIZ was maintained on Columbia ecotype of *A. thaliana*. Inoculation, spreading the conidia was carried out by touching the fully sporulating *Arabidopsis* leaves to the tested plants.

Two strains of the hemibiotrophic *Pseudomonas* bacteria were used: *P. syringae* pv. *syringae* 61 and *P. syringae* pv.*tomato* strain DC3000. These Pseudomonas strains are plant pathogens with differing host specificities and corresponding pathovar designations. *P. syringae* pv. *syringae* strain 61 elicits the hypersensitive response (HR) in nonhost plants.*Pseudomonas syringae* pv. *tomato* DC3000 (Pto DC3000) is the cause of bacterial speck disease on tomato and *Arabidopsis*, and gives a compatible reaction on Columbia ecotype. Bacterial cultures were maintained on nutrient agar at 30 °C. Cultures were transferred to fresh medium 16–24 h prior to use. Since we have evidence that even water injection itself can induce some membrane permeability changes, in the case of leakage measurements for infection all fully developed plant leaves were brushed either with water (control) or with bacterial suspension (10^8 cfu cm^{-3}). For measuring bacterial multiplication in plant tissue one centimetre diameter areas of fully expanded leaves in pre-flowering stage were infiltrated with bacterial suspensions or water as control using 1-ml needle-less syringe.

To have information on the effect of NRP protein on the reaction of plants to a typical necrotrophic pathogen infection with *Sclerotinia sclerotiorum* isolate Sz-24 (kindly provided by F. Virányi, Szent István University, Gödöllő, Hungary) was carried out as well. For visual evaluation, the fully developed leaves of the test plants were cut, placed on wet filter paper in Petri dishes and inoculated with 0.5 cm diameter agar culture disks containing the pathogen. Evaluation of the symptoms was carried out by measuring lesion development on the leaves. For leakage experiments, fully developed plant leaves were brushed either with water (control) or with *Sclerotinia* mycelial suspension.