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ARTICLE

Investigation of the regulation of the CBP20 gene in *Arabidopsis*

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ABSTRACT The *Arabidopsis* mutant line *cbp20* carries a lesion in the *Cap Binding Protein 20* gene, which is a member of the nuclear cap binding (nCBC) complex. The *cbp20* mutant displays elevated sensitivity to abscisic acid (ABA). Apparently this property leads to fast stomatal closure and thus improved drought tolerance. We found that this gene's transcription is elevated by exogenous application of a cytokinin hormone (BAP), suggesting a hormonal control over its expression. These data represent a new aspect of the regulation of the CBP20 gene and the nCBC complex.

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KEY WORDS

Arabidpopsis
drought stress
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ABA sensitivity

The *Arabidopsis* mutant line cbp20 carries a lesion in the Cap Binding Protein 20 gene, which is a member of the nuclear cap binding (nCBC) complex. The cbp20 mutant displays elevated sensitivity to abscisic acid (ABA). Apparently this property leads to fast stomatal closure and thus improved drought tolerance. We found that this gene's transcription is elevated by exogenous application of a cytokinin hormone (BAP), suggesting a hormonal control over its expression. These data represent a new aspect of the regulation of the CBP20 gene and the nCBC complex.

Introduction

Drought tolerance is a particularly important trait in nowadays agriculture due to the global warming effect and climate change.

We identified an *Arabidopsis* mutant with elevated drought tolerance by screening for mutations in pleiotropic traits (Papp et al. 2004). In our screening strategy we rigorously tested emerging mutants to different environmental stimuli, such as biotic or abiotic stresses.

This approach led to the isolation of a knockout mutation in a gene encoding the CBP20 subunit of the nuclear Cap Binding Complex (nCBC).

The *cbp20* mutant displays only mild pleiotropic morphological abnormalities, but elevated sensitivity to abscisic acid (ABA). Apparently this later property leads to fast stomatal closure and thus improved drought tolerance (Fig. 1). The nCBC complex consists of a handful of proteins, including the cap binding proteins CBP20, CBP80 and eIF4G (Izaurralde et al. 1994). CBP80 and CBP20 interact, forming the core of nCBC, which is able to bind the 5' cap structure. Both proteins are essential for nCBC function (Izaurralde et al. 1995).

Materials and Methods

Plant material and growth conditions: *Arabidopsis thaliana cv. Columbia* was used in the experiments. Plants were grown under short day light conditions (10 hours light, 14 hours dark periods) for four weeks after sowing. From the fifth week on long day illumination was applied (16 hours light, 8 hours dark). Relative humidity was kept at 65%, temperature was 21°C and and photon fluence rate was 120 µEinstein m^{-2} s^{-1}.

0,1 mM BAP + 0,01% Silwet or Silwet alone was applied as a foliar spray onto the leaves of the plants, followed by sampling after 24 hours.

Northern hybridization: Total RNA was prepared from treated and control 6-week-old plants. Tri-reagent (Sigma)

Figure 1. *cbp20* (left) and wild type (right) mutant *Arabidopsis* plants after water stress.

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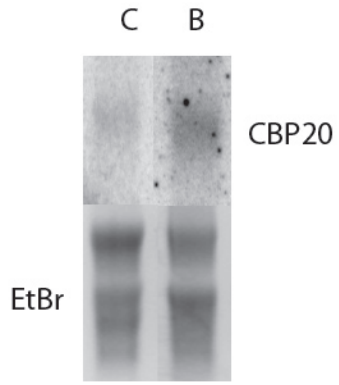

Figure 2. Northern hybridization of wild type Arabidopsis RNA samples - control (C) and after BAP (B) treatments, with the CBP20 cDNA as a probe.

was used according to the manufacturer's instructions. A total of 10 µg aliquots of RNA samples were run on denaturing formaldehyde-agarose gel, blotted onto HybondN membrane (Amersham-Pharmacia) and hybridized according to the instructions of the manufacturer. A full-lenght CBP cDNA fragment was labeled with ^{32}P by a Ready-To-Go kit (Amersham-Pharmacia) and used as probe.

Results and Discussion

In order to study the regulation of the CBP20 gene at the mRNA level we applied hormonal and stress treatments. These inculded physical stresses (e.g. cutting, infiltration of distilled water), hormonal treatments and infections with virulent and avirulent bacteria (data not shown). Among these treatments only a cytokinin (N-6-Benzylaminopurine, BAP) induced CBP20 transcription considerably (Fig. 2), while stress treatments and infections reduced its expression for a limited time (data not shown). In the publicly available Arabidopsis microarray datasets (Genevestigator) zeatin, a different cytokinin was used with no apparent effect on the gene's expression. Biotic stresses reduced CBP20 expression in the public microarray data in accordance with our results.

These results represent a new aspect of the regulation of a member of the nCBC complex in *Arabidopsis*. In animals the complex is subject to regulation by growth hormones by mean of phosphorylation of nCBC subunits. Whether this happens in plants remains to be investigated. Our results however show that growth hormones may contribute to nCBC control in plants too, although at a different level of regulation than in animals.

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