BIOLOGIA PLANTARUM 37 (1): 1-7, 1995

Expression of several genes encoding chaperone proteins in response to mechanical perturbation in *Bryonia dioica* internodes

J.P. GALAUD*, G. BARBE*, N. BOYER* and T. GASPAR**

*Physiologie végétale, 4, Rue Ledru, F - 63068 Clermont-Ferrand, France**
*Hormonologie végétale, Institut de Botanique B22, Sart Tilman, B- 4000 Liège, Belgium***

Abstract

Mechanical stress exerted on young *Bryonia dioica* internodes which resulted in reduced elongation and increased radial expansion induced a rapid and transient increase in specific mRNAs. Hybridizations were performed using ubiquitin, cyclophilin and heat-shock protein cDNAs as probes on RNA extracted at successive time intervals in control and rubbed internodes. Changes in ubiquitin and cyclophilin were rapidly enhanced after mechanical perturbation. Levels of mRNAs reached a maximum 0.5 h and 1.5 h after rubbing and then decreased. The heat shock protein gene was constitutively expressed; it was however slightly stimulated following the rubbing treatment. All the three genes encoded for molecular chaperones and they were regulated in response to environmental stimuli. The role of chaperones was discussed with regard to the plant response to several natural stresses.

Key words: cyclophilin, elongation, heat shock protein gene, hybridization, mRNA, radial expansion, ubiquitin

Introduction

A variety of external factors such as light, temperature, wind, gravity, abiotic and biotic agents, affect the morphogenesis of plants (Jaffe and Sorbes 1993). Mechanical manipulations may have effects similar to wind, especially in reducing elongation in woody and herbaceous species. For example, shaking or flexing greenhouse chrysanthemums reduced their elongation: height was directly controlled by mechanical stress. Such perturbations probably mimicked the effect of natural wind movements (Hammer *et al.* 1974).

Received 23 March 1994, *accepted* 14 July 1994.
Acknowledgments: We thank Dr. G. Burkard (Institut de Biologie Moléculaire des Plantes, Strasbourg, France) for providing the maize cDNAs encoding ubiquitin, cyclophilin and heat-shock protein.

In *Bryonia dioica*, mechanical stress exerted on young internodes resulted in reduced elongation and increased radial expansion (Boyer *et al.* 1979b). Previous studies have involved rapid increase of peroxidase activities (Boyer *et al.* 1979a), accelerated lignification (De Jaegher *et al.* 1985) and transient augmentation of ethylene production (Boyer *et al.* 1986, De Jaegher *et al.* 1987). This suggested that mechanical stimulus induced rapid modifications in gene expression. Analysis of 5-methylcytosine content of *Bryonia dioica* showed a rapid and transient decrease in DNA methylation after rubbing (Galaud *et al.* 1993a). In many cases, high levels of 5-methylcytosine have been found in unexpressed regions of the genome, whereas undermethylated genes were actively expressed (Amasino *et al.* 1984, Riggs 1989). These data indicated a negative correlation between the level of methylation and gene expression (Hepburn *et al.* 1987). Treatment of *Bryonia* plantlets with 5-azacytidine (a potent DNA anti-methylating agent) induced the same morphogenic response and indicated that DNA methylation was involved in this thigmomorphogenic process by affecting genes which control growth, peroxidase activity and ethylene pathway (Galaud *et al.* 1993b). Other works on stress showed rapid changes in gene expression with the induction of specific mRNAs (Guerrero *et al.* 1990) and enhancement of polysome formation (Schuster and Davies 1983). Among these specific mRNAs, several were identified to encode highly conserved proteins involved in the folding of cellular proteins. Such proteins were named chaperones. Therefore we have investigated here the expression of some genes encoding chaperone proteins such as ubiquitin, cyclophilin and heat-shock protein in response to a mechanical perturbation in *Bryonia dioica*. Ubiquitin is a highly conserved protein involved in several cellular processes ranging from the degradation of protein to the response to environmental stress (Van Nocker and Vierstra 1991). Cyclophilin is an ubiquitously expressed cytosolic peptidyl-prolyl *cis-trans* isomerase, which catalyses *in vitro* the *cis-trans* isomerisation of proline peptide bounds in small peptides and accelerates *in vivo* the slow folding of several proteins. Like ubiquitin and cyclophilin, the 70 kDa heat-shock proteins are an ubiquitous and highly conserved group of proteins (Hartman *et al.* 1992). Some members of each family are constitutively expressed, while others are regulated in response to environmental changes (Lindquist and Craig 1988).

Materials and methods

Plant material and culture conditions: *Bryonia dioica* Jacq. plants were grown in soil in 50 l containers in a controlled environment under 16 h photoperiod of 18 W m^{-2} photosynthetically active radiation provided by 40 W white daylight tubes (*Mazda LDL, TF 40*), 25 ± 1 °C, 60 ± 10 % relative humidity. The stimulus consisted of holding the last growing internode (± 15 mm) between the thumb and the forefinger and gently rubbing it back and forth for 3 s.

RNA isolation and northern blot analysis: Total RNA was extracted from terminal *Bryonia* internodes (3 g) using a guanidium thiocyanate method (Sambrook *et al.*

1989). Extractions were carried out at 0 h (control), 0.5 h, 1.5 h, 3 h, 6 h and 24 h after the mechanical stimulus. Total RNA (10 μg) was separated on formaldehyde gel and blotted to Hybond-N+ filter according to the manufacturer's specifications

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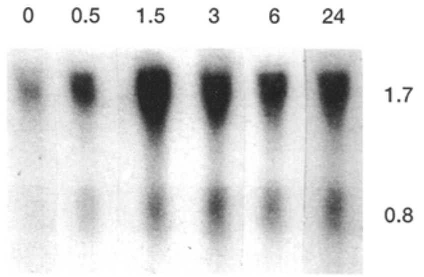

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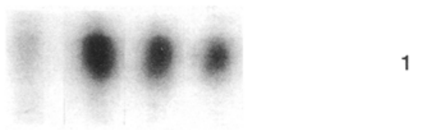

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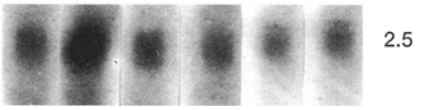

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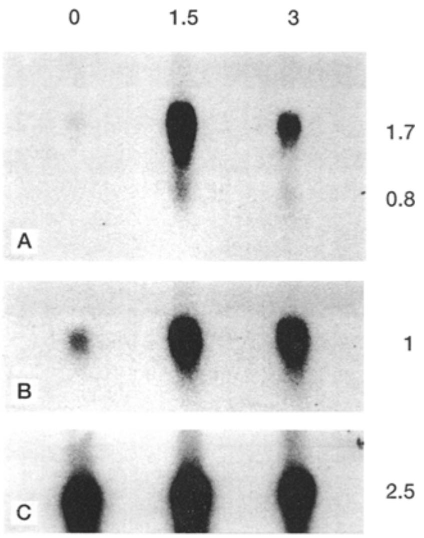

Fig. 1. Northern blot analysis of the time-course induction of ubiquitin gene expression following mechanical stimulus. Total RNA were purified from unrubbed *Bryonia* internodes (0 h) and from rubbed internodes at different times. Ten μg of total RNA was separated on a formaldehyde gel, blotted and probed using ^{32}P-labelled cDNA encoding ubiquitin. Size of RNAs transcripts is indicated on the right of the figure.

Fig. 2. Northern blot analysis of the time-course induction of cyclophilin gene expression following mechanical stimulus. Total RNA were purified from unrubbed *Bryonia* internodes (0 h) and from rubbed internodes at different times. Ten μg of total RNA was separated on a formaldehyde gel, blotted and probed using ^{32}P-labelled cDNA encoding cyclophilin. Size of RNAs transcripts is indicated on the right of the figure.

Fig. 3. Northern blot analysis of the time-course induction of heat-shock protein gene expression following mechanical stimulus. Total RNA were purified from unrubbed *Bryonia* internodes (0 h) and from rubbed internodes at different times. Ten μg of total RNA was separated on a formaldehyde gel, blotted and probed using ^{32}P-labelled cDNA encoding heat-shock protein. Size of RNAs transcripts is indicated on the right of the figure.

Fig. 4. Northern blot analysis of the time-course of induction of ubiquitin (A), cyclophilin (B) and heat-shock protein (C) expression following the mechanical stimulus. Poly(A+)RNA was purified from unrubbed *Bryonia* internodes (0 h) and from rubbed internodes at different times: 1.5 h and 3 h. Six μg of poly(A+)RNA was separated on a formaldehyde gel, blotted and probed using ^{32}P-labelled cDNAs. Size of RNAs transcripts is indicated on the right of the figure.

(*Amersham*). Poly(A+)RNA were purified from total RNA, extracted at various times after the rubbing treatment (0 h, 1.5 h and 3 h), by oligo (dT)-cellulose column, separated on formaldehyde gel and blotted to Hybond-N+ filters. Filters were probed with ^{32}P-labelled cDNA according to Sambrook *et al.* (1989).

Probes: The cDNA clones encoding ubiquitin, cyclophilin and heat-shock protein from maize were used as probes in northern analysis. Restriction fragments containing cDNA sequences were isolated from the vector DNA by agarose gel electrophoresis. These restriction fragments were purified from the gel using a prep-A-gene DNA purification kit (*Bio-Rad*).

Results

Changes in transcriptional activities of ubiquitin and cyclophilin genes in response to mechanical stimulus: Two different sizes of ubiquitin transcript were observed: the 1.7 kb mRNA was predominant and present in control internodes, whereas the 0.8 kb transcript was mechanically-induced (Fig. 1). The autoradiogram was subjected to a densitometer scanning analysis. The 1.7 kb transcript was present in control tissue and increased due to mechanical stimulation, 10-fold and 7-fold, after 1.5 and 3 h respectively, and was still present after 24 h. The 0.8 kb transcript was quite undetectable in control tissue, scarce after 0.5 h, but well visible from 1.5 h onwards.

Only one cyclophilin transcript of 1 kb length was observed in *Bryonia*. The cyclophilin gene was unexpressed in control internodes and highly stimulated following the rubbing treatment (Fig. 2). A maximum was reached in less than 30 min; thereafter the hybridization signal progressively decreased with time.

Mechanical perturbation and heat-shock proteins: In *Bryonia*, northern blot analysis with a hsp 70 probe (Fig. 3) showed the presence of a 2.5 kb transcript. The transcript was constitutively produced but the level increased slightly at 30 min after the mechanical stimulus.

Each experiment was repeated three times. The use of heterologous probes on total RNA could generate some artefacts. In order to confirm these results, hybridizations were performed on poly(A+)RNA purified from total RNA extracted from internode control (0 h), and excised 1.5 h and 3 h after the mechanical perturbation. Results obtained on poly(A+)RNA (Fig. 4) corroborate those observed on total RNA (Figs. 1, 2 and 3).

Discussion

Mechanical stress exerted on young *Bryonia dioica* internodes resulted in a rapid increase in both the steady-state transcript level and the rates of the transcription for ubiquitin and cyclophilin genes. Several reports clearly showed that wounding enhanced RNA polymerase activity (Kahl *et al.* 1982) and this was generally accompanied by an increase of polysome content (Nelson and Winkler 1987).

In *Bryonia*, after a mechanical perturbation, two ubiquitin transcripts of 1.7 kb and 0.8 kb were observed. This result could be related to those of Rickey and Belknap (1991) in potato and Genshick *et al.* (1992) in *Nicotiana sylvestris*: three and four transcripts were detected ranging from 0.8 kb to 1.9 kb and in all cases, ubiquitin genes were independently regulated in response to environmental changes. This suggest that whereas external stimuli induce a general transcriptional change, members of a same family cannot be enhanced by the same stimulus.

Like ubiquitin, cyclophilin gene expression was stimulated after the rubbing treatment in *Bryonia dioica*. mRNA transcripts reached a maximum in less than 1 h before decreasing progressively. In maize and in bean, the maximum was observed 7 h and 48 h after the application of a mercuric chloride solution (Marivet *et al.* 1992) and cyclophilin mRNA synthesis was activated differently by various stresses. In *Bryonia*, the rapid transcription of cyclophilin mRNA as well as ubiquitin might be related to previous studies on DNA methylation. A decrease of cytosine methylation was observed 1 h after the stimulus before return to its initial state in 3 h (Galaud *et al.* 1993a). The level of cytosine methylation is one component of a number of control mechanisms of gene expression (Riggs 1989). This suggests that in *Bryonia*, DNA methylation could, at least partly, play a role in the regulation of both ubiquitin and cyclophilin gene expression.

Northern blot realized with a cDNA encoding a 70 kDa heat-shock protein revealed the presence of a 2.5 kb transcript. It was constitutively present; however the transcription was slightly stimulated after rubbing, suggesting that hsp gene(s) could be regulated by stress conditions. The results suggested , as already reported by Lindquist and Craig (1988), that heat-shock proteins may also play a role in various developmental processes.

We reported here that among the abiotic stresses that a plant may encounter in its natural environment, one of them can stimulate several genes. A lot of them encode for different proteins involved in the protein folding and degradation. Ubiquitin, cyclophilin and hsp 70 are considered as molecular chaperones (Gething and Sambrook 1992). Molecular mechanisms of chaperone action is not understood in details, but this action appears to be directly stimulated in response to various stresses. The sequence analysis of ubiquitin (Hoffman *et al.* 1991), cyclophilin (Gasser *et al.* 1990, Bartling *et al.* 1992, Marivet *et al.* 1992) and hsp 70 (Cabané *et al.* 1993) indicated that a potent selective pressure existed in order to maintain the structure of these proteins among all the eukaryotes species, suggesting the important role played in normal cellular conditions or after environmental stimulations.

The plant response to stresses has been well studied and throughout these investigations, numerous results point to the predominant role of *1*) plasmalemma as a dynamic barrier (Muto 1993), and of *2*) calcium and calcium-binding proteins (Trewavas 1986, Poovaiah and Reddy 1987, Braam and Davis 1990, Galaud *et al.* 1993c, Thonat *et al.* 1993). Generally, in response to different stimuli, the same typical response may be observed during the initial steps of signal transduction, and the study of chaperone proteins as potential messengers appears to be preponderant in order to unravel mechanisms involved in these responses.

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