Plant Molecular Biology **26**: 1181–1189, 1994.

Bean cyclophilin gene expression during plant development and stress conditions

Jocelyne Marivet, Marcia Margis-Pinheiro, Pierre Frendo and Gérard Burkard *
Institut de Biologie Moléculaire des Plantes, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France (author for correspondence)*

Received 3 December 1993; accepted in revised form 20 September 1994

Key words: Peptidyl-prolyl *cis-trans* isomerase, ethylene-salicylic acid, alfalfa mosaic virus

Abstract

Cyclophilins (Cyp) are ubiquitous proteins with peptidyl-prolyl *cis-trans* isomerase activity that catalyses rotation of X-Pro peptide bonds and facilitates the folding of proteins; these enzymes are believed to play a role in *in vivo* protein folding. During development of normal bean plants, Cyp transcripts are first detected three days after beginning of germination and are present in all plant tissues examined. In a general way, higher amounts of Cyp mRNAs are found in developing tissues. Cyp mRNA accumulates in alfalfa mosaic virus-infected bean leaves and after ethephon and salicylic acid treatments. In response to a localized chemical treatment Cyp mRNA accumulation is observed in the untreated parts of the plants; however these changes in mRNA levels are restricted to the aerial part of the plant. A comparative study of Cyp mRNA accumulation in bean and maize in response to various external stimuli shows striking differences in profiles between the two plants. For instance, in response to heat shock, maize Cyp mRNA significantly accumulates, whereas no remaining mRNA is observed a few hours after the beginning of the heat stress in bean. Differences in mRNA accumulation profiles are also observed upon salt stress which induces the response earlier in maize than in bean, whereas the opposite situation is observed when plants are cold-stressed. All these findings further suggest that cyclophilin might be a stress-related protein.

Introduction

Cyclophilin, an ubiquitous protein, was first purified by Handschumacher and co-workers in 1984 from bovine thymocytes on the basis of its affinity for cyclosporin A (CsA) [13] a well known immunosuppressive agent. In the late 1980s, cyclophilin was found to be identical to an already described enzyme named peptidyl-prolyl *cis-trans* isomerase (PPIase) or rotamase (EC 5.2.1.8) [9, 29], that catalyses rotation of X-Pro peptide bonds and facilitates the folding of proteins *in vitro* and *in vivo* (for reviews see [2, 11]). As PPIase activity is inhibited by CsA, it was first postulated that the lost of this activity was the basis of the T cell inhibition by the drug. However, a number of evidences eroded that hypothesis, and at present it is believed that rather than inhibiting PPIase activity of cyclophilin, CsA becomes active as a complex with its cellular receptor and this complex promotes immunosuppression [23]. In view of some of its properties, it has been

suggested that cyclophilin might play *in vivo* an important role not only in protein folding, but also in trafficking and complex formation.

A great deal of work, essentially focused on mechanisms of immunosuppression by CsA, allowed the discovery of a family of highly conserved cyclophilin (Cyp) genes encoding abundant proteins with a common domain for CsA binding and enzyme activity, and surrounded by unique domains involved in organelle and membrane targeting (for reviews see [14, 30, 32]). Although Cyp genes have been described in various organisms, ranging from mammals to bacteria since several years, in higher plants, Cyp cDNA clones [3, 10, 21, 22] and proteins [18] have been isolated only recently. In a previous report, we have shown that in bean and maize plants Cyp genes are stress-responsive; indeed, accumulation of Cyp transcripts are observed after plants have been treated with mercuric chloride, a chemical elicitor [21].

In this paper we report on further investigation of bean Cyp gene expression, namely during germination and plant development; we also show changes occurring in Cyp mRNA levels upon virus infection, ethephon and salicylic acid treatments. We also report on the effect of a localized application of a chemical elicitor on Cyp mRNA levels in the untreated parts of a plant. Finally, a detailed analysis of Cyp mRNA accumulation in response to several external stimuli, showing striking differences when bean and maize plants are compared, is also presented. The accumulation of plant Cyp mRNAs in response to multiple external stimuli further suggests that cyclophilin may play an important and general role, not yet well defined, in plant defense mechanisms.

Materials and methods

Growth and stress conditions of plants

Bean (*Phaseolus vulgaris* L. cv. Saxa) and maize (*Zea mays* L. cv. INRA 258) plants were seeded in pots and grown in a greenhouse as previously described [21]. Seedlings were obtained from seeds germinated on wet Whatman 3 MM filters and placed in the dark at 22 °C. Prior to germination, seeds were sterilized for 10 min in a 2.5% calcium hypochlorite solution and rinsed several times with distilled water.

When 10–12 days old, plants were transferred to a growth chamber and maintained under the same controlled greenhouse conditions. To analyse the effects of external stimuli, different treatments were applied as follows:

- Leaves were sprayed once with a 0.2% (w/v) mercuric chloride solution which caused the appearance of necrotic lesions 3–4 days later.
- Leaves were wounded either by dusting with celite, gentle rubbing and rinsing with distilled water or by cutting with razor blades.
- Salt stress was performed by watering once the plants with 170 mM sodium chloride solution.
- Plants were irradiated twice, 0.5 h each time, with 254 nm UV light at an intensity of 0.8 W/m^2. Between the two UV light treatments, plants were returned for 0.5 h to the growth chamber.
- Plants were heat stressed at 42 °C for 4 h and then returned to the growth chamber.
- Cold treatments were performed in a cold room at 4 °C for several days.
- Leaves were sprayed once with a 10 mM salicylic acid solution.
- Leaves were sprayed once with MOPS buffer (0.02 M) containing 10 mM ethephon (2-chloroetylphosphonic acid) and plants incubated in closed plastic chambers. Control plants were sprayed with MOPS buffer and incubated under identical conditions.
- Plants were placed in the dark for 3 days and then returned to light into the growth chamber. After the dark period, maize leaves started to become yellowish, whereas bean leaves remained green.
- For virus infection of bean plants, both primary leaves of a plant were inoculated with a suspension of alfalfa mosaic virus (AlMV) by dusting leaves with celite, gentle rubbing and then rinsing with distilled water. The inoculum was obtained by grinding 1 g of infected tobacco leaf tissue (*Nicotiana tabacum* cv. xanthi

nc) in 1 ml of 0.02 M phosphate buffer pH 7 followed by a centrifugation at 1500 × *g* for 10 min. Infection caused the appearance of necrotic lesions 3–4 days later.

Leaves and seedlings were harvested at various time periods, frozen in liquid nitrogen and stored at −80 °C for subsequent RNA isolation.

RNA isolation and northern blot analyses

Total RNA was isolated from plant material using the phenol/SDS extraction and LiCl precipitation described by Ausubel *et al.* [1]. RNA samples (10 μg) were fractionated by electrophoresis on 1.2% formaldehyde-agarose gels, transferred onto Hybond N (Amersham) membranes and hybridized to ^{32}P-labelled inserts from cDNA clones corresponding to maize and bean cyclophilins [21] according to standard protocols [26]. To check integrity and verify whether different samples have been loaded in equivalent amounts, ethidium bromide has been added to the RNA samples prior to heating, which leads to markedly enhanced sensitivity of fluorescence detection [25]. This method allows to verify the quantitative transfer onto the membrane by scanning the membrane on a Shimadzu CS-9000 scanner. The amount of RNAs transferred onto the membrane has also been checked by using a bell pepper 25S RNA probe. Probes were generated by random primed labelling with [α-^{32}P]dCTP [8].

Results

Expression of bean cyclophilin gene during germination, and in various tissues of young and adult bean plants

The isolation and characterization of a bean Cyp cDNA used here as a probe to study the steady-state level of Cyp mRNA during normal development of unstressed bean plants has been described previously [21]. Total RNAs were extracted from whole bean embryos excised from dry and germinating seeds. Seedlings were collected every 12 h during 8 days after seeds were allowed to germinate. Northern blot analysis (Fig. 1, panel A) showed that bean Cyp mRNAs are not detectable in non-germinating embryos demonstrating that Cyp mRNAs are not stored in dry seeds. Cyp mRNAs were first detected about 3 days after beginning of germination and thereafter their level remained present in significantly higher amounts as compared to those found for instance in fully expended bean leaves (Fig. 1, panel C, lane 1).

Total RNAs were also extracted from various tissues and organs of young and adult bean plants and probed with bean Cyp cDNA (Fig. 1, panels B and C). Bean Cyp gene is constitutively expressed in all tissues of young and adult plants. However, depending on the tissues examined, differences were observed in the expression pattern. Thus, Cyp mRNA levels are higher in secondary trilobate leaves of young plants (panel B, lane sl), and also in roots, buds and inflorescence of adult

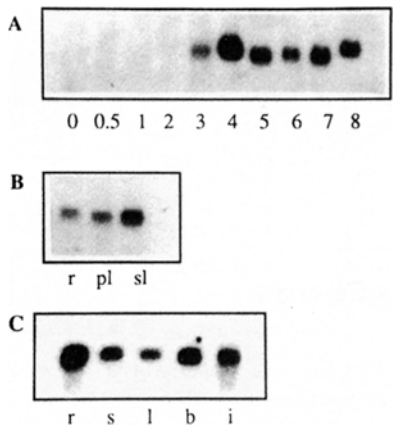

Fig. 1. Cyp gene expression during germination of bean seedlings and in various young and adult plants. Total RNAs (10 μg/lane), extracted from whole embryos and from the indicated tissues of young and adult plants, were separated on formaldehyde-agarose gels, transferred onto membranes and hybridized with ^{32}P-labelled insert from bean Cyp cDNA clone. A. Whole seedlings at different stages of germination from 0 to 8 days as indicated below each lane. B. Various tissues from young bean plants (10–12 days old): root (r), primary leaf (pl), secondary leaf (sl). C. Various tissues from adult bean plants (10 weeks old): root (r), stem (s), leaf (l), bud (b), inflorescence (i).

plants (panel C, lanes r, b, i) as compared to the levels found in fully expended leaves (panel C, lane l).

Bean Cyp mRNA accumulates upon virus infection

Abiotic stresses trigger changes in maize and bean Cyp mRNA levels [21]. To investigate the effect of a biotic stress, bean plants were inoculated with alfalfa mosaic virus using an inoculum dilution that usually induces about 300 small necrotic lesions on the inoculated leaves 3 to 4 days after inoculation [5]. The steady-state levels of bean Cyp mRNAs were analysed by northern hybridization using RNAs extracted at various time periods following inoculation. As depicted in Fig. 2A and B, higher levels of Cyp mRNAs are detected in both mock-inoculated and infected leaves as compared to normal leaves. Both diagrams represent the mean values of three different experiments, whereas the autoradiograms show the result obtained in a single experiment. In mock-inoculated leaves rubbed with celite and therefore wounded, two maxima of Cyp mRNA accumulation were observed, one at about 24 h, the second at 84 h after rubbing (Fig. 2A). In AlMV-inoculated leaves (Fig. 2B), the mRNA accumulation profile was somewhat different from that obtained by mock inoculation: Cyp mRNA accumulation reached a maximum 48 h after inoculation (a time point corresponding to a rather low Cyp mRNA level in mock-inoculated plants) and remained at high levels for several days.

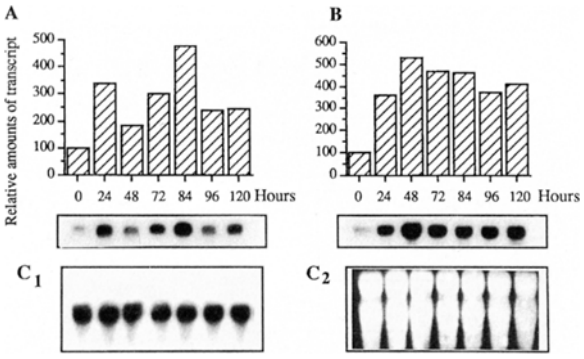

Fig. 2. Time course of accumulation of bean Cyp mRNA upon alfalfa mosaic virus infection. Northern blots from gels containing equal amounts (10 μg) of total RNAs isolated from mock-inoculated leaves (A) and from A1MV-inoculated leaves (B) at different time points after inoculation (indicated in hours below the diagrams). Each diagram represents the mean value of three different experiments performed with different RNA preparations; the autoradiograms below the diagrams show a single experiment. The amount of hybridizing RNA measured in unstressed plants has been taken to as the 100% value. Panel C1 shows the northern blot corresponding to the RNA from the mock-inoculated leaves hybridized with the bell pepper RNA probe. C2 shows the amount of RNA from mock-inoculated leaves that has been transferred onto the membrane.

Ethylene and salicylic acid treatments affect the constitutive level of Cyp mRNA

To investigate whether ethylene or salicylic acid (SA), have any effect on bean Cyp mRNA, bean leaves were sprayed with either ethephon, an ethylene releasing compound, or SA. Ethephon treatment resulted in a rather rapid increase above basal level of bean Cyp mRNA; amounts that remained at a higher level for several days (Fig. 3A). After SA treatment a two-phased accumulation of Cyp mRNA is observed: larger amounts of Cyp mRNA were found 6 h and between 48 h and 96 h after the beginning of treatment (Fig. 3B).

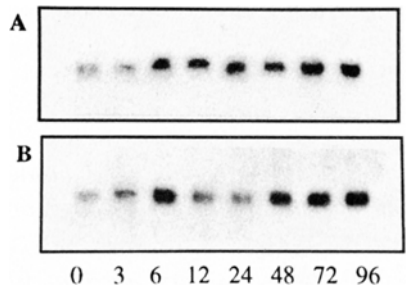

Fig. 3. Time course of accumulation of bean Cyp mRNA upon ethephon and salicylic acid treatments. Northern blots from gels containing equal amounts (10 μg) of total RNAs isolated from ethephon-treated leaves (panel A), and from salicylic acid-treated leaves (panel B) at different time points after onset of the treatment (in hours below each lane). The blots were hybridized to bean Cyp cDNA probe.

Effect of a local mercuric chloride treatment on Cyp mRNA levels in untreated parts of the plant

Mercuric chloride is a powerful inducer of various stress-related genes [5, 6, 24], and as previously observed, it stimulates the accumulation of bean Cyp mRNA [21]. In chemically treated plants the amount of Cyp transcripts accumulated proportionally with time to reach, like in virus-infected leaves, a maximum about 48 h after the onset of the chemical treatment [21]. In order to test the effect of a localized chemical treatment on Cyp mRNA levels in the untreated tissues, one of the two primary bean leaves was treated with the chemical, and RNAs were isolated from the treated leaf, the distal untreated leaf and the untreated roots and probed with bean Cyp cDNA. As shown in Fig. 4, Cyp mRNAs accumulated in the mercuric chloride-treated leaf to reach a maximum about 48 h after treatment and then returned to the basal level (Fig. 4, panel A). Cyp mRNAs also accumulates in the distal untreated leaf, with a delay of about 2 days when compared to the treated leaf (Fig. 4, panel B), whereas almost no change in Cyp mRNAs was detected in untreated roots (Fig. 4, panel C). Cyp mRNA accumulation has also been observed in the non-infected leaf upon A1MV infection of one of the two primary bean leaves (results not shown).

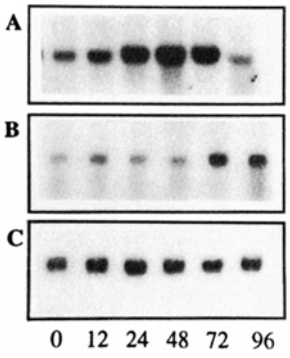

Fig. 4. Time course of accumulation of bean Cyp mRNA upon mercuric chloride treatment in treated and untreated parts of the plant. Northern blots from gels containing equal amounts (10 μg) of total RNAs isolated at different time points after onset of the treatment (in hours below each lane) from treated primary leaves (panel A), distal untreated leaves (panel B) and untreated roots (panel C). The blots were hybridized to bean Cyp cDNA probe.

Effects of various forms of stress on Cyp mRNA accumulation in bean and maize

We have already shown that, although Cyp mRNAs accumulate in both bean and maize in response to mercuric chloride treatment, differences were observed in accumulation profiles when both plants were compared [21]. To further investigate and compare the effect of other forms of abiotic stress, both plants were exposed to heat, cold and sodium chloride stresses, wounding, UV-light irradiation and dark/light treatment. Total RNAs were extracted from treated maize and bean leaves, harvested at different time points after the onset of the treatments. In the case of heat stress and dark/light treatment, RNAs were extracted from leaves harvested during and after the treatments. From heat-shocked plants total RNAs were isolated 1 h and 4 h after the beginning of the treatment, while plants were still maintained at 42 °C and also at various time points after plants were returned to normal growth conditions. For dark/light treatment, plants were transferred to a dark room and kept in the dark for 72 h, then returned to normal light conditions; RNAs were extracted at various time periods from plants maintained in the dark and from plants returned to light.

Figure 5 shows various time courses of Cyp mRNA accumulation in maize and bean, after plants have been subjected to different stresses; all the experiments have been repeated at least three times and gave the same patterns (all quantitative date given below are mean values from 3 different experiments). As shown, by these northern blot hybridization analyses, Cyp genes are constitutively expressed in both untreated plant species. Under stress conditions differences are observed in Cyp mRNA levels depending upon the stress and the plant species. During heat stress, maize Cyp mRNA significantly accumulated and in plants kept for 4 h at 42 °C the amounts of mRNA are 3 to 4 times higher than

those found at the beginning of the experiment (Fig. 5, IA). Once plants were returned at 22 °C, the levels of Cyp transcripts decreased to reach basal amounts after 3 h under normal conditions. In contrast, under the same stress conditions bean Cyp mRNAs became undetectable in plants maintained for 4 h at 42 °C (Fig. 5, IIA); when returned to normal temperature bean Cyp mRNA synthesis resumed, but the mRNA levels did not completely recover those found in control plants, at least during the duration of the experiment. In both plants Cyp mRNAs accumulated during cold stress (Fig. 5, IB and IIB) but maximum of accumulation appeared earlier in bean than in maize. The amount of maize Cyp mRNA accumulated proportionally with time to reach a level corresponding to a 4 time increase between 48 h and 96 h after exposure at 4 °C (Fig. 5, IB). A more rapid kinetic occurred in bean as maximum amounts (an about 3 time increase) of Cyp mRNA were already observed between 3 h and 6 h after treatment; the amount of bean Cyp mRNA returned to a basal level about 72 h after beginning of treatment (Fig. 5, IIB). In sodium chloride-treated maize Cyp mRNA accumulated (3 to 4 times) very rapidly (Fig. 5, IC) as compared to the slow kinetic observed in sodium chloride-treated bean plants, where 4 to 5 times higher levels of mRNA, as compared to time 0, are observed as late as 96 h after onset of the treatment (Fig. 5, IIC). When maize plants are kept in the dark for several days no changes in Cyp mRNA level are observed, neither during the dark period, nor after plants were returned to light (Fig. 5, ID). In bean plants, the amounts of mRNA remained at a low level during the dark period, but strongly accumulated once the plants were returned to light (4 times the initial level after 9 h light) (Fig. 5, IID).

Some variability can be observed among the northern signals of the different experiments at time 0 as seen in Fig. 5; this is mainly due to

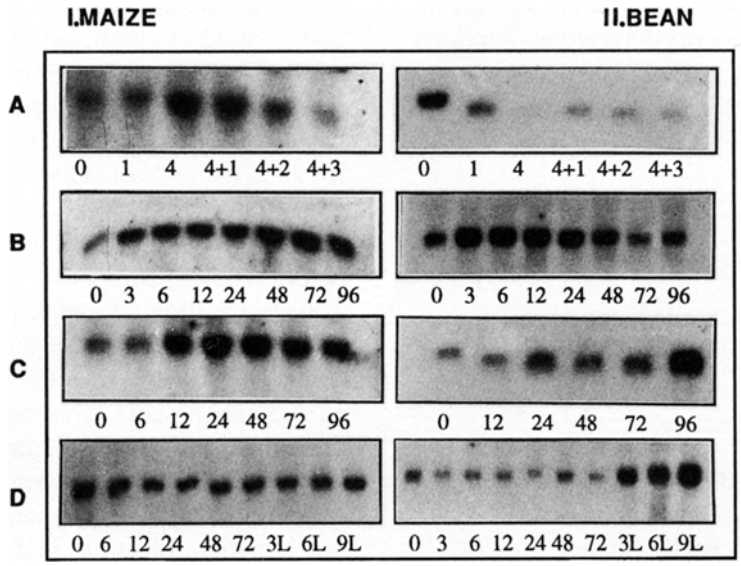

Fig. 5. Differences in Cyp mRNA accumulation in maize and bean plants in response to various forms of stress. Northern blots from gels containing equal amounts (10 μg) of total RNAs isolated from maize (I) and bean (II) leaves harvested at different time points (indicated in hours below each lane) after onset of the following stresses: heat-shock (A), cold (B), sodium chloride (C) and dark/light treatments (D). For heat-shock (A) total RNAs were isolated from plants kept for 1 h and 4 h at 42 °C and at three additional time points, once plants were returned under normal conditions (4 + 1 h = 4 h at 42 °C and 1 h at normal temperature). For dark/light treatment (D) plants were placed in the dark for 72 h and then returned to light for an additional 9 h period. RNAs were extracted during the dark and light (3L = 72 h dark and 3 h light) periods. The blots were hybridized to maize and bean Cyp cDNA probes. All experiments shown here have been repeated three times.

variations occurring in the specific activity of the different probes, and also due to the duration of autoradiogram exposures. The strength of the northern signals should therefore not be compared from one experiment to the other, however the comparison within a same experiment is valid as also shown in Fig. 5, ID. When the intensity of the northern signals corresponding to the 9 different RNA preparations shown on this figure, run on the same gel and hybridized with the same probe, were compared, it was found that the difference in intensity observed between the highest signal (time 0) and the lowest signal (time 24 h) did not exceed 20%. This value is far from those obtained (300 to 500%) when the stress triggers a response.

Cyp mRNAs accumulated in both maize and bean leaves, wounded by cutting with razor blades. However, whereas in maize mRNA levels reached a maximum about 24 h after wounding and then decreased to return to a basal level, in bean, as already observed in celite-rubbed leaves (see above), a two-phased accumulation profile is observed (results not shown). UV-light irradiation does not induce Cyp mRNA accumulation in maize [20], however, in UV-treated bean plants Cyp mRNAs accumulated and a maximum level was reached about 2 days after the onset of the stress (results not shown).

Discussion

The experiments described above show that in plants such as bean, Cyp gene is constitutively expressed in all tissues or organs examined, but higher levels of expression are observed in germinating seedlings, developing secondary leaves of young plants and in roots, buds and inflorescence of adult plants. These observations are consistent with those reported for other plant species. Thus, Cyp genes are highly expressed in young leaves, floral buds, growing shoots, stamens and anthers of tomato and *B. napus* [10] and in young tissues of tobacco [22]. All these results provide further evidence that cyclophilin may play a role in protein synthesis processes. Cyclophilins, which are believed to catalyse protein folding or other transconformational reactions in cells, might be needed in higher amounts to accelerate protein maturation in young tissues, where an active protein synthesis takes place. Surprisingly, significant amounts of bean Cyp transcripts were also found in tissues without high mitotic activity such as roots of adult plants. The functional significance of this observation has to be determined; although a higher constitutive expression may occur in roots, the possibility of a localized stress response cannot be ruled out. Indeed, alteration in Cyp gene expression is not only related to developmental conditions, but is also induced in response to various stresses like chemicals (mercuric chloride), as demonstrated previously [21], and A1MV infection.

Accumulation of Cyp mRNA has been observed in untreated parts of a bean plant in response to a localized chemical stress. The changes in Cyp mRNA amounts in untreated tissues began after the maximum of accumulation of Cyp mRNA was reached in the stressed part of the plant. Like for wound-inducible genes [15], this accumulation is limited to the aerial part of the plant and no changes in Cyp mRNA levels could be detected in the untreated roots. Molecules such as salicylic acid or ethylene, a phytohormone, are thought to act as endogenous signals in stress responses. Indeed, these compounds usually accumulate systemically after a pathogen or pest attack, move throughout the plant, induce the synthesis of defense-related proteins and enhance resistance to pathogens or pests [7]. In our experiments, exogenously applied ethephon and SA to the whole plant, led to a significant accumulation of bean Cyp mRNA in bean leaf tissues. All these observations are consistent with our first statement that cyclophilin might play a role in defense mechanisms [21].

A general survey of Cyp gene expression by northern blotting in bean and maize indicates that, although Cyp mRNA accumulation is stimulated in both plants in response to various stresses, striking differences were observed when bean and maize mRNA accumulation profiles were compared. In response to heat shock, maize Cyp

mRNA is accumulated, whereas in bean Cyp gene is not longer expressed during the heat treatment. An observation with relevance to our finding in maize has recently been made showing that the transcription of two yeast Cyp genes is induced by heat [28]. In the same report it was also demonstrated that the induction of one of the two yeast genes is mediated through a *cis*-acting sequence similar to the well-characterized heat shock element (for reviews see [12, 27]). Preliminary results obtained by sequencing a maize genomic clone indicate the presence of such heat shock elements in the promoter region of this gene, suggesting that the heat stimulation observed in maize could be mediated by these elements (J. Marivet, unpublished). It is well known that in higher plants, the effects of heat stress on protein synthesis is diverse (see reviews [16, 31]): for instance, in bean the expression of a chitinase gene (P4-chitinase) is induced by heat stress [20], whereas for other proteins, such as bean P3-chitinase gene [19] or Cyp gene, expression is completely blocked. Whether this difference in Cyp gene expression observed between the two plants has a functional significance and, for instance, results in a better accommodation of maize than bean to higher temperatures is not yet clear. We need to do further experiments before we can evaluate the effect, if any, of a rapid induction of Cyp gene expression on plant resistance to heat.

Dark/light treatment has an effect only on bean Cyp mRNA which accumulates once the plants are returned to light, suggesting that changes occurring during darkness might be responsible for turning on the signal. Differences in accumulation profiles were also observed in response to other types of stress. Salt stress induced the response earlier in maize than in bean, whereas the opposite situation is observed when plants are cold stressed. In both plants, wounding results in a rapid accumulation of Cyp mRNAs.

In summary, the results shown here suggest that Cyp gene expression might be partly regulated by stress. They are also consistent with the hypothesis that cyclophilin has an important role to play throughout the life cycle of a plant. During normal life, the peptidyl *cis-trans* isomerase activity might be needed to accelerate the processing of native polypeptides. In diseased plants cyclophilin might function as a 'chaperone-like molecule' in order to decrease the risk of proteolytic degradation or to avoid aggregation, reactions that take place during a stress. In stressed plants higher amounts of cyclophilin might also be needed to accelerate the folding step and therefore the maturation processes of newly synthesized proteins known to be induced during stress and believed to play protective functions (for reviews see [4, 16, 17]). That bean and maize respond differently at the level of Cyp mRNAs to a given environmental stimulus might reflect differential sensitivities of the two plant species to a given stress, and/or differences in the regulatory elements located in their promoter and/or distinct signal transduction pathways for Cyp gene activation.

References

1. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K: Current Protocols in Molecular Biology, vol. 4. Greene Publishing Associates/ Wiley-Interscience, New York (1987).
2. Bang H, Fischer G: Slow conformational changes in protein folding can be accelerated by enzymes. Bimed Biochem Acta 50: 137–142 (1991).
3. Bartling D, Heese A, Weiler EW: Nucleotide sequence of a cDNA encoding an *Arabidopsis* cyclophilin-like protein. Plant Mol Biol 19: 529–530 (1992).
4. Bowles DJ: Defense-related proteins in higher plants. Annu Rev Biochem 59: 873–907 (1990).
5. de Tapia M, Bergman P, Awade A, Burkard G: Analysis of acid extractable bean leaf proteins induced by mercuric chloride treatment and alfalfa mosaic virus infection. Partial purification and characterization. Plant Sci 45: 167–177 (1986).
6. Didierjean L: Clonage et caractérisation d'ADNc de protéines de stress chez le maïs. Effets de différentes formes de stress abiotiques sur l'expression de leurs gènes. Thèse Université Louis Pasteur, Strasbourg (1992).
7. Enyedi AJ, Yalpani N, Silverman P, Raskin I: Signal molecules in systemic plant resistance to pathogens and pests. Cell 70: 879–886 (1992).
8. Feinberg AP, Vogelstein A: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132: 6–13 (1983).

9. Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, Schmid FX: Cyclophilin and peptidyl-prolyl *cis-trans* isomerase are probably identical proteins. Nature 337: 476–478 (1989).
10. Gasser CS, Gunning DA, Budelier KA, Brown SM: Structure and expression of cytosolic cyclophilin/peptidyl-proplyl *cis-trans* isomerase of higher plants and production of active tomato cyclophilin in *E. coli*. Proc Natl Acad Sci USA 87: 9519–9523 (1990).
11. Gething MJ, Sambrook J: Protein folding in the cell. Nature 355: 33–45 (1992).
12. Gurley WB, Key JL: Transcriptional regulation of the heat-shock response: a plant perspective. Biochemistry 30: 1–11 (1991).
13. Handschumacher RE, Harding MW, Rice J, Drugge RJ: Cyclophilin: A specific cytosolic binding protein for cyclosporin A. Science 226: 544–546 (1984).
14. Heitman J, Movva NR, Hall MN: Proline isomerase at the crossroads of protein folding, signal transduction and immunosuppression. New Biol 4: 448–460 (1992).
15. Hildmann T, Ebneth M, Pena-Cortes H, Sanchez-Serrano JJ, Willmitzer L, Prat S: General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding. Plant Cell 4: 1157–1170 (1992).
16. Ho THD, Sachs MM: Stress-induced proteins: characterization and the regulation of their synthesis. Academic Press, 15: 347–378 (1989).
17. Linthorst HJM: Pathogenesis-related proteins of plants. Crit Rev Plant Sci 10: 123–150 (1991).
18. Luan S, Weiwei L, Rusnak F, Assman SM, Schreiber SL: Immunosuppressants implicate protein phosphatase regulation of K^+ channels cells. Proc Natl Acad Sci USA 90: 2202–2206 (1993).
19. Margis-Pinheiro M: Les chitinases de haricot (*Phaseolus vulgaris*): clonage des ADNc de deux chitinases acides induites par stress chimique ou par infection virale. Etude de leur structure et de l'expression de leurs gènes en réponse à divers types de stress. Ph. D. thesis, University Louis Pasteur Strasbourg – France (1993).
20. Margis-Pinheiro M, Marivet J, Burkard G: Bean class IV chitinase gene: structure, developmental expression and induction by heat stress. Plant Sci 98: 163–173. (1994).
21. Marivet J, Frendo P, Burkard G: Effects of abiotic stresses on cyclophilin gene expression in maize and bean and sequence analysis of bean cyclophilin cDNA. Plant Sci 84: 171–178 (1992).
22. Marty I, Brugidou C, Chartier Y, Meyer Y: Growth-related gene expression in *Nicotiana tabacum* mesophyll protoplasts. Plant J (1993).
23. Mckeon F: When worlds collide: immunosuppressants meet protein phosphatases. Cell 66: 823–826 (1991).
24. Nasser W, de Tapia M, Kauffmann S, Montasser-Koushari S, Burkard G: Identification and characterization of maize pathogenesis-related proteins. Four maize PR proteins are chitinases. Plant Mol Biol 11: 529–538 (1988).
25. Rosen KM, Villa-Komeroff L: An alternative method for the visualization of RNA in formaldehyde agarose gels. Focus 12: 23–24 (1990).
26. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).
27. Sorger PK: Heat shock factor and the heat shock response. Cell 65: 363–366 (1991).
28. Sykes K, Gething MJ, Sambrook J: Proline isomerases function during heat shock. Proc Natl Acad Sci USA 90: 5853–5857 (1993).
29. Takahashi N, Hayano T, Suzuki M: Peptidyl-prolyl *cis-trans* isomerase is the cyclosporine A-binding protein cyclophilin. Nature 337: 373–375 (1989).
30. Trandinh CC, Pao GM, Saier MH: Structural and evolutionary relationships among the immunophilins: two ubiquitous families of peptidyl-prolyl *cis-trans* isomerase. Faseb J 6: 3410–3420 (1992).
31. Vierling E: The roles of heat shock proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 42: 579–620 (1991).
32. Walsh CT, Zydowsky LD, Mckeon FD: Cyclosporin-A, the cyclophilin class of peptidylprolyl isomerases, and blockade of T-cell signal transduction. J Biol Chem 267: 13115–13118 (1992).