Short Communication

The Level of mRNA Transcribed from psaL, Which Encodes a Subunit of Photosystem I, Is Increased by Cytokinin in Darkness in Etiolated Cotyledons of Cucumber

Tomoko Toyama¹, Haruhiko Teramoto² and Go Takeba²

¹ Department of Botany, Faculty of Science, Kyoto University, Kyoto, 606-01 Japan
² Laboratory of Applied Biology, Faculty of Living Science, Kyoto Prefectural University, Kyoto, 606 Japan

A cDNA clone for an mRNA whose level increased within 2 h of the start of treatment with N⁶-benzyladenine in etiolated cotyledons of cucumber was isolated by differential hybridization. The cDNA was homologous to psaL, which encodes subunit XI (PSI-L) of photosystem I. The accumulation of psal mRNA was specifically induced by cytokinins or light.

Key words: cDNA (cytokinin-induced mRNA) — Cucumber — Cytokinin — Photosystem I — psaL gene.

Cytokinins are phytohormones that have been extensively studied and they have well-known physiological effects. They induce greening in various dicotyledons (Dei and Tsuji 1978), and they promote the formation of both etioplasts and chloroplasts (Longo et al. 1979, 1986). However, the molecular mechanisms of their actions remain unknown and it is important to identify those genes whose expression is regulated by cytokinins. It has been reported that levels of some mRNAs and proteins are modulated by cytokinins (Stabel et al. 1990, Crowell and Amasino 1991, Teramoto et al. 1993). For example, cytokinins induce the accumulation of the mRNAs for the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and the light-harvesting a/b protein in etiolated cotyledons of cucumber (Ohya and Suzuki 1991) and Cucurbita pepo (Lerbs et al. 1984), as well as in Lemna gibba upon the transfer of plants from light to darkness (Flores and Tobin 1986) after 12 h to several days of treatment. Expression of the apoprotein of phytochrome (Cotton et al. 1990), of phosphoenolpyruvate carboxylase (Schmitt and Piepenbrock 1992, Sugiharto et al. 1992) and of carbonic anhydrase (Sugiharto et al. 1992) is also affected by cytokinins. Crowell et al. (1990) isolated 20 cDNAs that corresponded to mRNAs that had accumulated 1 to 4 h after the addition of zeatin to a culture of soybean cells that had been starved of a cytokinin, but the genes corresponding to the cDNA clones were not completely identified.

In previous studies, we used differential hybridization to isolate cDNA clones for cytokinin-repressed genes [CR9, CR20 and some CRR (cytokinin-repressed rapidly) clones] in cucumber (Teramoto et al. 1994, 1995, Toyama et al. 1995). In excised cotyledons of cucumber, the levels of the transcripts that corresponded to these clones decreased within 2 to 4 h after the start of treatment with N⁶-benzyladenine (BA) in darkness. Some of the CRR clones were homologous to those for catalase, 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), and a lectin (Toyama et al. 1995). In the present study, we isolated a cDNA clone for a cytokinin-inducible gene which had not previously been identified as a gene whose expression is modulated by cytokinins. The cDNA clone was homologous to the psaL gene, which encodes a subunit of photosystem I (PSI), namely, subunit XI (PSI-L).

Differential hybridization was performed as described previously (Toyama et al. 1995). The cDNA library was constructed from excised cotyledons of cucumber (Cucumis sativus L. cv. Aonagajibai) that had been incubated in water for 18 h in darkness. Hybridization was performed with 32P-labeled first-strand cDNA that had been reverse transcribed from poly(A)⁺ RNA, extracted from cotyledons after treatment with BA or water for 2 h after an 18-h preincubation in water. cDNA clones that hybridized preferentially with the 'BA-treated' probe, as compared with the 'control' probe, were isolated. One of the clones was sequenced and the nucleotide sequence of the cDNA fragment was presented in Figure 1. The nucleotide sequence is almost the same as that of the psaL gene of cucumber which was isolated by Iwasaki et al. (GenBank accession no. D50456). However, our sequence differs from the sequence reported by Iwasaki et al. by the presence of T at nucleotide positions 40, 676 and 683 instead of C. Moreover, the change at nucleotide position 40, which is part of the codon for amino acid residue 8, results in a change from threonine to isoleucine in the encoded protein (Fig. 1). A search of databases revealed homologies of 74.9% and 76.3% to amino acid sequences deduced from cDNA
Cytokinin increases psaL mRNA in darkness

Fig. 1  Nucleotide and deduced amino-acid sequence of the cDNA for the psaL gene of cucumber. The dots indicate the nucleotides that are replaced by C in the sequence of the psaL gene of cucumber reported by Iwasaki et al. (GenBank accession no. D50456). The resultant changed amino acid residue is underlined.

clones from barley (Okkels et al. 1991) and spinach (Flieger et al. 1993), respectively.

Northern blot analysis was performed to examine the changes in the level of the mRNA transcribed from psaL upon treatment with BA or light. Figure 2A shows the time courses of the BA-induced and light-induced changes in the level of this transcript. Hybridization with the psaL cDNA probe revealed a major transcript of 0.9 kb, which was similar in size to the transcripts of psaL in spinach (Flieger et al. 1993) and barley (Okkels et al. 1991). The level of psaL mRNA increased slightly 2 h after application of BA and it reached to a level that was about four times as high as the level in the control samples after 24 h. The level of psaL mRNA also began to increase 2 h after exposure to light and again reached to a level that was about four times as high as the level in the control samples that had been incubated with water in darkness after 24 h (Fig. 2A). In a control experiment, we also examined hybridization with a cDNA designated NC2 (negative control 2) as the probe. NC2 cDNA was obtained as a clone that corresponded to a transcript whose level did not change upon treatment of cotyledons with BA (Toyama et al. 1995). The level of the tran-
Cytokinin increases psaL mRNA in darkness

Fig. 2 Changes in the levels of mRNAs that correspond to psaL (A) and NC2 (B) in cucumber cotyledons during treatment with BA or light. Cucumber seeds were allowed to germinate for 4 d at 28°C in darkness. Excised cotyledons were incubated with water for 18 h and then with 10 μM BA in darkness (BA) or with water in the light (Light) for indicated times at 28°C. Cotyledons were also incubated with water for 0, 2, and 24 h after an 18-h preincubation (Water). Thirty micrograms of total RNA that had been extracted from cotyledons of each sample were subjected to electrophoresis and analyzed by Northern hybridization with 32P-labeled cDNA of the psaL gene of cucumber, as described previously (Toyama et al. 1995), by the method of Ausubel et al. (1991). Northern blot hybridization with a 32P-labeled fragment of NC2 cDNA was also performed as a control. NC2 is a cDNA that corresponds to an mRNA whose level is unchanged by treatment with BA. The lengths of transcripts were estimated from mobilities relative to those of rRNAs. Each panel shows representative results from one of several independent experiments.

We compared the effects of treatment of cotyledons with various other compounds on the level of psaL mRNA. Kinetin, a cytokinin, was as effective at 10⁻⁵ M as BA at the same concentration in inducing the accumulation of psaL mRNA (Fig. 3A). By contrast, adenine, which has no cytokinin activity; 2,4-D, an auxin; and ABA did not affect the level of psaL mRNA in darkness. As anticipated, the level of NC2 mRNA did not change significantly during treatment with these compounds (Fig. 3B). Thus, the effects of cytokinins on the accumulation of psaL transcripts were specific.

Kusnetsov et al. (1994) studied the effects of cytokinins and ABA on the levels of many chloroplast proteins and the corresponding mRNAs in dark- and light-grown cotyledons of lupine (Lupinus luteus). They used 15 gene-specific probes for plastid proteins and corresponding antisera. Cytokinins affected the synthesis and accumulation of the various proteins in a protein-specific manner. BA strongly promoted the accumulation of cytochrome b₅₅₉ and of subunit IV of the cytochrome b/f complex, but it had little effect on levels of cytochrome b₆ and of the large subunit of RuBisCO. Kusnetsov et al. (1994) also suggested different modes of interaction between phytohormones and light in the expression of the proteins. Thus, characterization of the effects of cytokinins on each individual protein in plastids is necessary if we are to elucidate the molecular mechanisms of cytokinins and to determine the correlation between the effects of cytokinins and light since plastids contain numerous protein complexes and enzymes. In the present study, we demonstrated that cytokinins caused significant increases in the level of psaL mRNA in darkness. The effects of cytokinins mimicked those of light. These results suggest that cytokinins and light might act through a common signal-transduction pathway to control the expression of the psaL gene. Kusnetsov et al. (1994) discussed...
only one protein component of PSI, the P_700 chlorophyll a apoprotein, and there has been only one report of the effects of cytokinins on the accumulation of the subunits of PSI, even though PSI is a very important complex that catalyzes the transfer of electrons from plastocyanin to ferredoxin (Margulis 1989). Therefore, more studies of the responses of other constituents of PSI to cytokinins and light are necessary for an accurate picture of the action of cytokinins. Our results provide a small piece of such a picture.

A trimeric quaternary structure of PSI in cyanobacteria has been characterized (Ford et al. 1988, Krauss et al. 1993, Kruip et al. 1993, van der Staay et al. 1993), and Chitnis and Chitnis (1993) showed that the PSI-L protein encoded by the psaL gene is necessary for trimerization of PSI and might constitute the trimer-forming domain in the structure of PSI. Although trimers of PSI have not been found in vivo in higher plants, PSI-L probably plays an important role as a constituent of PSI in such plants.

The authors gratefully acknowledge the use of the facilities of the Laboratory of Radioisotopes, Kyoto Prefectural University, for the radiolabeling experiments described in this report.

**References**


(Received April 10, 1996; Accepted July 31, 1996)