Conclusion
Our understanding on galactolipid (MGDG and digalactosyldiacylglycerol) metabolism benefitted strongly from recent advances in the identification of sequences coding for MGDG synthase ([6,7] and K. Awai, E. Maréchal, M. A. Block, D. Brun, T. Nasuda, H. Shimada, K. I. Takamiya, H. Ohta and J. Joyard, unpublished work) and galactolipid-producing enzymes (DGD1 [13]). Interestingly, much functional and structural information was accumulated before the primary sequences were deciphered, opening the possibility of correlating fine biochemical analyses with molecular and physiological data.

References

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Transcriptional regulation by light and phytohormones of the MGD gene in cucumber
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Abstract
Monogalactosyldiacylglycerol (MGDG) synthase catalyses formation of MGDG, a major structural lipid of chloroplasts. We have already cloned a cDNA for the synthase from a cucumber cDNA library and shown that expression of this gene is regulated by light and a phytohormone, cytokinin. In the present study, we report the molecular basis for transcriptional regulation by light and cytokinin in detail. First, in terms of the enzyme activity, gradual increases in activity mediated by light and cytokinin treatments were observed. At the same time, however, the changes in the mRNA level showed different profiles, with a transient peak during the early stages of light and cytokinin treatment. The interval between the peak level of mRNA and enzyme activation implies the existence of a post-transcriptional regulatory system.

Key words: cytokinin, galactolipid, light response, MGD synthase.
Abbreviations used: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; BA, 6-benzyladenine; LUC, luciferase.
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In addition, a genomic clone of MGDG synthase isolated from a cucumber genome library was used for a motif search in databases, and this revealed that putative cis-acting elements for light and phytohormones exist in the 5’-upstream region of the MGDG cucumber gene. Detailed analysis of this region for light- and cytokinin-responsive activity was performed using a −90 truncated 35 S minimal promoter/luciferase (LUC) reporter gene. It resulted in high levels of LUC expression in etiolated cucumber cotyledons in response to illumination and cytokinin treatment. These results indicate that this 5’-upstream region is involved in light- and cytokinin-enhanced MGDG gene expression, and that light- and cytokinin-responsive enhancements of the MGDG synthase activity are regulated, at least in part, at the level of transcription.

Introduction
Thylakoid membranes are the site of photosynthesis, and contain the photosynthetic apparatus, namely photosystems I and II, but about 40 %
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(w/w) of their constituents are lipids. In addition, the greater part of the thylakoid lipids, almost 80\% (w/w), are galactolipids, such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). Therefore the physicochemical properties of thylakoid membranes are mainly governed by these galactolipids.

The final step of MGDG synthesis is catalysed by MGDG synthase (UDP-galactose:1,2-diacylglycerol 3-β-D-galactosyltransferase; EC 2.4.1.46). This enzyme transfers a galactose from UDP-galactose to 1,2-diacylglycerol in the chloroplast envelope [1,2]. We have found that MGDG synthase activity in cucumber seedlings dramatically increased concomitantly with the accumulation of the two major galactolipids, MGDG and DGDG, during chloroplast development [3]. We also revealed that the expression of this gene was regulated by light and a phytohormone, cytokinin [4]. To clarify the mechanism that regulates MGDG synthesis during plastid development, we investigated in detail the expression of the MGDG synthase gene (MGD) in germinating cucumber cotyledons, their relationship to the change in the enzyme activity, and the accumulation of galactolipids by illumination and cytokinin treatment. In the present study, we report the molecular basis for the transcriptional regulation by light and cytokinin in detail.

Materials and methods
Preparation of plant material
Cucumber seeds (Cucumis sativus L. cv. Aonagaijibai) were sown on wet vermiculite, and grown at 27 °C in darkness. After 4 days, cotyledons were cut off and soaked in water on a dish with a piece of 3MM Cr paper (Whatman Biosystems Ltd., Maidstone, Kent, U.K.) for 12–15 h to eliminate any unexpected change caused by the excision. They were then treated with 6-benzyladenine (BA) or illumination. Ten cotyledons for determination of the mRNA level, 20 for the enzyme assay and 30 for the lipid analysis were collected at the times indicated. We prepared another set of samples, which were illuminated directly on etiolated seedlings.

Assay procedure for MGDG synthase activity
MGDG synthase activity was measured as described by Shimojima et al. [5].

Northern blot hybridization
Total isolated RNA (10 μg/lane) was denatured, subjected to electrophoresis on a 1.2 % (w/v) agarose gel and transferred to a nylon membrane. The blot was hybridized with a [α-32P]dCTP-labelled 429 bp (1167–1595) fragment of the MGDG synthase gene at 65 °C. After hybridization, the blots were washed with 2 × SSC (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate)/0.1 % (w/v) SDS for 5 min, followed by two washes with 0.2 × SSC/0.1 % SDS for 20 min. The membrane was exposed to an imaging plate (Fuji Photo Film Co., Tokyo, Japan), and analysed with BAS 2000 (Fuji Photo Film Co.).

Promoter assay
A firefly-luciferase (LUC) gene with a minimal promoter region (90 bp) of the cauliflower mosaic virus 35 S promoter was adjoined to a 5'-upstream region of the MGD gene in a pHF221 plasmid. This plasmid DNA was introduced into cucumber cotyledons of 4-day-old etiolated seedlings by particle bombardment. To correct the variance of transformation efficiency in each bombardment, a 35 S-Remilla-LUC gene was co-introduced. Their expressions were analysed according to the dual-luciferase assay system (Promega). In order to eliminate an unexpected increase in the expression by bombardment, they were pretreated in darkness for 15 h and then subjected to BA treatment or illumination. All the procedures were performed in darkness.

Results and discussion
The level of mRNA of MGDG synthase showed a transient increase during 3–6 h of BA treatment, followed by an immediate decrease to the initial level (Figure 1). At the same time, however,

![Figure 1](image)

**Effects of BA and illumination on the level of MGDG synthase mRNA**

Cucumber seeds were germinated and seedlings were grown in the dark for 4 days. They were then treated with BA or light. Ten cotyledons were excised at the times indicated, and the total RNA was prepared. Of the total RNA, 10 μg was applied to each lane. Amounts of MGDG synthase mRNA were detected by Northern blot hybridization.
enzyme activity behaved differently: it increased in accordance with the treatment time of BA, and the increase after 12 h of BA treatment was especially significant (Y. Yamaryo, Y. Yamamoro, D. Kanai, K. Awai, T. Masuda, H. Shimada, K.-i. Takamiya and H. Ohta, unpublished work).

In the same manner, the effects of illumination on mRNA level of MGDG synthase and enzyme activity were determined. mRNA showed a transient increase at 3 h of illumination (Figure 1). In the case of the enzyme activity, a noteworthy increase after 24 h of illumination was observed (Y. Yamaryo, Y. Yamamoro, D. Kanai, K. Awai, T. Masuda, H. Shimada, K.-i. Takamiya and H. Ohta, unpublished work). Here again, the existence of the time lag between an increase in the mRNA level and enzyme activity was found.

To clarify the mechanism for the induction of gene expression, promoter assaying was performed as a detailed analysis. A plasmid construct, containing the 5'-upstream region of a MGD/LUC gene fusion, was utilized for the assay. The chimaeric gene was introduced into etiolated cucumber cotyledons by particle bombardment. The promoter activities were analysed in terms of the transient expression of the reporter gene in response to BA and illumination. The transient assays clearly demonstrated the elevated levels of LUC expression in response to BA and light (Y. Yamaryo, Y. Yamamoro, D. Kanai, K. Awai, T. Masuda, H. Shimada, K.-i. Takamiya and H. Ohta, unpublished work). From these results, it was revealed that the mRNA level of MGDG synthase showed a transient increase in response to both BA treatment and illumination. Moreover, there was a time lag between the peak of the mRNA level and the enzyme activation. Promoter assay with chimaeric genes showed the existence of light- and cytokinin-responsive elements in the 5'-upstream region. Consequently, it may be inferred that the activation of MGDG synthase is regulated, at least in part, at the post-transcription level, in addition to regulation at the transcription level.

References

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The role of triacylglycerol as a reservoir of polyunsaturated fatty acids for the rapid production of chloroplastic lipids in certain microalgae
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Abstract
Many microalgae are known to accumulate triacylglycerols (TAGs), especially under nitrate starvation. Generally, these TAGs are predominantly constructed of saturated and mono-unsaturated fatty acids. In contrast, the TAGs of the red microalga Porphyridium cruentum are rich in arachidonic acid (AA, 20:4ω6) and eicosapentaenoic acid (EPA, 20:5ω3). A mutant of this alga, impaired of growth at suboptimal temperatures, was shown to have reduced levels of EPA and of the eukaryotic molecular species of monogalactosyldiacylglycerol (MGDG) and an elevated level of TAG. Labelling experiments have shown that labelling of wild-type TAGs decreased, whereas that of the mutant remained high. Contemporarily, eukaryotic MGDG of the mutant was less labelled. Similarly, TAGs of the green alga T12, which can grow at a low temperature, are extremely rich in AA. We have labelled exponentially growing cultures of T12 kept at 25 °C with radioactive AA and cultivated the cultures for a further 12 h at 25 °C, 12 °C or 4 °C. At low

Key words: arachidonic acid, LC PUFA, long-chain PUFA, Porphyridium cruentum.
Abbreviations used: TAG, triacylglycerol; PUFA, polyunsaturated fatty acid; AA, arachidonic acid.
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