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学位論文題目	Isolation and characterization of the promoter regions of plastidic phosphate translocator genes and a polyubiquitin gene from *Mesembryanthemum crystallinum*（アイスプラントからのプラスチド型リン酸輸送体遺伝子とポリユビキチン遺伝子のプロモーター領域の単離と機能解析）
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論文の要約

Background:

Crassulacean acid metabolism (CAM) is a type of photosynthesis that evolved in some plants as an adaptation to arid condition. *Mesembryanthemum crystalinum* is a rare species that has a unique feature of switching from C_3 photosynthesis to CAM by salt or drought stress. In CAM, the metabolic pathways are located in multiple compartments of the mesophyll cells (i.e. chloroplasts, cytosol, mitochondria, and vacuole). Therefore, a number of intracellular transport processes are required, particularly across the chloroplast envelope. Each metabolite exchange is mediated by a specific transport protein. In the case of phosphate/phosphate ester exchange across the chloroplast envelope, there are four subfamilies of phosphate translocators: triose phosphate/phosphate translocator (TPT), phosphoenolpyruvate/phosphate translocator (PPT), glucose-6- phosphate/phosphate translocator (GPT) and pentose phosphate/phosphate translocator. Specific genes have been found in *M. crystallinum*, from the first 3 subfamilies: *McTPT1*, *McPPT1*, *McGPT1,* and *McGPT2*. Among these plastidic phosphate translocator genes, *McGPT2* is most highly induced during CAM induction, which makes it an excellent model case for an investigation of CAM-specific gene expression mechanisms.

The Aims of This Study:

In this study, the promoter regions of plastidic phosphate transporters *McTPT1*, *McGPT1*, and *McGPT2* have been isolated and sequenced. In an attempt to develop a transient assay system for the study of these promoter regions, the use of transient assay by using microprojectile bombardment and dual-luciferase reporter assay (DLRA) have been investigated. In DLRA, CaMV 35S promoter gave a large fluctuation at the expression level and was not suitable as an internal control. Among the strong constitutive plant promoters, the polyubiquitin promoters have been used extensively for directing transgene expression and have received the most widespread attention. Therefore, to find out a strong, constitutive and native promoter in *M. crystallinum*, the promoter region of the *McUBI1* has been isolated.

Experimental Procedure and Main Results:

The isolated polyubiquitin gene designated as *McUBI1* comprised 7 repeats of the ubiquitin coding-unit with a C-terminal additional phenylalanine codon. A 4.0 kb 5'-upstream region of *McUBI1* was isolated by primer walking. A 725 bp intron (hereafter referred to as leader intron) was found at the immediate 5'-side of the translation initiation codon in the second exon.

To find out the effect of the 5' UTR intron on gene expression, an intronless *McUBI1* promoter was compared with the whole *McUBI1* promoter (including the 5' UTR intron) in *M. crystallinum* and also in another dicot plant, *Nicotiana tabacum*. Moreover, the intronless and with intron *McUBI1* promoter activities were also compared with the CaMV 35S promoter both in *M. crystallinum* and *N. tabacum*. I observed a high expression level from the full length *McUBI1* promoter, which was more stable than that from the CaMV 35S promoter in *M. crystallinum*. Both the intronless and with intron *McUBI1* promoters were also highly expressed in *N. tabacum*, whose expression level was comparable with the CaMV 35S promoter. The deletion of the 5' UTR intron reduced the *McUBI1* promoter activity by two fold in *M. crystallinum*, whereas it had no significant effect in *N.tabacum*.

By using the intronless*McUBI1* as an internal control, the regulation of the *McGPT2* promoter was investigated with a deletion series of the *McGPT2* promoter region. The full-length *McGPT2* promoter (–2603 bp*McGPT2* promoter) showed slightly but significantly higher expression in the CAM leaf tissue than in the C3. The –2603 bp *McGPT2* promoter contained two long inverted repeats: one is IR1 from –2498 bp to –1979 bp and the other is IR2 from –1753 bp to –1407 bp. Deletion of most 5' region including IR1 from the –2603 bp*McGPT2* promoter stimulated the promoter activity especially in the CAM tissue, suggesting that IR1 is a suppressor of the promoter activity mainly in the CAM tissue. On the other hand, deletion of another inverted repeats IR2 resulted a dramatic loss of the promoter activity in the CAM tissue, suggesting that IR2 had a positive inducer role in CAM induction. In the 1-kb downstream of IR2, other regions of a strong silencer and an enhancer were also found. Finally the smallest –239 bp upstream region still showed salt-inducibility, although the expression level was rather low even in the CAM tissue. Thus the *McGPT2* 5'-upstream region contains a very complex regulatory elements. I also found that the *McGPT1* promoter was salt-inducible but the *McTPT1* promoter was not, by using the microprojectile bombardment and DLRA. All the 5'-deletion constructs of *McGPT2* promoter responded to day/night regulation except the shortest construct PF5, the activity of which remained unchanged at dark period.

Discussion and Conclusions:

In the first part, I found that the isolated *McUBI1* promoter was highly and steadily expressed and was more suitable as an internal control in *M. crystallinum* for DLRA than the CaMV 35S promoter. In second part, I used the *McUBI1*promoter as an internal control and analyzed the regulatory mechanism of plastidic phosphate translocator genes. I concentrated on *McGPT2* promoter and found that the 5'-upstream region of *McGPT2* promoter contains a complex set of regulatory elements that have both silencer and enhancer activities. In the *McGPT2* promoter, two long inverted repeat sequences were found to be an important factor for salt-inducible gene expression. To the best of my knowledge there is no clear report on inverted repeat mediated salt-responsive regulation of plant promoters. Elucidation of the detail of such regulatory mechanism in plants will be a noble chance in plant molecular biology. Day/night expression pattern of *McGPT2* promoter also revealed that the 5'-upstream region of *McGPT2* maintains the circadian clock-controlled pattern of regulation.