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Foreign Gene Expression in Barley Coleoptile Epidermis: An Improved System for Gene Transfer and *in situ* Detection of Gene Expression by Dual Microinjection

Yoshinori MATSUDA, Hideyoshi TOYODA, Yasushi NOGI, Takayuki TAMAI and Seiji OUCHI

Laboratory of Plant Pathology, Faculty of Agriculture Kinki University, Nara 631, Japan

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The foreign genes, β-glucuronidase gene and TMV coat protein gene both ligated with the CaMV 35S promoter and nopalinsynthase terminator, were introduced into the inner epidermal cells of this tissue, using a barley coleoptile/microinjection system. The expression of the introduced genes was successfully examined by cytochemical identification of the transcription and translation products of the genes. Transcripts of the introduced genes were *in situ* hybridized to photobiotin conjugated antisense RNAs which were secondarily introduced by a pricking microinjection, and the translation products were detected by a complex formation with injected enzyme-conjugated antibodies. With a dual microinjection method, sense RNAs of these genes could be introduced and their translation products were detected at the same frequency (more than 80% of successfully injected cells) as those in DNA-injected cells. Thus, the present system, a combination of microinjection and *in situ* cytochemical techniques, allows introduction of foreign genes into barley coleoptile epidermal cells and precise detection of foreign gene expression in a higher plant cell system.

Introduction

Various materials including a foreign gene have been microinjected into epidermal cells of barley coleoptile tissue to determine their effect on cellular activity and to evaluate their function in the injected cells[1,2], and it has been demonstrated that coleoptile epidermis is a useful material for analyzing foreign gene expression in higher plant cells. In order to expand the applicability of a barley coleoptile/microinjection system, some methods have been elaborated, especially for improving detection of gene expression. The gene expression has commonly been detected by Northern or dot blot analysis. These analyses are highly sensitive, but insufficient for resolution of spatial distribution of gene expression, because these analyses measure the overall transcription in the tissues[3]. From this point of view, the authors have extended a microinjection method to an *in situ* cytochemistry for estimating gene expression at the injected site[4,5]. In the present study, a dual microinjection method, by which foreign genes and labeled probes or antibodies can be effectively introduced into target cells, will be presented for evaluating precisely the expression of foreign genes using a barley coleoptile system.

Materials and Methods

1. *Plant and microinjection method*

Inner coleoptile epidermis was prepared from detached coleoptile tissues of 10-day-old seedlings

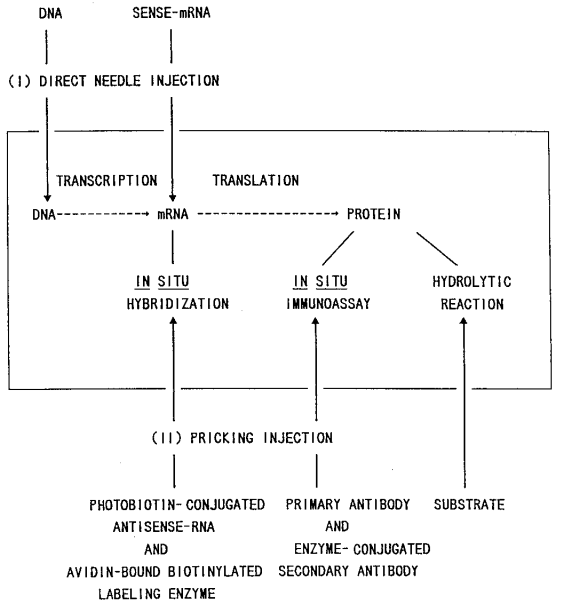

Fig. 1 A dual microinjection method used for introduction of foreign genes and RNA probes or antibodies.

of barley (*Hordeum vulgare* L, cv. Kobinkatagi) by the method described previously[1,2]. Microinjection of coleoptile epidermal cells was also conducted by the method described previously using an Olympus injectoscope[6]. **Fig. 1** summarizes the present microinjection method that utilizes two different injections (dual microinjection); i) a direct injection of plasmid DNAs and sense RNAs through a glass needle[7], and ii) a pricking introduction[8], in which cells were punctured with a needle in a hybridization buffer containing labeled antisense RNA probes, or in a solution containing specific antibodies. A direct injection of plasmids or RNAs was conducted according to the method described previously[1]. An autoclaved glass needle (inner tip diameter, 0.2 μm) containing 1 $\mu g/\mu l$ DNA or RNA was inserted into the cell, and the injection material was forced out of the needle by a constant pressure (2.5 kg/cm^2) of nitrogen gas. After 24 hr of incubation, survival rates of injected cells were examined before fixation. Survival of the injected cells was determined by checking the continuity of cytoplasmic streaming (CS)[1,2]. The cells maintaining CS after injection were regarded as successfully injected.

2. *Injection materials*

The genetic materials used for injection were β-glucuronidase (GUS) gene and coat protein (CP) gene of tobacco mosaic virus (TMV, OM-strain), and their sesne RNA. The plasmid pBI121 (purchased from Clontech Laboratories, Palo Alto, CA, USA) contains the GUS gene linked with the cauliflower mosaic virus (CaMV) 35S promoter and nopalin synthase terminator[9] (**Fig. 2**). The CP gene was isolated from cDNAs from TMV-RNA. The cDNA synthesis was carried out using a random primer (Takara Biomedicals, Kyoto, Japan) according to the standard protocols of Gubler and Hoffman[10]. The cDNAs were amplified by polymerase chain reaction (PCR) in the presence of artificially synthesized primers (37-44 nucleotides) (**Fig. 3**). The nucleotide sequences for PCR primers were designed so as to contain the sequences complementary to both terminal sequences of the CP-coding region[11] and some restriction endonuclease digestion sites attached to the complementary sequences. In addition, the upstream sequences from the initiation codon of the CP gene were constructed completely homologous to those of the GUS gene, thereby allowing functional positioning of the CP gene in the present vector. These primers were synthesized with an

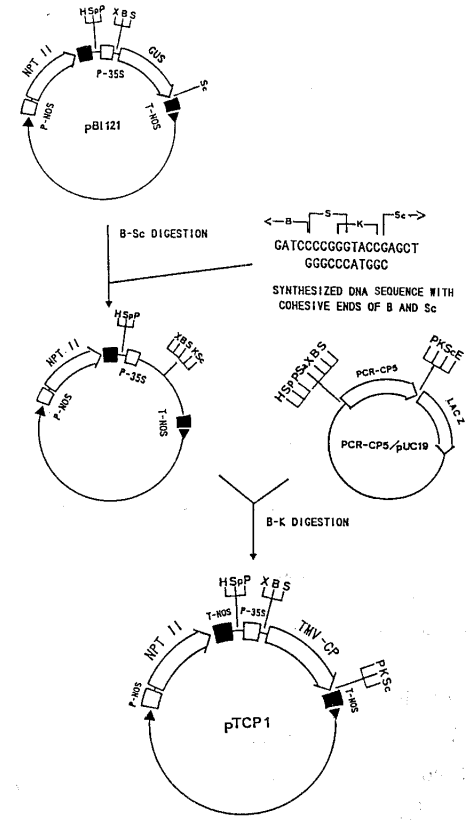

Fig. 2 Construction of a plasmid vector pTCP1 carrying a TMV-CP and NPT II genes inserted between the border seqences (▲) of T-DNA of Ti-plasmid.
Abbreviation, H ; *Hind* III, Sp ; *Sph* I, P ; *Pst* I, X ; *Xba* I, B ; *Bam* HI, S ; *Sma* I, Sc ; *Sac* I, K ; *Kpn* I, Sa ; *Sal* I, E ; Eco RI.

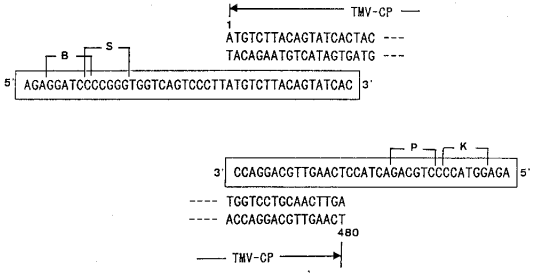

Fig. 3 Synthesized primers used for amplification of TMV-CP gene by PCR.
The cleavage sites for restriction endonucleases *Bam* HI (B) and *Sma* I (S), and *Pst* I (P) and *Kpn* I (K) were attached to each primer in order to promote the subcloning of amplified PCR product.

aid of an Applied Biosystems 391 DNA Synthesizer. The PCR products obtained were digested with restriction endonucleases (*Bam* HI and *Kpn* I) and cloned into a plasmid pUC19. The DNA sequences of the clones were determined by the dideoxy method using M13 phage vector[12] by an Applied Biosystems 370A DNA sequencer. The CP gene obtained was replaced with GUS gene of pBI121 for constructing a plasmid vector pTCP1 according to the cloning method shown in **Fig. 2**. Namely, the plasmid pBI121 was digested with *Bam* HI and *Sac* I to excise the GUS gene from the vector, ligated with the synthesized oligomer with cohesive ends of *Bam* HI and *Sac* I, and linked

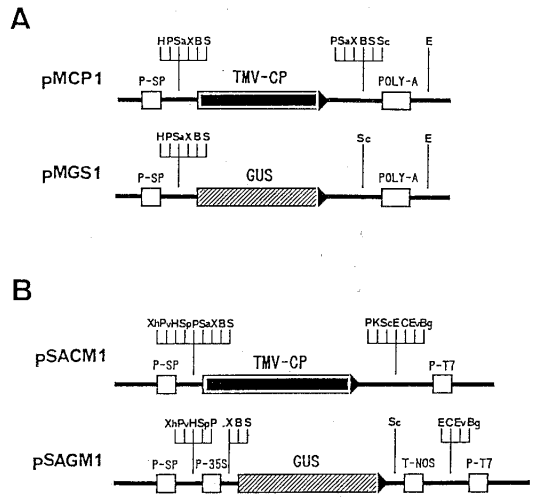

Fig. 4 Plasmid vectors used for *in vitro*-transcription of sense RNAs (A) and probe RNA strands (B). Abbreviation, Xh ; *Xho* I, Pv ; *Pvu* II, C ; *Cla* I, Ev ; *Eco* RV, Bg ; *Bgl* II. Refer to the legend of **Fig. 2** for other restriction endonucleases.

with *Bam* HI-*Kpn* I fragment (CP gene) of PCR-CP5/pUC19.

To obtain sense RNAs of GUS and CP genes, the authors constructed new plasmids (pMCP1 and pMGS1), in which these genes were inserted into polylinker sites of a plasmid vector pSP64-poly (A) (Promega Co., Madison, USA) (**Fig. 4-A**). Sense RNA strands were then synthesized by *in vitro* transcription of the insert from the promoter sequence (SP6 promoter) or this vector.

The *in vitro* transcription was conducted using the Riboprobe System (Promega Co.) by Melton's method[13]. The plasmid (1 $\mu g/\mu l$) was enzymatically linealized and mixed with 0.8 unit/μl SP6 RNA polymerase and a nucleotide mixture (5mM of ATP, CTP, UTP, and 0.5 mM GTP). The transcription of sense RNA was initiated in the presence of 5 mM cap analogue, p^1-5′-(7-methyl)-guanosine-p^3-5′-guanosine triphosphate. Synthesized RNAs were deproteinized, ethanol-precipitated, and collected by centrifugation.

3. *Detection of gene expression*

i) *In situ* hybridization

For detecting transcripts of introduced genes, antisense CP-and GUS-RNAs were prepared from pSACM1 or pSAGM1 (**Fig. 4-B**), respectively, and used as probes for *in situ* hybridization. In addition, sense RNAs were similarly produced from these plasmids for testing a non-specific binding of probes. Both RNA strands were produced by bidirectionally transcribing the DNAs from two promoter sequences (SP-6 and T-7 promoters) oppositely oriented in this vector. The procedures for *in vitro*-transcription were similar to those mentioned above, except for the absence of a cap analogue and the addition of a 10-fold concentration of GTP. The strands obtained were labeled with photobiotin (Photoprobe system ; Vector Laboratory, Burlingame, CA, USA) by the method of Forster *et al.*[14]. The DNA or RNA (1 $\mu g/\mu l$) was dissolved in sterilized water and mixed with an equal concentration of photobiotin, and incubated for 20 min. on ice during exposure to light from a tungsten lamp. The excess nonconjugated photobiotin was eliminated by extraction with 2-butanol and the photobiotin-conjugated nucleic acid was obtained by ethanol precipitation.

The procedures of Raikhel *et al.*[3] were slightly modified for fixation and *in situ* hybridization. The epidermis was transferred to a glass slide 24 hr after the first injection, covered with 4% paraformaldehyde dissolved in PBS buffer (10 mM phosphate buffer, pH 7.0, containing 0.85%

NaCl), and incubated for 30 min at 4°C for fixation. Fixed epidermis was repeatedly washed with PBS and then with sterile water treated with diethyl pyrocarbonate. The specimen was further washed with 100 mM triethanolamine containing 0.25% acetic anhydride, dehydrated with a series of 70-100% of ethanol, and air-dried. The epidermis was covered with a hybridization buffer containing 50% deionized formamide, 2×SSC (300 mM NaCl, 30 mM sodium citrate, pH 7.0), 1% bovine serum albumin (BSA), and 10% dextran sulfate, and all of epidermal cells were punctured with a glass needle (0.7 μm of outer tip-diameter). A cover solution was changed by a buffer containing 0.5 $\mu g/\mu l$ sonicated salmon sperm DNA (heated at 95°C for 10 min) and 2 $\mu g/\mu l$ *Eschericia coli* tRNA, and the specimen was kept at 37°C overnight in a humidified chamber sealed with parafilm. After washing with 50% formamide and then stepwise with 2×and 1×SSC, the specimen was covered with a hybridization buffer containing 5 $ng/\mu l$ of a photobiotin-conjugated probe, incubated at 37°C overnight, and then repeatedly rinsed with TTBS buffer (100 mM Tris-HCl, pH 7.5, containing 150 mM NaCl and 0.1% Tween 20). To achieve the formation of an avidin-mediated complex between the biotinylated probe and enzyme, the epidermis was treated for 3 hr with avidin-bound biotinylated alkaline phosphatase (ABA-phosphatase) dissolved in TTBS supplied by the manufacturer (Vector Laboratories) (*in vitro*-labeling of photobiotin-conjugated probe with ABA-phosphatase). The specimen was repeatedly washed with TTBS and then with 100 mM Tris-HCl, pH 9.5, containing 100 mM NaCl and 5 mM $MgCl_2$, and incubated for 1 hr in dark with the substrate solution, containing 330 μg nitro blue tetrazolium (NBT) and 167 μg 5-bromo-4-chloro-3-indorylphosphate (BCIP) in 1 ml of TTBS buffer. The enzyme reaction was stopped by the addition of sterilized water. The positive color-generating reaction produced a brownish color at the site of *in situ* hybridization.

ii) *In situ* immunoassay

For detection of translation products of the introduced genes, the fixed epidermis was alternately washed with 100 mM phosphate buffer (pH 7.0) and pricked in a buffer containing 1% BSA, and incubated at 37°C for 1 hr. The cover solution was then substituted by a buffer containing 10 $\mu g/ml$ of rabbit anti-GUS-antibody or anti-TMV-antibody and further incubated for 1 hr. After washing with phosphate buffer, the specimen was treated with 10 $\mu g/ml$ of goat anti-rabbit IgG-antibody conjugated with alkaline phosphatase. After 3 hr of incubation, the specimen was repeatedly washed with phosphate buffer in order to eliminate excess antibody, and reacted with the substrates (NBT and BCIP) of this enzyme.

Results and Discussion

A direct introduction of a foreign gene (β-glucuronidase gene) into barley coleoptile epidermal cells has been described in a previous work[1]. The expression of the introduced gene was detected by histochemical examination of enzymatic activity of the translated β-glucuronidase. However, this detection method cannot be used for analyzing the expression of a gene encoding non-hydrolytic enzyme or structural protein such as a viral coat protein. In order to expand the applicability of the microinjection method, it is thus essential to establish an alternative method for effective and precise identification of gene expression. Consequently the present work put an emphasis on developing a methodology for detecting products of gene expression. Namely, transcripts were identified by *in situ* hybridization to antisense RNA probes and translation products by *in situ* immunoassay with enzyme-conjugated antibodies. An *in situ* hybridization has been developed for identification of transcripts primarily in sectioned cellular or tissue specimens of higher animal and plants[3,15,16]. Therefore, the authors have developed an alternative strategy, by

which DNA or RNA probes labeled with non-radioactive photobiotin were injected into non-sectioned fungal cells to detect cytoplasmic rRNA[4] or mRNA[5] in various fungal infection structures. The method has been further improved for detection of gene products in higher plant cells. As a result, the authors have devised a dual microinjection method, which allows introduction of a foreign gene and subsequent introduction of the corresponding RNA probe or antibody by pricking. In this study, in addition to the GUS gene, the TMV-CP gene was used as a foreign gene to be introduced into barley coleoptile epidermal cells, and the expression of these genes were verified at the stages of transcription and translation using a barley coleoptile/dual microinjection system.

The first step of this study was to construct a plasmid carrying the CP gene of TMV by modifying the plasmid pBI121 which is commercially available. The coding region of CP gene was amplified from cDNAs synthesized from TMV-RNA by PCR. The insertion of the PCR product to the cloning vector was facilitated by cleaving the restriction enzyme sites attached to the PCR primers (see **Fig. 3**). Some PCR clones were selected at random, and the total DNA sequences determined were compared with the data reported previously by Takamatsu *et al.*[11]. The present clone (PCR-CP5) involved 480 nucleotides and its sequence was completely identical with those of the original OM strain of TMV (data not shown). The formation of antigen-antibody complex between the translation product and anti-TMV-antibody was confirmed by Western blot analysis of pMCP1-product *in vitro*-translated using a rabbit reticulocyte lysate for cell-free protein synthesis (Nippon Gene, Tokyo, Japan) (data not shown). Thus, the CP gene isolated in this study was found to encode an immunologically detectable coat protein of TMV, suggesting that it can be used for analysis of gene expression in coleoptile tissues.

To confirm the possible expression of a foreign gene introduced into coleoptile epidermal cells, an enzymatic activity of β-glucuronidase was histochemically determined in the GUS gene-injected cells according to the method described previously[1]. The data revealed that more than 70% (75.3 ± 3.1, average of three separate experiments) of successfully injected cells showed positive reaction resulting from hydrolysis of the substrate (5-bromo-4-chloro-3-indolyl-β-D-glucuronide) by the translated β-glucuronidase. These results confirmed the previouse finding[1] that the microinjected gene could be functionally expressed in the coleoptile epidermal cells. Under the present microinjection condition, therefore, the GUS and CP genes were introduced into coleoptile epidermal cells, and the gene products were *in situ* detected by photobiotin-labeled probes or enzyme-conjugated antibodies introduced secondarily by a pricking method.

Prior to actual *in situ* hybridization, an ABA-phosphatase was used to test non-specific binding with proteins in the cells, into which a photobiotin-labeled probe had not been introduced. In this experiment, the cells were pricked in a probe-free solution and then treated with ABA-phosphatase. The result indicated that a positive color-generating reaction was not observed in the absence of a photobiotin-labeled probe (**Table 1**), suggesting that BSA used for protein-blocking suppressed effectively non-specific binding of ABA-phosphatase.

In addition, photobiotin-conjugated sense RNAs were introduced into gene-injected cells and then treated with ABA-phosphatase, since the sense mRNA has been commonly utilized in order to examine the non-specific binding of a probe[3]. Any detectable reaction was not observed in these cells even when the reaction time for complex-formation between hybridized probe and ABA-phosphatase was prolonged to 5 hr (**Table 1**). These results indicate that the positive reaction could be produced only when transcribed mRNAs were *in situ* hybridized with corresponding photobiotin-conjugated antisense RNA strands and subjected to *in vivo* labeling with ABA-phosphatase.

In the previous paper[4], the authors demonstrated that the puncture of cells by pricking was

Table 1. Detection of transcripts of foreign genes microinjected into barley coleptile epidermal cells by *in situ* hybridization*[1].

Genes injected	Pricking	Materials in pricking solution	No. of cells showing positive reaction by *in situ* hybridization			
			1	2	3*[2]	
pBI121	+	ABA-P only	0/66	0/41	0/30*[3]	0*[4]
pBI121	+	sense GUS-mRNA	0/43	0/43	0/41	0
pTCP1	+	sense CP-mRNA	0/58	0/59	0/54	0
pBI121	−	antisense GUS-mRNA	3/63	2/71	1/65	3.0
pTCP1	−	antisense CP-mRNA	2/71	1/60	3/78	2.9
pBI121	+	antisense GUS-mRNA	63/63	45/49	49/59	88.3
pTCP1	+	antisense CP-mRNA	40/46	50/61	54/62	85.2

*1 Coleoptile epidermal cells were injected with the plasmids indicated and incubated at 20°C. After 24 hr, the cells were fixed and then pricked in a solution containing photobiotin-conjugated sense or antisense RNA strands. After incubation, avidin-bound biotinylated alkaline phosphatase (ABA-P) was treated for 3 hr for conjugation with hybridized probes. The positive hybridization was detected by color-generating reaction resulting from hydrolysis of the substrates by a labeling enzyme.

*2 Numbers of experiments.

*3 Total number of successfully injected cells; cells maintaining cytoplasmic streaming for 24 hr after injection.

*4 Averaged percentage of gene-injected cells showing positive reaction *in situ* hybridization.

essential to introduce macromolecules such as nucleic acid probes or proteins into nonsectioned specimen. In this experiment, the necessity of puncturing was confirmed by pricking the gene-injected cells in a probe-containing solution. More than 80% of pricked cells showed positive reaction, whereas the rates (less than 5%) of the positive reaction in non-pricked cells were negligible (**Table 1**). **Fig. 5** shows the positive *in situ* hybridization signal in CP-gene injected coleoptile epidermal cells after introduction of photobiotin-conjugated antisense CP-RNA probe by pricking. The similar results were obtained when GUS gene-injected cells were pricked in a solution containing photobiotin-conjugated antisense GUS-RNA probe (**Table 1**). Thus, the expression of introduced foreign genes could be successfully detected at the transcriptional level, and the rates of the expression were considerably stable, compared with those determined by β-glucuronidase assay.

The present experiments clearly demonstrated that the transcripts could be specifically detected

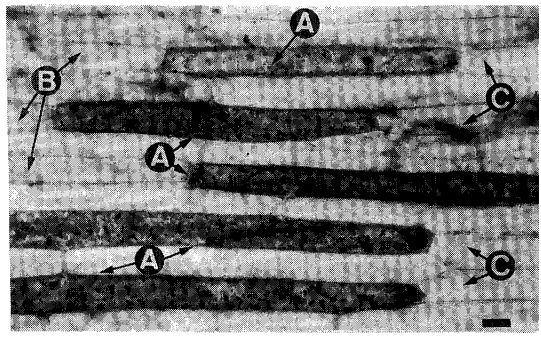

Fig. 5 Detection of transcription of CP-gene in pTCP1-injected barley coleoptile epidermal cells. Note that positive *in situ* hybridization signal was detected only in the cells which had been injected with a plasmid, followed by pricking introducion of photobiotin-conjugated antisense CP-RNA (A), but not in the pricked cell without injection (B) or the gene-injected cell without pricking injection of the probe (C). Bar represents 20 μm.

Table 2. Detection of translation products of foreign genes and their sense RNAs microinjected into barley coleoptile epidermal cells by *in situ* immunoassay*1

Genetic materials injected	Pricking	Antibodies in pricking solution		No. of cells showing positive reaction			
		1 st	2 nd	1	2	3*2	
non	−	−	+	0/46	0/53	0/39*3	0*4
pBI121	+	+	+	36/46	50/53	35/39	87. 7
pTCP1	+	+	+	35/37	33/40	45/46	91. 9
sense GUS-mRNA	+	+	+	53/57	38/39	51/55	94. 0
sense CP-mRNA	+	+	+	33/34	37/49	49/58	84. 4

*1 Coleoptile cells were injected with genetic materials indicated and incubated for 24 hr. After fixiation, the cells were reacted with the 1st antibody (anti-GUS-antibody for pBI121-injection and anti-TMV-antibody for pTCP1-injection) and then with the antibody (anti-rabbit IgG-antibody) conjugated with alkaline phosphatase. The positive reaction was detected by hydrolytic color -regenerating reaction of the labeling enzyme.

*2 Numbers of experiments.

*3 Total number of successfully injected cells; cells maintaining cytoplasmic streaming after injection.

*4 Averaged percentage of injected cells showing positive reaction.

by employing the microinjection method the authors invented. This method will be applicable for detection of translation products of introduced genes by simply replacing hybridization buffer with a solution containing enzyme-linked antibody. In fact, both the gene-injected and non-injected cells were pricked in antibody-containing solution and then reacted with the substrates of the conjugated enzyme. The result indicated that positive reaction of the conjugated enzyme was detected only in gene-injected cells, but not in the cells into which the gene had not been primarily injected (**Table 2**).

Moreover, this method allowed us to examine an efficiency of translation of sense mRNAs microinjected into coleoptile epidermal cells. The sense mRNAs of CP and GUS genes were *in vitro* -translated from pMCP1 and pMGS1, respectively. During the present procedure for RNA synthesis, mRNAs produced were attached to a cap structure and poly A-tail, because it has been generally recognized that these structures can enhance the stability and effective transcription initiation of mRNAs in eukaryotic cells[17,18]. The present result indicated that the rates of the positive reaction were stably high regardless of the types of injected mRNAs and were comparable to those in the cells injected with pBI121 or pTCP (**Table 2**).

To examine the duration necessary for expression of introduced genes, the gene-injected epidermis was incubated for various periods of time and then subjected to an *in situ* hybridization analysis. Alternatively, the injected epidermis was floated onto hormone-free Murashige-Skoog[19] (MS) medium containing 100 μg/m*l* kanamycin at the various periods after injection, and the survival of injected cells was determined on the basis of cytoplasmic streaming. As shown in **Fig. 2**, the plasmid pTCP1 used in this study contains the neomycin phosphotransferase (NPT II) gene which confers kanamycin resistance to the cells. With a barley coleoptile system, the expression of NPT II could be examined by testing microscopically the resistance of the gene-injected cells against kanamycin added to a medium, because cell survival can be easily determined by observing cytoplasmic streaming of coleoptile epidermal cells. The duration necessary for gene expression in pTCP1-injected cells, as assayed by *in situ* hybridization detection and antibiotic-resistance, was shown in **Fig. 6**. The data clearly indicated that the number of positively hybridized cells exponentially increased during the first 3-9 hr and reached the maximum (approximately 70-75% of successfully injected cells) 24 hr after injection, and this maximum level was kept constant

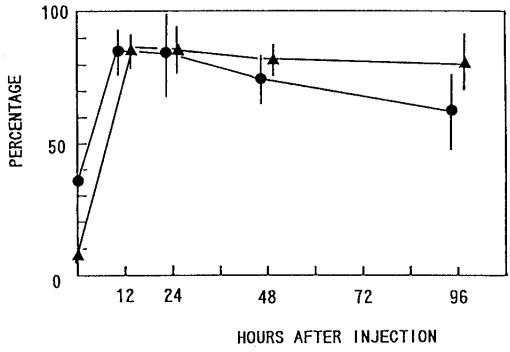

Fig. 6 Duration necessary for expression of TMV-CP (-▲-) and NPT II (-●-) genes injected into barley coleoptile epidermal cells.
The pTCP1-injected epidermis was incubated for various periods and fixed for *in situ* hybridization or alternatively floated on a hormone-free MS medium containing 100 $\mu g/ml$ kanamycin. The expression of the TMV-CP gene was evaluated by *in situ* hybridization to photobiotin-conjugated antisense CP-RNA and the NPT II gene by testing resistance (cytoplasmic streaming) of the injected cells against an antibiotic.

throughout the experimental period of 96 hr. A similar result was obtained when resistance of the injected cells to kanamycin (cytoplasmic streaming) was examined, suggesting that the NPT II gene would be expressed in barley coleoptile epidermal cells, although the expression of this gene was not substantially elucidated in this study.

In the present study, it was not clear whether the injected DNAs were integrated into host chromosomes or not. However, the integration of the genes into chromosomes may not be prerequisite for expression of introduced genes[1]. According to the present strategy, the foreign genes microinjected into barley coleoptile epidermal cells could be functionally expressed regardless of gene sources. This implies that a barley coleoptile system can be used as a model system in higher plants for the detailed analysis of foreign gene expression. Is fact, using a barley coleoptile and the present microinjection method, the authors have isolated a specific promoter sequence responsible for infection from barley chromosomal DNA, microinjected a chimeric GUS gene linked with this sequence, and successfully detected the expression of the chimeric GUS gene only in pathogen-invaded coleoptile epidermal cells (unpublished data). Thus, the method described in this paper will certainly provide a promising experimental basis for analyzing and manipulating gene expression in a higher plant cell system.

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《和文要約》

オオムギ子葉鞘細胞における外来遺伝子の発現解析システム：マイクロインジェクションによる遺伝子の導入と *in situ* 検出

松田克礼・豊田秀吉・野城　康・玉井隆行・大内成志

近畿大学農学部

オオムギの子葉鞘細胞系を利用し，マイクロインジェクション法による外来遺伝子の導入と *in situ* 検出法を組合わせ，高等植物における遺伝子発現解析システムを確立した．導入する外来遺伝子としては，GUS 遺伝子および TMV の外被蛋白質遺伝子を使用し，CaMV の 35S プロモーターと NOS ターミネーターに接続して子葉鞘細胞に注入した．導入遺伝子の転写は，フォトビオチン標識したアンチセンス mRNA プローブをプリッキング法で二次導入し，*in situ* ハイブリダイゼーションによって検出した．また，遺伝子の翻訳産物については，遺伝子導入細胞に一次抗体（抗 TMV 抗体もしくは GUS 抗体）を注入し，さらに酵素標識抗体を二次注入して検出した．以上の結果，子葉鞘細胞に導入した遺伝子の発現が転写ならびに翻訳の段階で検出され，その検出頻度は導入遺伝子の種類にかかわらず 80%以上であった．同様の結果は，*in vitro* で合成した mRNA を注入した場合にも認められ，本法が高等植物の細胞レベルにおける遺伝子発現解析システムとして有効であることが示された．