Note

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Large-Scale Identification of Elicitor-Responsive Genes in Suspension-Cultured Rice Cells by DNA Microarray

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Abstract

To isolate genes responsive to *N*-acetylchitooligosaccharide elicitor in suspension-cultured rice cells, we screened rice ESTs by DNA microarray analysis, and were able to identify novel elicitor-responsive genes. Sequencing analysis of three ESTs revealed that the up-regulated genes include a transcription factor, *Myb*, small G-protein, *Rac*, and calmodulin. The results indicated the genome-wide change of gene expression in response to *N*-acetylchitooligosaccharide elicitor.

Keywords: DNA microarray, *N*-acetylchitooligosaccharide elicitor, rice cell

Higher plants are capable of perceiving the invasion of pathogenic microorganisms and evoke a set of defense reactions, accompanying the dynamic change of gene expression. Characterization of the defense-related genes is crucial to understand the molecular mechanisms of host resistance. Elicitors and suspension-cultured cells have been proven to be excellent tools to analyze the defense reactions (Nürnberger and Scheel, 2001). *N*-acetylchitooligosaccharide works as a potent elicitor at subnanomolar concentration in suspension-cultured rice cells inducing a variety of defense reactions such as production of reactive oxygen species (Kuchitsu *et al.*, 1995) and phytoalexins (Yamada *et al.*, 1993). In this experimental system, we have isolated and characterized novel elicitor-responsive genes by subtractive hybridization (Minami *et al.*, 1996; Takai *et al.*, 2001).

The use of DNA microarray analysis for the identification and characterization of large numbers of cDNAs is rapidly becoming one of the most commonly used genetic tools for dissecting environmental and developmental responses in plants. In *Arabidopsis*, numerous laboratories have characterized the expression patterns of thousands of cDNAs in response to a variety of environmental cues, including development (Schaffer *et al.*, 2001), stress (Desikan *et al.*, 2001; Seki *et al.*, 2001; Thimm *et al.*, 2001) and defense (Petersen *et al.*, 2000; Schenk *et al.*, 2000). Similarly, in rice, the use of DNA microarray analysis is of particular biological, agronomic and economic interest as rice is the staple food for more than 3 billion people worldwide, and a broad based insight into rice functional genomics could provide the information necessary for developing cultivars better suited for sustained agriculture.

In order to analyze the gene expression at early stage of elicitation in rice cells, we employed DNA microarray analysis and identified a number of unique mRNAs regulated by *N*-acetylchitooligosaccharide elicitor.

DNA tips were prepared as previously described (Yazaki *et al.*, 2000). Identification of cDNAs which are up regulated in response to *N*-acetylchitoheptaose was performed as follows: Total RNA was isolated from suspension-cultured rice cells (*Oryza sativa* L. cv. Nipponbare) elicitor-treated (1 μg ml^{-1}) for 0 or 15 min by SDS-phenol method (Watanabe and Price, 1982). Poly (A)$^+$-mRNA was further purified using oligo-dT cellulose. Cy5-dCTP-labeled cDNA probes were prepared according to the standard method shown in http://microarray.rice.dna.affrc.go.jp. Hybridizations were carried out at 42 ℃ in the dark overnight. After hybridizations, the glass slides were washed in

1 x SSC/0.2% SDS for 10 min at 55 ℃ in the dark, followed by washing in 0.1 x SSC/0.2% SDS for 10 min at 55 ℃ twice in the dark. A final wash was performed using 0.1 x SSC for 1 min at room temperature. Hybridizations were analyzed using an Array Scanner Generation III Microarray Scanner (Pharmacia Biotech). Scatter plots were generated using Array Gauge Software version 1.2 (Fuji Film Company, Tokyo, Japan) and Microsoft Excel (Microsoft, Redmond, WA). To select clones for northern analysis from 1265 EST array, the ratio of treated/control for each spot, their average (Av) and standard deviation (SD) were calculated and cut-off was done according to Av ± SD. Further analysis of reproducibility, a list of clones and accession numbers can be viewed at http://microarray.rice.dna.affrc.go.jp.

Ten μg total RNA was subjected to northern blot hybridization as described by Thomas (1983) using a Biodyne nylon membrane (Pall BioSupport). As a loading control of RNA, 25S rRNA gene was used (Takaiwa *et al.*, 1984).

We probed DNA tips on which randomly selected and independent 1265 rice ESTs with Cy5-labeled-cDNA prepared from rice cells treated with *N*-acetylchitoheptaose for 15 min. Of 14 ESTs selected as candidates for elicitor-responsive genes, 7 ESTs were shown to be up-regulated by addition of *N*-acetylchitoheptaose (**Fig. 1**). Complete sequence analysis showed that these included ESTs identical to *OsMyb8* (accession # Y11414) and *OsRac* (AB029508; Ono *et al.*, 2001), or related to *OsCAM-2* (calmodulin; AB060552), all of which are considered to be involved in the transcriptional regulation or signal transduction. In this experiment, the results of northern analysis of 7 ESTs were not consistent with the results of microarray experiments, due likely to the subtle difference in the mRNA population after elicitor-treatment for 15 min.

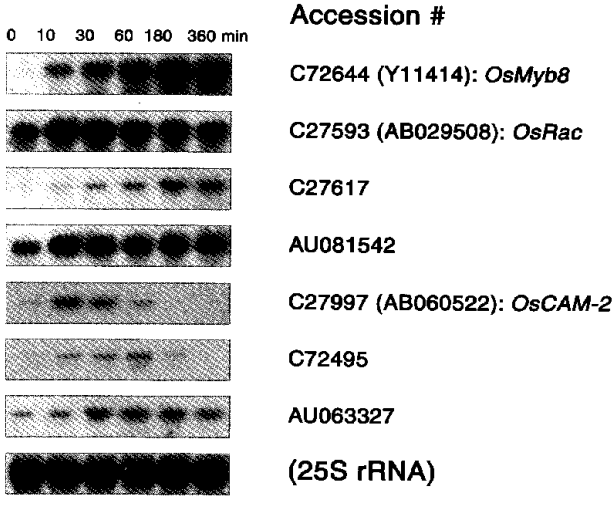

Fig. 1. Northern blot analysis of ESTs identified by microarray analysis.
Total RNAs from rice suspension-cultured cells treated with *N*-acetylchitooligosaccharide for 0 to 6 h were analyzed by northern blot hybridization using 7 ESTs selected by microarray analysis and preliminary northern blot hybridization. Rice 25S rDNA was used as a probe to verify equal loading of RNA. The GenBank Accession numbers and annotations for the ESTs are indicated in the figure.

To get further insight into the change of gene expression during defense response induced by *N*-acetylchitooligosaccharide, we surveyed 8987 of rice ESTs arrayed on slide glasses with the cDNA probe prepared from the cells elicitor-treated for 0, 30 and 120 min. We chose these time points, expecting the enlarged extent of gene activation/repression. **Fig. 2** shows the scatter plot. For the vast majority, the level of mRNAs remains unchanged at 30 min after elicitor treatment, whereas more conspicuous change of mRNA levels at 120 min was observed.

Stress-responsive genes in plants often show multi-responses to other environmental cues as have already been shown for the previously characterized elicitor-responsive genes in rice. For example, we have shown that EL2 and EL3, two novel early responsive genes, are also responsive to propionic acid that induces cytoplasmic acidification, a protein phosphatase inhibitor, calyculin A (He *et al.*, 1998), or protein synthesis inhibitor, cycloheximide (Nishimura *et al.*, 2001). Expression-analysis by DNA microarray would reveal the profile of gene expression in response to these stimuli.

Defense reaction is considered to be the result of coordinated change of cellular metabolisms, and repression of gene expression is likely to occur. In

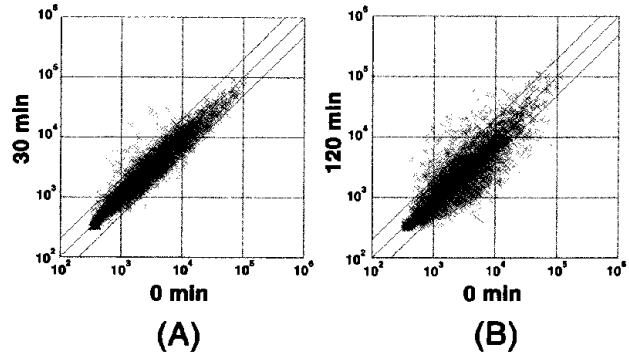

Fig. 2. Scattered plot analysis of 8987 microarray hybridization.
Normalized channel intensities of each ESTs were plotted with signals from non-treated (X-axis) versus elicitor-treated for 30 min (A) or 120 min (B). The diagonal lines represent 2-fold induction/repression ratio cut-offs.

fact, the decrease of the small subunit of ribulose 1,5-bisphosphate carboxylase was observed both in mRNA and protein levels in potato leaves infected or treated with pathogen or elicitor (Kombrink and Hahlbrock, 1990). In parsley suspension cells treated with elicitor, the repression of cell cycle-related genes was observed (Logemann *et al.*, 1995). Random selection of ESTs has an advantage that we can identify down-regulated genes as well as up-regulated genes. However, little is known about the genes repressed during the defense response. DNA microarray analysis is a powerful tool to analyze the network of gene function through genome wide profiling of the expression pattern of genes.

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References

Desikan, R., A. -H. -Mackerness, S., Hancock, J. T., Neill, S. J., 2001. Regulation of the Arabidopsis Transcriptome by Oxidative Stress. Plant Physiol., **127**: 159-172

He, D. -Y., Yazaki, Y., Nishizawa, Y., Takai, R., Yamada, K., Sakano, K., Shibuya, N, Minami, E., 1998. Gene activation by cytoplasmic acidification in suspension-cultured rice cells in response to the potent elicitor, *N*-acetylchitoheptaose. Mol. Plant Microbe Interact., **11**: 1167-1174.

Kombrink, E, Hahlbrock, K., 1990. Rapid, systemic repression of ribulose 1,5-bisphosphate carboxylase small subunit mRNA synthesis in fungus-infected or elicitor-treated potato leaves. Planta, **181**: 216-219

Kuchitsu K., Kosaka H., Shiga T., Shibuya, N., 1995. EPR evidence for generation of hydroxyl radical triggered by *N*-acetylchitooligosaccharide elicitor and a protein phosphatase inhibitor in suspension-cultured rice cells. Protoplasma, **188**: 138-142.

Logemann, E, Wu, S. C., Schröder, J., Schmelzer, E., Somssich, I. E., Hahlbrock, K., 1995. Gene activation by UV light, fungal elicitor or fungal infection in *Petroselinum crispum* is correlated with repression of cell cycle-related genes. Plant J., **8**: 865-876.

Minami, E., Kuchitsu, K., He, D. -Y., Kouchi, H., Midoh, N., Ohtsuki, Y., Shibuya, N., 1996. Two novel genes rapidly and transiently activated in suspension-cultured rice cells by treatment with *N*-acetylchitoheptaose, a biotic elicitor for phytoalexin production. Plant Cell Physiol., **37**: 563-567.

Nishimura, N., Tanabe, S., He, D. -Y., Yokota, T., Shibuya, N., Minami, E., 2001. Recognition of *N*-acetylchitooligosaccharide elicitor by rice protoplasts. Plant Physiol. Biochem., **39**: 1105-1110

Nürnberger, T., Scheel, D., 2001. Signal transduction in the plant immune response. Trends Plant Sci., **6**: 372-379.

Ono E., Wong H. L., Kawasaki T., Hasegawa M., Kodama O., Shimamoto K., 2001. Essential role of the small GTPase Rac in disease resistance of rice. Proc. Natl. Acad. Sci. U. S. A., **98**: 759-764.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H. B., Lcay, M., Austin, M. J., Parker, J. E., Sharma, S. B, Klessig, D. F., Martienssen, R., Mattsson, O., Jensen, A. B., and Mundy, J., 2000. *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. Cell, **103**: 1111-1120.

Schaffer, R., Landgraf, J., Accerbi, M., Simon, V. V., Larson, M., and Wisman, E., 2001. Microarray analysis of diurnal and circadian-regulated genes in *Arabidopsis*. Plant Cell, **13**: 113-123.

Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Sommerville, S. C., Manners, J. M., 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc. Natl. Acad. Sci. U. S. A., **97**: 11655-11660.

Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y., Shinozaki, K., 2001. Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell, **13**: 61-72.

Takai, R., Hasegawa, K., Kaku, H., Shibuya, N, Minami, E., 2001. Isolation and analysis of expression mechanisms of a rice gene, *EL5*, which shows structural similarity to ATL family from *Arabidopsis*, in response to *N*-acetylchitooligosaccharide elicitor. Plant Sci., **160**: 577-583.

Takaiwa, F., Oono, K., Sugiura, M., 1984. The complete nucleotide sequence of a rice 17S rRNA gene. Nucleic Acids Res., **12**: 5441-5448.

Thimm, O., Essigmann, B., Kloska, S., Altmann, T., Buckhout, T. J., 2001. Response of *Arabidopsis* to iron deficiency stress as revealed by microarray analysis. Plant Physiol., **127**: 1030-1043.

Thomas, P. S., 1983. Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. Methods Enzymol., **100**: 255-266

Watanabe, A., Price, C. A., 1982. Translation of mRNAs for subunits of chloroplast coupling factor 1 in spinach. Proc. Natl. Acad. Sci. U. S. A., **79**: 6304-6308.

Yamada, A., Shibuya, N., Kodama, O. Akatsuka, T., 1993. Induction of phytoalexin formation in suspension-cultured rice cells by *N*-acetylchitooligosaccharides. Biosci., Biotechnol., Biochem., **57**: 405-409.

Yazaki, J., Kishimoto, N., Nakamura, K., Fujii, F., Shimbo, K., Otsuka, Y., Wu, J., Yamamoto, K., Sasaki, T., Kikuchi, S., 2000. Embarking on rice functional genomics via cDNA microarray: Use of 3' UTR for specific gene expression analysis. DNA Res., **7**: 367-370.