RNA Silencing in Higher Plants and Functional Genomics

Project Leader:

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1. Objective:

The development of practical systems to identify gene functions based on RNA silencing (RNA interference; RNAi) as well as its application to the characterization of genome networks in higher plant systems have been investigated. The molecular bases of RNAi in plant cells were also investigated to contribute to the establishment of the next generation of genetic engineering with more controlled gene expression.

2. Summary

2-1. Development of simple transient RNAi system for the functional genomics :

An efficient transient RNAi method with artificially synthesized dsRNA has been developed. (Fumihiko Sato, Ei-ichiro Fukusaki)

This transient RNAi system has been successfully used to identify several trans-factors and previously unidentified biosynthetic genes in medicinally important isoquinoline alkaloid biosynthesis in *Coptis japonica* cells. (Fumihiko Sato)

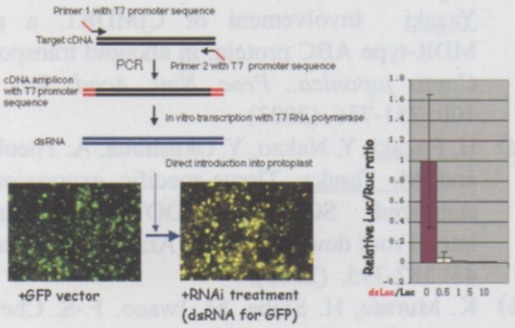

Fig.1. Development of transient RNAi system in *Coptis japonica* protoplasts.

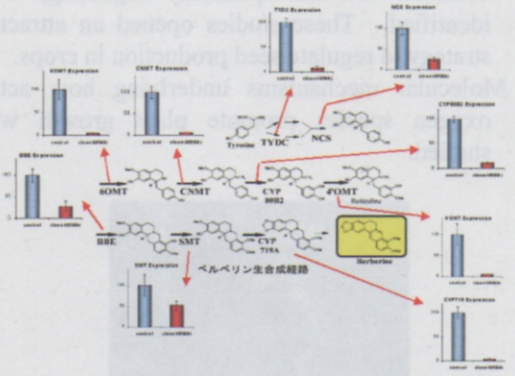

Fig.2. Application of transient RNAi has revealed a unique transcriptional network in isoquinoline alkaloid biosynthesis.

2-2. Development of differential RNAi (dRNAi), comprehensive RNAi for gene families, and a novel gene-substitution method based on dRNAi for functional genomics :

The differential RNAi (dRNAi) method for the gene-specific silencing of gene-families has been established using specific double-stranded (ds)RNA designed for a gene-specific region such as the 3' untranslated region (UTR) of transcript. Furthermore, a unique 37 bp-RNAi system for the comprehensive silencing of whole gene families in stable transformants has been established. Using dRNAi, the physiological functions of important photosynthetic PsbP in tobacco, chalcone synthase in anthocyanin synthesis in petunia, and Rac genes in signal transduction of pathogenesis in rice have been successfully characterized. (Fumihiko Sato, Ei-ichiro Fukusaki, Kazuyuki Isshiki, Ko Shimamoto)

A novel gene-substitution method by the ectopic expression of a complementary (or substitutional) expression vector in dRNAi host plants has been developed to substitute the gene function in transgenic plants. (Fumihiko Sato)

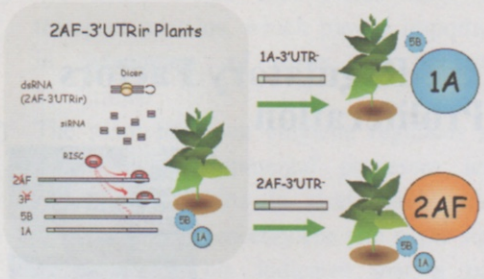

Fig.3. Effective gene-substitution method based on differential RNAi has been developed.

2-3. Development of efficient RNAi vectors for rice functional genomics :

Several RNAi vectors, such as practical RNAi vector (pANDA) based on the Gateway system, those for the quantitative control of RNAi using splicing mutation, and those for cell-specific silencing in endosperm have been developed. Using these vectors, *Wx* mRNA function was efficiently and quantitatively disrupted to modify the food quality of rice grain. pANDA vector was also successfully used for the characterization of a flowering-time control gene and disease-resistance genes in rice. (Kimiko Ito, Kazuyuki Isshiki, Ko Shimamoto)

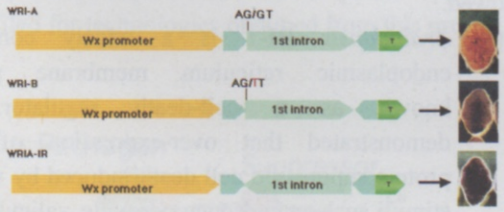

Fig.4. Cell-specific and qualitatively controlled RNAi vector has been developed to modify the amylose content in rice grain

2-4. RNAi and metabolic engineering :

RNAi has been successfully used for metabolic engineering in an isoquinoline alkaloid biosynthetic pathway to produce an important intermediate, reticuline, which is accumulated in transgenic cells. These novel cell lines have provided insight into understanding the generation of new biosynthetic pathways by metabolic engineering. (Fumihiko Sato)

2-5. Molecular characterization of DNA methylation :

The characterization of the low DNA-methylation mutant *ddm1* in Arabidopsis has revealed that several endogeneous genes as well as transposons can be de-suppressed by the reduction of DNA-methylation. Further analysis in rice also indicated the existence of a novel system of gene regulation/recognition in plant cells. (Tetsuji Kakutani)

2-6. RNAi and virus suppressors :

RNAi in plants is known to help defend against virus infection. On the other hand, viruses have RNAi suppressors to counteract RNAi. The NSs protein of *Tomato spotted wilt virus* has been characterized as the first RNAi suppressor in negative-strand RNA viruses, and it has been shown that NSs could interfere with multiple steps in the RNAi pathway. Also, a novel mechanism of RNAi suppression by *Red clover necrotic mosaic virus*, which requires multiple viral components, has been identified. (Kazuyuki Mise, Tetsuro Okuno)

3. Concluding Remarks

Novel RNAi methods (dRNAi, comprehensive RNAi, transient RNAi etc.) have been developed for functional genomics in plant. The application of these methods has revealed functional networks of genes. Further development of the gene-substitution method based on dRNAi could provide new tools for the development of plant functions using a more rational approach. Detailed characterization of the RNAi mechanism could also provide the molecular basis for the next generation of gene engineering.

4. Primary Publications

(1) Joseph G. Dubouzet, Takashi Morishige, Nanae Fujii, Chuang-Il An, Ei-ichiro Fukusaki, Kentaro Ifuku and Fumihiko Sato: "Transient RNA Silencing of Scoulerine 9-*O*-methyltransferase Expression by Double Stranded RNA in *Coptis japonica* Protoplasts", *Biosci. Biotech. Biochem.*, 69, 63-70 (2005).

(2) Kentaro Ifuku, Yumiko Yamamoto, Taka-aki Ono, Seiko Ishihara, and Fumihiko Sato: "PsbP Protein, but not PsdQ Protein, is Essential for the Regulation and Stabilization of Photosystem II in Higher Plants", *Plant Physiol.* 139, 1175-1184 (2005).

(3) Ryosuke Hayama, Shuji Yokoi, Shojiro Tamaki, Masahiro Yano and Ko Shimamoto: "Adaptation of Photoperiodic Control Pathways Produces Short-day Flowering in Rice", *Nature* 422, 719-722 (2003).

(4) Tetsu Kinoshita, Asuka Miura, Yeonhee Choi, Yuki Kinoshita, Xiaofeng Cao, Steven E. Jacobsen, Robert L. Fischer and Tetsuji Kakutani: "One-way Control of *FWA* Imprinting in *Arabidopsis* Endosperm by DNA Methylation", *Science*, 303, 521-523 (2004).

(5) Masahiro Tatsuta, Hiroyuki Mizumoto, Masanori Kaido, Kazuyuki Mise and Tetsuro Okuno: "The *Red clover necrotic mosaic virus* RNA2 *trans*-activator is also a *cis*-acting RNA2 Replication Element", *J. Virol.* 79, 978-986 (2005).