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Prospect of plant molecular cytogenetics in the 21st century

Yasuhiko Mukai

Laboratory of Plant Molecular Genetics, Division of Natural Science,
Osaka Kyoiku University, Kashiwara, Osaka 582-8582 Japan

Abstract

The genomes of Arabidopsis and rice have been fully sequenced. Genomic sequencing provides global information about genome structure and organization. A comprehensive research account of our recent studies conducted on genome painting, comparative genomics and genome fusion is provided in order to project the prospects of plant cytogenetic research in post-genomics era. Genome analysis by GISH using genome painting is demonstrated as an excellent means suitable for visualization of a whole genome, since total genomic DNA representing the overall molecular composition of the genome is used as a probe. FISH on extended DNA fibers has been developed for high-resolution FISH and has contributed to determining the copy number and order of genes. We have also mapped a number of genes involving starch synthesis on wheat chromosomes by FISH and compared the position of these genes on linkage map of rice. Macro synteny between wheat and rice can be observed by comparing the location of these genes in spite of the fact that the size of DNA per chromosome differs by 20 fold in two. Moreover, to approach our goal towards making bread and udon noodles from rice flour in future by incorporating bread making and the noodle qualities in rice, we

have been successful in introducing large genomic DNA fragments containing agronomically important genes of wheat into a rice by successive introduction of large insert BAC clones, there by expanding genetic variability in rice. We call this method genome fusion.

Keywords : molecular cytogenetics; visualization of genome information; comparative genomics; genomic in situ hybridization; FISH; chromosome painting; synteny; genome fusion

1. Introduction

In the past one decade plant cytogenetics has undergone tremendous transition from conventional chromosome analysis to modern molecular cytogenetic applications. The conventional staining techniques by carmine and Feulgen have now been refined by the use of fluorescent dyes. With the aid of Fluorescence In Situ Hybridization (FISH) techniques, it has now been made possible to physically visualize genes and DNA sequences under a microscope on chromosomes and extended DNA fibers. The Genomic In Situ Hybridization (GISH) has provided new dimensions to accurately underpin the genome donors in natural polyploids based on direct genomic painting, omitting the need to test the validity by raising artificial hybrids. As such, the scientific developments are progressing very fast, and newer and newer techniques and their refinements are emerging on the scientific horizon in the field of molecular cytogenetics.

Classical cytogenetics has contributed to plant breeding. Molecular cytogenetics combining traditional cytological techniques with innovative molecular-biological technologies opens new field in plant breeding as its practical outcome. Here we review the progress and prospects of plant molecular cytogenetics with a multidisciplinary approach appropriate for the 21st century.

2. Visualization of genome information

In the late 1980s FISH was first applied to visualize DNA sequences on the plant chromosomes (Yamamoto and Mukai 1989). Since then the technique has proved to be remarkable in genome analysis of plants to assess the homology between genomes and to locate the position of different genes and DNA sequences on individual chromosomes or extended DNA fiber. With the improvement in detection sensitivity and resolution of FISH technique it is now possible to even detect unique sequences, as small as 1kb. Mapping of genes or DNA sequences on extended DNA fiber and intact cloned DNA has increased the resolution of FISH technique (Fukui et al. 2001). It has been successful in revealing the position of agronomically important genes on chromosomes of crop plants through FISH techniques (Li et al. 2003; Turnbull et al. 2003).

2.1 Genome analysis at a glance

GISH has been one of the excellent technologies suitable for the visualization of a whole genome as the total genomic DNA as a probe reflects the overall molecular composition of the genome (Jauhar 1996). The classical method of genome analysis, like pairing of chromosomes at meiosis and pollen fertility of F1 hybrids have various limitations. The GISH technique has overcome all these problems and successfully discriminated the genomes of many polyploid species including wheat (Mukai et al. 1993), oat (Chen and Armstrong 1994), finger millet (Bisht and Mukai 2001), cotton (Hanson et al. 1995), peanut (Raina and Mukai 1999), tobacco (Kenton et al. 1993), coffee (Raina et al. 1998) and banana (DHont et al. 2000). GISH visualized genome homology between polyploid species and their progenitors and gave supplement information on the genomic origin of the polyploids. Now, it is expected that GISH will also prove a promising tool for the

analysis of genomes of woody species in which it takes long time to observe the chromosome association at meiosis.

2.2 Chromosome painting

Through FISH analysis using human chromosome-specific painting probes, the syntenic relationship between human and other mammalian karyotypes has been studied. This comparative chromosome painting was termed zoo-FISH (Scherthan et al. 1994; Fronicke and Scherthan 1997). We are now working on chromosome painting in plants using chromosome specific BAC library and multi-color FISH, although there is a negative view that it is difficult to achieve chromosome painting because of the dispersed distribution of repetitive sequences (Fuchs et al. 1996). Chromosomal homologies between individual rice chromosomes and other cereal karyotypes could be established by using a new approach termed cereal-FISH. Pooled BAC DNA clones derived from individual rice chromosomes are used as probes for FISH on chromosomes of certain cereals. Apart from visualizing cytogenetic homologies of rice and cereal chromosomes, cereal-FISH refines the comparative maps constructed by molecular gene mapping of individual locus. Cereal-FISH also allows to study the karyotypic evolution and opens new avenues for genomics by facilitating the extrapolation of results from the rice genome project. Recently, chromosome painting was successful in *Arabidopsis thaliana* chromosomes by using Arabidopsis BAC clones as probes (Lysak et al. 2001, 2003).

2.3 FISH on DNA fibers

In physical mapping studies, FISH on extended DNA fiber from interphase nuclei is a useful tool for determining the sizes of target DNA sequences, the

order of genes or clones and their distances in a large chromosomal region (Fransz et al. 1996). Currently, fiber FISH has indicated the potential for tracing the target sequences with lengths of up to 2.0 Mb on single extended DNA fibers, a spatial resolution of 1 kb between adjacent targets, and detection sensitivity of a target of as small as 700 bp in plants (Yamamoto and Mukai 1998; de Jong et al. 1999). In transgenic plants, fiber FISH can physically map the transgenes directly on extended DNA fibers and this method is complementary to PCR, Southern blot and sequence analyses (Jackson et al. 2001).

Two new technologies, molecular combing method (Michalet et al. 1997) and digital mapping (Jackson et al.1999), have further enabled direct FISH mapping to purified BAC or lambda phage DNA clones. Analysis of the FISH on intact cloned BAC DNA demonstrated that three genes of starch branching enzyme I (SBEI) were clustered in wheat within 30 kb of DNA (Suzuki et al. 2003).

3. Comparative genomics and gene synteny

Genomics, a branch of genetics concerned with the systematic molecular characterization of whole genome, is important for obtaining an overview of the genome organization and to provide the basic information for isolating specific genes or DNA sequences. At present, several projects in many higher plants are at various stages of sequencing and genome analysis.

Genomic sequencing provides information about global genome structure and organization, regulatory regions, transposable elements, and non-coding sequences. Comparative genomics is the study of the similarities and differences in structure and function of genetic information across taxa at the DNA level using molecular tools (Paterson et al. 2000). In cereals, that cover most of the worlds food and feed crops, a consensus map of twelve grass genomes is now available and it represents the chromosome segment of each genome relative to

rice on the basis of mapping of anchor DNA markers (Gale and Devos 1998; Devos and Gale 2000). However, the comparison of gene location along chromosomes has been mainly conducted in terms of genetic map and not physical map. Therefore we need to determine the accurate position of genes of interest by physical mapping by FISH.

We have mapped a number of genes involved in starch synthesis on wheat chromosomes by FISH and compared with the position of these genes on linkage map of rice (Rahman et al. 1997, 2001, 2003; Li et al. 2003). The results indicated that gene synteny was well conserved (Fig.1). Despite the size differences in genomes of wheat and rice, gene synteny is conserved, even in large blocks often comprising entire chromosome. In comparison to rice, large amount of DNA is lying in between the genes on the chromosomes of wheat (Fig.2).

Cereal genomes are highly variable in size, ranging from diploid species with 415 Mb in rice to 16,000 Mb in hexaploid wheat. Comparative genomic analysis

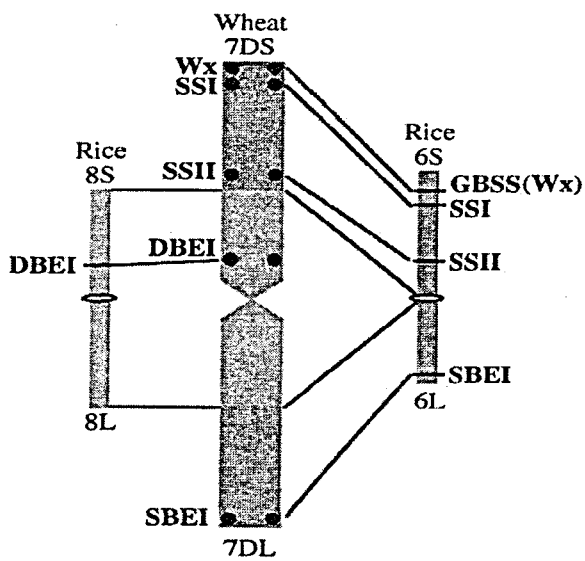

Fig. 1. Physical map of genes controlling starch synthesis in wheat, vis-a-vis linkage map in rice.

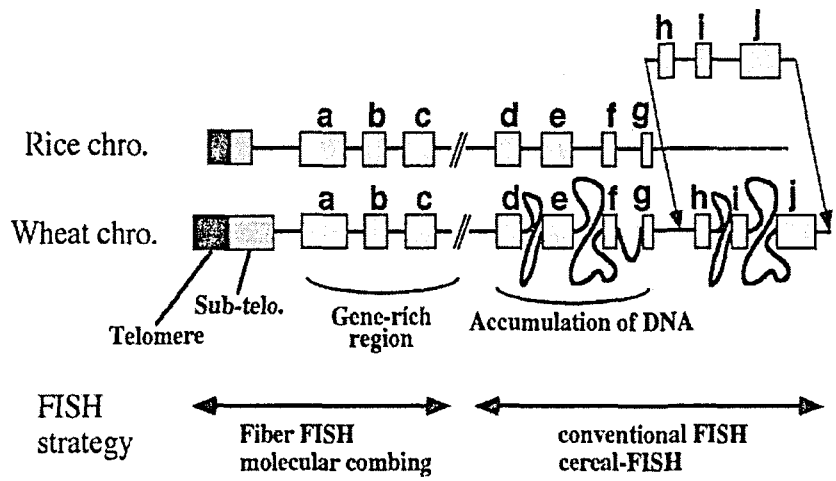

Fig. 2. Genome organization in wheat and rice.

at the genetic-map level has shown extensive conservation of gene order between different grass genomes in many chromosomal regions. However, little is known about the gene organization in grass genomes at the microlevel. Comparison of gene-coding regions between cereals show that the distance between the genes is correlated with the genome size. Recent analysis of large, orthologous genomic fragments from maize, rice, and sorghum genomes demonstrated that the gene density varies in correlation with the genome size (Bennetzen et al. 1998). On the other hand, Feuillet and Keller (1999) reported that comparison of the gene composition at orthologous loci in wheat, barley, and rice revealed a maximal gene density of one gene per 45 kb, very similar to the gene density in *Arabidopsis thaliana*. They concluded that small and large grass genomes contain regions that are highly enriched in genes with very little or no repetitive DNA. The comparison of the gene organization suggested various genome rearrangements during the evolution of the different grass species.

4. Aplication of molecular cytogenetic research to plant breeding

4.1 An innovating system in plant breeding

On of the important tasks in plant breeding is to introduce a set of agronomically useful genes from a certain crop into another distant crop overcoming the barrier of reproductive isolation. For the revolutionary improvement of a crop, a new system which can transform a number of genes controlling many functions in plants is indispensable. Therefore, it is of urgent necessity for practical use of plant genomics to establish a transformation system that introduces large fragment of plant genomes into other crops (Shibata and Liu 2000).

To complement this work, FISH provides a useful tool in determining the position of integration of the genome and its size. Currently where transgenes land are unpredictable when they are introduced into an organism and what effect this has on the expression of transgenes. So, it is highly desirable to check for location of the transgenes using FISH, an elegant and rapid means of identifying chromosomal locations of genes.

4.2 Genome fusion: successive introduction of large genome fragments

In order to expand genetic variability in rice, we are trying to introduce the large genomic DNA fragments containing agronomically important genes of wheat into rice. Our strategy is illustrated in Fig 3. We plan to breed rice with wheat genome by successive introduction of huge DNA fragments such as BAC clones. We call this method "genome fusion". In the first step of our research, we aim to develop a system which can introduce a mass wheat genome efficiently and a simple method of detecting the introduced genome or genes in transgenics. We focus on the introduction of three types of gene clusters: starch synthesis-related genes, grain hardness-related genes and prolamin-related genes. Our goal is to make bread and udon noodles from rice flour in future by incorporating bread

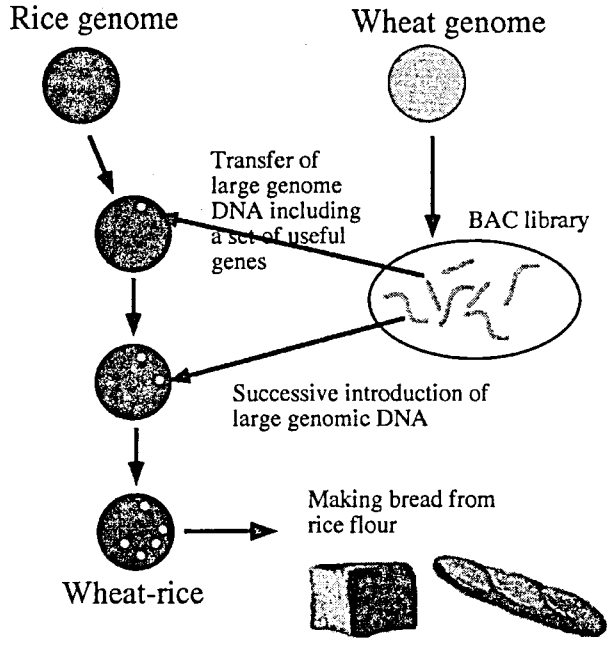

Fig. 3. Production of new rice with wheat genome by genome fusion.

making nature and the noodle aptitude to rice. It is useful also to environmental preservation by not only contributing to consumption expansion of rice by aiming at conversion into the rice from wheat flour, but also maintaining a paddy field by rice cultivation.

A 75 kb of *Aegilops squarrosa* (the D genome donor to common wheat) genome insert containing a gene related to starch synthesis was transformed to rice by *Agrobacterium* mediated transformation method using a binary cosmid vector. The presence of the transgenes was confirmed in regenerated plants by PCR analysis using primers for a part of the gene. Subsequently, to detect wheat DNA fragments cytologically, we carried out FISH experiments using the original large DNA fragment as a probe in transgenic rice. Two hybridization signals were observed in metaphase chromosomes in thomozygous T2 plants. Most signals appeared at the terminal or distal regions of rice chromosomes. The fragments of

wheat genome DNA were stably transmitted to offspring and the transgenes were expressed in rice.

5. Concluding remarks

Molecular approach in plant cytogenetics is widening the knowledge in plant genomics and accelerating breeding research. The direction of plant cytogenetic research in the 21st century could be summarized as follows: (1) from chromosome level to DNA fiber level; (2) from individual analysis to comparative analysis; (3) from structural analysis to functional analysis; (4) from segregated comprehension to integrated comprehension; (5) from basic study to applied study; and (6) towards multidisciplinary.

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