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Advances in rice chromosome research, 1995-2000

K. Fukui

The most significant accomplishment in rice chromosome research during the last 5 years has been the development of fluorescence *in situ* hybridization (FISH) methods. Now, these methods are routinely used to localize genes of practical importance on rice chromosomes. Three new rice genomes of G, H, and J were designated 36 years after the assignment of the F genome in 1961. The orientation of the molecular linkage map of rice has been attained for the first time using secondary trisomics and telotrisomics. Advances in rice chromosome research are summarized by (1) molecular cytology, (2) genome- and chromosome-related research, and (3) new technologies.

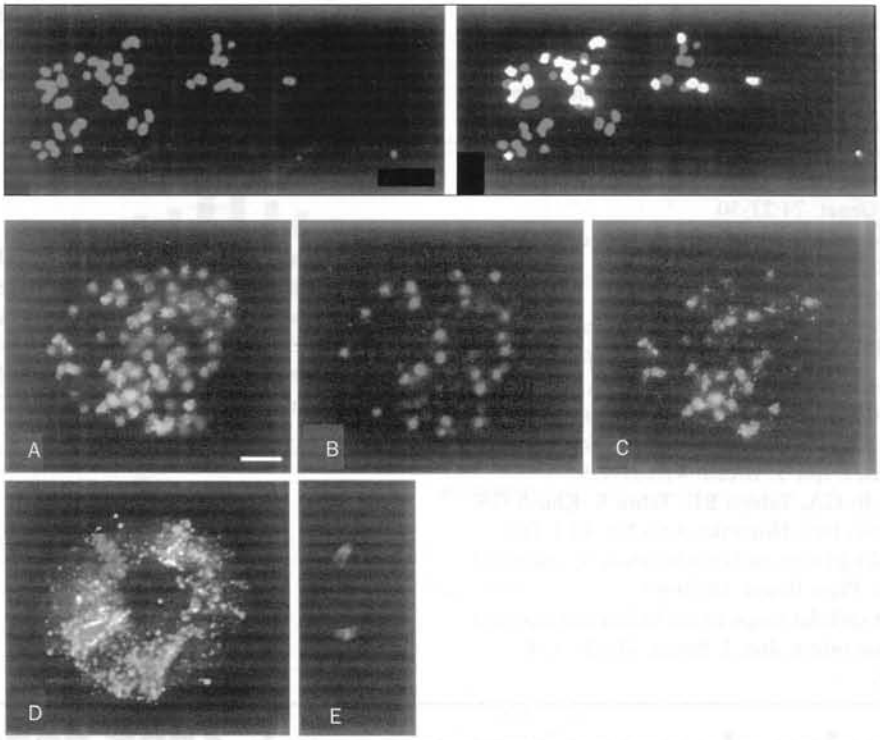
Advances in rice chromosome research during the last few years have been characterized by three features. First, dramatic improvement was attained in molecular cytology, expanding the target material from chromosomes to DNA fibers. Single-copy genes can be localized on rice chromosomes. Moreover, genomic in *situ* hybridization (GISH) became useful in breeding programs. Second, three new rice genomes were assigned by Southern hybridization methods instead of conventional cytological observation of the meiotic configuration of homologous chromosomes. The rice centromeres have been mapped on linkage maps and the orientation of the molecular linkage map was presented. Third, new technologies have continuously been developed in the field of rice chromosome research. The cloning of a functional domain of rice chromosomes, the development of new software to analyze rice pachytene chromosomes, and the identification of rice chromosome organization within a nucleus will be reviewed. The effectiveness of the molecular cytological approaches to rice genome research has been reviewed earlier (Fukui and Ohmido 2000a).

Advances in molecular cytology

Fluorescence *in situ* hybridization (FISH)

The first reproducible rice FISH results were reported in 1994 using 45S rDNA as the probe (Fukui et al 1994). Then, the development of FISH methods in rice chromosome research went far beyond what we had expected (Fukui 1996). Major advances in FISH on rice chromosomes include (1) the target nucleotide sequences were broadened from repeated genes, such as ribosomal RNA genes, to single-copy DNA sequences; (2) the target materials were broadened from somatic chromosomes to DNA fibers; and (3) a single color was used to detect fluorescent signals before; now, many colors can be used simultaneously.

As a result, several useful genes, which are sometimes single-copy genes, were physically mapped on the rice chromosomes. Useful genes, *Gm2*, *Pib*, *Xa21*, and an RFLP marker (Xnp247), etc., have successfully been visualized on rice chromosomes. The genes detected were often located at the termi-



nal regions, suggesting the concentrated localization of transcriptionally active genes at the terminal regions of rice chromosomes. Shishido et al (1996) detected the third 45S rDNA locus in *Oryza eichingeri* (CC) by FISH. A peculiar distribution of rDNA loci has been found in rice genomes different from collinearity among rice and other cereal species.

FISH on extended DNA fibers (EDF-FISH)

Recent visualization methods allow us to directly see even single DNA molecules under a fluorescence microscope. Extension of repetitive sequences was visualized on DNA fibers by the FISH method targeting DNA fibers. After FISH on extended DNA fibers (EDF-FISH) using tandem repeat sequence A (TrsA) and telomere sequences as probes, dot-like fluorescent signal tracks of TrsA and telomere sequences were visualized (Ohmido et al 2001). By using EDF-FISH, the amounts of four different repetitive sequences of 45S rDNA, 5S rDNA, telomere sequences, and TrsA between indica and japonica rice were compared. As a result, indica showed higher contents of all four repetitive sequences. It is thus concluded that the FISH method, especially on DNA fibers, contributes much to quantitative analyses of the copy number of the genes and repetitive sequences.

Genomic *in situ* hybridization (GISH)

GISH is the method of painting chromosomes belonging to different genomes with different colors. Although GISH in rice was developed earlier by Fukui et al (1997), it has made much progress more recently. These authors detected the fluorescent signal from the genomic DNA of *O. officinalis* (CC) on the C-genome chromosomes in *O. minuta* (BBCC) and *O. latifolia* (CCDD). They have shown that the phylogenetic distance between the C and D genomes is closer than that between B and C by comparing fluorescence intensity among the chromosomes in different genomes. Multicolored GISH in rice was used to detect the specific elimination of chromosomes in the B and C genomes in somatic hybrids with A (*O. sativa*), B, and C (*O. punctata*) genomes. The three different genomes were painted differently and chromosome reduction in the specific genomes was unequivocally revealed. GISH is also used to monitor chromosomal behavior in wide crosses (Abbasi et al 1999).

Advances in genome- and chromosome-related research

New rice genomes and genome organization

Three new genomes were assigned by molecular means. Six genomes from A to F had been reported and accepted to date. After the assignment of the F genome in 1961, no report appeared until the G (*O. meyeriana*), H, and J (*O. ridleyi*) genomes were reported (Aggarwal et al 1997). These three new genomes were assigned not by conventional hybridization experiments followed by observation of meiotic configuration but by genomic DNA hybridization.

Dong and Jiang (1998) reported a three-dimensional organization of rice chromosomes within a rice nucleus. Using the FISH method with telomere- and centromere-specific sequences, they found no fixed allocation of rice chromosomes within a nucleus (Rabl pattern) but a more or less random distribution of telomeres and centromeres within a nucleus. This

tendency is common among plant species with a smaller genome size. Telomere sequences within a nucleus have shown random distribution.

Centromere mapping, introgression lines, and the rice B chromosome

Using secondary trisomics, telotrisomics, and RFLP markers, the positions of the centromeres on rice chromosomes were located and the correct orientation of linkage maps obtained (Singh et al 1996). Wang et al (2000) also mapped centromeric regions on the molecular linkage map using centromere-associated sequences.

Efforts in developing alien introgression lines continue by using all the wild species that represent the A, B, C, BC, CD, E, F, G, and HJ genomes at IRRI. A series of hybrids and monosomic alien addition lines have successfully been produced through an embryo rescue method, especially after remote hybridization. Several useful traits—such as cytoplasmic male sterility and resistance genes for grassy stunt virus, bacterial blight, blast, brown planthopper, etc.—have been introduced (Brar and Khush 1997). These efforts are urgently needed to broaden the rice gene pool by introgressing genes from diverse sources.

Rice B chromosomes were found in a rice aneuploid variation among the progenies of triploid Zhongxian 3037 (Cheng et al 2000). Molecular markers on all 24 arms of the rice chromosomes did not show any dosage effects; therefore, B chromosomes might not have originated from any A-chromosome fragments.

Advances in new technologies in rice chromosome research

The use of imaging technologies in the construction of the rice somatic chromosome map and pachytene chromosome map, cloning of functional domains of rice chromosomes or centromeres, precise measurement of genome sizes of several rice species by flow cytometry, and other technologies have been reported in the last few years. Uozu et al (1997) reported genome sizes of several rice species and revealed a strict relationship between genome size and chromosome morphology. A quantitative chromosome map of indica rice (IR36) has been constructed by means of image analysis methods. Imaging methods have been shown to be effective to quantitatively develop the rice somatic chromosome map (Fukui and Iijima 1991). The third-generation image-analyzing system, CHIAS 3, has been developed. Furthermore, software for the analysis of pachytene chromosomes is being developed. Based on the preliminary analysis of rice chromosome 9 with a nucleolar organizing region (NOR), large differences have been found among three maps prepared from somatic chromosomes, pachytene chromosomes, and a molecular map, especially at the satellite regions. The result indicates that different maps contain different biological information and that integration of these maps is important.

Rice centromeric sequences are being analyzed to produce a rice artificial chromosome. All the rice chromosomes have an *Arabidopsis*-type telomere at the ends of the chromosomes. Cloning of a functional centromere is an important step toward constructing rice artificial chromosomes. Nonomura and Kurata (1999) cloned a 264-bp sequence (RCS1516) by PCR using CENP-B box-like sequences (CBLS). They characterized the structure of a 14-kb centromere sequence in the rice genome that includes 1.9-kb direct repeats.

In conclusion, rice chromosome research has developed rapidly, introducing new technologies and giving new results. We anticipate that rice chromosome research will become more advanced and will provide indispensable information for rice breeding, genetics, and genome research.

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Achievements in rice cytogenetics

Hsin-Kan Wu and Ming-Hong Gu

Rice chromosomes are so small that it was difficult to peruse any cytogenetic data before 1960. In 1978, Dr. Kurata of Kyushu University invented a technique that revealed rice somatic prometaphase chromosomes with clear-cut centromere position and a total length varying from a few to more than 10 microns. This technique, in combination with that for pachytene chromosomes explored in 1987, yielded many basic results: (1) the karyotypes of several cultivated and wild rice species showed specific differences, (2) several sets of rice translocations were identified and used to correspond the genetic linkage groups to chromosomes identified in the karyotype and to map morphological marker genes, and (3) two series of rice primary trisomics have been examined. The invention of rice chromosome techniques can be regarded as the first milestone in rice cytogenetics and the second was the construction of a molecular linkage map in 1988. The designation of the map was based on an approved agreement in 1990 at IRRI. The molecular maps have been expanded and a new saturated map, consisting of 2,300 DNA markers, has been released in Japan. These maps are the basis for mapping of genes, marker-assisted selection, comparative genomics, map-based gene isolation, and the like. Mapping of rice single-copy markers or genes has lagged behind that of repetitive sequences. Fluorescent *in situ* hybridization (FISH) with nonisotope probes was found superior to that with isotope probes. Achieving reliable signals of a single-copy gene below 10 kb has remained difficult. However, bacterial artificial chromosome (BAC) clones containing a target single-copy gene produce good-quality signals on rice chromosomes. Thus, FISH with BACs can be used to locate single-copy genes. Its future use in rice is promising, especially when detection schemes with high resolution are adopted. As the complete rice genome sequence becomes available, the use of functional genes would be substantially facilitated on the basis of cytogenetic knowledge accumulated in the past few decades—from the chromosome to the DNA level.

Rice chromosomes are so small that it was difficult to peruse any conventional cytogenetic studies, except for counting the number of chromosomes specific to a species. Shastry et al (1960) first suggested the use of rice chromosomes at the pachytene stage to number chromosomes in descending order. However, the order was found to be substantially different from that found in three other laboratories (Oka and Wu 1988). Seven years later, Wu (1967) explored the double mordant technique for rice pachytene chromosome preparations. When this technique was used in combination with that invented by Kurata and Omura (1978) for mitotic prometaphase chromosomes, many useful results were obtained.

- The karyotypes of several cultivated and wild rice species were analyzed (Wu and Li 1964, Kurata and Omura 1978, Chen and Wu 1982, Chung and Wu 1987, Chung et al 1993a). Variation was specific to the species. In general, the 12 pairs of cultivated rice can be well recognized. Chromosome 4 is unique in morphology, with a heterochromatic short arm and the biggest long arm to short arm ratio (L/S). Chromosomes 8 and 10 are the nucleolar chromosomes (in the case of indica; there is only one in japonica, chromosome 8) attached to the nucleolus with their short arm ends. Chromosome 10 is more metacentric. Undoubtedly, the defined karyotypes are the basis for identifying rice translocations and trisomics and for physical mapping of rice genes.
- Several previously reported rice translocations (Nishimura 1961) were identified. These lines have been used to relate rice genetic linkage groups to chromosomes and to map rice genes (Iwata and Omura 1971a,b, Chung and Wu 1994, Sato et al 1973, 1975, Chen et al 1982).
- At least two series of rice primary trisomics, one in indica and one in japonica, were produced. The identification of the extra chromosomes involved in the two trisomic series was respectively reported by Khush et al (1984) and Iwata and Omura (1984). However, because of differences in chromosome preparation technique, discrepancies occurred among the identification groups (Khush at al 1984, Kurata 1988, Chung and Wu 1987, Wu and Chung 1988) of