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Introduction

The age of classical cytogenetics has, however, been largely superseded by the implementation of DNA techniques during the past few decades. *In situ* hybridization (ISH) is now recognized as an important technique in many areas of molecular biological research and its associated clinical studies. The technique is used to locate the physical position of a known DNA sequence on a chromosome. In this technique, treating the cells that have been squashed on a slide denatures DNA within the cell. The squashed cells can then be incubated in a solution of labeled DNA, whose position on a chromosome, we are interested in knowing. Repeated or unique DNA sequences, isolated from an organism or artificially synthesized, can be utilized as radioactively labelledor biotinylated probes for a study of the location of these sequences on the chromosomes.

FISH and GISH Techniques

In a modification of *in situ* hybridization technique,a fluorescent molecule is deposited at the site of *in situ* hybridization. The sites located will exhibit fluorescence and can be photographed with a fluorescent microscope. Thus, precise physical location of genes or DNA can be visualized on chromosomes. The technique is popularly described as FISH (Fluorescence *in situ* hybridization). The advantages of FISH over ISH are faster detection, higher resolution, sensitivity and speed. There has been considerable refinement in the technique sinceits development in the area of human molecular cytogenetics about a decade ago. A variety of probe- labelling schemes are now available for simultaneous detection of two or more sequences in the same nucleus.

There are two methods for multicolour FISH. The indirect method uses biotin, digoxigenin and dinitrophenol (DNP) as reporter molecules. They are detected by fluorochrome-conjugated avidin or antibodies. Fluorochrome-labelled nucleotides are used for probe labeling

in the direct method. The direct coupling of reporter molecules like fluorochromes to probes eliminates the need for immunocytochemical detection. Thus, the direct method has two advantages over indirect method, i.e., better resolution and speed. DNA sequences using only three fluorescent dyes by labelling a probe with more than one hapten and detecting with more than one fluorochrome. Using three haptens (single, double, and triple labelling) and three fluorochromes, in principle, a total of seven probes should be resolvable. Using variable ratios of each hapten could further increase the total number of probes, which could be detected. When total genomic DNA (consisting of the entire nuclear DNA of a plant species) is used as a probe in hybridization experiments to chromosomal DNA *in situ*, the technique becomes known as GISH (Genomic *in situ* hybridization). Repeated sequences, which comprise 40-95% of the genomic DNA in higher plants reanneal more rapidly than the unique sequences of the genome. Genomic hybridization method examines the inter-species distribution and organization of these sequences. It involves extraction of genomic DNA from one of the species of interest, for use as a probe by either Southern hybridization to DNA digests or *in situ* hybridization to chromosome preparations from the species or hybrids being studied. Many of the DNA sequences within the two or more genomes under investigation may be sufficiently different so that genomic probing discriminates them.

Applications of FISH and GISH

Some very useful studies have been conducted utilizing these techniques both in animals and plants. Initially, studies made involved repeated DNA including sat-DNA from *Drosophila* and mouse. The application of *in situ* hybridization techniques in plants has lagged behind compared to its use in mammalian cytogenetics. However, they are now finding increasing application in plants, especially in the breeding programmes. Although the first published work was within *Tritiaceae*, the method has been successful in other families of both monocotyledons and dicotyledons. Some of the applications of these techniques are cited below.

Chromosome Mapping

The utilization of *in situ* hybridization technology is of particular interest to those engaged in chromosome walking or genome mapping projects. FISH has been utilized in many plants to identify chromosome accurately, using species-specific repetitive sequences, ribosomal genes and even unique sequences. Because of their universal occurrence and redundancy, the ribosomal genes are of great value for karyotype analysis and comparative studies of genome organizations. FISH techniques using florochrome allows the visualization of multigenic families, such as 5S and 18S-5.8S-26S ribosomal RNA genes for their location on chromosomes. Physical localization of multicopy gene families, such as 5S and 18S-26 rRNA genes have been reported in wheat, tomato, barley, garlic and in *Aegilopes umbellulata*. In cotton, multicopy genes were mapped on specific chromosomes in meiosis. Recently, FISH been used for the physical mapping of ribosomal genes, microsatellite and transposable DNA sequences on sugar beet chromosome.

A digoxigenin-labelled 5S rDNA probe containing the 5S rRNA genes and the adjacent intergeneric spacer was used for *in situ* hybridization to metaphase and interphase chromosomes of a trisomic stock from sugar beet. Three chromosomes of primary trisomic line IV revealed the signals close to the centromere. Polymorphism of 5S rDNA repeats in a segregating population was used to map genetically the 5S rRNA genes within a cluster of markers in linkage group II of sugar beet. The concentration of genetic markers around the centromeres presumably reflected the suppressed recombination frequency in centromeric region. The correlation of physical and genetic data allowed the assignment of a linkage group to sugar beet cc IV according to line of the primary trisomic.

Genome Analysis (GISH)

Genomic *in situ* hybridization (GISH) permits characterization of the genome and chromosome of hybrid plants, allopolyploid species and recombinant breeding lines. Thus, the ancestry of hybrid and polyploid species can be elucidated by genomic southern and *in situ* hybridization. In essence, the analysis involves hybridization of labelled genomic DNA from suggested ancestors or relatives to chromosome spreads or southern blots of DNA from the species under investigation. Hybridization strength, uniformity, and presence of positive or negative bands are then assessed to indicate relationships. Traditionally, genome relationship was analyzed by study of chromosome painting but there may be several limitations of chromosome pairing. The amount of pairing not only depends on the degree of homology between the pairing chromosomes but also on genetic and environmental factors. Multicolour FISH (mFISH) using total genomic DNA probes is a promising approach for simultaneously discriminating each genome in natural or artificial amphidiploids. It uses various fluorescence dyes to represent different painting probes at the same time. Moreover, this technique is powerful tool for investigating genome homology between polyploid species and their diploid progenitors. Using fluorescent probes produced by shearing the total genomic DNA of a particular progenitor species, it may be possible to identify all chromosomes belonging to a particular genome of the amphidiploids.

Phylogenetic Relationship

GISH offers new opportunities in phylogenetic and taxonomic studies for determining and testing genomic relationship of wild and cultivated plant species. It gives unique information about similarities between DNA from related species. Furthermore, it provides data about the physical distribution of sequences, which are common or differ between the species being probed and the species used to supply the probe DNA. Together, the information can be used to support and develop theories aboutphylogenic, hybridization, and diversification of plant species. Since plant breeding involves genomic reconstitutions, these informations help to plan Analysis of Somaclonal Variations Somaclonal variations arising in tissue culture have been looked upon as a novel source of genetic variation for crop improvement.

Tissue culture phases impose stress, and induce instability (chromosome breakage and the DNA transposition) leading to karyotyping changes. Genetic instability may be associated with the fraction of repeated sequences of DNA present in the plant genome. Analysis of genetic variation in the regenerated plants is necessary for identification and utilization of the proper somaclonal variation for crop improvement. Examination of the chromosomal distribution of 5S and 18S-26S rRNA is useful in identifying the types of genomic changes that might occur during *in vitro* culture.

Physical map showing the localization of 5S and 18S-26S rRNA genes was constructed by bi-colour FISH in amphidiploid *Allium wakegi* cultivar and an amphidiploid tissue culture regenerant[10]. A rhodamine labelled 5S rDNA and a biotin labelled 18S-26SrDNA were used as probes. The signals of 5S rDNA were detected on the intercalary region of short arm in chromosomes 9 (two region) and 15 (one region). The signals of 18S-26S rDNA were detected on the terminal region of short arm of chromosome 10 and at same regions of chromosomes 6 and 14 including the satellite. In an amphidiploid regenerant, homologous chromosomes were identified by chromosomallocalization of rRNA gene families.

Detection of Alien Chromatin

Interspecific and intergeneric crosses have been made in plants with the aim of transferring desirable traits, such as disease and pest resistance from wild or related species into cultivated species. Following hybridization if the donor has at least one genome in common with the recipient, and then the recombination between the homologous genome in common with the recipient, then recombination between homologous genome can readily take place and, through several cycles of backcrossing and selection, the desired trait can be transferred. If the donor and the recipient genomes are not homologous, the preferred method of handling such hybrid is to continue backcrossing and chromosome screening to produce series of addition or substitution lines of the genome of the donor parent. Chromosome medicated alien gene transfers through hybridization have resulted in the genetic improvement of many crops. Recently, development of direct gene transfer methods have further helped to engineer genes of importance into crops.

In plant breeding programme, alien chromosome, chromosome segments, and genes can be identified and characterized by FISH and GISH. They can be visualized and counted in wide hybrids andamphidiploids, not only in high quality metaphase spreads, but also within interphase nuclei. Subsequently, alien chromosomes can be followed through backcrosses and recombinant lines. FISH technology has been used to identify partial amphidiploids derived from crosses of wheat with *Thinopyrum intermedium* and *Lophopyrum elongatum* with the resistance to BYDV and wheat streak mosaic virus.

Detection of Chromosomal Aberration

The identification of structural abnormalities by routine and high-resolution cytogenetic studies plays an important role in diagnosis and treatment of disease. However, this analysis is

relatively gross and only permits the visual diagnosis of aberrations ofsingle chromosome bands on the order of seven million or so base pairs. ISH technique has felicitated the diagnosis and identification of chromosomal aberrations particularly for human and animal chromosomes. Using chromosome-specific DNA libraries, it permits the identification of small chromosome aberrations, which are not readily detected by standard high resolution banding techniques. Therefore, this technique may be used in prenatal and postnatal cytogenetic studies. For example, women who have an increased risk of carrying abnormal fetuses can undergo cytogeneticanalysis of fetal cells to rule out chromosomal aberrations but it requires time. In that case, FISH can provide a rapid and accurate identification for the most common autosomal trisomics and sex chromosome abnormalities. With mFISH analysis all 24 human chromosomes can be hybridized using fluorochrome labelled chromosome specific DNA libraries. The advantage of this staining method is the demonstration of structural aberrations, which cannot be detected by conventional staining techniques. The STARFISH system is another excellent method for the identification of single human chromosomes, and allows the detection of translocations and insertionson metaphase chromosomes.

Recently developed m Band FISH technique yields high resolution multi-colour banding based on region- specific partial chromosome paints. The use of m Band FISH was tested to determine the inter- and intra-arm chromosome exchanges in human. Altogether, seven overlapping microdissection DNA libraries of chromosome 5 were constructed, 2 within the p-arm and 5 within the q-arm. These techniques may also be used to identify marker chromosomes, clarify translocations, define chromosome duplications, or analyze complex chromosome rearrangements. Analysis at both meiotic prophase and metaphase 1 gives maximum amount of information about genetic relationships between homologous and homeologous chromosomes in a hybrid, or species where there may be duplications by identifying pairing partners at the early meiotic stages. In many polyploid species, there are intergenomic translocations, which are clearly shown by GISH.

Chromosome Organization at Interphase Nuclei

Simultaneous visualization of total genomic and highly repeated DNA as probes is also useful for investigating chromosome organization in the interphase nucleus, orientation of telomeres and centromeres, spatial location of individual chromosomes, and the relationship between chromatin decondensation and gene expression. The structural organization ofinterphase nuclei using a range of examples from the plants, animals and fungi and showed nuclear organization to be an important phenomenon in cell differentiation and development. FISH was used to simultaneously visualize specific chromosomal regions and functional nuclear domains and to elucidate the relationship between specific chromosome arrangements and nuclear functions especially the extent to which changes in higher-order nuclear organization are implicated in the etiopathogenesis of human disease. A few studies have investigated the arrangement of telomeres or centromeres in interphase cells by *in situ* hybridization. Plant telomeric sequences have been cloned from *Arabidopsis thaliana* and tomato, but no plant

centromeric sequences have been cloned. The translocation line of wheat, 4AS-6RL.4AL has a good centromere marker. A tiny segment of rye chromatin is inserted near the centromere of wheat chromosome 4A.

Centromere-specific multi-colour FISH (cenM-FISH) is a new technique that allows the simultaneous characterization of all human centromeres by using labelled centromeric satellite DNA as probes. This approach allows the rapid identification of all human centromeres by their individual pseudo-colouring in one single step. Barley interphase nuclei showed strong polar arrangement of chromosomes with telomeres and centromeres located at the opposite nuclear poles (Rabl-orientation), as shown by two-colour FISH experiments using the barley subtelomeric 118 bp repeat HvTO1 and a BAC containing centromere- specific retroelements and satellite sequences.

Chromosome Specific Painting in Plants

Determination of karyotype based on chromosome size, centromeric index and banding patterns is limited by the similar morphology of chromosomes in many species. FISH or PRINS (primed *in situ* labelling) overcomes these limitations by providing specific labelling patterns useful for discrimination of similar chromosomes. Additional cytogenetic landmarks can be obtained using species- or genus- specific satellite repeats that are often amplified to high copy numbers and form discrete bands or spots on chromosomes. These repeats frequently occur at a higher number of genomic loci and may therefore produce signals characteristic for each chromosome within the karyotype. Successful painting of a specific plant chromosome wthin its own genome. Dissected isochromosomes for the long arm of chromosome 5 of the wheat B genome (5BL) were amplified and used as probes. Hybridization signal data suggested that chromosome and homoeologous group-specific sequences are more abundant in 5BL than in genome specific sequences. FISH had been used to analyse the structure of the rye B chromosome. GISH demonstrated high level of similarity between A and B chromosomes of rye. The B-specific repeat families D1100 and E 3900 were analysed in terms of their physical location and possible contiguity.

These techniques have been useful for the simultaneous mapping of different DNA sequences and genome allocation of genes of interest. The genome probing methods supplement data from other methods of genomic analysis, gives complementary and novel data about genomes and their relationships, including identification of parents or ancestors in unknown crosses or in polyploid species, information about genomic regions which have diversified between species, and enabling clear identification of pairing partners at meiosis and evolutionary translocations between genomes in polyploid and hybrids. Application of FISH to somaclonal variants is a useful tool for identifying and understanding chromosomal changes during the tissue culture process.

However, there are some limitations of these techniques. Multicolour FISH can only be used successfully on polyploids with at least one known progenitor species. Closely related genomes in certain allopolyploids cannot be discriminated using GISH technique. Multicolour FISH is less sensitive and shows a lower degree of detection resolution thansingle colour FISH due to multiple exposure photographs. Advances in mammalian genetics involving the use of techniques outlined above provide promise for future progress in plant molecular cytogenetic research. There is no doubt that the applications of these techniques will multiply in coming years and enable the investigation of even more difficult problems of biology.

Reference:

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