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Review

High resolution free chromatin/DNA fiber fluorescent in situ hybridization

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Abstract

High resolution chromatin/DNA fiber fluorescent in situ hybridisation (FISH) is a powerful system for physical mapping and genome research. With direct visualisation of molecular probes along released chromatin or DNA fiber, fiber FISH has become the method of choice to order genes or DNA markers within chromosomal regions of interest. Combined with DNA-protein in situ codetection fiber FISH shall play a more important role for analysis of genome function. In this paper the concept and technical developments of fiber FISH are reviewed with the emphasis of comparison on the various protocols. Future challenges are also discussed along with the highlights of the successful applications achieved by fiber FISH methodology. © 1998 Elsevier Science B.V.

Keywords: Fluorescent in situ hybridization (FISH); Physical mapping; Chromatin fibers; DNA fibers; Chromosomes; Gene mapping; Genome analysis; Detection; Reviews

Contents

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1. Introduction

Fluorescent in situ hybridization (FISH) technology is one of the most powerful and versatile experimental systems to be developed in recent years. It combines the accuracy of molecular hybridization with fluorescence tagging and the classical description of cytology to provide direct and precise research tools for gene mapping, chromosomal structure study and genome analysis [1]. The original in situ hybridization system was established by Gall and Pardue almost 30 years ago using radioactively labelled probes to detect DNA targets within nucleoli [2]. Various repetitive probes have been used successfully since then [3–5]. Since the development of single copy gene detection a decade later, in situ hybridization has become an important tool for human gene mapping [6,7]. Despite its success, conventional isotopic in situ hybridization technology has great limitations in terms of time and the ability to precisely localize targets due to the use of photographic emulsion and the high background caused by the sensitive isotopic probes. Fluorescence tagging was developed to replace isotopic labelling. In this new detection system, the probe is labelled with biotin or other molecules before hybridization to target DNA. Since these molecules are then tagged with fluorescent markers to visualize specific sequences in situ, the technique is called fluorescent in situ hybridization (FISH) [8–14]. The high resolution FISH mapping discussed in this paper performs FISH detection on released, less condensed, and linearized chromatin or DNA fibers, generating ultra high resolution mapping data compared with conventional chromosome-based FISH mapping. This novel approach also provides an ideal tool for chromosome and genome structure studies [15–21].

2. Why fluorescent in situ hybridization?

With its simplicity and its capacity for allowing direct visualization, FISH has become the methodology of choice for current genome and medical research [1,12–14]. It is appreciated the most when combined information is needed both from molecular biology and cytology. Some of these research aspects are listed in Table 1 [1].

3. Why high-resolution FISH?

With the rapid development of human genome project, the density of mapped genes along each chromosome is increasing dramatically. To group these genes and to order them within the same chromosomal region, and particularly, to construct a fine integrated genetic and physical map a high-resolution cytogenetic anchor map is essential. In addition, it appears that there are gaps in the current physical maps due to the unstable or ‘unclonable’ region related to current cloning methodology. A straightforward high-resolution approach is needed to define the size of gaps, to order contigs within gaps, and to provide quality control during map-sequence-assembly step of large-scale DNA sequencing [21,22]. The resolution of chromosome-based FISH mapping, however, is limited to approximately 1–3 megabases (Mb), which makes it difficult to achieve this goal. Obviously, the need to improve mapping resolution is a key issue for extending the capability of FISH to the analyze genome structure and to contribute more to the genome project.

4. Concept of high-resolution FISH

The resolution of FISH depends on the target of hybridization. The DNA molecule is packaged to approximately 10 000 times compaction in the metaphase chromosome. Thus, it is extremely difficult to determine the order or even the physical distance among DNA fragments within a 1–3 Mb using such a structure. Less condensed prophase chromosomes or elongated chromosomes have been used recently to increase mapping resolution [23,24]. Higher resolution can be obtained by using the much less condensed chromatin of interphase nuclei [12–14]. Similarly, surface-spread pachytene chromosomes as well as interphase pronuclei have been used as targets to achieve high-resolution FISH [25,26].

The use of released chromatin fibers for FISH mapping takes advantage of the removal of the spatial arrangement of the chromatin in the nucleus [15,16]. While the three-dimensional organization of the chromatin is reduced to two dimensions in the linear fiber, the physical mapping information is preserved. Mapping on chromatin fibers not only

Table 1
FISH applications

A	*Detection of chromosomal numerical and structural aberration*
	Clinical diagnosis: trisomy, translocation, deletion, duplication and inversion
	Cell line characterization
	Marker chromosome identification
	Expanded trinucleotide repeat detection
	Whole genome scan by comparative genomic hybridization
B	*Gene/physical mapping*
	Single fragments of DNA mapping to correlate gene loci to disease related phenotypes
	Gene cluster/families/function-related-genes
	Ordering DNA markers/probes
	Large scale physical mapping with chromosomal region/whole chromosome/entire genome
C	*Nuclear architecture/interactions among chromatin domains*
	Organization of interphase chromatin
	Distribution patterns for centromeric/telomeric region
	Chromatin domain position and gene expression
D	*Chromosome structure and behavior study*
	Repeat sequences and chromosome banding
	High order structure of the mitotic/meiotic chromosomes
	Organization of repetitive sequences at the centromere
	Telomere structure and function
	B Chromosomes, ring chromosomes, micro-chromosomes and double minute chromosomes
	Chromosome pairing, synapsis and segregation
E	*Studies of biological processes*
	DNA replication
	RNA processing
	Gene amplification
	Gene integration
	Gene expression/regulation
	Chromatin elimination
F	*Chromosome evolution*
	Interspecific conservation of specific sequences
	Syntenic conservation
	Chromosomal fusion and division
	Karyotypic analysis of allopolyploidy

provides maximum mapping resolution, it also simplifies data presentation comparing with interphase FISH mapping, especially when used to analyze large areas of the genome with a number of markers. This approach is also very useful for studying chromosome and genome structure, for it opens a window to directly visualize DNA and protein interaction at various level of condensation, allowing one to trace the process of high order structure formation.

Certain reagents in chromosome preparations of human blood cultures can increase the frequency of 'rope' and 'spindle'-like chromatin, which are chromatin released from the more familiarly packaged chromosomes and interphase nuclei. These structures have been known at various terms as 'free chromatin', 'sister unit fiber' and 'uncompleted-packing-mitotic figure' [27–32]. In 1987, these fibers were recognized as candidates for in situ hybridization mapping [33]. However, due to their unconventional morphology, the fibers were suspected to be non-chromatin artifacts generated in the slide preparations and were ignored for a few decades. Questions were raised about the legitimacy of these fibers and its application for gene mapping, including whether or not the gene order can be preserved on the released chromatin fiber. Can FISH be effectively performed on extended chromatin/DNA structure?

Which method should be used for releasing chromatin? It was not until the Human Genome Project generated a demand for novel methodologies able to perform high-resolution gene mapping that interest in the free chromatin fiber was resurrected.

The high-resolution FISH mapping on released free chromatin fiber was introduced by our laboratory in the early 1990s [34]. This work demonstrated the feasibility of FISH detection along released free chromatin fiber and opened up the possibility of using even more decondensed DNA fibers to order genes or DNA fragments.

5. Methodology development for fiber FISH

The original protocol for releasing chromatin fibers was based on the observation that certain chemical reagents could interfere with the chromosomal condensation process and 'free' chromatin fibers from interphase nuclei [27–32]. The first demonstration of FISH on chromatin fibers used fibers released from human–hamster hybrid cell to trace the fate of human chromatin in hamster background. The FITC-highlighted human chromosome 7 appeared as a single, continuous extended chromatin fiber distinct from the hamster chromatin. These data, plus the results of high-resolution FISH detection using alpha satellite repeats specific to human chromosomes 7, 11 and Y, clearly demonstrated the power of extended chromatin fiber mapping [34].

Further modifications immediately followed, which simplified the releasing of the fiber and improved the resolution. To establish a protocol suitable for all type of cultured cells, alkaline releasing buffer was introduced to open the nuclear envelope since nuclear membrane is unstable under high pH condition [35]. Released chromatin fibers were used to map single copy probes in the cystic fibrosis gene region, within which the physical distances among probes were known by restriction mapping. There was a nice correlation between the expected and observed physical distances in free chromatin FISH mapping.

An alternative approach was also established by using FISH detection on DNA 'halo' preparations [36]. When nuclei are treated with detergent and high salt, released DNA loops are visualized as a halo surrounding the nuclear matrix after DNA-specific staining [37]. These DNA loops can be used as targets for high-resolution FISH. Since the released DNA loops are still attached to the nuclear matrix, the range of mapping is somewhat limited by the size of the loops. In addition, the loop size varies and may not be evenly distributed throughout the genome. Therefore, it may be difficult to study certain regions using the halo preparation. Further, it has been shown that releasing behavior of specific DNA loops is related to the activity of gene expression [38]. Such function-related variation may not be the best system for physical mapping.

More straightforward releasing methods were introduced by different groups based on the use of alkaline solution, nonionic detergents, sodium dodecyl sulfate (SDS) and with or without mechanical force to stretch chromatin or DNA on glass slides [24,39–43]. Protocols developed by Windle's group and Sheer's group are relatively popular due to their simplicity of DNA fiber preparation [39,41]. Different from previous protocols which maintain the chromatin structure at a certain degree [35,36], these two methods use force to stretch the DNA molecule to its maximum length regardless of the chromatin structure. After releasing DNA by lysing the nuclei with SDS, the movement of an aqueous drop of DNA across a glass slide can pull the DNA molecules into a long stream [39]. Alternatively, DNA fibers can be released from fixed cells by lysis in alkaline conditions and subsequently linearizing the DNA by mechanic stretching with the edge of coverslide [41]. In theory, all these protocols should generate linearilized-naked DNA after cell lysis and disassociation of chromatin proteins. In practice, however, nonuniform chromatin/DNA fibers occur at various frequencies. Fortunately, probes themselves can be used as internal controls to judge the degree of release of the DNA fibers.

To obtain more control in the cell lysis process and to reduce the interference from each lysed cell, cells were agarose-embedded and lysed with proteinase K to ensure that the released long DNA was well preserved in the agarose. After melting the agarose block by microwave irradiation, the DNA fibers were extended mechanically on slide by drawing the edge of another microscope slide across

the one on which the released DNA was ready for linearization [44].

In contrast to previous protocols of linearization of chromatin and DNA fiber from nonfixed and fixed cells, the protocol of 'molecular combing' used isolated DNA molecule to generate linear DNA fibers rather than lysed cells. With this protocol, DNA in solution is linearized by binding one end of the DNA molecule to a silanated glass slide. The DNA molecules are then stretched during evaporation of the solution [22,45,46]. It has also been shown that this procedure is useful for determining the distances on extended genomic DNA [47]. In fact, before the invention of molecular combing, the isolated DNA molecules were stretched on a solid surface and cut by restriction enzyme. This protocol is refereed to as optical mapping, [48,49] another example of using released linearilized structure for physical mapping.

The key difference between the high-resolution FISH protocols is the method of release and the linearization of the chromatin or DNA fiber. FISH detection is essentially the same as chromosome-based FISH. Various names have been used to describe released chromatin or DNA fibers generated by different protocols including free chromatin, free DNA, extended chromatin fiber, extended DNA fiber, elongated chromatin or DNA fiber, DNA halo, single molecule, and individual stretched DNA molecules. Recently, it has been suggested that all high-resolution chromatin and DNA fiber FISH methods (summarized in Table 2) can be simply referred to as fiber-FISH [19].

In practice, it is necessary to differentiate between 'free chromatin fiber FISH' and 'DNA fiber FISH' to facilitate the interpretation of the mapping data. 'Free chromatin' should be used to describe artificially released chromatin fibers to differentiate them from the nonrelease interphase 'chromatin fiber' commonly referred to in the literature.

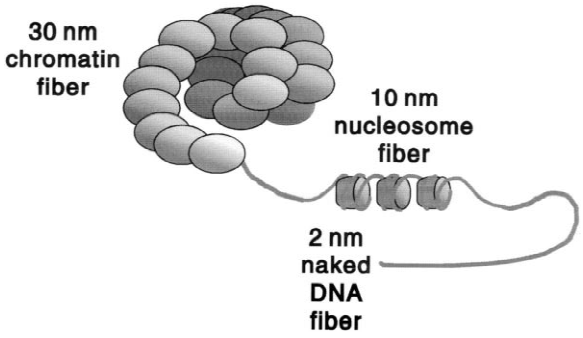

Fig. 1. Relationship between the free chromatin fiber, the nucleosome fiber and the naked DNA fiber. Each represents a change in the level of chromatin packaging where the DNA fiber is the least condensed. When the DNA fiber accumulates histone proteins, it condenses to the nucleosome structure. This nucleosome fiber becomes even more condensed as it coils on itself, forming the chromatin fiber.

DNA fibers can be generated from various types of cells including noncultured cells and sperm from species varying from yeast to human [50–53].

Free chromatin fibers have a width of 30 nm. When stripped of some proteins and reduced to their nucleosome structure, these fibers have a width of 10 nm and are referred to as 'nucleosome fibers'. When all nucleosomal histones are removed, only the 2-nm wide naked DNA fiber remains (Fig. 1).

Originally, free chromatin fibers were produced by alkaline treatment [35]. However, this treatment also

Table 2
List of fiber-FISH methodologies

Name of fiber	Method of release	Structural basis	Reference
Free chromatin fiber	Inhibitor of topo II	30-nm chromatin	[34]
Free chromatin fiber (+DNA)	Alkaline releasing	30-nm chromatin+DNA fiber	[35]
DNA halo	High salt	DNA fiber+mixed chromatin	[36]
DIRVISH	SDS	DNA fiber+mixed chromatin	[39]
Optical mapping	Molten agarose gel	Naked DNA	[48]
Extended DNA	Lysis+histone depleting	DNA fiber+mixed chromatin	[40]
Free DNA fiber	Mechanic force	DNA fiber+mixed chromatin	[42]
DNA fiber	Lysis in gel block, mechanic force	DNA fiber+mixed chromatin	[44]
Molecular combing	Water–air phase interaction	Naked DNA+broken DNA	[45]

resulted in the release of DNA fibers. Other protocols developed to strip the chromatin to DNA fibers also produced fibers with a range of decondensation between free chromatin fiber and naked DNA (Table 2). It was therefore necessary to build into the methodology a series of internal controls to determine the nature of fiber in question. To this end, probes of known length are used to indicate the degree to which the fiber has been stripped. Since at least two probes are used to determine physical mapping distance, the morphology of the test probes themselves can also indicate the degree of release of the fiber. For example, when a cosmid probe hybridizes to a chromatin fiber, the signal is a spot. However, a linear signal is produced by the cosmid probe on a DNA fiber. Thus, since the size of the probe is usually known, the length of the probe signal can be used to gauge the degree of stripping of the fiber as well as the physical mapping distance between the two markers. The known probes can service as reference probes.

6. FISH detection on fibers

While fiber preparation is the main difficulty in fiber FISH technology, attachment of the fluorescent probe needs care as well. For fiber FISH, DNA denaturation times must be extended from the normal 1–2 min to as long as 4–5 min. Since there are no chromosomal morphological features that can be used to distinguish true signals from background, it is helpful to use multiple color FISH labelling to detect different probes. For this same reason, reference probes are worth using if available.

Specialized equipment may be necessary for FISH mapping on fibers. Faint signals over a wide range of distances may require the use of a highly sensitive charged-couple device (CCD) camera, and in many cases, quantitative analysis and image processing is required.

7. Applications of high-resolution fiber FISH

To illustrate the utility of fiber FISH technology in both physical mapping and chromosome/genome structure studies, four types of applications are summarized below:

7.1. Physical mapping

Fiber FISH technology was originally designed for high-resolution physical mapping which can bridge the gap between cytological and molecular approaches. Cytological studies of metaphase chromosomes can map probes of a few megabases while molecular sequencing and Southern blotting can be used to analyze a few kilobases. Fiber FISH mapping handles intermediate distances since it is able to distinguish two probes separated by 1 kilobase (kb) on a DNA fiber or to cover a few megabases on a free chromatin fiber.

The accuracy of fiber FISH mapping has been confirmed by restriction mapping [44,54,55]. Quantitative DNA fiber mapping experiments have mapped a 49-kb molecule with a precision of 1 kb and a 740-kb strand with an accuracy of 5 kb [46,47].

The time required for fiber FISH mapping is significantly less than that for conventional restriction mapping. Under optimal circumstances, fiber FISH mapping can determine the size of the gap between two markers in 1 or 2 days [18]. In certain problematic areas of the genome, fibre FISH may be the only technique which can circumvent the problems caused by certain repetitive sequences, restriction sites and methylation status.

The advantages of fiber FISH makes it of use to confirm and in some cases, redefine the physical map [56,57]. It is particularly useful in defining the size of both the gaps and the overlaps between contigs which facilitates the integrated mapping efforts. The accumulation of physical mapping data generated by conventional approaches is punctuated by many regions in the human genome that have an underrepresentation of genes and probes. Gaps exist in almost all the YAC-based physical maps, in both the gene-rich and gene-poor regions. An estimation of the sizes of such gaps would be helpful in estimating the quality of the map, since a good map should have a minimal number of gaps. To this end, fiber FISH mapping can speed up the assembly of such maps as well as serving as a quality control during the assembly process.

Fiber FISH has also been used in constructing high-resolution physical maps in plants. Extended DNA fibers prepared from interphase nuclei of tomato and *Arabidopsis thaliana* proved to be excellent targets for high-resolution FISH mapping [50,51]. With this method, different sizes of DNA fragments, hitherto restricted to being mapped by conventional molecular mapping techniques, were defined at high-resolution in a very short time.

7.2. Positional cloning of disease genes

Physical mapping is only a step towards the identification of genes. Linkage analysis can assign genes to their chromosomal region within few megabases. However, to clone such genes, it is necessary to convert genetic distances to physical ones by first physically mapping genetic markers and then to estimate the actual distance between them. A high-resolution map is developed as more and more markers are placed within a region along the chromosome.

Not only can fiber FISH be used in the development of such maps, it can also be the means of identifying or excluding genes or chromosomal regions directly involved in a specific biological process. For instance, if a critical region is defined by two closely related markers, these markers can act as guide posts during FISH analysis on a DNA/chromatin fiber. Simultaneous hybridization of test cDNAs which are differently colored will place the test probes either within the two guide posts or outside of it. In the latter case, the test probe may be excluded as a candidate gene. Specific examples of this type of research abound in the recent literature [54,56,58–60].

7.3. Genome structure studies

Genome structure studies require comprehensive methods which not only generate high-resolution data but also provide wide coverage. High-resolution methodology ensures the mapping quality of each small area. Coverage, on the other hand, allows an overview of a greater region. Since one must study genome structure over a large region but in fine detail, fiber FISH is the tool adaptable to both criteria.

In the study of the mechanism of gene amplification, the repetitive and complex arrangement of amplification arrays makes it both tedious and difficult to characterize the amplified structure using standard cloning and restriction mapping techniques. Fiber FISH allows direct visualization of this structure by using a combination of different probes tagged to variously colored fluorochromes [39]. This multicolored fiber FISH permits the determination of the number of amplicons, the orientation of each amplified fragment within the cluster, the size of the amplified gene and the variation among the different cells and/or generations.

Not only is fibre FISH useful in studying amplification, it is also a powerful approach for deletion analysis of large regions of the chromosome. Florijn et al. have demonstrated this application by mapping two Duchenne muscular dystrophy deletion break points on DNA fibers with an accuracy of 1–2 kb [61]. Thus, fiber FISH allows rapid visual identification of deletions, translocations and duplication in clinical studies. Examples can be founded for mapping translocation breakpoints within a 50-kb region involving Rieger Syndrome [62].

Fiber FISH has proved its worth in tracing the evolutionary conservation or otherwise in genome structure among species. One example is the conservation analysis of syntony between regions of the genome of puffer fish and humans [63]. Fiber FISH comparisons of three genes, cFOS, S31iii125 and S20I15, showed their order to be the same in both species, despite the fact that these three genes are within a 12.4-kb region in Fugu but are stretched over 600 kb in humans. If other regions exhibit this conservation of gene order between these two species, it may be possible to use the puffer fish as a model to clone candidate genes for loci involved in specific human diseases.

Determination of the size and structure of foreign insertions in mammalian genome is of importance in the study of the integrated viral genome, the relationship between these inserts and the host and the impact of integration on the high-order structure of the host chromosome. Fiber FISH has been used to investigate the integrated Epstein–Barr virus (EBV) genome in the Burkitt's lymphoma cell line, Namalwa [64]. Thus, fiber FISH can readily be used to obtain a detailed picture of the viral DNA

structure generated during the integration process. In a similar vein, the insert size of numerous transgenic mice has been established by fiber FISH measurements [65]. In conjunction with information on meiotic chromatin loop size, fiber FISH played an important role in establishing that the pattern of chromatin packaging of both foreign and native sequences is strongly influenced by the position of integration on the chromosome. Sites close to the end of the chromosome are packaged into much smaller loops than regions which are interstitial.

7.4. Chromosome structure and function

Direct visualization of DNA sequences on condensed chromosomes reveals only limited information due to the tight packaging of the chromosome. For example, FISH studies of the major and minor satellites of the mouse centromeric region on metaphase chromosomes produce overlapping signals. However, the precise relationship between these two types of repeating sequences is resolved by two color FISH detection on released chromatin fibers. Such studies show that the major and minor satellite sequences are not interspersed but exist as separate entities [15]. Haaf and Ward also used FISH detection on chromatin fibers to confirm data from PFGE (pulse field gel electrophoresis) that on human chromosome 7, two distinct α-satellite arrays, D7Z1 and D7Z2, are not interspersed but are separated by several hundred kilobases [24]. Similarly, the orientation of repetitive elements, PTRS-25, PTRA 20 and PTRA-25, in the human chromosome 15 centromeric region was determined with fiber FISH [15]. While PFGE produces equivalent results, fiber FISH requires much less time. More examples of this type of analysis can be found in the recent literature on human chromosome 22, the human and chimpanzee Y chromosome and human chromosome 4 [66–68].

In the study of packaging of telomeric sequences at the ends of meiotic chromosomes, fiber FISH analysis recognized length difference in the TTAGGG sequences of mouse, rat and Chinese hamster [64]. In addition, the methodology was sensitive enough to reveal length differences of individual telomeres of the same animal by tagging specific chromosomes with identifying subtelomeric probes.

The identification of replication origins on yeast artificial chromosomes with fiber FISH showed that cloned human DNA in yeast is capable of initiating its own replication [52]. Combined with the detection of BrdU incorporation, chromatin fibre FISH allows the detailed study of the spatial and temporal distribution of DNA replication sites [69].

8. Future challenges

With its capacity for multicolor labelling on chromosomes or DNA fibers at various stages of condensation, FISH has been an invaluable investigative tool [1,35,70]. It has met the increasing demands of studies of genome function which are more difficult than these of physical mapping. This has been accomplished by the ability of FISH to detect combined chromatin/DNA targets at multiple levels of resolution and coverage. Table 3 summarizes some of these targets, the resolution that can be achieved with such targets and an indication of the advantages and limitations of each. With the information presented in this table, one should be able to determine the combination of FISH detection to use in order to answer a specific biological question.

DNA–protein codetection is the latest research tool which incorporates the power of FISH with immunodetection to study chromosome and genome structure [69,73]. Released free chromatin fibers rather than protein-dissociated DNA fibers are the ideal target for this type of research since DNA–protein interaction can be visualized and characterized in situ at a high level of resolution. Thus DNA–protein codetection promises to play an important role in future genome research [1,74].

In order to use fiber FISH methodology correctly, one must understand the structural basis of DNA fibers, nucleosome fibers, free chromatin fibers as well as the intermediate structures that exist between these states of decondensation (Fig. 1). Only by targeting the correct type of fiber can FISH be used most efficiently (Fig. 2). FISH methodology itself requires further improvement in order to increase its sensitivity to detect smaller DNA targets. Small fragments ranging from a few hundred base pairs to

Table 3
Comparison among various FISH detection approaches

Name of target	Coverage	Advantages	Disadvantages	Detailed protocols
Metaphase prophase chromosomes	>2 Mb — whole chromosome	Chromosomal morphology Telomere-centromere orientation Mapping genes on chromosomal bands	Low resolution	[71]
Elongated chromosome	>200 kb — whole chromosome	Telomere-centromere orientation Order probes along chromosome at higher resolution DNA–protein interaction	No chromosomal Morphology	[24]
Meiotic chromosome	300–500 kb	Chromosomal morphology Resolution better than using mitotic chromosomes	Limited material for human	[25]
Interphase	50–200 kb	Study chromatin domain interaction order probes and measure distance	Three dimensions, coverage limited Resolution lower than fiber FISH	[72]
Free chromatin fiber	20–5 kb	DNA–protein interaction broad coverage	Resolution lower than DNA fiber	[16,17]
DNA fiber	1–300 kb	Highest mapping resolution	Coverage is limited Proteins are depleted	[16,18]

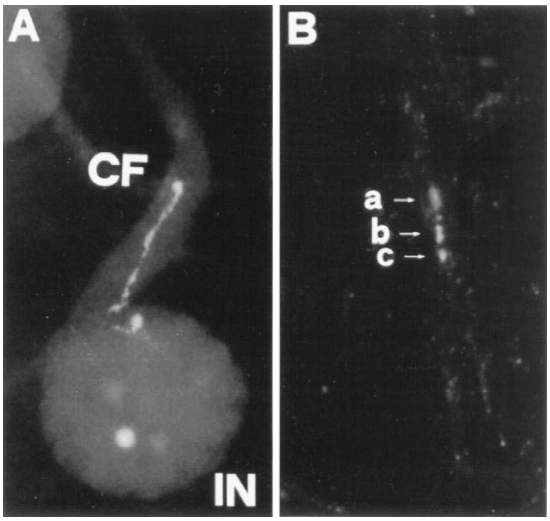

Fig. 2. Comparison of free chromatin fiber FISH and DNA fiber FISH. (A) Free chromatin fiber FISH: the morphologies of FISH signals of a 2-Mb array of human α-satellite DNA. There are two nuclei in this micrograph. The round interphase nucleus (IN) displays two α-satellite DNA signals as round yellow spots. The other nucleus is not intact and has released its chromatin as free chromatin fiber (CF). The α-satellite signal on free chromatin now appears linear. (B) DNA fiber FISH: this micrograph displays FISH signals of three probes on a 50-kb region which are visualized by red (a), green (b) and yellow (c) fluorochrome, respectively.

1 kb can already be successfully mapped on banded chromosomes [71,75,76]. However, there is a need to be able to target even smaller fragments reliably. Recent developments using tyramide-based ultra-sensitive detection methods may present a possible solution to this problem [77]. With a sensitive detection system, fiber FISH will make significant contributions not only to the physical mapping studies in the human genome project but also to the understanding of the high order structure of the chromosome and the genome.

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