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Molecular Cytogenetics Using Fluorescence *In Situ* Hybridization

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MOLECULAR CYTOGENETICS USING FLUORESCENCE *IN SITU* HYBRIDIZATION

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INTRODUCTION

Fluorescence *in situ* hybridization (FISH) with chromosome-specific probes enables several new areas of cytogenetic investigation by allowing visual determination of the presence and normality of specific genetic sequences in single metaphase or interphase cells. In this approach, termed molecular cytogenetics, the genetic loci to be analyzed are made microscopically visible in single cells using *in situ* hybridization with nucleic acid probes specific to these loci [26]. To accomplish this, the DNA in the target cells is made single stranded by thermal denaturation and incubated with single-stranded, chemically modified probe under conditions where the probe will anneal only with DNA sequences to which it has high DNA sequence homology. The bound probe is then made visible by treatment with a fluorescent reagent such as fluorescein that binds to the chemical modification carried by the probe. The DNA to which the probe does not bind is made visible by staining with a dye such as propidium iodide that fluoresces at a wavelength different from that of the reagent used for probe visualization. We show in this report that probes are now available that make this technique useful for biological dosimetry, prenatal diagnosis and cancer biology.

NUCLEIC ACID PROBES

The utility of FISH in enhanced by the increasing availability of probes specific for medically or biologically interesting genetic loci. Two general classes of probes are being used in molecular cytogenetic studies. These target a) DNA sequences that are highly repeated in a limited part of the genome and b) DNA sequences distributed along whole chromosomes or at specific loci associated with genetic disease.

Probes for repeated sequences: These probes target DNA sequences that are tandemly repeated several hundred to several thousand times [4,29,30] near the centromeres of one chromosome type . Thus, the sequence to which the probe is homologous may range in size up to several megabases (Figure 1a). The sequences targeted by the probe are tightly localized so that the hybridization domains that

Figure 1. Panel a. Schematic illustration showing the characteristics of probes for chromosome-specific repeated DNA sequences. Panel b. FISH to a human metaphase spread and an interphase nucleus with a probe for a repeated DNA sequence on chromosome 11. The hybridization domains show as light gray regions and the material counter-stained with the DNA specific dye show as dark gray regions.

result from FISH with these probes are intense and localized in a small area in metaphase spreads and in interphase nuclei. Figure 1b, for example, shows hybridization with a probe for chromosome 11 to human interphase and metaphase preparations. The locations and number of the copies of the chromosome 11 centromere can be quickly and accurately determined by visual analysis of the these preparations. Probes of this type now exist for almost all of the human chromosomes and are used for chromosome enumeration and for detection of numerical aberrations.

Composite probes: These probes are designed to stain entire chromosomes or regions thereof. Figure 2a indicates that these probes are comprised of large numbers of elements with DNA sequence homology to different parts of the target chromosome type. The elements of these probes may target either single copy sequences or sequences that are repeated on the target chromosome type. Typically, these probes are derived from chromosome-specific recombinant DNA libraries [6,7,11,17,23,27]. Figure 2b, for example, shows hybridization with a whole chromosome probe for chromosome 4 to a human metaphase spread. The two

copies of chromosome 4 are clearly visible and uniformly stained. These probes are particularly useful for detection of structural chromosome aberrations and for analysis of the chromosomal origin of the material involved in subtle structural aberrations.

Figure 2. Panel a. Schematic illustration showing the characteristics of composite probes for whole chromosomes or subregions thereof. Panel b. FISH to a human metaphase spread with a whole-chromosome probe for chromosome 4. The hybridization domains show as light gray regions and the regions counter-stained with the DNA specific dye show as dark gray regions.

Probes also can be selected to bind to specific genetic loci [13,18,19,25]. The regions targeted by these probes may be as small as 10 kb in size. However reliable hybridization usually requires probes that target sequences of several tens to hundred of kilobases. These probes are usually selected to reveal specific genetic defects. For example, the probe might target a sequence at the locus 21q22.3 to facilitate detection of a partial trisomy involving this part of chromosome 21 (strongly associated with Down syndrome [18]). Alternately, the probe might target as DNA sequences such as the BCR and ABL genes whose fusion is diagnostic for chronic myelogeneous leukemia [13,25].

APPLICATIONS

FISH has proved useful in several biological and medical areas including biological dosimetry, prenatal diagnosis and cancer biology. Some of these applications are illustrated in the following sections.

Biological dosimetry: The frequency of structural chromosome aberrations (e.g. dicentrics and translocations) has long been known to increase with increasing exposure to DNA damaging agents such as ionizing radiation. Furthermore, the relationship between dose and aberration frequency is well known for several classes of radiation so that an estimate of the level of exposure can be determined by assessment of the frequency of chromosome aberrations in the peripheral blood of exposed individuals. Dicentric chromosome frequencies are typically measured for this purpose since these distinctively shaped chromosomes can be scored rapidly; especially if the centromeres are distinctly stained using FISH with a probe that binds to all chromosome centromeres [20]. Rapid scoring is important in biological dosimetry to allow detection of the low frequency aberrations that may be induced by occupational exposure and/or to facilitate analysis of large exposed populations. However, assessment of the degree of exposure based solely on dicentric chromosome frequency is difficult for chronically exposed populations or at long times after acute exposure because dicentric chromosomes are lost with time. Aberrations such as reciprocal translocations are more stable with time. However, these have proved difficult to score using conventional cytogenetic techniques. FISH with whole chromosome probes facilitates translocation analysis since translocations between a chromosome type(s) targeted by a whole chromosome probe and another nontarget chromosome can be recognized immediately [23,30]. This is illustrated in Figure 3a which shows a translocation between one copy of chromosome 4 (stained by FISH) and another chromosome type. The efficiency with which chromosome translocations can be detected can be increased by hybridizing simultaneously with probes for several chromosomes. In fact, approximately half of all translocations can be detected by using probes for chromosomes 1 through 5. This technique has been shown to be useful for assessment of genetic damage more than 40 years after exposure (Lucas et al; manuscript in preparation).

Prenatal diagnosis: The most common genetic diseases that are detected prenatally are caused by the occurrence of an extra copy of a somatic or sex chromosome. The chromosomes most frequently involved are 21, 18, 13, X and Y. These chromosomes aberrations are usually detected by analysis of banded metaphase spreads. This process, while highly accurate, is time consuming and expensive. FISH with chromosome-specific probes for these chromosomes

Figure 3. Panel a. FISH with a whole chromosome probe for chromosome 4 to a human metaphase spread carrying a translocation involving chromosome 4. Panel b. FISH with a whole chromosome probe for chromosome 21 to a human interphase amniocyte that is trisomic for chromosome 21. In both panels, the arrows indicate the regions targeted by the probe.

facilitates diagnosis of these diseases by allowing analysis of aneuploidy in interphase nuclei [5,12,15,16,18,23.24]. This may eliminate the need for expensive, time consuming cell culture. In addition, it minimizes the effort and skill needed for analysis since the number of chromosomes targeted by the probe is determined simply by counting the number of distinct domains in the interphase nuclei. Figure 3b, for example, shows hybridization with a whole chromosome probe for chromosome 21 to interphase amniocytes from a fetus that is trisomic for chromosome 21. The three domains for chromosome 21 are clearly visible.

Tumor biology: The detection and characterization of chromosome aberrations associated with human malignancies is important because such studies allow identification of genetic changes that may be diagnostically or prognostically informative. In addition, assessment of the specific genetic loci associated with selected malignancies may guide the search for the molecular cause of the disease. The search for informative aberrations is now accomplished by analysis of

metaphase spreads and by molecular analysis of specific loci. Such studies have led to detection of aberrations such as the t(8;14) translocation associated with Burkitt's lymphoma, the loss of 13q14 associated with retinoblastoma and the loss of heterozygosity involving chromosome 17 associated with solid tumor progression. However, these approaches are limited in several important ways. Conventional cytogenetic studies cannot be applied without cell culture. Thus, the results obtained using this approach reveal information only about cells that can be stimulated into mitosis. These cells may not be representative of the whole tumor and sometimes cannot be obtained during therapy. In addition, results obtained by analysis of metaphase spreads cannot be correlated with individual cell phenotype since phenotypic markers are usually lost in mitosis. Similarly, molecular analyses using in vitro DNA amplification or Southern blotting start with bulk DNA isolated from many tumor cells so that genotype-phenotype correlations for individual cells are lost. FISH with chromosome-specific probes extends these studies by allowing analysis of specific loci in individual interphase cells without culture in vitro. For example, FISH with repeat-sequence probes for specific chromosome centromeres has facilitated analysis of aneuploidy in a variety of malignancies including hemopoietic malignancies [1,2], neural tumors [8,21], breast cancer [10], bladder cancer [14] and gastric tumors [28]. Such probes have proved useful for detection of residual leukemic cells after therapy or bone marrow transplantation [3,24,31]. FISH with chromosome-specific probes also has been used to detect specific structural aberrations such as the fusion between the BCR gene normally on chromosome 22 and the ABL gene normally on chromosome 9 that occurs in chronic myelogeneous leukemia [13,25], a chromosome 16 inversion in acute nonlymphocytic leukemia [9], and a deletion on chromosome 8 associated with hereditary spherocytosis [19]. This technique is particularly powerful since it allows analysis of multiple loci in the same cells [22] thereby facilitating investigation of the serial genetic changes associated with disease progression and detection of low frequency residual malignant cells.

CONCLUSION

FISH with chromosome-specific probes now facilitates biological dosimetry, prenatal diagnosis and tumor biology by allowing visual detection of numerical and structural aberrations in metaphase spreads and in interphase nuclei. The power of this approach should increase as the number of locus-specific probes increases as a result of the physical mapping aspects of the international Human Genome Project.

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(a)

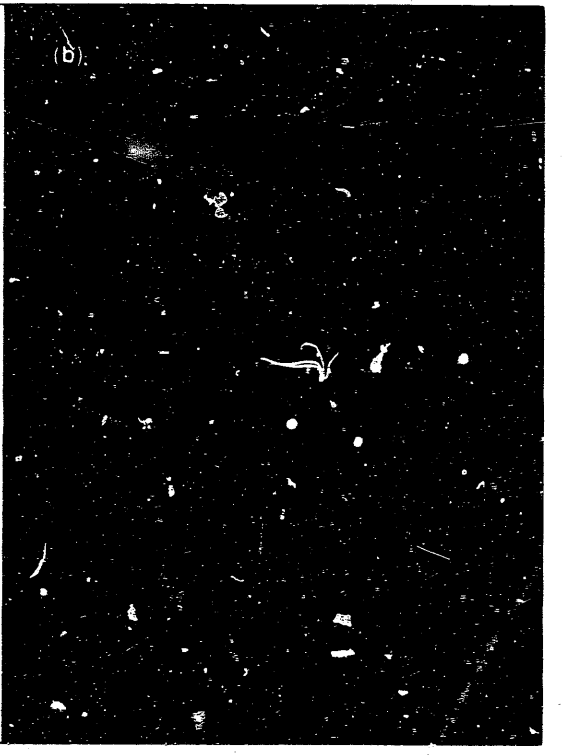
Chromosome-specific composite probe

--atcg--tacc--caat--ggtt--taaa--ggcc-- Target

--tagc-- --ccaa-- --ccgg-- Probe

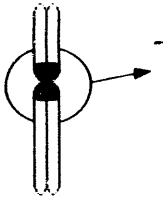
- Probes are distributed along the chromosome
- Hybridization intensity is moderate
- Probes are constructed from chromosome specific libraries

(b)



(a)

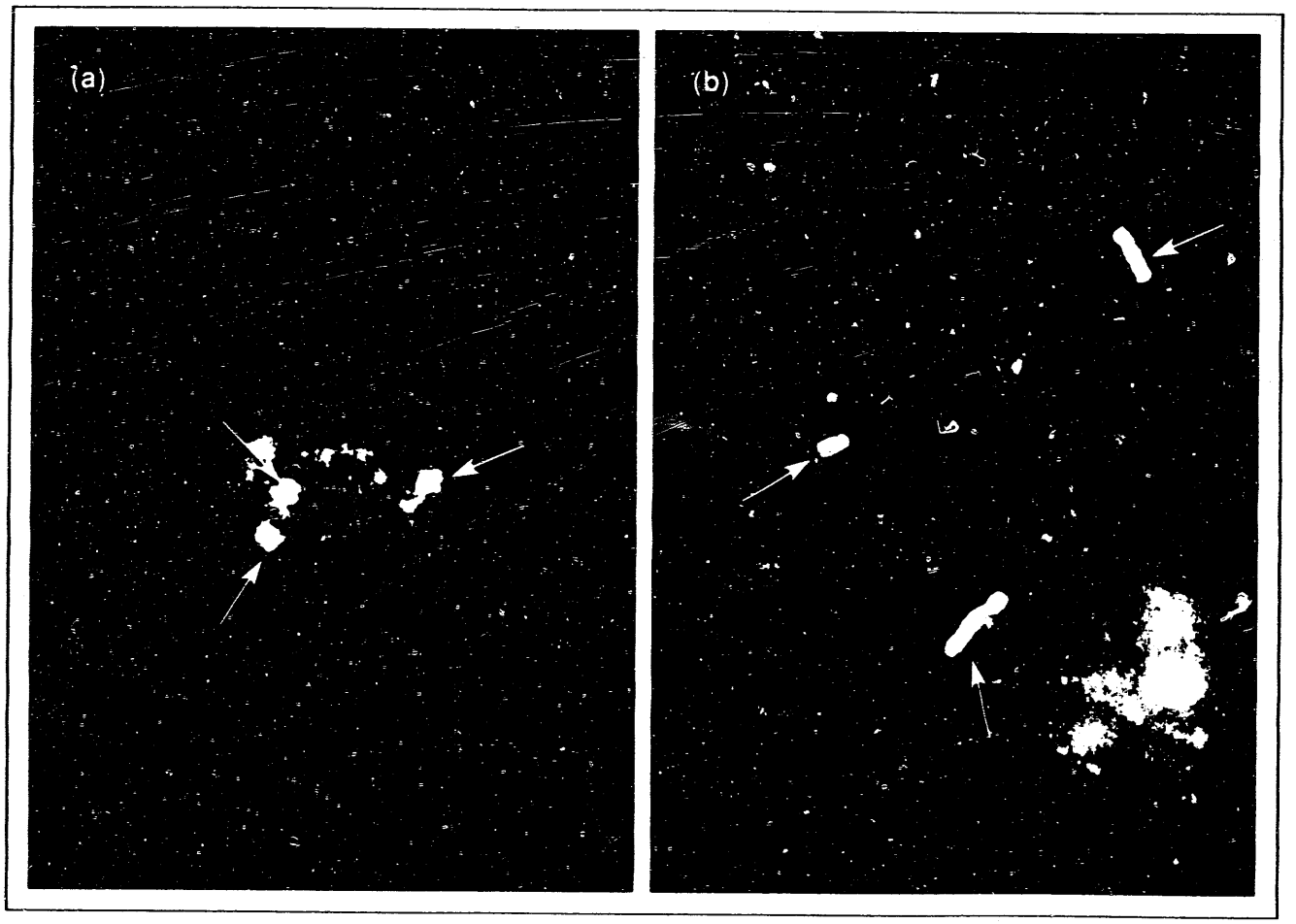
Chromosome-specific repeat sequence probes



--atcg--atcg--atcg--atcg--atcg--atcg-- Target

--tagc-- Probe

- Large target allows intense hybridization
- Target is tightly localized
- Probes available for ~2/3 of human chromosomes



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