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Genetics

Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization

(*in situ* hybridization/biotin labeling/hybrid cells/chromosome-specific staining)

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ABSTRACT **This report describes the use of fluorescence *in situ* hybridization for chromosome classification and detection of chromosome aberrations. Biotin-labeled DNA was hybridized to target chromosomes and subsequently rendered fluorescent by successive treatments with fluorescein-labeled avidin and biotinylated anti-avidin antibody. Human chromosomes in human–hamster hybrid cell lines were intensely and uniformly stained in metaphase spreads and interphase nuclei when human genomic DNA was used as a probe. Interspecies translocations were detected easily at metaphase. The human-specific fluorescence intensity from cell nuclei and chromosomes was proportional to the amount of target human DNA. Human Y chromosomes were fluorescently stained in metaphase and interphase nuclei by using a 0.8-kilobase DNA probe specific for the Y chromosome. Cells from males were 40 times brighter than those from females. Both Y chromosomal domains were visible in most interphase nuclei of XYY amniocytes. Human 28S ribosomal RNA genes on metaphase chromosomes were distinctly stained by using a 1.5-kilobase DNA probe.**

Major advances in cytogenetics have occurred as new methods of distinguishing between chromosome types have been developed. Banding procedures allow identification of individual chromosomes within a species (1, 2), and the G-11 procedure (3) distinguishes human and rodent chromosomes. However, these techniques require metaphase spreads and produce only subtle differences between chromosomes, so that classification is labor intensive and can be accomplished only by highly trained observers. Analysis would be simplified by development of more distinctive chromosome staining procedures, especially if these could be applied to interphase cells. Progress in several areas is now making this possible: (*i*) Development of *in situ* hybridization for sensitive detection of specific nucleic acid sequences in metaphase or interphase cells. Radioactively labeled probes are detected autoradiographically and chemically modified probes are detected by enzymatic activity or fluorescence (4–6). (*ii*) Discovery that individual chromosomes are localized in interphase cells (7). (*iii*) Identification of nucleic acid sequences homologous to extended chromosome regions (8–17, 47).

In this paper, we demonstrate the use of *in situ* hybridization and fluorescence detection (called fluorescence hybridization) for (*i*) labeling human chromosomes in metaphase and interphase human–hamster hybrid cells, (*ii*) detecting interspecies translocations, (*iii*) labeling specific human chromosomes in metaphase and interphase human cells, and (*iv*) quantitating the amount of target DNA sequence. We also show that high-contrast fluorescence hybridization is possible when probes for which the target sequence is on the order of 50 kilobases (kb) are used.

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MATERIALS AND METHODS

Cell Lines. Human–hamster cell lines UV20HL4, UV20HL-21-27, and UV20HL21-29 were developed by Larry Thompson at the Lawrence Livermore National Laboratory. They contain human chromosomes 1, 4, 5, 6, 11, 14, 15, 16, 19, and 21; 4, 8, and 21; and 8 and 12, respectively, as confirmed by isozyme and banding analysis (18), and flow karyotyping (19). Human amniocytes (47,XYY) were provided by M. Golbus at the University of California, San Francisco.

Sample Preparation. Exponentially growing UV20HL21-29 cells grown in T150 culture flasks (Corning) were irradiated at room temperature with californium-252 fission neutrons at a dose rate of 0.0042 Gy/min to total doses of 0.05, 0.1, 0.3, 0.6, and 1.2 Gy. After irradiation they were grown for 2–3 weeks to allow loss of the most unstable aberrations.

Metaphase spreads for the hybrid cells and the amniocytes were prepared from cells shaken from monolayer cultures after treatment for 4–12 hr with Colcemid (0.1 μg/ml). Human lymphocyte chromosomes were prepared as described by Harper *et al.* (20). Unstimulated human lymphocytes were separated from peripheral blood with Histopaque 1077 (Sigma). Metaphase and interphase cells were fixed in methanol/acetic acid (3:1, vol/vol) and dropped onto cleaned microscope slides. Slides were stored in a nitrogen atmosphere at −20°C.

Probes. Human genomic DNA was isolated from peripheral blood lymphocytes. The human Y-specific probe pY431A, 800 base pairs in pBR322, was supplied by K. Smith (Howard Hughes Medical Institute, Johns Hopkins University). The human 28S ribosomal RNA probe pAbb (21), 1.5 kb in pBR322, was obtained from R. Schmickel (University of Pennsylvania). Probe DNA was labeled by nick-translation with biotin-dUTP (Bethesda Research Laboratories) according to the instructions of the supplier. A trace of [^{3}H]dATP was added to allow determination of the degree of biotin incorporation. Between 13% and 30% of the thymidine was substituted. Labeled probe was separated from the reaction by using spin columns filled with Sephadex G-50 swollen in 50 mM Tris·HCl/1 mM EDTA/0.1% $NaDodSO_4$, pH 7.5 (22). Total plasmid DNA was used for the hybridizations with the cloned probes.

***In Situ* Hybridization.** The hybridization protocol followed that of Harper *et al.* (20) with modifications. Slides carrying interphase cells, metaphase spreads, or both were removed from the nitrogen, heated to 65°C for 4 hr in air, treated with RNase (Sigma) [100 μg/ml in 2× SSC for 1 hr at 37°C (1× SSC is 0.15 M NaCl/0.015 M sodium citrate)], dehydrated in an ethanol series, denatured [70% (vol/vol) formamide/2× SSC (final concentration), pH 7, at 70°C for 2 min], and dehydrated in a 4°C ethanol series. They were then treated with proteinase K [60 ng/ml in 20 mM Tris·HCl/2 mM $CaCl_2$, pH 7.5, at 37°C for 7.5 min (23)] and dehydrated. The proteinase

Abbreviation: kb, kilobase(s).

K concentration was adjusted so that almost no phase-contrast microscopic image of the chromosomes remained on the dry slide. The hybridization mix consisted of (final concentrations) 50% formamide, 2× SSC, 10% dextran sulfate, carrier DNA (sonicated herring sperm DNA) at 500 μg/ml, and biotin-labeled human genomic DNA at 2 μg/ml, human Y-specific DNA at 0.4 μg/ml, or ribosomal DNA at 0.2 μg/ml. This mixture was applied to the slides under a glass coverslip (3 μl/cm^2) and sealed with rubber cement. After overnight incubation at 37°C, the slides were washed at 45°C (50% formamide/2× SSC, pH 7, three times, 3 min each; followed by 2× SSC, pH 7, five times, 2 min each) and immersed in BN buffer (0.1 M sodium bicarbonate, 0.05% Nonidet P-40, pH 8). The slides were never allowed to dry after this point.

For the genomic probes, it was adequate to use an abbreviated protocol, omitting the slide heating, RNase, and proteinase K steps, reducing the hybridization incubation to 2 hr, and omitting all but one of the posthybridization washes of each type.

Cytochemical Detection. The slides were removed from the BN buffer and incubated for 5 min at room temperature with BN buffer containing 5% nonfat dry milk (Carnation) (24) and 0.02% NaN_3 (5 μl/cm^2 under plastic coverslips). The coverslips were removed, the excess liquid was briefly drained, and fluorescein-avidin DCS (3 μg/ml in BN buffer with 5% milk and 0.02% NaN_3) was applied (5 μl/cm^2). The coverslips were put back in their original places and the slides were incubated 20 min at 37°C. They were then washed three times (2 min each) in BN buffer at 45°C. The intensity of biotin-linked fluorescence was amplified by adding a layer of biotinylated goat anti-avidin antibody (5 μg/ml in BN buffer with 5% goat serum and 0.02% NaN_3), followed, after washing as above, by another layer of fluorescein-avidin DCS. Fluorescein-avidin DCS, goat anti-avidin, and goat serum were all from Vector Laboratories (Burlingame, CA). After washing in BN buffer and draining the excess liquid from the slide, a fluorescence anti-fade solution, *p*-phenylenediamine (25) (1.5 μl/cm^2 of coverslip) was added. A thin layer produced optimal microscopic imaging. The DNA counterstain [4,6-diamidino-2-phenylindole (DAPI) or propidium iodide] was included in the anti-fade solution at 0.25–0.5 μg/ml.

For genomic probes it was adequate to reduce the avidin and antibody incubations to 10 min and use only two short washes between steps. This allowed completion of the entire fluorescence hybridization procedure in less than 3 hr.

Microscopy and Quantitative Measurement. The red-fluorescing DNA-specific dye propidium iodide was used to allow simultaneous observation of hybridized probe and total DNA. The fluorescein and propidium iodide were excited at 450–490 nm (Zeiss filter combination 487709). Excitation at 546 nm (Zeiss filter combination 487715) allowed observation of the propidium iodide alone. 4,6-Diamidino-2-phenylindole, a blue fluorescent DNA-specific stain excited in the ultraviolet (Zeiss filter combination 487701), was used as the counterstain for quantitative measurements so that biotin-labeled and total DNA could be observed separately.

Ektachrome ASA 400 color slide film and type R direct positive printing were used for all photographs. This film renders the green fluorescein emission as yellow when the DNA is counterstained with the red-fluorescing propidium iodide.

Fluorescence intensities were measured by using FLEX, a quantitative fluorescence microscope system (26, 27). FLEX consists of a Zeiss fluorescence microscope equipped with a SIT TV camera (Cohu model 4410SIT, San Diego, CA) controlled by a digital image processor (Quantex model DS-12, Sunnyvale, CA). The Quantex processor is interfaced to an LSI-11 microprocessor. Integration of the total fluorescence intensity of subregions of an image was performed by the computer.

RESULTS

Hybrid Studies. The fluorescence hybridization of human genomic DNA to a metaphase spread and a nucleus from the hybrid line UV20HL21-29 is shown in Fig. 1 *Left*. The majority of these cells contain one copy each of human chromosomes 8 and 12. The two human chromosomes are immediately apparent in the metaphase spread, as are their domains in the interphase nucleus (28, 29).

Identification of structural chromosome changes such as interspecies translocations was straightforward, even when very small. Fig. 1 *Right* shows a neutron-induced hamster–human chromosome translocation, which resulted in a derivative chromosome that is red at one end (hamster) and yellow at the other (human). Scoring of such aberrations was accomplished readily by untrained observers and has clear potential for automation. Fig. 2 shows the frequency of translocations between human and hamster chromosomes measured in line UV20HL21-29 after irradiation with californium-252 neutrons at doses ranging from 0.05 to 1.2 Gy. Metaphases were found and scored at a rate of well over 100 per hr, more than an order of magnitude faster than possible for banded spreads.

Three different experiments were conducted to determine if the relationship between fluorescence intensity and target DNA content was quantitative. (*i*) We measured the probe-linked fluorescence from individual human chromosomes in metaphase spreads of a hybrid line (UV20HL21-27) that contains one copy each of chromosomes 4, 8, and 21. The intensities for each chromosome within a given spread were summed and the fraction due to each was calculated. The respective intensities (mean ± SD) were 0.56 ± 0.09, 0.34 ± 0.09, and 0.11 ± 0.02. The relative DNA contents of these chromosomes are 0.50, 0.38, and 0.12 (30, 31). (*ii*) We measured the probe fluorescence from nuclei from the hybrid cell lines UV20HL21-29 and UV20HL4, which differ in their proportion of human DNA content by a factor of 4.5 (30, 31). The measured probe fluorescence intensities differed by a factor of 5.2 ± 2. (*iii*) We compared the probe-linked fluorescence and the number of distinct chromosome domains in interphase cell nuclei of line UV20HL21-29. Cells with 0, 1, and 2 human chromosomes occurred because of the karyotypic instability of the line. The two human chromosomes in this line differ in DNA content by only 8% (30, 31), so that the human DNA content of the nucleus is approximately proportional to the number of domains. The relative intensity of cells with one domain was 0.57 ± 0.12, while that for cells with two was 1.0 ± 0.3. Fluorescence from cells without a domain was not detectable. In both of the whole-cell measurements, probe fluorescence was normalized to nuclear DNA content (cell cycle position) by dividing by the 4,6-diamidino-2-phenylindole intensity of the nucleus.

Human Studies. Similar results can be obtained in human cells by using human chromosome-specific probes. Fig. 3*a* shows the fluorescence hybridization to a metaphase spread, using a highly specific probe for the human Y chromosome. An average male human cell contains about 10^3 copies of a DNA sequence homologous to this probe on the Y chromosome. In addition there are small regions homologous to the probe on several autosomes. Lowering the post-hybridization wash temperature to 42°C increased autosomal binding. Fig. 3*b* shows the hybridization of this probe to nuclei from unstimulated male lymphocytes. All but 2 of 1000 sequentially observed male lymphocytes showed a single bright fluorescent spot, along with some weak background fluorescence presumably due to the autosomal binding evident in Fig. 3*a*. One nucleus contained no spots and one contained

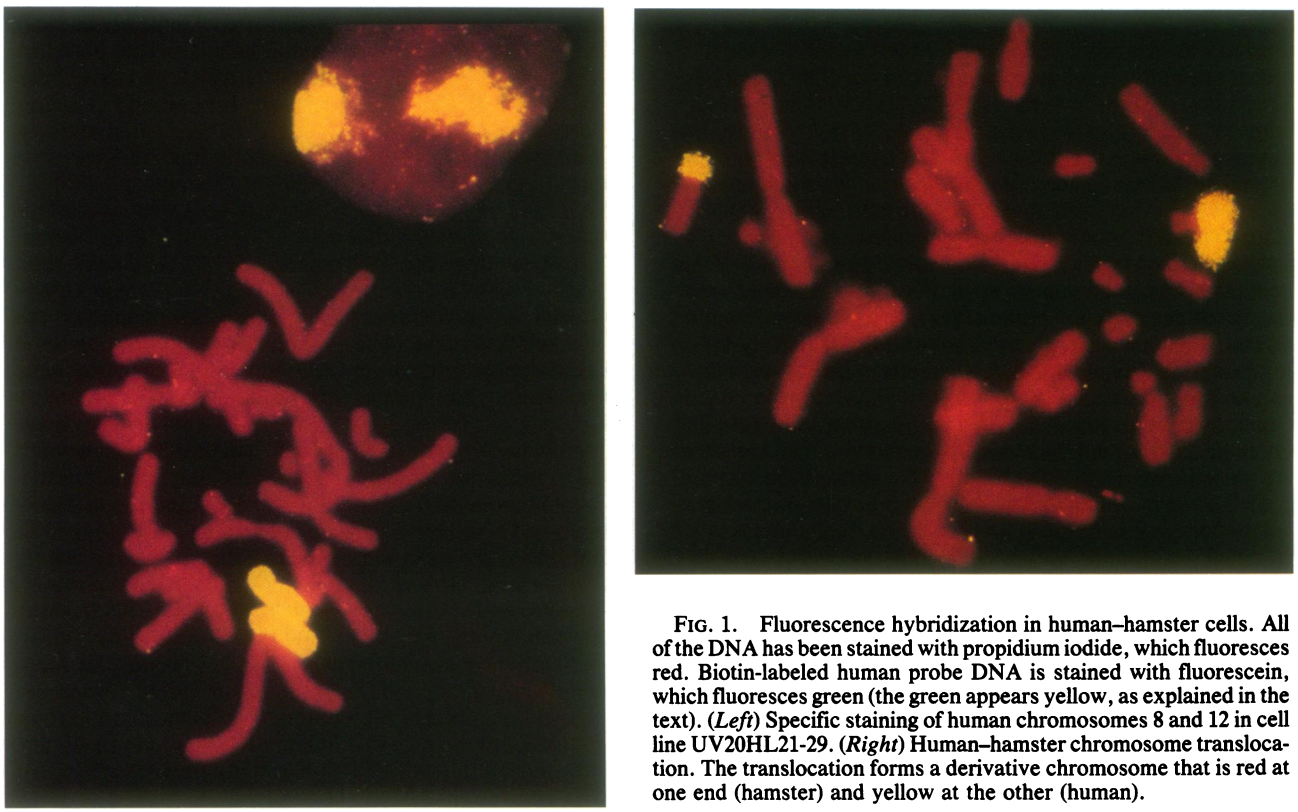

FIG. 1. Fluorescence hybridization in human–hamster cells. All of the DNA has been stained with propidium iodide, which fluoresces red. Biotin-labeled human probe DNA is stained with fluorescein, which fluoresces green (the green appears yellow, as explained in the text). (*Left*) Specific staining of human chromosomes 8 and 12 in cell line UV20HL21-29. (*Right*) Human–hamster chromosome translocation. The translocation forms a derivative chromosome that is red at one end (hamster) and yellow at the other (human).

two. No female lymphocyte showed the bright fluorescent domain, but all showed the autosomal binding. On average, the male nuclei fluoresced 40 times more intensely than the female nuclei (1.0 ± 0.4 vs. 0.02 ± 0.03). Fig. 3*c* shows the hybridization of this probe to XYY amniocytes. The two Y chromosomes are visible in most cells, along with some autosomal binding.

Much smaller targets are detectable. Fig. 3*d* shows the hybridization of the 28S ribosomal DNA probe pAbb to a human metaphase. Between 100 and 200 copies of this 1.5-kb sequence are present in the haploid genome, distributed with unknown proportions among chromosomes 13, 14, 15, 21, and 22 (32). Thus 10 chromosomes are expected to appear labeled in each metaphase, and each chromatid will contain, on average, 20–40 copies of the target DNA sequence (i.e., 30–60 kb). Fig. 3*d* shows clear fluorescence hybridization on 8 chromosomes, with distinct labeling of many individual chromatids.

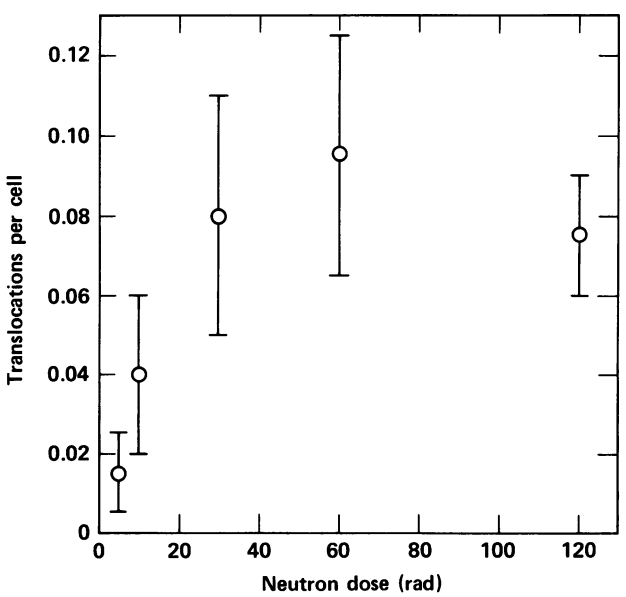

FIG. 2. Dose–response curve for neutron-induced human–hamster chromosome translocations. Error bars show SD. One rad = 0.01 Gy.

DISCUSSION

Fluorescence hybridization depends critically on accessibility of the target DNA sequence(s) to the hybridization reagents (probe, avidin, and antibody) and on the degree to which nonspecific reagent binding can be suppressed. The accessibility to reagents is dependent on the specimen preparation and storage. Fluorescence hybridization is most intense when the specimens are fresh; however, the chromosomes appear fluffy after hybridization (Fig. 1 *Right*). With increased storage time in air the chromosomes remain compact and the hybridization intensity decreases. A reasonable compromise is reached after about a week (Fig. 1 *Left*). After several months, hybridization is visible only on chromosome surfaces. We now preserve specimens in a nitrogen atmosphere at −20°C and heat them in air before hybridization. Fig. 1 *Right* illustrates an additional aspect of the accessibility issue. The widths of the yellow (probe) images of the chromosomes are greater than the propidium (DNA) images. This suggests that a diffuse halo of DNA around each chromosome is more accessible to the hybridization reagents than the more concentrated DNA in the chromosome interior. The stoichiometry of fluorescence hybridization can be affected by such differential accessibility, requiring control of sample preparation and storage for quantitative measurement.

Reducing the nonspecific binding increases sensitivity by allowing increased amplification using biotinylated anti-avidin. In our protocol we are able to amplify at least twice (three layers of avidin). We have measured approximately a

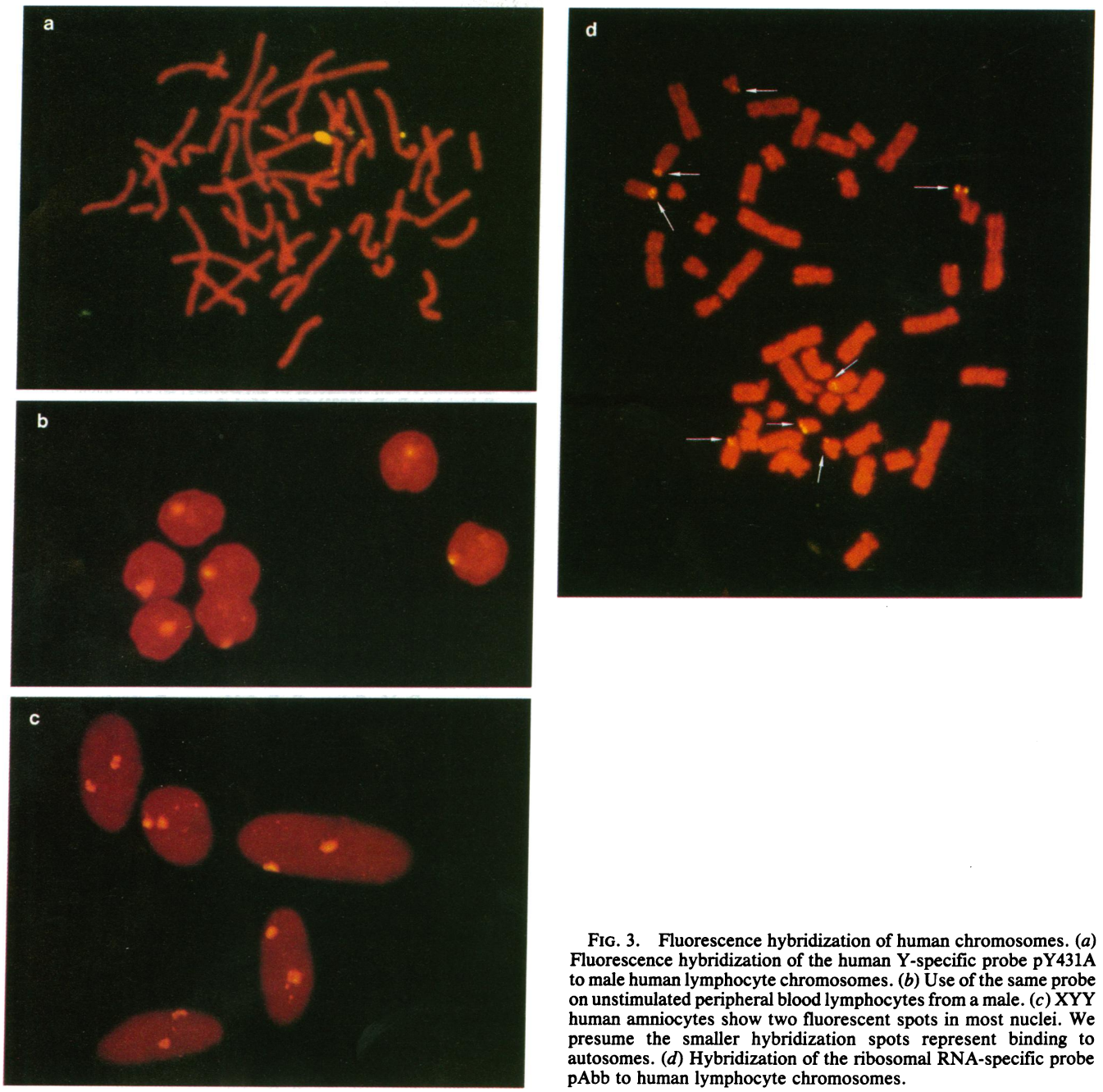

FIG. 3. Fluorescence hybridization of human chromosomes. (*a*) Fluorescence hybridization of the human Y-specific probe pY431A to male human lymphocyte chromosomes. (*b*) Use of the same probe on unstimulated peripheral blood lymphocytes from a male. (*c*) XYY human amniocytes show two fluorescent spots in most nuclei. We presume the smaller hybridization spots represent binding to autosomes. (*d*) Hybridization of the ribosomal RNA-specific probe pAbb to human lymphocyte chromosomes.

6-fold intensity increase with each amplification, and each avidin molecule has approximately 6 fluorescein molecules. Thus double amplification (Fig. 3*d*) should result in a complex of about 200 fluorescein molecules for each detected biotin. Thus there is the potential to put 1000 fluorescein molecules on probes several hundred base pairs in length. This should be visible microscopically. The estimated sensitivity achieved with the ribosomal DNA probe, 30–60 kb, is considerably poorer, presumably due to reagent accessibility difficulties.

There is some evidence that the actual detection limit is somewhat lower than 30–60 kb, which was estimated from the average target per chromatid. In Fig. 3*d* the two chromatids of each labeled chromosome are approximately equal in intensity, while variation among chromosomes is large. This interchromosomal variation may be due to the amount of target present, indicating detection in the vicinity of 20 kb. This sensitivity is comparable to that reported for enzyme-based nonradioactive techniques (33, 34) and for a related fluorescence technique (35).

Fluorescence hybridization with genomic DNA has proven to be a powerful tool for identification of human chromosomes in human–hamster hybrid cells and for detection of interspecies chromosome rearrangements. The entire procedure can be completed in 3 hr for many applications. This method is far superior to G-11 staining for detection of small DNA segments from one species inserted into the genome of another. The ability to rapidly identify interspecies translocations also suggests the utility of hybrid cell systems for studies of cell response to low-dose radiation or other agents.

Human chromosome-specific repetitive probes, of which a number are known for the sex chromosomes and autosomes (8–16, 47), will be useful for analysis of aneuploidy in metaphase and interphase cells (Fig. 3). This will have a major impact in such areas as detection of diagnostically important aneuploidy in human malignancies and prenatal samples (48) and determination of the frequency of aneuploid

cells as a measure of mutagen-induced genetic damage. The stoichiometry of fluorescence hybridization suggests the possibility of detecting homogeneously occurring chromosome-specific aneuploidy by fluorescence intensity measurements. However, variability makes detection of rare aneuploid cells in an otherwise normal population unreliable at this time.

Probes whose binding is confined to chromosomal subregions are most useful in interphase aneuploidy detection since overlaps of labeled regions will be minimized. However, these probes are not as useful for detecting translocations since the rearrangement usually will not occur in the middle of the labeled chromosomal segment, although it may occasionally happen (36). Detection of translocations between human metaphase chromosomes may be possible by using cocktails of chromosome-specific sequences that hybridize more or less uniformly along the chromosome. A translocation would then appear as in Fig. 1 *Right*. The availability of chromosome-specific recombinant DNA libraries made from sorted chromosomes (37–42) may facilitate production of suitable probes.

The sensitivity achieved with the probe for the ribosomal genes indicates that probes for gene clusters also may be useful for chromosome identification and aneuploidy detection. In addition, nucleic acid probes (43, 44) coupled with fluorescence image reconstruction techniques (45) will facilitate exploration of the structure of interphase nuclei.

In conclusion, fluorescent chromosome staining using chromosome-specific nucleic acid probes facilitates cytogenetic analysis where speed, high contrast labeling, and quantitation are important. Potential applications include detection of chromosome-specific aneuploidy in metaphase and interphase cells, quantification of the frequency of chromosome translocations and/or aneuploidy as a measure of induced genetic damage, and detection of diagnostically and prognostically important chromosomal lesions. Depending on the aberration, its detection may be by visual fluorescence microscopy or quantitative fluorescence microscopy as we have described here or by flow cytometry (46).

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1. Caspersson, T., Farber, S., Foley, C. E., Kudynowski, J., Modest, E. J., Simonsson, E., Waugh, U. & Zech, L. (1968) *Exp. Cell Res.* **49**, 219–222.
2. Nilsson, B. (1973) *Hereditas* **73**, 259–270.
3. Burgenhout, W. (1975) *Humangenetik* **29**, 229–231.
4. Langer, P. R., Waldrop, A. A. & Ward, D. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6633–6637.
5. Landegent, J. E., Jansen in de Wal, N., Baan, R. A., Hoeijmakers, J. H. J. & van der Ploeg, M. (1984) *Exp. Cell Res.* **153**, 61–72.
6. Bauman, J., Wiegant, J., Borst, P. & van Duijn, P. (1980) *Exp. Cell Res.* **138**, 485–490.
7. Zorn, C., Cremer, C., Cremer, T. & Zimmer, J. (1979) *Exp. Cell Res.* **124**, 111–119.
8. Devine, E. A., Nolin, S. L., Houck, G. E., Jr., Jenkins, E. C. & Brown, W. T. (1985) *Am. J. Hum. Genet.* **37**, 114–123.
9. Lau, Y. F. & Schonberg, S. (1984) *Am. J. Hum. Genet.* **36**, 1394–1396.
10. Graham, G. J., Hall, T. J. & Cummings, M. R. (1984) *Am. J. Hum. Genet.* **36**, 25–35.
11. Devilee, P., Cremer, T., Bakker, E., Wapenaar, M. C., Kievits, T., Slicker, W. A. T., Slagboom, P. E., Scholl, H. P., Hager, H. D., Stevenson, A. F. G. & Pearson, P. L. (1985) *Cytogenet. Cell Genet.* **40**, 616 (abstr.).
12. Yang, T. P., Hansen, S. K., Oishi, K. K., Ryder, O. A. & Hamkalo, B. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6593–6597.
13. Willard, H. F. (1985) *Am. J. Hum. Genet.* **37**, 524–532.
14. Waye, J. S. & Willard, H. F. (1985) *Am. J. Hum. Genet.* **37**, A182 (abstr.).
15. Jabs, E. W., Wolf, S. F. & Migeon, B. R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4884–4888.
16. Jabs, E. W. & Persico, M. C. (1985) *Am. J. Hum. Genet.* **37**, A158 (abstr.).
17. Gusella, J. F., Keys, C., Varsanyi-Breiner, A., Kao, F.-T., Jones, C., Puck, T. T. & Housman, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2829–2833.
18. Thompson, L. H., Mooney, C. L., Burkhart-Schultz, K., Carrano, A. V. & Siciliano, M. J. (1985) *Somatic Cell Mol. Genet.* **11**, 87–92.
19. Gray, J. W. & Langlois, R. G. (1986) *Annu. Rev. Biophys.* **15**, 195–235. Annual Reviews, (Palo Alto, CA).
20. Harper, M. E., Ullrich, A. & Saunders, G. F. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4458–4460.
21. Erickson, J. M., Rushford, C. L., Dorney, D. J., Wilson, G. N. & Schmickel, R. D. (1981) *Gene* **16**, 1–9.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 466.
23. van Prooijen-Knegt, A. C., van Hoek, J. F. M., Bauman, J. G. J., van Duijn, P., Wool, I. G. & van der Ploeg, M. (1982) *Exp. Cell Res.* **141**, 397–407.
24. Duhamel, R. C. & Johnson, D. A. (1985) *J. Histochem. Cytochem.* **33**, 711–714.
25. Johnson, G. D. & de C. Noguieie Aroujo, J. G. M. (1981) *J. Immunol. Methods* **43**, 349–350.
26. Balhorn, R., Kellaris, K., Corzett, M. & Clancy, C. (1985) *Gamete Res.* **12**, 411–422.
27. Young, I. T. (1983) in *Application of Digital Image Processing* (Society of Photo-Optical Engineers, Bellingham, WA), pp. 326–335.
28. Durnam, D. M., Gelinas, R. E. & Myerson, D. (1985) *Somatic Cell Mol. Genet.* **11**, 571–577.
29. Schardin, M., Cremer, T., Hager, H. D. & Lang, M. (1985) *Hum. Genet.* **71**, 281–287.
30. Mendelsohn, M. L., Mayall, B. H., Bogart, E., Moore, D. H., II, & Perry, B. H. (1973) *Science* **179**, 1126–1129.
31. Gray, J. W., Langlois, R. G., Carrano, A. V., Burkhart-Schultz, K. & van Dilla, M. A. (1979) *Chromosoma* **73**, 9–27.
32. Schmickel, R. D. (1973) *Pediatr. Res.* **7**, 5–12.
33. Landegent, J. E., Jansen in de Wal, N., van Ommen, G.-J. B., Baas, F., de Vijlder, J. J. M., van Duijn, P. & van der Ploeg, M. (1985) *Nature (London)* **317**, 175–177.
34. Manuelidis, L. & Ward, D. C. (1984) *Chromosoma (Berlin)* **91**, 28–38.
35. Albertson, D. G. (1985) *EMBO J.* **4**, 2493–2498.
36. Lau, Y. F., King, K. L. & Donnell, S. N. (1985) *Hum. Genet.* **69**, 102–105.
37. Müller, C. R., Davies, K. E., Cremer, C., Rappold, G., Gray, J. W. & Ropers, H. H. (1983) *Hum. Genet.* **64**, 110–115.
38. Krumlauf, R., Jeanpierre, M. & Young, B. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2971–2975.
39. Davies, K. E., Young, B. D., Elles, R. G., Hill, M. E. & Williamson, R. (1982) *Nature (London)* **293**, 374–376.
40. Distech, C. M., Kunkel, L. M., Lojewski, A., Orkin, S. H., Eisenhard, M., Sahor, E., Travis, B. & Latt, S. A. (1982) *Cytometry* **2**, 282–286.
41. Laland, M., Dyrja, T. P., Schreck, R. R., Shipley, J., Flint, A. & Latt, S. A. (1984) *Cancer Genet. Cytogenet.* **13**, 283–295.
42. Van Dilla, M. L. & Deaven, L. L. (1984) *DNA* **4**, 75 (abstr.).
43. Rappold, G. A., Cremer, T., Hager, H. D., Davies, K. E., Müller, C. R. & Yang, T. (1984) *Hum. Genet.* **67**, 317–325.
44. Manuelidis, L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3123–3127.
45. Mathog, D., Hochstrasser, M., Gruenbaum, Y., Saumweber, H. & Sedat, J. (1984) *Nature (London)* **308**, 414–421.
46. Trask, B., van den Engh, G., Landegent, J., Jansen in de Wal, N. & van der Ploeg, M. (1985) *Science* **230**, 1401–1403.
47. Cooke, H. J., Schmidtke, J. & Gosden, J. R. (1982) *Chromosoma* **87**, 491, 502.
48. Burns, J., Chan, V. T. W., Jonasson, J. A., Fleming, K. A., Taylor, S., McGee, J. O. D. (1985) *J. Clin. Pathol.* **38**, 1085–1092.