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Human sex determination by *in situ* hybridization using non-radioactive probes

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INTRODUCTION

In human genetics, sex determination is of definite interest, primarily in the field of recessive X-linked diseases. Prenatal diagnosis is the main instance of investigation. *In situ* hybridization using specific DNA probes appears to be more accurate than other labeling techniques (*eg*, quinacrine staining). Data obtained with this latter staining technique were not very reliable because other brightly fluorescing spots could be misinterpreted as being Y-chromosomal material. The method of *in situ* hybridization with ^{3}H-labeled probes has the disadvantages of long exposure time, the need for an isotope laboratory and safety problems. Therefore, different non-radioactive labeling and detection systems have been developed that now enable rapid and reliable analysis of *in situ* hybridization signals.

The most widespread non-radioactive labeling system is based on the modification of the probe with the hapten, biotin (Langer *et al*, 1981). Significant 'amplification' of the signal can be attained by using a second layer, biotinylated anti-avidin antibody, and a third layer, fluorochrome-coupled avidin (Pinkel *et al*, 1986). Another labeling and detection system based on digoxigenin (Seibl *et al*, 1991; Arnold, Seibl, Kessler and Wienberg, submitted) has been commercially available for about a year. Results indicate that this technique, because of its simplicity and the distinct signals it produces, is well suited for the determination of the sex chromosomes as well as for other cytogenetic problems (*eg*, Jauch *et al*, 1990).

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MATERIALS AND METHODS

Chromosome preparation

Metaphase spreads from amniotic fluid cells were prepared according to standard procedures. After washing the slides in phosphate-buffered saline (PBS) (3×, 3 min each) to remove traces of acetic acid and passing them through an ethanol series, the slides were stored in 70% ethanol at 4°C until *in situ* hybridization was performed.

Labeling procedure

A human satellite probe specific for the Y chromosome (DYS16; Arneman *et al*, 1985) was labeled with digoxigenin by random-primed enzyme labeling (Feinberg and Vogelstein, 1984).

In situ *hybridization*

The hybridization mixture contains 65% formamide, 10% dextran sulfate, 2×SSC (SSC: 150 mM sodium chloride; 15 mM sodium citrate, pH 7.0), 0.5 mg/ml sonicated salmon sperm DNA and 1 ng/μl of the digoxigenin (DIG)-labeled probe. After dehydration with ethanol and air drying, 50 μl of the hybridization mixture were placed on the slides; these were covered with a 24 × 60 mm coverslip and sealed with rubber cement (Fixogum). The probe and the chromosomes were denatured together at 73°C for 12 min in a waterbath. *In situ* hybridization was performed overnight in an incubator at 42°C. After incubation, the coverslips were removed and the slides were washed 3× in 50% formamide: 2×SSC, pH 7.0 (1:1), at 37°C for 5 min each. An additional wash was performed in 0.1×SSC (3×, 5 min each).

Detection procedures

Slides were preincubated with 5% bovine serum albumin in 4×SSC, 0.2% Tween 20 at 37°C for 20 min, then rinsed in 4×SSC, 0.2% Tween 20.

Detection with peroxidase (POD)

The DIG–POD conjugate was diluted 1:100 in 4×SSC, 0.2% Tween 20. 500 μl of the solution were placed on the slides and incubated for 45 min at 37°C in a black box. To remove non-specifically bound antibodies, slides were rinsed three times in PBS, 0.2% Tween 20 and PBS once (5 min each) and stained with DAB (1.39 mM diaminobenzidine, 0.05% (v/v) H_2O_2, in PBS) for 10 min. After incubation slides were washed extensively in PBS and counterstained in 3% Giemsa for 5 min.

All preparations were analyzed with phase–contrast optics, which easily detect the dark brown hybridization signals.

RESULTS AND DISCUSSION

In this article we present our results on *in situ* hybridization of DNA probes to chromosomes and cell nuclei. The digoxigenin-labeled DNA probes gave strong hybridization signals on chromosomes and interphase nuclei with all immunological systems used and with both phase–contrast and fluorescence microscopy. In all experiments, we were able to demonstrate the hybridization signal on the Y chromosome and the results were obtained within two days. For human chromosomes,

specific probes for the X and Y chromosomes are commercially available. Using the digoxigenin and biotin system, it is possible to visualize the two probes together in different colors.

The non-radioactive *in situ* hybridization technique with chromosome-specific DNA probes appears to be a good method to add to classical human cytogenetic estimation of aneuploidy in metaphase spreads or interphase nuclei (Guttenbach and Schmid, 1990). Chromosomal *in situ* suppression (CISS) hybridization (Cremer *et al*, 1988) of human chromosome libraries provides a new tool for translocation detection (Jauch *et al*, 1990) as well as for comparative chromosome mapping (Wienberg *et al*, 1990). *In situ* hybridization of pools of DNA sequences established from specific chromosomal subregions (Lichter *et al*, 1988) or CISS hybridization of DNA clones established from the chromosome region of interest, such as yeast artificial chromosome clones or cosmid clones (Lichter *et al*, 1990) will enable direct band-to-band comparisons between species at the DNA level (Arnold, unpublished results). This can also be achieved by using probes from microdissected chromosomal bands (Luedecke *et al*, 1989).

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