Jpn J Human Genet 39, 255–258, 1994

Short Communication

A CHROMOSOME PAINTING METHOD FOR HUMAN SPERM CHROMOSOMES USING FLUORESCENT *IN SITU* HYBRIDIZATION

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Summary A method of chromosome painting on human sperm chromosomes using fluorescent *in situ* hybridization (FISH) is introduced. Sperm chromosome slides were prepared after *in vitro* fertilization of hamster eggs with human spermatozoa. The slides were treated by RNase A before FISH. Chromosome 4 was clearly and specifically painted in a majority of sperm-derived metaphase plates after an application of whole chromosome painting DNA probes of this chromosome. This is the first report of successful painting on human sperm chromosomes.

Key Words human sperm chromosome, RNase A treatment, chromosome painting

In addition to chromosome banding analysis, the use of fluorescent *in situ* hybridization with chromosome-specific DNA libraries (chromosome painting) is becoming increasingly widespread in somatic cytogenetics because it allows a rapid and striking visualization of numerical and structural chromosome anomalies. Needless to say, this technique is a powerful tool in human sperm cytogenetics as well, especially for detecting chromosomal rearrangements in spermatozoa and analyzing meiotic chromosome segregation in translocation carriers.

In analyzing sperm chromosomes, cytoplasm has to be preserved to prevent artificial loss of chromosomes (Mikamo and Kamiguchi, 1983). When there exist cytoplasmic substances, the background fluorescence blurs chromosomes. As

Received February 22, 1994; Revised version accepted April 4, 1994.
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a result painting human sperm chromosomes covered by ooplasm becomes less successful.

To our knowledge, there is no report on successful use of painting method in human sperm chromosome analysis. In this paper, we describe our newly developed method for human sperm chromosome painting.

1. Human sperm chromosome preparation. In this experiment, frozen-thawed spermatozoa and frozen-thawed hamster oocytes were used for interspecific *in vitro* fertilization. The semen sample from a healthy Japanese man had been cryopreserved in TEST yolk buffer (TYB) with glycerol (Irvine Sci, Santa Ana, CA) following the prescription. Hamster oocytes had been cryopreserved according to the method described by Tateno *et al.* (1992).

After thawing the semen sample, we collected motile spermatozoa by means of continuous-step Percoll density gradient centrifugation (Kaneko *et al.*, 1987). Then these spermatozoa were treated with 10 μM of ionophore A23187 for 5–7 min and cultured for 1.5–2.0 h to allow capacitation.

When spermatozoa were adequately capacitated, frozen oocytes were thawed, rehydrated and prepared for interspecific *in vitro* fertilization, as described by Tateno *et al.* (1992). The procedures for insemination and culture of fertilized ova were performed according to the method by Kamiguchi and Mikamo (1986). Chromosome slides of metaphasic ova were prepared by the gradual fixation-air drying (GF-AD) method (Mikamo and Kamiguchi, 1983). The chromosome slides were kept in deep freezer (−125°C) until the time of hybridization.

2. Pre-treatment with RNase A. Cytoplasmic substances were removed by RNase A according to the method described by Hayata (1993). Slides were rinsed in the distilled water, then treated with 1 mg/ml RNase A (RNase A, Sigma Chemical Co., St. Louis, MO) in Tris EDTA buffer (1 mM Tris-HCl, pH 7.4, 0.01 mM ethylenediaminetriacetic acid) at 37°C for 15 min. The slides were washed through in tap water, and dipped in methanol: acetic acid (3:1) mixture and methanol alone for about 2 min each, and then dried under the strong air current at the room temperature.

3. Chromosome painting. Chromosome painting was done using a whole chromosome painting system kits, purchased from Life Technologies Inc., Gibco BRL, MD, U.S.A. *In situ* hybridization was performed according to the instruction manual provided by the company. In brief, slides were denatured in 70% formamide/2×SSC for 3 min at 70°C, and then immediately put into 70% ethanol. After dehydration through 85 and 100% ethanol, those slides were dried in air. SpectrumGreen bound whole chromosome painting DNA probes of chromosome 4 and human Cot-1 blocking DNA in the hybridization buffer were denatured at 70°C for 5 min, and then applied onto the target area of each slide under a coverslip. Hybridization was performed for 16–18 h at 37°C in moist chamber in dark. After the hybridization, the slides were washed five times at 45°C, *i.e.*, three times

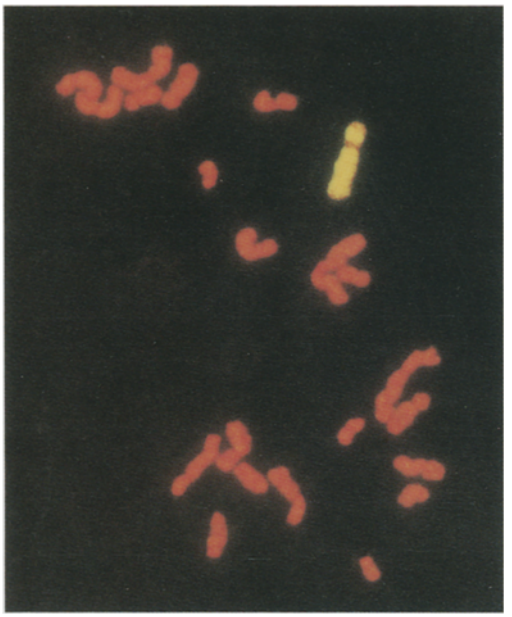

Fig. 1. Human sperm chromosomes visualized in a golden hamster ovum. Chromosome 4 distinctively painted with SpectrumGreen bound DNA probes. Counter stain was done with propidium iodide.

(10 min each) in 50% formamide/2×SSC, once in 2×SSC (pH 6.3) (10 min) and once in 0.1% NP-40/2×SSC (10 min). The slides were then counterstained with DAPI (4,6-diamidino-2-phenylindole) or with PI (propidium iodide).

As shown in Fig. 1, the sperm chromosome 4 was painted clearly and specifically. The frequency of the painted sperm chromosome 4 was 68.8% (11/16) in the slides treated by RNase A while 54.5% (12/22) in the slides without RNase A treatment.

As mentioned earlier, the GF-AD method is highly reliable for detecting aneuploidy and structural anomalies such as breaks and fragments because all chromosomes are maintained within the flattened dry ooplasm (Mikamo and Kamiguchi, 1983). In the present experiment, it seems that removal of ooplasmic materials by RNase A makes hybridization more successful.

The use of chromosome painting technique as well as banding method in human gamete cytogenetics will provide additional information effectively about causal mechanisms of chromosome anomalies in man. In this context, further experiments using DNA probes specific to the other chromosomes will be performed.

Acknowledgments The authors thank Dr. Reiko Kanda (Safety Analysis Unit, NIRS, Japan) for her kind help.

This research work was supported by a grant from the Science and Technology Agency of Japan.

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