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Application of laser scanning image cytometry for human chromosome microdissection and DNA analysis

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INTRODUCTION

Recent progress in molecular biology have increased the use of chromosomal microdissection in human genetics (Kao and Yu, 1991; Seki et al., 1993). Indeed, generation of DNA probes from genome is of great interest in Genetics. This objective can be obtained by using microdissection and amplification of a desired chromosomal region (Ludecke et al., 1989; Meltzer et al., 1992). We describe here application of laser scanning image cytometry for human chromosome microdissection and DNA analysis.

MATERIALS AND METHODS

Chromosome preparation

Phytohemagglutinin-stimulated human lymphocyte cultures were established from healthy donors. Metaphase spreads were prepared according to our standard protocols.

Laser microdissection

The laser microdissection of the chromosomes was performed on chromosomes stained with Giemsa 0.5%. Conditions adopted for current microdissection were obtained with

an argon ion laser (Coherent 92-5, Palo Alto, Ca, USA) emitted beam at 488 nm, with a 50 mW power reduced to 12.5 mW by an acousto optic modulator. A 100X immersion oil objective lens allowed to obtain a laser spot diameter of 0.2 μm . The effective power at the plane of focus was determined using a power meter field Master with a detector head LM-2 (Coherent). For manual burning, the displacement of laser beam was controlled with a mouse by a computer and directly monitored by a TV display. All these experimental conditions were realised by using the image cytometer ACAS 570 (Meridian, Okemos, Mi, USA).

RESULTS

Laser microdissection was performed on non stained and stained chromosomes with Giemsa 0.5% and on GTG banded chromosomes. On non stained chromosomes, aspect of the chromosomes was not modified, whatever the laser power used. The concentration and staining time of Giemsa were important parameters to visualize the effects of the laser beam on the chromosomes. Burning of chromosomes with intense staining led to a blackening of the chromosomes, and with weak staining to a whitening of the chromosomes. All the chromosomes of one metaphase except one precise chromosomal region have been burnt in a few minutes.

DISCUSSION

The method described allows a precise and rapid microdissection of any chromosomal region from a metaphase. A laser scanning image cytometer have been used to microdissect chromosome. Laser is based on the fact that at high photon densities light can liquefy, evaporate or break down optically biological material (Letokhov, 1985). To obtain an optimal laser power stability in these weak values and to attenuate laser beam irradiations, we used an acoustico optic modulator. The effects of these different laser beam powers were analysed by the alterations of the stained chromosome. A weak Giemsa staining was necessary to visualize the action of the laser beam on the chromosomes. With no stained chromosomes, we did not observe alterations of DNA. However, the effects of the laser beam were not similar using different Giemsa

concentrations. With a low concentration of Giemsa, staining disappeared, and the destruction of the chromosomes was easily visualised. In opposition, a high concentration of Giemsa led to a blackening of the chromosomes and did not allow the visual differentiation between the burnt and non burnt chromosomes. When the light power used was high, the whitening of burnt chromosomes was not similar to that observed in our finally adopted experimental conditions. Laser power under 5 mW was not sufficient to destroy the chromosome. Destruction of all the metaphase excepted one chromosomal fragment was extremely rapid and required about 2 min. To destroy chromosomes, the laser burning intensity was lower than the usually generally used 100-300 mW laser power, corresponding probably to a lower power in the plane of sample to kill whole cells by the same system (Schindler et al., 1985). Size of the laser beam depended with the objective utilized. With a X100 immersion oil objective, it was of 0.2 μm . This value corresponds approximatively to 30 megabases in metaphase (Monajembashi et al., 1986). The experiments presented in this work demonstrate the feasibility and simplicity of laser microdissection of the chromosomes.

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