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Nonisotopic In Situ Hybridization for Mapping Oncogenic Sequences

P. F. Ambros [1], C. R. Bartram [2], O. A. Haas [1], H. I. Karlic [3], and H. Gadner [1]

A. Introduction

In situ hybridization techniques are currently the most direct way of determining the localization and quantification of repetitive sequences or unique genes, e.g. oncogenes, in tissues or on chromosomes. This method involves the annealing of labeled polynucleotide sequences to chromosomal or cellular preparations whose DNA or RNA has been denatured (or otherwise exposed) to enable hybridization with the labeled probe. Along with autoradiographic techniques [5, 6], the use of nonisotopic in situ hybridization methods has brought a better resolution of the signal and a considerable shortening of the procedure.

The rapid biotin/streptavidin method [8] combined with a specific microscopic set up [7] is a powerful tool for locating unique sequences on metaphase chromosomes [1, 2]. Using biotinylated DNA probes and a streptavidin-peroxidase detection system we are now able to trace cellular oncogenes on human chromosomes by in situ hybridization with DNA probes less than 2 kb in size. Sequential staining of the preparations with chromomycin A_3 and DA/DAPI [10] allows unequivocal chromosome identification and an exact assignment of the in situ hybridization signals in the target chromosomes.

In the present study we applied this high-resolution in situ hybridization technique to chromosomes of the human leukemia cell line K562. This cell line, originating from a patient with CML in blast crisis, has turned out to be an ideal model for molecular biological studies: A marker chromosome contains nearly identical amplified *c-abl*/*5'bcr* and lambda light-chain constant-region immunoglobulin genes (C_L) [3]. The aim of our study was to analyze the exact positions of the *c-abl* and *5'bcr* sequences in the genome.

B. Material and Methods

Two pUC plasmids (kindly provided by G. Grosveld) were used, one containing a 1.8-kb *Eco*RI *c-abl*, and the other one a 2.0-kb *Bgl* II/*Hind*III *5'bcr* insert. Labeling of the DNA probes was done with biotinylated dUTP (ENZO Biochem., Inc., New York) with an Amersham Nick-Translation Kit.

K562 cells grown according to standard techniques (RPMI 1640 supplemented with 10% FCS) were used for chromosome preparations. The in situ hybridization protocol was performed as described previously in detail [1, 2]. Briefly, after RNase treatment and dehydration of the slides, the hybridization solution was applied. Slides were covered with ethanol-cleaned glass coverslips and sealed with rubber cement. Denaturation was done at 75°–78 °C for 10 min in a humid chamber and followed by overnight incubation at 37 °C in the same chamber. After extensive washing in several steps with 2 × SSC (2xSSC/50% formamide), PBS, and PBS with 0.1% triton, signal detection of the la-

[1] St. Anna Kinderspital, Kinderspitalg. 6, A-1090 Vienna, Austria
[2] Universitätskinderklinik Ulm, Prittwitzstr. 43, D-7900 Ulm, Federal Republic of Germany
[2] Ludwig-Boltzmann Inst. f. Leukämieforschung Hanusch-Krankenhaus Vienna

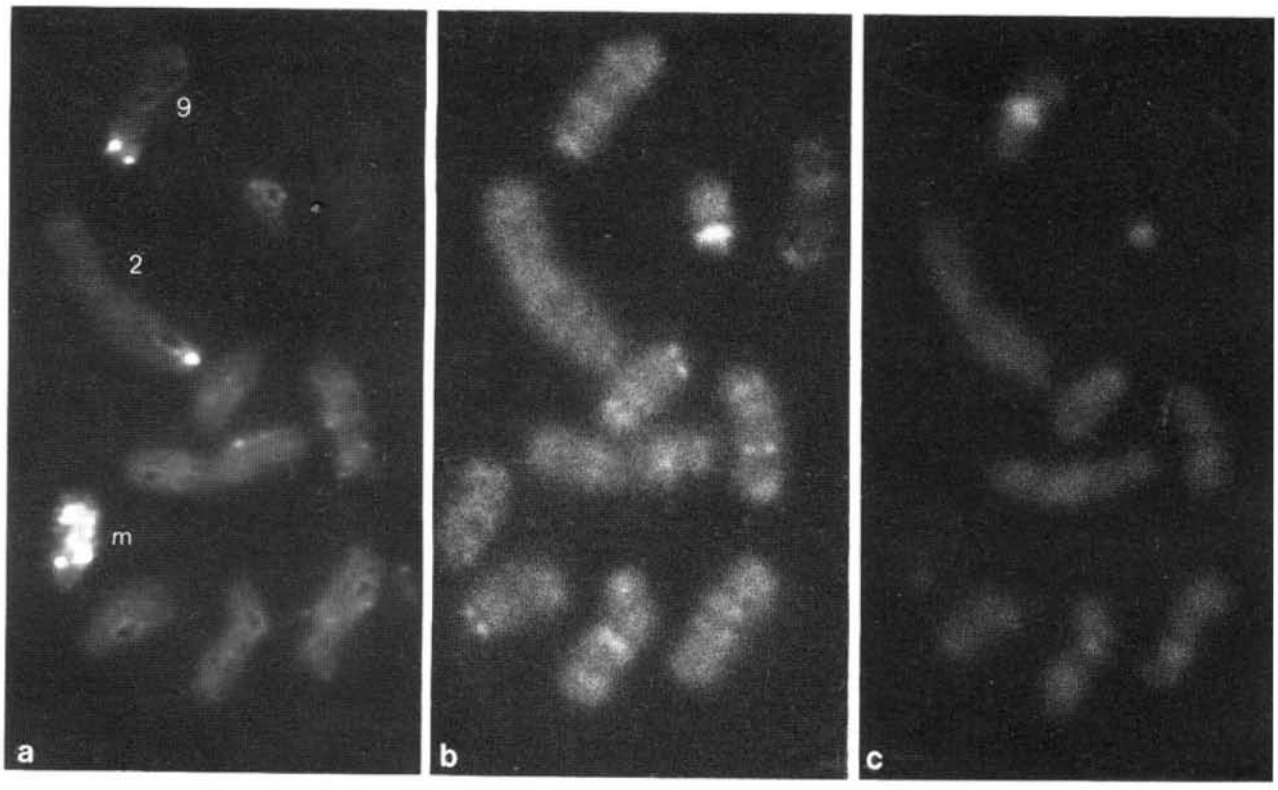

Fig. 1. a Partial chromosome spread from a K562 cell after hybridization with a biotinylated c-abl probe and detection of the signals with peroxidase-labeled streptavidin, which were visualized with a reflection-contrast microscope on chromosome 9 (9q34) chromosome 2 (2q37) and on a marker chromosome. **b** R-banding of the same cell with chromomycin A_3. **c** Distamycin A/DAPI banding

beled DNA probes was performed as described in the DETEK I-hrp signal-generating systems instruction manual (ENZO Biochem., Inc., New York). The diaminobenzidine development was carried out for 5 min at room temperature.

After analysis of the slides with reflection-contrast microscopy [7] chromomycin-distamycin-DAPI staining was performed with a slight modification according to Schweizer [10].

C. Results and Discussion

Nonisotopic detection systems such as the rapid biotin/streptavidin system offer marked advantages over autoradiographic methods currently in use: In view of the considerably shortened procedure, in situ hybridization is no longer restricted to research laboratories and has proven to be a reliable tool for diagnostic and routine work. An increased resolving potential, highlighting sin-

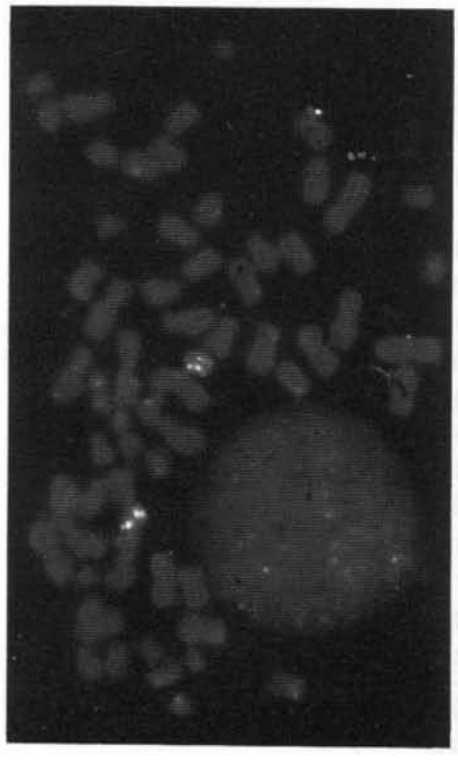

Fig. 2. Chromosome spread from a K562 cell after hybridization with a biotinylated *5'bcr* probe and peroxidase-streptavidin detection

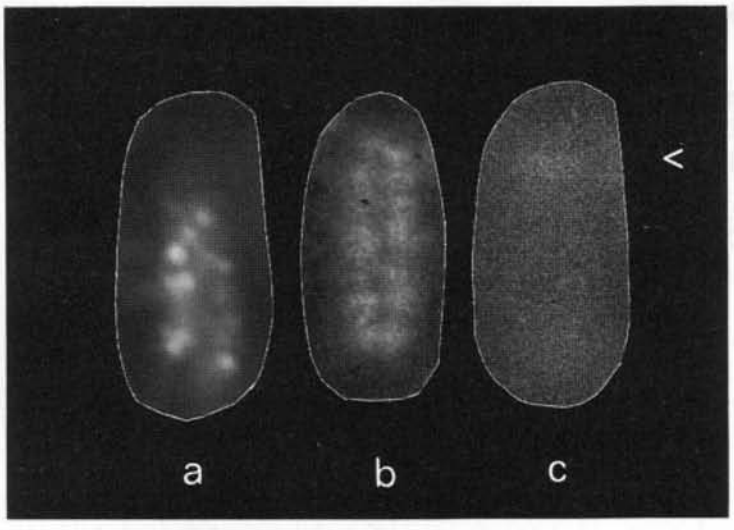

Fig. 3 a–c. Marker chromosome probed with a biotinylated *c-abl* probe **a**, and sequentially stained with chromomycin A_3 **b** and DA/DAPI **c**, indicating the centromere position (*arrow*). The bright spots in **a** represent the saltatory amplified *c-abl* sequences on this chromosome

gle sequences down to less than 2 kb, gives more detailed information about chromosome organization and gene localization.

Using the biotin-HRP-streptavidin/reflection-contrast microscopy technique and sequential staining of the chromosomes with chromomycin/distamycin/DAPI after the signal detection, we demonstrated that both the *c-abl* oncogene (1.8kb *Eco*RI fragment) and the *5'bcr* (2.0kb *Bgl*II/*Hin*dIII fragment) are located on the same acrocentric marker chromosome (presumably derived from a Ph^1 chromosome) in the human K562 cell line (see Figs. 1–3), amplified four to seven times.

Besides the germline position of the *c-abl* oncogene on chromosome 9 (9q34), this DNA probe hybridized to a specific site on chromosome 2 (2q37), indicating a further translocation in the genome (Fig. 1). In addition to the rearranged position of this gene, the site of *5'bcr* on 22q was also clearly visible by this technique (data not shown in detail).

In accordance with molecular biological [3] and in situ hybridization data obtained previously with radioactively labeled *c-abl* and c-lambda probes [11], we clearly identified an amplification of the *c-abl* oncogene and *5'bcr* gene on the Ph^1-like marker chromosome. The most striking information gained from this study is a result of the increased resolution obtained by this specific technique: the amplified *c-abl* sequences also include the second molecular hallmark of CML, the *bcr* sequences [a region on chromosome 22 (22q) within which the majority of Ph^1 breakpoints are clustered], in a saltatory pattern, interspersed with other DNA segments (Fig. 3). A further translocation of the *c-abl* oncogene could be seen in a terminal position of the long arm of chromosome 2 in this specific cell line.

This method has considerable advantages over time-consuming autoradiographic in situ hybridization techniques and expensive molecular biological assays, and recent data show that the methods presented in this study will be useful for future routine diagnosis of unusual translocations in CML or other leukemias and tumors.

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