Acta virol. **33** : 582—585, 1989

AN ALTERNATIVE APPROACH FOR INDUCTION OF CHROMOSOMAL BANDING AND PRESERVATION OF CHROMOSOMAL MORPHOLOGY FOR VIRUS DNA MAPPING BY IN SITU HYBRIDIZATION

A. MINCHEVA

Research Institute of Infectious and Parasitic Diseases, Department of Virology, Medical Academy, Sofia 1233, Bulgaria

Received December 2, 1987

Summary. — I report on the use of acetic/saline/Giemsa technique for the induction of chromosome banding and an additional fixation (prior hybridization) to preserve the chromosome morphology as regards in situ hybridization under stringent conditions. This approach results in high quality banding resolusion for grain localization of integrated sites of virus DNA sequences on both metaphase and prometaphase chromosomes.

Key words: *in situ hybridization*; *stringent conditions*; *acetic/saline/Giemsa* (*ASG*) *band staining technique*; *human papillomavirus*

In situ hybridization is a powerful method for quantitation and localization of nucleic sequences on chromosomes or interfase nuclei. Recent advances in this methodology have allowed detection of single genomes of viruses in cells (Haase *et al.*, 1982), single genes in chromosomes (Gerhard *et al.*, 1981; Harper and Saunders, 1981) and low copy number mRNA molecules in individual cells (Harper *et al.*, 1986).

For identification of chromosomes Q- or G-banding patterns can be produced before or after hybridization (Gosden *et al.*, 1975; Chandler and Yunis, 1978; Popescu *et al.*, 1985). There are disadvantages to the type of banding used as well as the time of banding. Banding prior hybridization requires very time consuming work: the photographs of the same metaphase cell should be made before hybridization and after the autoradiogram is developed. But there arise problems with banding after stringent hybridization conditions.

We were concerned with the chromosomal locations of human papillomavirus (HPV) 16 or 18 DNAs in a few cervical cancer cell lines mapped by *in situ* hybridization of HPV 16/18 DNA probes (Mincheva *et al.*, 1987).

One major problem in the course of these studies was the severe damage of chromosomal morphology during the different steps of *in situ* hybridization under stringent conditions and the loss of their stainability. Despite the use of various chromosome banding methods — before or after hybridization (Wang and Fedoroff, 1972; Chandler and Yunis, 1978; Popescu *et al.*,

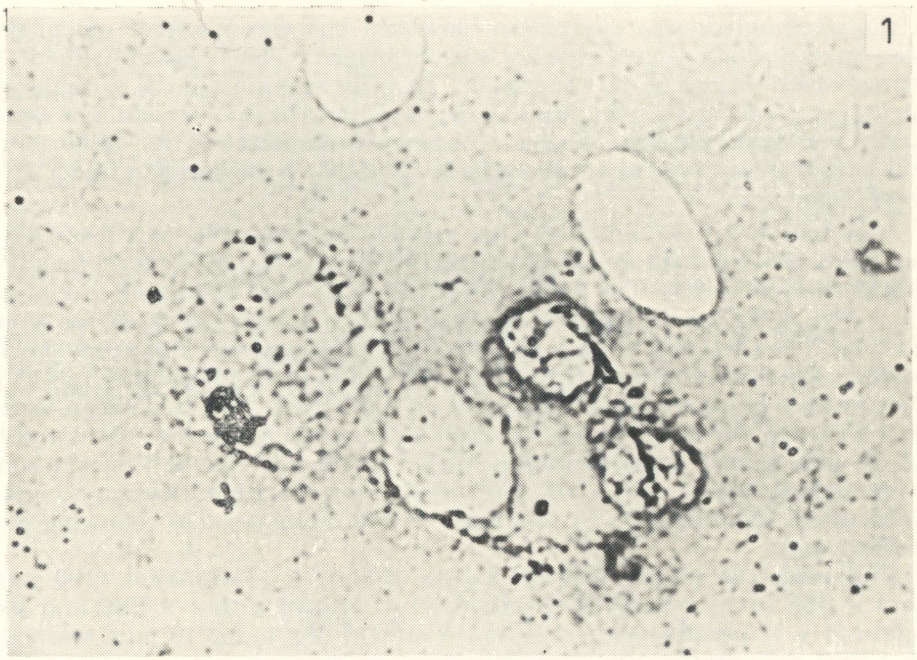

Fig. 1.
Severe damage of a metaphase cell after *in situ* hybridization and loss of her stainability

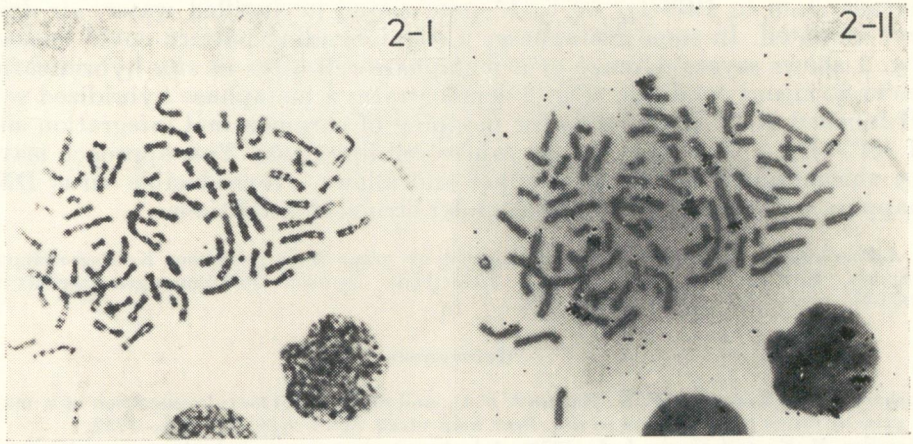

Fig. 2.
A metaphase from Caski cells, hybridized *in situ* with ^{3}H-HPV 16 DNA: ASG-photograph and matching autoradiograph following hybridization

1985) no banding was observed even when the chromosomal morphology was intact (after a second fixation was performed). This is possibly due to the stringent conditions of the experiment. In order to maintain the chromosomal structure intact with good stainability we used the ASG technique and an additional pretreatment of slides (prior to hybridization) for induction of chromosome banding as an alternative to *in situ* hybridization under stringent conditions.

Chromosomes were obtained from actively growing cells of a few cervical cell lines. Air dried chromosome preparations were stained by ASG technique (Summer *et al.*, 1971). The freshly prepared slides were incubated in 2 × SSC (1 × SSC is 0.15 mol/l NaCl, 0.015 mol/l sodium citrate) at 60 °C for 45 min to 2 hr. Then the slides were washed twice in phosphate buffer pH 6.8 and stained for 7 min with 2 % Giemsa stain (Merck) in phosphate buffer, pH 6.8, washed twice with distilled water and air dried. The incubation time in 2 × SSC and Giemsa staining for a cell culture is cell line dependent and has to be determined experimentally. Well spread ASG banded chromosomes were photographed with a dry lens prior to *in situ* hybridization to enable subsequent identification by comparison with chromosomes following autoradiography.

Following the initial treatment with RNase A (SIGMA) — 100 μg/ml in 2 × SSC for 1 hr at 37 °C the slides were washed with 2 × SSC and fixed again in 4 % paraformaldehyde and 5 mmol/l $MgCl_2$ for 10 min, washed in 2 × SSC dehydrated via a graded series of ethanol and processes *in situ* hybridization as described (Mincheva *et al.*, 1987). After hybridization the slides were washed in 50 % formamide, 50 % 2 × SSC pH 7.0 at 40 °C for 2 min, then a few other washings were performed (20 min each) in 2 × SSC at room temperature. After autoradiography the slides were stained in 2 % Giemsa stain in PBS for 10 to 15 min, rinsed in distilled water, air dried and mounted. In some metaphases a slight banding pattern could be seen. Fig. 1 shows severe damage of a metaphase cell after *in situ* hybridization under stringent condition. Fig. 2 demonstrates a metaphase hybridized with ^{3}H-labelled HPV DNA probe for mapping of chromosomal integration sites of HPV DNA 16 in the cervical cancer cell line Caski. This approach leaves the chromosomal morphology intact and allows a reproducible virus DNA mapping by *in situ* hybridization under stringent conditions.

Acknowledgements. This work was supported by grant from Deutsches Kresbsforschungszentrum, Institut für Virusforschung, Heidelberg, Deutsche Forschungsgemeinschaft (Gi 128/I-I).

References

Gerhard, D. S., Kawasaki, E. S., Bancroft, F. C., and Szabo, P. (1981): Localization of a unique gene by direct hybridization *in situ*. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3755—3759.

Chandler, M. E., and Yunis, J. J. (1978): A high resolution *in situ* hybridization technique for the direct visualization of labelled G-banded early metaphase and prophase chromosomes. *Cytogenet. Cell Genet.* **22**, 352—356.

Gosden, J. R., Mitchell, A. R., Buckland, A. R., Clayton, P., and Evans, H. J. (1975): The location of four human satellite DNAs on human chromosomes. *Exp. Cell. Res.* **92**, 148—158.

Haase, A. T., Stowring, L., Harris, J. P., Traynor, B., Ventura, P., Peluso, R., and Brahic, M. (1982): Visna DNA synthesis and the tempo of infection *in vitro*. *Virology* **119**, 399–410.

Harper, M. E., and Saunders, G. R. (1981): Location of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. *Chromosoma* **83**, 431–439.

Harper, M. E., Marselle, L. M., Gallo, R. C., and Wong-Stahl, F. (1986): Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by *in situ* hybridization. *Proc. natn. Acad. Sci. U.S.A.* **83**, 772–776.

Mincheva, A., Gissmann, L., and zur Hausen, H. (1987): Chromosomal integration sites of human papillomavirus DNA in three cervical cancer cell lines mapped by *in situ* hybridization. *Med. Microbiol. Immunol.* **176**, 245–256.

Popescu, N. C., Amsbaugh, S. C., Swan, D. C., and Di Paolo, J. A. (1985): Induction of chromosome banding by trypsin/EDTA for gene mapping by *in situ* hybridization. *Cytogenet. Cell Genet.* **39**, 73–74.

Sumner, A., Evans, H., and Buckland, R. (1971): New technique for distinguishing between human chromosomes. *Nature* **232**, 31–32.

Wang, H., and Fedoroff, S. (1972): Banding in human chromosomes treated with trypsin. *Nature* (New Biol.) **235**, 52–53.