Increased silver stainability of metaphase chromosomes from rat hepatocytes during in vivo hepatocarcinogenesis

M. Kirsch-Volders, A. Deleener, M. Lans and J. de Gerlache

Laboratory for Human Genetics, Free University of Brussels, Pleinlaan, 2, B–1050 Brussels (Belgium), and Unit of Biochemical Toxicology and Cancerology, University of Louvain, UCL 7369, B–1200 Brussels (Belgium), 8 August 1984

Summary. Silver stainability of metaphase chromosomes was studied in hepatocytes obtained from rats exposed or not to a partial or complete carcinogenic treatment with diethylnitrosamine and phenobarbital. An increased hyperstaining is reported in the carcinogen-treated animals.

Key words. Silver strain; hepatocyte; chromosome; carcinogen.

There is evidence that silver-staining of metaphase chromosomes is essentially located on the nucleolar organizer regions (NORs) bearing the rDNA genes[1]; moreover the intensity of the staining is very well but not strictly correlated with the rate of rRNA synthesis which took place in the previous interphase[3,9]. Since tumor cells are known to present enlarged nucleoli and modifications of gene and chromosome numbers, it was our aim to estimate the rate of rRNA synthesis by silver staining of the NORs in hepatocytes obtained after in vivo induction of liver cancer by known carcinogens. In this report, we describe the hyper-silver stainability of rat chromosomes after a combined treatment with diethylnitrosamine, 2-aminofluorene and phenobarbital.

Material and methods. The experimental protocol used and further referred to as Gerlans treatment is described by Lans et al.[5]. Male Wistar rats, outbred Wi (IOPS AF) HAM, IFFACREDO, France, were injected i.p. with a single necrogenic dose of diethylnitrosamine (200 mg/kg dissolved in 0.9% NaCl). 2 weeks later, a selection similar to that described by Solt and Farber[10] was applied by giving the animals a concentration of 0.03% 2-acetylaminofluorene (2-AAF) in their diet for 2 weeks. In the middle of this period, a necrogenic dose of CCl_4 (2 ml/kg, 1/1, v/v in corn oil) was given per os in order to stimulate the selective division of the initiated cells. The animals received a basal diet for 1 week and thereafter a 0.05% phenobarbital (PB) supplemented diet up to the end of the experiment. Untreated animals were submitted to a $^2/_3$ partial hepatectomy.

To isolate parenchymal cells at different times, rats were submitted to a chemical enzymatic liver perfusion using the method originally devised by Friend as modified by Krack et al.[4]. By this procedure the preneoplastic nodules can be separated from the surrounding parenchymal cells. After the enzymatic liver perfusion performed on one treated or untreated animal, the cell suspension obtained was incubated with Colcemide (0.1 mg/ml) for 1.5 h at 37 °C. Air-dried slides were obtained by routine processing through hypotonic solution (15 min; 0.075 M KCl), fixation (3:1/methanol:acetic acid) and spreading of the cells on precleaned slides. Silver-staining was performed on 1-week-old cytological preparations by an incubation with 50% $AgNO_3$ solution (1 g/2 ml H_2O) for 24 h at 56 °C.

Results. Silver-stained metaphases of hepatocytes from rats which received different treatments were compared:

1) Control rats which were killed 24 h after partial hepatectomy as mitotic stimulus.

2) Rats which received the Gerlans treatment until exposure to PB. No PB treatment was performed and the animals were killed at the end of the selection procedure.

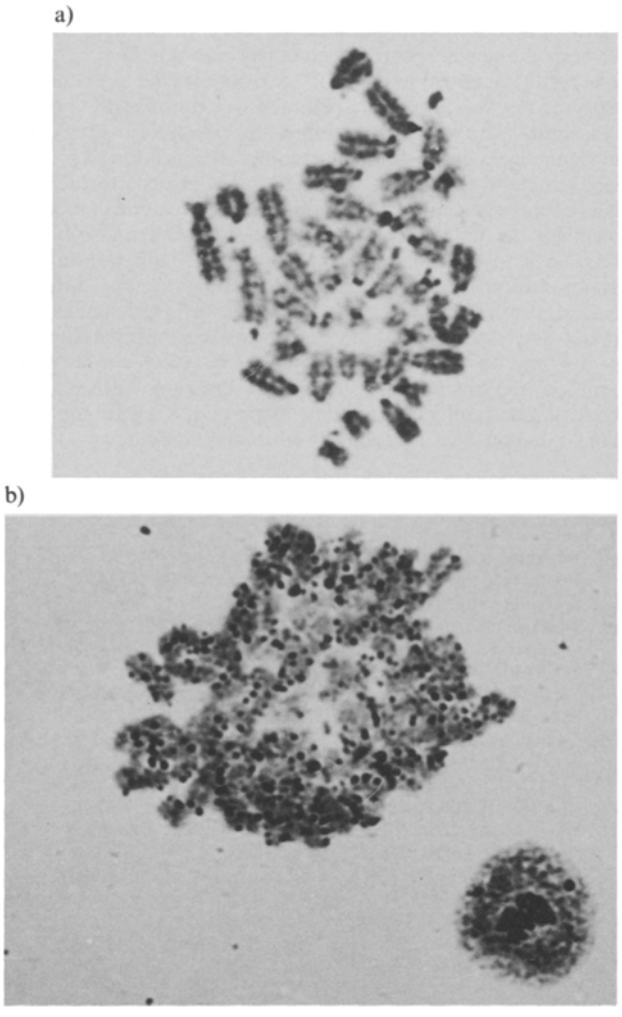


Figure 1. Ag-stained metaphases from rats which received only a 2-day PB treatment.

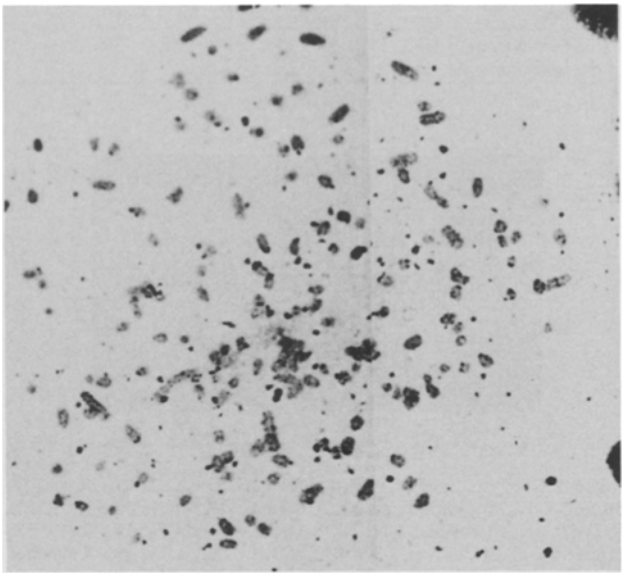


Figure 2. Ag-stained metaphase from polyploid hepatocytes treated by Gerlans procedure.

3) Rats which received only 2 days of PB treatment (fig. 1).
4) Rats which received the Gerlans treatment including PB treatment and were killed 48 h after the start of PB treatment (fig. 2).
Examination of the metaphases showed that in the control rats, even after hepatectomy, the silver-staining was relatively low and confined to the NOR regions. On the contrary, in the carcinogen-treated rats, different types of silver-stained metaphases are observed. In some metaphases, silver staining is seen, as expected, only on the NORs (fig. 1a), but in other metaphases, a very intensive silver staining is present on the whole chromosomes (fig. 1b) and sometimes also in the nucleo-(cyto-)plasm (fig. 2).
These observations are relevant for diploïd cells and for polyploïd cells as well (fig. 2). Moreover, the unexpected silver-staining found in the nucleo-(cyto-)plasm cannot possibly be considered as a technical artefact since two neighboring cells may or may not present this nucleoplasmic silver-staining. Quantification of the data presented in relation to the carcinogen treatment given is difficult as long as the biological significance of the observed data is not understood.

Discussion. The present interpretation of silver-staining on chromosomes is that, under standardized conditions, silver nitrate binds acidic highly phosphorylated proteins B_{23} and C_{23}[6-8] which must be closely associated with nascent rRNA and with rDNA. One of the possible candidates might be the RNA polymerase I[11]. However since a strict relationship cannot be established between silver-staining and transcriptional activity, it is suggested that silver-staining may detect the presence of a protein (or set of proteins) responsible for chromatin decondensation[2]. In the light of these suggestions, our data may indicate that carcinogens like phenobarbital and 2-acetylaminofluorene induce a quick decondensation of chromatin. Silver nitrate would in this case stain not only the NOR regions but also all decondensing chromosome regions. These findings need confirmation in other systems with different carcinogens before a causative relationship can be proved between carcinogens and chromosome decondensation.
As far as the nucleo-(cyto-)plasmic silver-staining is concerned, the interpretation is difficult. Of course one might think of free rDNA copies or free 'decondensing proteins' but further molecular studies are needed to understand the findings.

1 Goodpasture, C., and Bloom, S. E., Chromosoma *53* (1975) 37.
2 Hernandez-Verdun, D., Biol. cell. *49* (1983) 191.
3 Hofgartner, F. J., Krone, W., and Jain, K., Human Genet. *47* (1979) 329.
4 Krack, G., Gravier, O., Roberfroid, M., and Mercier, M., Biochim. biophys. Acta *632* (1980) 619.
5 Lans, M., de Gerlache, J., Taper, M., Preat, V., and Roberfroid, M., Carcinogenesis *4* (1983) 141.
6 Lischwe, M. A., Richards, R. L., Busch, R. K., and Busch, H., Exp. Cell Res. *136* (1981) 101.
7 Lischwe, M. A., Smetana, K., Olson, M. O. J., and Busch, H., Life Sci. *25* (1979) 701.
8 Mamrack, M. D., Olson, M. O. J., and Busch, H., Biochemistry *18* (1979) 3381.
9 Paweletz, N., Siels, W., and Lettre, R., Z. Zellforsch. *76* (1967) 577.
10 Solt, D. B., Medline, A., and Farber, E., Am. J. Path. *88* (1977) 595.
11 Williams, M. A., Kleinschmidt, J. A., Krohne, G., and Franke, W. W., Exp. Cell Res. *137* (1982) 341.

0014-4754/85/091182-02$1.50 + 0.20/0

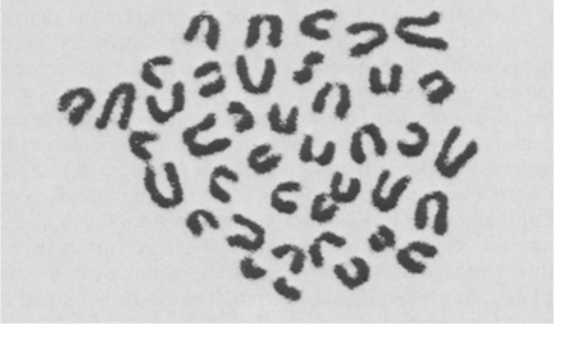
A method for karyotyping mouse blastocyst embryos developing from in vivo and in vitro fertilized eggs

W. Garside and N. Hillman

Department of Biology, Temple University, Philadelphia (Pennsylvania 19122, USA), 15 August 1984

Summary. A method for karyotyping blastocyst-staged mouse embryos is described. The use of this protocol results in the recovery of a high percentage (> 70%) of readable karyotypes and can be completed rapidly.
Key words. Karyotype; mouse blastocyst.

There are two published methods for karyotyping preimplantation staged mouse embryos, one for cleavage-staged embryos[2] and one for blastocyst-staged embryos[3]. Both have disadvantages. We have found that the former method yields a low percentage of readable karyotypes (approximately 25%), and the latter results in the loss of a large number of the embryos because of the extensive length of time required for fixation.
The method we describe here is a modified version of that described by Takagi et al.[3]. With this modified protocol embryos are not lost and a high percentage of readable karyotypes are obtained. The method is fast and reproducible.
Procedure. Blastocyst embryos, developing from eggs fertilized either in vivo or in vitro[4,5] have been karyotyped. Following fertilization, 2-cell embryos, removed either from excised oviducts or from insemination dishes, are placed into 200 µl drops of standard egg culture medium (SECM)[6] under paraffin oil contained in 10 × 35 mm tissue culture dishes (Falcon Plastics). The dishes are placed into a continuous gas flow incubator (5% CO_2 in air) maintained at 37 °C. The embryos are allowed to continue development until they reach the early blastocyst stage[7].
At this stage, the embryos are transferred to 150–200 µl drops of SECM containing 1 µg/ml Colcemid (Sigma Chemical Co.). The drops of supplemented culture medium are under paraffin oil in 10 × 35 mm tissue culture dishes. The dishes are returned to the incubator for 3–4 h. After treatment, the embryos are divided into groups of 10, each of which is transferred to a depression in a spot test plate containing a hypotonic solution (deionized water: SECM; 2:1) which causes the cells to swell. After 5 min at



A representative air-dried, giemsa stained preparation of mouse blastocyst chromosomes. × 2900.