UPTAKE OF MAMMALIAN CHROMOSOMES BY MAMMALIAN CELLS

M. CHORĄŻY, M.D., A. BENDICH, Ph.D., E. BORENFREUND, Ph.D., O. L. ITTENSOHN, and D. J. HUTCHISON, Ph.D.

From the Division of Experimental Chemotherapy and Division of Nucleoprotein Chemistry, Sloan-Kettering Institute for Cancer Research, and the Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York. Dr. Chorąży's present address is the Department of Tumor Biology, Institute of Oncology, Gliwice, Poland

ABSTRACT

Chromosomes isolated from mouse leukemia L1210 cells were taken up by mouse macrophages, HeLa cells, and rat embryo fibroblasts following simple exposure *in vitro*. The process, which resembles pinocytosis or phagocytosis, was traced by autoradiography of chromosomes prelabeled with thymidine-H^3, and by staining techniques and phase contrast microscopy. During the first six hours, the uptake of chromosomes was restricted to the cytoplasm, but there was some evidence of penetration into the nucleus after 16 and 26 hours of exposure. Treatment of rat fibroblasts with glucose and insulin markedly enhanced the uptake of chromosomes, whereas iodoacetate inhibited their penetration.

INTRODUCTION

New genetic determinants have been acquired by bacterial and mammalian cells after exposure to deoxyribonucleic acid (DNA) isolated from other cells or from viruses. Although the acquired traits in certain instances could be demonstrated unequivocally, it is not yet clear whether the genetic alterations were mediated by intact DNA. Furthermore, it has not yet been established in any instance whether DNA as ordinarily isolated is in as native a condition as that in a viable cell. DNA is fragile and is exposed to degrading and denaturing conditions during isolation and handling.

It can be expected that the DNA in the intact isolated chromosome would be in a more native form, less susceptible to modifications than DNA itself. A procedure for the isolation of mammalian chromosomes was developed (11) and studies of their possible uptake by mammalian cells were initiated.

MATERIALS AND METHODS

Chromosomes

Metaphase chromosomes were isolated from mouse lymphocytic leukemia L1210 cells by the procedure described in the preceeding paper (11). For the penetration studies, chromosomes were suspended in 0.25 M sucrose in 0.1 M acetate buffer, pH 5.6, containing 0.003 M $CaCl_2$.

For autoradiographic studies, chromosomes were labeled with thymidine-H^3 in the following manner. Mice of DBA_2 or BDF_1 (F_1 of ♀ C57Bl × ♂ DBA_2) strains bearing L1210 leukemia in the ascitic form were injected intraperitoneally, on the 3rd and 4th day of tumor growth, with 20 μc of thymidine-H^3 (specific activity 3.0 C/mM; Schwarz BioResearch, Inc., Orangeburg, New York) per mouse. On the 4th day of tumor growth, colchicine (1 μg/gm body weight) was injected intraperitoneally and the ascites cells were harvested 17 hours later. The labeled chromosomes were isolated by the method

of Chorąży *et al.* (11). The final preparation was washed 4 times with 0.25 M sucrose in 0.1 M acetate buffer, pH 5.6, containing 0.003 M $CaCl_2$ and 0.025 per cent non-radioactive thymidine. An autoradiograph of isolated labeled chromosomes is shown in Fig. 1.

Recipient Cells

Mouse peritoneal macrophages, HeLa cells, and and rat embryo-fibroblasts[1] were used for *in vitro* uptake studies. Recipient cells were maintained in tissue culture medium 1B, a modification of 1B7 medium (32) consisting of a 25 per cent increased concentration of non-essential amino acids and vitamins and a substitution of calf serum for fetal bovine serum in cultures of HeLa cells and macrophages. Macrophages were collected from the peritoneal cavity of DBA_2 mice and maintained *in vitro* according to the procedure of Goodman and Koprowski (21).

The cells were seeded as monolayers on coverslides in Leighton tubes two days before the uptake experiments were performed. To a tube containing cells in 2.0 ml of medium, 0.1 ml of a chromosome suspension was added and the cells were incubated for designated periods of time at 37° with occasional shaking. As controls, the same cell lines were exposed to 0.1 or 0.5 μc of thymidine-H^3 per ml of medium. In several experiments, cells were exposed to non-labeled chromosomes, fixed, and then stained with methyl green pyronin or Feulgen reagent. In other experiments, observations were made by phase-contrast microscopy.

Autoradiography

For autoradiographic studies, cells which had been exposed to labeled chromosomes were washed twice with 0.14 M NaCl containing 0.003 M $CaCl_2$ and 0.025 per cent of non-radioactive thymidine, rinsed once in distilled water, fixed in 95 per cent ethanol–glacial acetic acid (3,1), washed in 80 per cent and 50 per cent ethanol, rinsed in water, and then mounted on microscope slides. The slides were then covered with Kodak Ar-10 stripping film and exposed thereto in the dark for 3 to 4 weeks. After development, the preparations were stained with methyl green pyronin and examined microscopically.

Stimulation and Inhibition of Phagocytosis

In order to stimulate the phagocytic activity of the recipient cells, glucose and insulin at a final concentration of 2.5 per cent and 0.1 unit per ml of tissue culture medium were added (29). These compounds were added simultaneously to the culture a few minutes before the addition of the chromosome preparation. The cultures were incubated for 1, 3, and 6 hours, respectively. In order to inhibit phagocytosis, iodoacetate (29) at a final concentration of 1×10^{-4} M was added and the cells exposed to chromosomes for the same periods of time.

RESULTS

Macrophages

Mouse macrophages exposed to the labeled chromosomes for 1 to 6 hours showed extensive incorporation of radioactive components as judged by the distribution of silver grains in autoradiographs. After 3 hours of incubation, silver grains were seen over the cytoplasm of several of the cells but not over the nuclei. The uptake by the cells was more pronounced when the incubation time was extended to 6 hours (Fig. 2), and at that time some of the cells also showed labeling over the nuclei. This is of particular interest because of the failure of these cells to incorporate thymidine-H^3 in the control series. Silver grains were seen over the nuclei of macrophages which were exposed for 16 hours to labeled chromosomes and these grains were usually randomly dispersed over the nucleus as well as the cytoplasm (Fig. 3). This distribution of labeling was still apparent after 26 hours of incubation. Macrophages exposed to chromosomes for 1 hour, 3 hours, and 6 hours and then stained with methyl green pyronin showed distinct evidence of the incorporation of chromosome particles which were often located in vacuoles.

HeLa Cells

HeLa cells, after 1 hour and 3 hours of exposure to labeled chromosomes, in most instances showed chromosomes attached to the cell surface, and only a small percentage of cells showed phagocytic activity. However, the number of cells with clumps of silver grains over the cytoplasm increased after 6 hours of exposure. The silver grains in some cells were localized also over the nucleus. In control cells incubated with thymidine-H^3, grains were randomly dispersed over the nucleus.

HeLa cells incubated with labeled chromosomes for 16 hours very often showed silver grains over the nucleus that were situated very near to the nucleolus (Fig. 4). However, the percentage of cells labeled in this manner was small.

[1] Rat fibroblasts were obtained through the courtesy of Dr. J. Fogh.

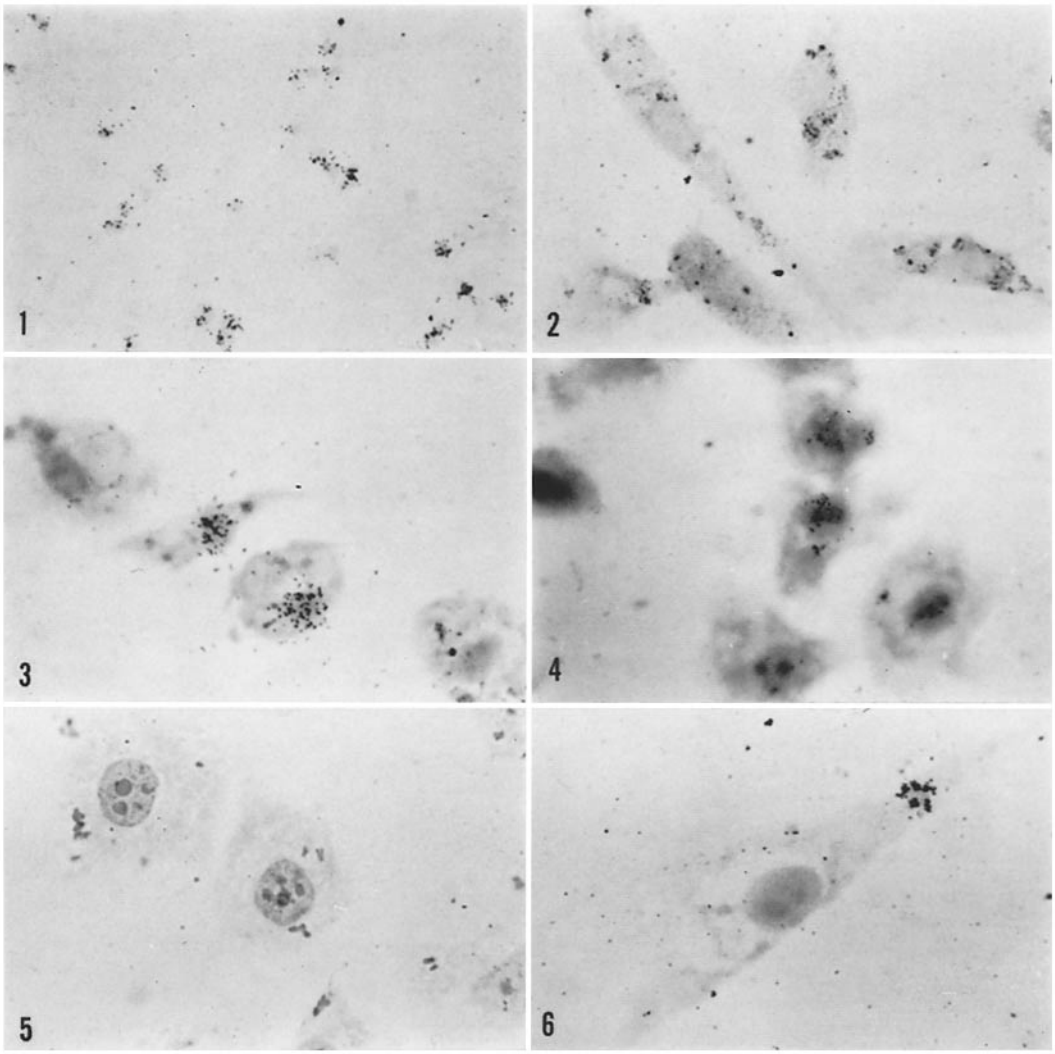

FIGURE 1 Isolated chromosomes labeled with thymidine-H^3. Autoradiograph. Methyl green pyronin. × 700.

FIGURE 2 Autoradiograph of mouse macrophages exposed to labeled chromosomes for 6 hours. Note labeling mainly in the cytoplasm. Methyl green pyronin. × 700.

FIGURE 3 Autoradiograph of macrophages exposed to labeled chromosomes for 16 hours. Note labeling over the nuclei. Methyl green pyronin. × 700.

FIGURE 4 Autoradiograph of HeLa cells exposed to labeled chromosomes for 16 hours. Note labeling of the nuclei. Methyl green pyronin. × 700.

FIGURE 5 HeLa cells exposed to non-labeled chromosomes for 1 hour. Methyl green pyronin. × 700.

FIGURE 6 Autoradiograph of rat embryo fibroblast exposed to labeled chromosomes for 1 hour. Methyl green pyronin. × 700.

The results obtained by the staining technique paralleled those obtained by autoradiography. Only in a small percentage of HeLa cells stained with methyl green pyronin was it possible to find engulfed chromosomes. The morphology of the incorporated chromosomes was very well preserved (Fig. 5).

Fibroblasts exposed to non-labeled chromosomes for 1 hour, 3 hours, and 6 hours and then stained with methyl green pyronin gave results similar to those obtained by autoradiography. Only a small percentage of the cells possessed phagocytic activity since only few of them showed engulfed chromosomes (Fig. 8).

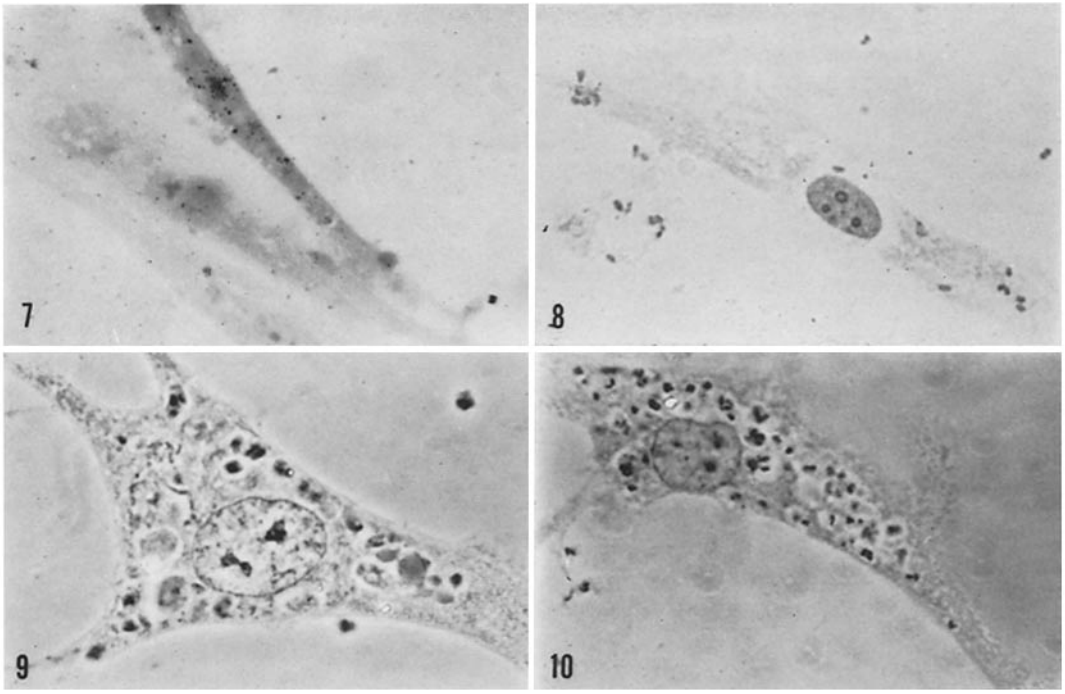

FIGURE 7 Autoradiograph of fibroblasts exposed to labeled chromosomes for 16 hours. Methyl green pyronin. × 700.

FIGURE 8 Fibroblast exposed to non-labeled chromosomes for 1 hour. Methyl green pyronin. × 700.

FIGURE 9 Fibroblast exposed to non-labeled chromosomes in the presence of glucose and insulin for 6 hours. Phase contrast. × 700.

FIGURE 10 As above. × 700.

Fibroblasts

Rat embryo fibroblasts exposed to labeled chromosomes showed discrete clumps of silver grains distributed over the cytoplasm. Grains were often found over the elongated processes of cells exposed for one hour (Fig. 6). After prolonged exposure (16 hours), dispersed labeling was often seen over the nucleus (Fig. 7), although occasionally the silver grains were limited to only a small area over the nucleus.

Effect of Glucose, Insulin, and Iodoacetate

Glucose and insulin, when added simultaneously to the incubation medium, exerted a stimulatory effect on the uptake of chromosomes by fibroblasts (Figs. 9 and 10). Some of the engulfed chromosomes were still recognizable after 6 hours of exposure. The process of engulfing of the chromosomes seemed to proceed from the end of elongated processes of the cell. The incorporated particles are then drawn into the body of the

cell. Although a stimulatory effect of glucose and insulin was observed in most experiments, it was not reproducible in every experiment. Cells treated with iodoacetate had a rather different appearance: the cytoplasm of these cells was foam-like and no chromosomes could be seen inside the cells. Treatment of macrophages and HeLa cells with glucose and insulin did not lead to an increased number of engulfed particles.

DISCUSSION

We were encouraged to study the uptake of chromosomes by cells since it is well known that many other types of particulate matter also are ingested by cells. For example, cells in tissue culture can engulf bacteria (38), dye aggregates (22), nucleoli (27), and other structures such as inorganic particulates, *i.e.* asbestos (15) and colloidal gold (23), by a process analogous to phagocytosis or pinocytosis. A large variety of cells similarly exhibit such activity (4, 6, 25, 34, 36) and, according to Gey (cited in Harris, reference 24), pinocytosis is a characteristic activity shown by all cells which are cultivated *in vitro*. Exceptions to this generality have been noted, however (26). Uptake of serum proteins, albumins, histones and protamines has also been observed (7, 8, 16). However, the "infection" of cells by ribonucleic acids isolated from viruses is of particular interest since a biological consequence of this intrusion can be clearly observed: the production of viruses. Reviews of this subject have appeared recently (13, 17). This has also been demonstrated with the carcinogenic DNA isolated from polyoma (14), papilloma (28), and SV40 (20) viruses.

The incorporation of extraneous DNA into mammalian cells has been studied extensively (3, 5, 9, 10, 12, 18, 19, 30, 35, 37, 39, 41, 42) and the engulfed DNA can be located in the cell nucleus (5, 19). There have been many failures to demonstrate the phenomenon of transformation of mammalian cells (2, 31, 33). Recently, however, Szybalska and Szybalski (40) have presented evidence that DNA-mediated transformation may be achieved in mammalian cells under the appropriate circumstances. The intruding DNA may be subject to interference by cellular nucleic acids or altered by cellular enzymes. These effects could possibly be circumvented with isolated chromosomes.

Uptake of chromosomes by mammalian cells could be demonstrated by autoradiography when thymidine-H^3-labeled chromosomes were used. The label could be located almost exclusively in the cytoplasm after 6 hours of exposure (Figs. 2 and 6), and possible penetration into the nuclei may be observed after 16 and 26 hours (Figs. 3, 4, and 7). Since the pattern of labeling was different from that obtained with thymidine-H^3 itself, it can be inferred from the cytoplasmic uptake that extensive destruction or digestion had not attended the uptake of chromosomes at least during 6 hours. The morphology of the inclusion bodies permitted their recognition as chromosomes (see Figs. 5, 8, and 10) comparable in appearance to those isolated from L1210 cells. In many instances (Figs. 8 and 10) these bodies were contained in discernible vacuoles within the cytoplasm, much as if the cellular incorporation had proceeded by pinocytosis or phagocytosis.

The biological (if not chemical) identity of DNA isolated from tumor-inducing viruses (1, 14, 20, 28) is often retained following penetration or infection of mammalian cells. If extensive alteration or destruction of such nucleic acids had attended their penetration of mammalian cells by a process akin to pinocytosis, the consequent appearance of new virus or transformed cells would not have been expected. Thus, there is no reason *a priori* to exclude the possibility that some new biological activity or property may appear in cells which have acquired new chromosomes by the process of pinocytosis or phagocytosis. It is hoped that the studies described here may furnish experimental approaches to a further understanding of the chemical basis of genetics.

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