Autoradiographic Study of the Penetration of Non-Histone Chromatin Proteins into Differentiating Cells[1]

C. Mathieu[2], P. Ferrer, M. H. Dupuy and J. P. Zalta[3]

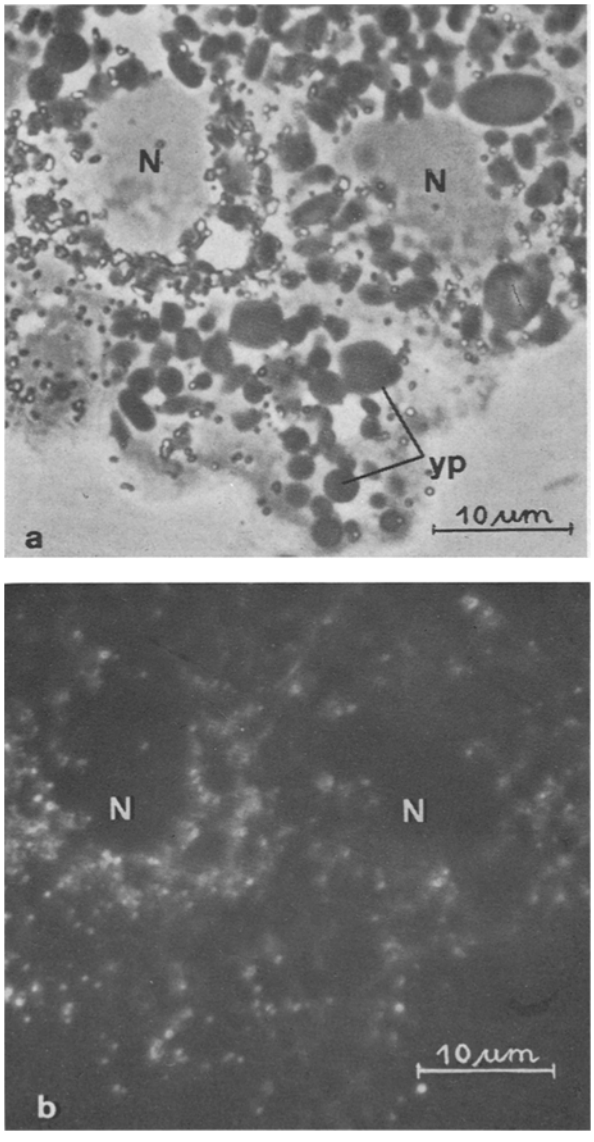
Centre de Recherches de Biochimie et de Génétique Cellulaire du C.N.R.S., 118 Route de Narbonne, F–31077 Toulouse (France), 7 July 1975.

Summary. Attention has previously been drawn to a specific effect of NHCP on embryonic *Pleurodeles* cell differentiation. With a modified NHCP labelling technique, autoradiography has revealed a cytoplasmic concentration of labelled NHCP and has not revealed any difference between homospecific and heterospecific NHCP penetration.

It has been clearly demonstrated that the non-histone chromatin proteins (NHCP) play a role in the control of gene expression in eukaryotes[4,5]. There is evidence that they stimulate RNA synthesis in non-cellular systems[6–8] and, at transcription level on isolated chromatin, have a tissue specificity in agreement with the tissue from which they are extracted; some of them bind specifically to DNA[8]. Moreover, they exhibit species and tissue specificity as revealed by acrylamide gel electrophoresis patterns[9–11], and their synthesis varies during the cell cycle in relation to gene activity[12,13].

^{125}I labelled NHCP distribution after penetration into Zajdela ascitic hepatoma cells growing in vitro

	Attached Zajdela hepatoma cells (2 experiments)		Non-attached Zajdela hepatoma cells (1 experiment)
Cytoplasmic fraction	12,600 cpm	9,800 cpm	51,500 cpm
Nuclear fraction	120 cpm	135 cpm	1,250 cpm



Autoradiograms showing ^{125}I labelled NHCP localization in *Pleurodeles* cells. a) Phase contrast micrograph of the preparation; b) Silver grains revealed in light dark field. YP, yolk platelets; N, nuclei.

Kruh[14] has shown the existence of nuclear acidic proteins that specifically inhibit differentiated cell protein synthesis in an in vitro acellular system. Our previous work[15] on the biological effects of NHCP extracted from *Pleurodeles waltlii* (Michah) hepatic cells enabled us to show clearly a specific NHCP inhibitory effect on the morphological differentiation of embryonic cells of the same species. It is therefore important to study the way in which they act and where they are localized after entering the cells. Two culture systems were used. With the first, using embryonic amphibian cells (*Pleurodeles waltlii*) which react in in vitro culture,

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[2] C. Mathieu: to whom reprint requests should by addressed Centre de Recherches de Biochimie et de Génétique Cellulaires du C.N.R.S., 118 Route de Narbonne, F–31077 Toulouse, France.

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[4] G. S. Stein, T. C. Spelsberg and L. J. Kleinsmith, Science *183*, 817 (1974).

[5] G. S. Stein, G. Hunter and L. Lavie, Biochem. J. *139*, 71 (1974).

[6] M. Kamiyama and T. Y. Wang, Biochim. biophys. Acta *228*, 563 (1971).

[7] N. C. Kostraba and T. Y. Wang, Int. J. Biochem. *1*, 327 (1970).

[8] C. S. Teng, C. T. Teng and V. G. Allfrey, J. biol. Chem. *246*, 3597 (1971).

[9] K. Wakabayashi, S. Wang, G. Hord and L. S. Hnilica, FEBS Lett. *32*, 46 (1973).

[10] E. M. Wilson and T. C. Spelsberg, Biochim. biophys. Acta *322*, 145 (1973).

[11] F. C. Wu, S. C. R. Elgin and L. C. Hood, Biochemistry *12*, 2792 (1973).

[12] G. Stein and R. Baserga, Biochem. biophys. Res. Commun. *41*, 715 (1970).

[13] G. S. Stein and K. W. Borun, J. Cell Biol. *52*, 292 (1972).

[14] J. Kruh, L. Tichonicky and H. Wajcman, Biochim. biophys. Acta *195*, 549 (1969).

[15] C. Mathieu, A. M. Duprat, M. H. Dupuy, P. Ferrer, J. P. Zalta and J. C. Beetschen, J. Embryol. exp. Morph. *33*, 1051 (1975).

only an autoradiographic approach to the problem of penetration is possible. With the second, in which Zajdela ascitic hepatoma cells are incubated in the presence of labelled NHCP, the labelling distribution inside the cells can be studied by means of a biochemical extraction method.

Materials and methods. Nuclei from hepatic cells of adult *Pleurodeles* and from Zajdela ascitic hepatoma cells are isolated as described in [16]. The chromatin and then the NHCP are isolated according to a modification by KRUH [14] of WANG's method [17]. NHCP can be kept frozen at −80 °C in 5×10^{-2} *M tris* HCl (pH 8.5). All operations are performed at 4 °C.

NHCP labelling. ^{125}I labelling of NHCP is performed by an adaptation of the enzymatic method (use of solid state bovine lactoperoxidase) of David [18]. Free iodine is separated from labelled NHCP by means of chromatography on a (15 × 0.9 cm) G 15 Sephadex column. All operations, except labelling, are performed at 4 °C. The exposed cells are embryonic cells, obtained from medullary plate and the chordomesodermal region of *Pleurodeles waltlii* embryos at the neurula stage. After disaggregation the isolated cells are cultivated according the technique of Duprat [19] in plastic micro-chambers. The above cells are treated with Barth's solution, without bovine serum albumin (BSA) but with labelled NHCP extracted from hepatic *Pleurodeles* (homospecific proteins) cells or from Zajdela ascitic hepatoma (heterospecific proteins) cells, with an active protein concentration of 50 µg/ml culture medium [15]. After the period of treatment, they are washed with Barth's solution, fixed in situ for 24 h with 5% formol in Sorensen's 0.1 *M* phosphate buffer at pH 7.4. The cells are embedded in epon. Sections about 1500 Å thick are placed for optical autoradiography on slides (previously covered with gelatin and dessicated) and then coated with a 50 µm Ilford L4 emulsion layer. After 1 to 3 weeks' exposure (at 4 °C in a dehydrated atmosphere), specimens are developed for 20 min at 14 °C.

Zajdela ascitic hepatoma cell labelling. Cells cultivated in 5 ml flasks or in spinners in Glasgow medium are incubated at 37 °C for 6 h in the presence of ^{125}INa labelled NHCP (50 µg/ml medium). The nuclei are extracted as described in [16]. Cytoplasmic and nuclear fractions are isolated and their radioactivity determined.

Results and discussion. The NHCP labelling technique using iodination allows specific activities between 0.5 and 2.5×10^6 dpm/µg to be obtained. The labelling is stable in our incubation conditions for periods at least as long as those of our penetration studies. In any case, the radioactivity precipitated by trichloracetic acid does not decrease over this period when maintained at 4 °C (taking into consideration the decrease in ^{125}INa radioactivity). Our modified iodination method does not change NHCP biological activity, since homospecific labelled NHCP added to a culture medium at a concentration of 50 µg/ml was previously found to inhibit morphological differentiation of neurons and alter muscular cell differentiation [15]. From autoradiography carried out on sections 1500 Å thick, it is seen that almost all the nuclei are hardly labelled at all, unlike the cytoplasm which is very significantly labelled (Figure). Even after 16 h incubation of cells in the presence of NHCP, no significant nuclear labelling is observed. This agrees with the results of the study of ^{125}I labelled NHCP ascitic (Zajdela) hepatoma penetration in the cells of the same tissue. Almost all the radioactivity is localized in the cytoplasmic fraction (Table) as cellular fractionation and determination of the radioactivity of the different fractions show. Although preliminary results lead us to think that most of the proteins are labelled with high specific activity, we cannot exclude the hypothesis that some of them are scarcely labelled at all and thus escape monitoring in autoradiography. In addition, using the autoradiographic technique on whole cells treated and fixed on culture chambers without detaching them from their support, we cannot conclude that there is a difference in labelling between the various cellular types, whereas on the biological tests, only the neurons are sensitive to NHCP action [15]. Furthermore, heterospecific labelled NHCP penetration showed a cytoplasmic labelling as great as labelling obtained with homospecific NHCP; but it has previously been shown that heterospecific NHCP does not act on morphological cellular differentiation. These results thus give evidence within the limits of our autoradiographic technique for a) penetration and concentration of proteins at the cytoplasmic level and not at the nuclear level, which raises the question of how they act [15]. b) Homospecific and heterospecific proteins penetrate the cells equally well, although the heterospecific ones have no action on cell differentiation, unlike the homospecific ones.

[16] J. ZALTA, J. P. ZALTA and R. SIMARD, J. Cell Biol. *51*, 563 (1971).
[17] T. Y. WANG, J. biol. Chem. *242*, 1220 (1967).
[18] G. S. DAVID, Biochem. biophys. Res. Commun. *48*, 464 (1972).
[19] A. M. DUPRAT, J. Embryol. exp. Morph. *24*, 119 (1970).

Cell Types Originating from Kidney Explants of Young and Old Mice

J. LIPETZ and R. E. BOSWELL

Department of Biological Sciences, Drexel University, Philadelphia (Pennsylvania 19104, USA), 29 July 1975.

Summary. Explants from young and old mouse kidneys give rise to two different cell types when placed in organ culture dishes. The two cell types differ in morphology and ability to grow in vitro. Explants from young mice give rise to one predominant cell type; those from old mice give rise to another. Our data supports the mosaic theory of aging.

Since the initial reports by CARREL and BURROWS [1] that cells emigrate more rapidly from explants taken from young animals than those taken from older animals, this phenomenon, referred to as 'latency', has been studied in the hope of gaining insights into the general problem of organismal and cellular aging [2–4].

EPHRUSSI and LACASSAGNE [5] noted that different cell types emigrated from explants taken from young and old animals, as did SOUKUPOVA et al. [6]. A majority of these studies have been qualitative, made on explants cultured in plasma clots or hanging drops, and generally only continued for a few hours or days. Our experiments