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Uptake of RNA precursors in normal and virus-transformed cells

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Biochimica. — *Uptake of* RNA *precursors in normal and virus-transformed cells* (*). Nota di TAMILLA EREMENKO, TONINO MENNA e PIETRO VOLPE, presentata (**) dal Socio A. RUFFO.

RIASSUNTO. — Il trasporto totale di uridina in cellule NRK trasformate con RSV era più alto di quello che si osservava in cellule NRK normali. La trascrizione, però, non veniva influenzata in modo apparente da questo aumento di trasporto. Inoltre, per le cellule NRK normali, si verificava una correlazione inversamente proporzionale tra il peso molecolare e/o la carica dei precursori (dall'U all'UTP) e il loro reale trasporto. Tale correlazione inversa, che peraltro si verificava anche per le cellule HeLa in presenza o in assenza di anidride carbonica, scompariva per le cellule NRK RSV-trasformate.

1. INTRODUCTION

It was found that contact-inhibited cells incorporate UDR, and then insert it into RNA, more slowly than do rapidly growing cells [1]. This led to two suggestions: on the one hand, that inhibition of the rate of transcription could be a controlling element in contact inhibition of cell proliferation [2] and, on the other, that only the decreased entry of UDR into the cell, rather than a decreased rate of RNA synthesis, is responsible for the reduced rate of incorporation into RNA of contact-inhibited cells [3].

The present investigation, confirming that the total entry of UDR (uridine) into contact-inhibited cells is lower than that into virus-transformed cells, suggests that RNA precursor uptake and transcription are rather independent processes in transformed cells. In addition, we demonstrated that in normal cells there exists an inverse correlation between the molecular weight and/or charge of the RNA precursors (from U to UTP) and their capacity to penetrate the cell membrane which is not found in transformed cells.

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2. MATERIALS AND METHODS

2.1) *Cell cultivation.*

HeLa cells (mycoplasma-free) were grown as spinner suspension at 37 °C in Joklik-modified minimum essential medium, without Ca^{++}, supplemented with 10% calf serum under magnetic stirring and a continuous flow of 5% carbon dioxide in air [4]. Fresh medium was substituted every 2 days, maintaining the culture between 2.5 and 5 $\times 10^5$ cells/ml. The primary lines of NRK cells and NRK cells transformed with RSV (mycoplasma-free) were grown as monolayers ay 37 °C in Eagle minimum essential medium, with Ca^{++}, supplemented with 10% foetal calf serum under a continuous flow of 5% CO_2 in air [5]. The medium, in this case, was maintained unchanged for about 100 hours during the cell culture growth cycle, whereas the new cell cultures were always implanted starting from confluent monolayers.

2.2) *Cell radiolabelling.*

Before incubation with the labelled RNA precursors, samples of various cells were washed twice in Hanks salt solution, resuspended in this same solution at the concentration of 1 $\times 10^6$ cells/ml and then subdivided into the needed portions for detection of the RNA precursor uptake curves, as specified in the legends to the figures. For cell labelling all radioactive RNA precursors were used at the final concentration of 1 μCi/ml $\times 10^{-6}$ cells.

2.3) *Cell disruption.*

After labelling, at the times chosen for detection of the RNA precursor uptake curves, the cells were fractionated with 10% TCA to yield the precipitable material containing the newly synthesized RNA, and the soluble material containing the pool free, non-incorporated, precursors transported through the membranes.

2.4) *Chemicals.*

The Joklik-modified and the Eagle minimum essential media as well as adult and foetal calf sera were obtained from Grand Island Biological Company, New York, USA. Before use, the sera were heated for 2 hours at 56 °C and filtered through Seitz EKSII [6]. Trypsin (ICN Pharmaceuticals, Cleveland Ohio, USA) was diluted in TD-solution [5]. The radioactive products were furnished by the Radiochemical Centre, Amersham, England: ^{3}H-5-uracil, 27 Ci/mmol; ^{3}H-5-uridine, 25 Ci/mmol; ^{3}H-5-uridine-5′-monophosphate, 11.2 Ci/mmol; ^{3}H-5-uridine-5′-diphosphate, 22 Ci/mmol; ^{3}H-5-uridine-5′-triphosphate, 13.6 Ci/mmol.

3. Results

3.1) *Kinetics of incorporation of* RNA *precursors by Hela cells under different experimental conditions.*

Fig. 1 shows that, in 6 hours of uptake, the amount of radiolabelled RNA precursors transported through the HeLa cell plasma membrane was inversely proportional to their molecular weight and/or charge, i.e. it was the highest for ^{3}H-U and the lowest for ^{3}H-UTP. This phenomenon did not seem to vary as a function of aeration with 5% carbon dioxide. In fact, the general fashion of all RNA precursor uptake curves was the same both in experimental cells (where the radioactive substances entered the external membrane under a flux of 5% CO_2 in air) and in control cells (in the absence of CO_2). The amounts of precursors non-incorporated (open circles) and incorporated (shaded areas) were also inversely proportional to their molecular weights and/or charges. Nevertheless, the maximal rate of uptake of these substances by the cells took place over about 30 min (from ^{3}H-U to ^{3}H-UDP) to 2 hours (^{3}H-UTP) decreasing again in about 6 hours. The corresponding maximal rate of their incorporation into RNA took place later, when the uptake was practically finished.

3.2) *Kinetics of incorporation of* RNA *precursors by* NRK *and* RSV-*transformed* NRK *cells.*

The inverse correlation observed in HeLa cells between the degree of structural complexity of the RNA precursors and their capacity to penetrate the cell membrane appeared to be a common feature also for normal NRK cells treated with radioactive substances in the absence of 5% carbon dioxide in air (fig. 2, I-V). However, in these cells there were no differences between the uptake behaviours of the five RNA precursors since they all showed the maximal point of penetration into the cell at later stages (about 4 hours).

In normal NRK cells, the maximal incorporation of precursors into RNA (shaded areas) roughly corresponded to the maximal point of total uptake. NRK cells, unlike HeLa cells (fig. 1), showed no increase, at later stages, in the incorporation of procursors into RNA corresponding to the decrease in their total uptake.

There were unequivocal features which made NRK cells transformed from normal, different with respect to the RNA precursor uptake. For transformed cells the inverse correlation mentioned no longer existed. After transformation, the uptake per cell of all RNA precursors, except for U (fig. 2, VI), was much higher than that observed for normal cells. Actually, in transformed cells, the inverse correlation of the degree of uptake to the molecular comple-

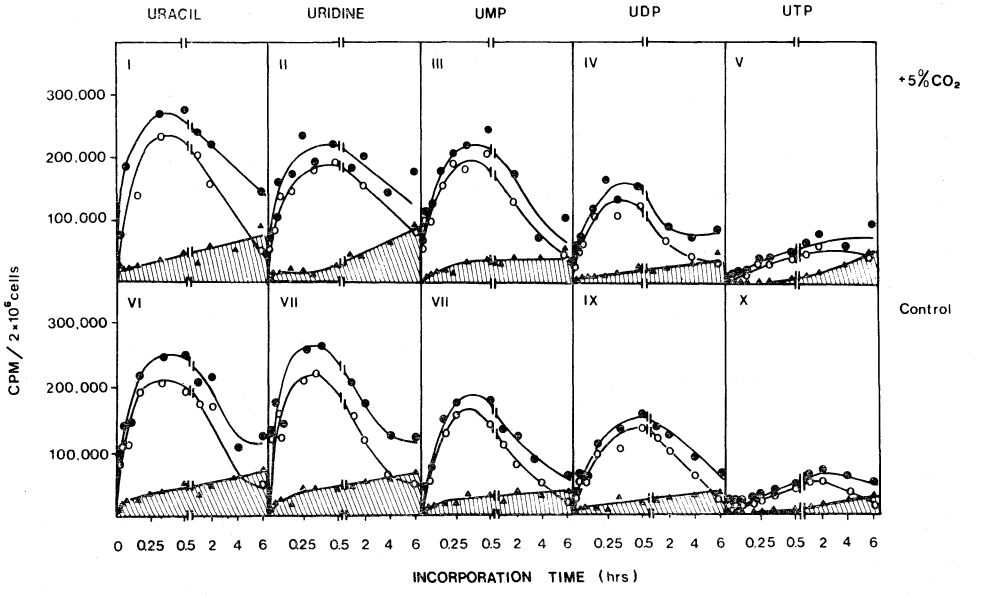

Fig. 1. Kinetics of incorporation of RNA precursors by HeLa cells. For each precursor (^{3}H-uracil, ^{3}H-uridine, ^{3}H-UMP, ^{3}H-UDP and ^{3}H-UTP), 25×10^6 cells were withdrawn from the culture suspension, resuspended in 24.75 ml Hanks and mixed with 0.25 ml of the same solution containing 25 μCi of radioactive material. The cell suspension obtained (1 μCi/11 $\times$ 10^6 cells) was then incubated for 6 hours at 37°C under continuous shaking. To stop radioactive incorporation at the indicated times during 6 hours, 2 ml of experimental suspension (with 2×10^6 labelled cells) were withdrawn and quickly replaced in a centrifuge tube containing 5 ml of chilled Hanks. Cell harvesting was achieved in 6 min at 2,000 rev./min. Hence, the cell pellet was washed twice in 5 ml of chilled Hanks, recentrifuged under the same conditions and treated for 30 min in the cold with 1 ml 20% trichloroacetic acid. The TCA-lysate was fractionated by centrifuging for 6 min at 2,000 rev./min. The supernatant was replaced directly in a vial containing 10 ml Insta-gel, while the precipitate was washed with 8 drops of water which, after centrifuging again for 6 min at 2,000 rev./min, was added to the same vial to test the TCA-soluble radioactivity yielded. The washed TCA-precipitable fraction was dissolved in 10 ml Insta-gel and also tested for radioactivity. Above (I-V), there are the kinetics of incorporation of the different RNA precursors as measured under a continuous flow of 5% CO_2 in air; below (VI-X), there are the control kinetics measured in the absence of CO_2. The values represent the mean of three experiments. (●—●) Total radioactivity; (○—○) TCA-soluble radioactivity; (—▲) TCA-precipitable radioactivity (shaded areas).

xity remained only for mono-, di- and triphosphoribonucleosides (fig. 2, VIII-X). From U to UMP, this correlation was, instead, direct (fig. 2, VI-VIII). In any case a gradual increase of incorporation of U, UDR and UMP into RNA did not correspond to the gradual increase of uptake, and an increased RNA synthesis did not correspond to the increased uptake of UDP and UTP in transformed cells (fig. 2, IX, X) when compared to that in normal cells (fig. 2, IV, V).

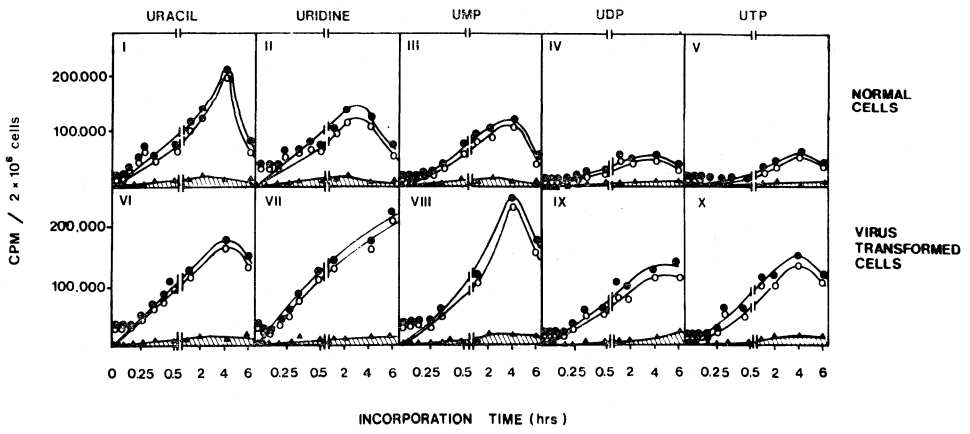

Fig. 2. Kinetics of incorporation of RNA precursors by NRK and RSA-transformed NRK cells. The uptake curves for all five precursors examined (^{3}H-U, ^{3}H-UDR, ^{3}H-UMP, ^{3}H-UDP and ^{3}H-UTP) were followed both in normal (I-V) and virus-transformed (VI-X) cells, using the same procedure described in the legend to fig. 1. However, the incorporation of the radioactive material was only allowed to occur in the absence of CO_2. The values represent the mean of three experiments. (●—●) Total radioactivity transported into the cells; (○—○) TCA-soluble radioactivity; (▾ — ▾) TCA-precipitable radioactivity (shadedareas).

4. Discussion

The initial aim of this work was to verify whether the transport of UDR (which is the commonly used precursor for cell or virus RNA synthesis, since a very small portion of it is incorporated into DNA) in transformed cells is higher than that in normal cells, as claimed by Levine and co-workers [1]. It is indeed shown that in 6 hours after pulsing the uptake of UDR was enhanced in virus-transformed cells (fig. 2, VII) However, the net synthesis of RNA, while low, still remained the same for both NRK and RSV-transformed NRK cells In transformed cells the synthesis of RNA tended to plateau at the end of the pulse, while the free precursor in the pool continued to increase. All this suggested that after transformation the UDR uptake, rather than depending on transcription, depends to a certain extent on variation of permeability of the cell membrane.

Next, whereas the uptake of the other RNA precursors—except for U—was roughly doubled after transformation, an inverse correlation between the structural complexity of these precursors and their levels of uptake through the cell membrane was found in different cell types, e.g. HeLa and NRK cells, both in the presence and in the absence of carbon dioxide. Of course, one could not estimate whether all molecules such as UTP were taken up as such or whether in part they were first broken down by the cell membrane. Anyway, the phenomenon revealed by fig. 2 represented a credible criterion for comparing normal and virus-transformed cells. For RSV-transformed NRK cells, the

inverse correlation remained only for mono-, di- and triphosphoribonucleosides; from U to UMP the correlation of the degree of uptake to the structural complexity was instead direct. In addition, the gradual increase of uptake of U, UDR and UMP by transformed cells did not correspond to a gradual increase of their incorporation into RNA. This strongly supported the idea that the RNA precursor uptake and the RNA biosynthesis become rather independent processes in transformed cells. In other words, it appeared that the transformed cell membrane loses normal transport control, whereas the overall uptake of precursors is increased. Such increase, in turn, conformed fairly well with the fact that the metabolic lability of triphosphonucleosides is in general increased upon viral transformation, causing reductions in the UTP pool [7]. Probably, after viral transformation, the decreased UTP pool would cause by feed-back an increased UTP uptake.

We suppose that in RSV-transformed NRK cells the proteins involved in the active transport of nucleotides could be phosphorylated by the $pp60^{src}$ transforming enzyme [8], thus modifying the normal uptake shown in figure 2, I-V to that of fig. 2, VI-X. The aim of the further analysis is, therefore, to verify whether the normal condition is conserved after infection of the NRK cells with *src*-defective [9] virus mutants.

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