FURTHER EXPERIMENTS ON THE ROLE OF
THE NUCLEOLUS IN THE TRANSFER OF RNA
FROM NUCLEUS TO CYTOPLASM

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SUMMARY

The role of the nucleolus in the transfer of RNA from nucleus to cytoplasm was examined by means of experiments in which the nucleolus or other parts of the nucleus were inactivated by a microbeam of ultraviolet light. These experiments confirmed previous work in showing that such inactivation of the nucleolus inhibited the appearance in the cytoplasm, not only of ribosomal RNA, but also of other types of RNA made in the nucleus, in particular transfer RNA.

INTRODUCTION

It is generally thought that nucleoli are the sites of synthesis of ribosomal RNA. Recent work has, however, indicated that nucleoli might also have a role in controlling the transfer of informational RNA from the nucleus to the cytoplasm (Harris, Sidebottom, Grace & Bramwell, 1969; Deák, Sidebottom & Harris, 1972).

The irradiation of the nucleolus with a microbeam of ultraviolet light reduces cytoplasmic RNA labelling (Perry, Hell & Errera, 1961; Takeda, Naruse & Yatani, 1967). This reduction was thought to be due to inhibition of the synthesis of ribosomal RNA and its transfer to the cytoplasm (Perry et al. 1961; Takeda et al. 1967). However, Sidebottom & Harris (1969) have recently shown that erythrocyte nuclei reactivated in heterokaryons do not contribute detectable amounts of labelled RNA to the cytoplasm of the cell before the nuclei develop nucleoli; and they have also shown that intensive inactivation of the nucleolus in a nucleus that contains only one such structure reduces cytoplasmic RNA labelling to very low levels. These results were thought to indicate that the nucleolus might be required not only for the synthesis of ribosomal RNA, but also for the transfer of other types of nuclear RNA to the cytoplasm of the cell. However, no detailed study has yet been done to determine whether the reduction in cytoplasmic labelling observed by Sidebottom & Harris after nucleolar inactivation could be accounted for simply by the abolition of ribosomal RNA synthesis. This question is examined in the present paper.
MATERIALS AND METHODS

Cells

BSC-1 cells, a line derived from Cercopithecus aethiops kidney cells (Hopps, Bernheim, Nisalak, Tjio & Smadel, 1963), were used for all experiments. They were maintained in Eagle's Minimal Essential Medium (Eagle, 1959) containing 10 % foetal calf serum (Flow Laboratories, Irvine, Scotland).

Ultraviolet microbeam

The apparatus was constructed by Sidebottom and was used, with minor modifications, as described by Sidebottom & Harris (1969). Nucleoli and other parts of the nucleus of BSC-1 cells were irradiated by a beam 4 μm in diameter, whole nuclei by a beam 25 μm in diameter. The dose of irradiation was varied by varying the time of exposure to the beam.

Localization of cells

A chromium grid was shadowed on to the quartz coverslips used in the microbeam experiments (details of the procedure will be published elsewhere). The position of the cells relative to the chromium grid was recorded before they were irradiated so that cells could be found again.

Radioactive labelling of cells

Cells were irradiated with the microbeam at room temperature and then incubated at 37 °C for 1 h. They were then exposed to tritiated uridine (specific activity 29 Ci/mM, obtained from the Radiochemical Centre, Amersham, Bucks.) at a concentration of 20 μCi/ml for 2.5 h at 37 °C, rinsed in phosphate-buffered saline at pH 7.3 and fixed in methanol. The fixed preparations were prepared for autoradiography.

Autoradiography

Autoradiography was done by the method of Messier & Leblond (1957). Coverslips were mounted on slides with D.P.X. (G. T. Gurr, London). Acid-soluble precursors were extracted with 5 % trichloroacetic acid at 4 °C for 30 min and the slides were then washed. Slides were dipped into K5 liquid emulsion (Ilford Ltd., Ilford, Essex) diluted 2:1 (v/v) with distilled water, and then left to dry at room temperature for 2 h. Autoradiographs were exposed for 4-8 days and then developed with Kodak D19B developer (Kodak Ltd., Hemel Hempstead, Herts.) and fixed with ‘Fix-Sol’ (Johnsons, Hendon, London) diluted 1:3 with distilled water.

The developed autoradiographs were stained for 5-10 min in 10 % Giemsa stain (G. T. Gurr) in distilled water buffered to pH 6-8.

Chemical analysis of labelled cytoplasmic RNA

Labelling of cells. Ten 20-oz (approx. 570-ml) medical flats, each containing 2-3 × 10⁶ cells, were left for 1 h at room temperature, incubated at 37 °C for 1 h and then exposed to tritiated uridine at a concentration of 2.5 μCi/ml for 2.5 h.

Extraction procedures. After the cells were labelled, the medium was decanted and the cells harvested in a solution of ethylene diaminetetraacetic acid (EDTA) at a concentration of 0.025 % in phosphate-buffered saline at pH 7.3. The cells were then washed twice with saline and 5 × 10⁷ HeLa cells were added as carrier. The cytoplasmic RNA was extracted by the method of Bramwell & Harris (1967).

Polyacrylamide gel electrophoresis. The method used was essentially that described by Loening (1967) except that 6 M urea was included in the gels. These were composed of 2.8 % acrylamide (recrystallized, Kodak, London) containing 0.14 % ethylene diacyrate. The gels were subjected to electrophoresis for 2 h at 100 V at 4 °C in an 0.02 M sodium acetate—0.02 M sodium dihydrogen phosphate—0.001 M EDTA buffer at pH 5.4. After the optical density had been scanned, the
gels were cut into 2-mm slices which were digested in 0.3 M lithium hydroxide for 30 min at 37 °C. Scintillation fluid containing CAB-O-Sil (Packard, Wembley) was added and the radioactivity in the samples counted.

RESULTS

Effects of microbeam irradiation on uridine incorporation into RNA

Five groups of cells were compared: (1) unirradiated cells; (2) cells in which the whole nucleus was irradiated with the ultraviolet microbeam; (3) cells with nuclei containing a solitary nucleolus which was irradiated; (4) cells with nuclei containing 2 nucleoli one of which was irradiated; (5) cells in which a non-nucleolar part of the nucleus (nucleoplasm) was irradiated. Several doses of irradiation were given and approximately thirty cells were counted for each dose in each group. Cytoplasmic RNA labelling after irradiation of different parts of the nucleus was expressed as a percentage of the level of labelling found in unirradiated cells. Some residual cytoplasmic RNA labelling occurs even in cells in which the whole nucleus has been irradiated. A correction was therefore made to allow for this residual labelling.

Irradiation of the nucleolus inhibits the incorporation of uridine into nucleolar RNA (Fig. 1). A dose of 6 s inhibits incorporation by 95%; increasing the dose beyond 6 s does not eliminate the residual 5%.

\[ \text{Fig. 1. Effect of increasing the dose of nucleolar irradiation on nucleolar RNA labelling.} \]

Irradiating any part of the nucleus causes a partial inhibition of nucleoplasmic labelling (Fig. 2). Inhibition reaches a maximum level with a dose of 6 s. Irradiating a solitary nucleolus causes much more extensive inhibition of nucleoplasmic labelling than irradiating an area of nucleoplasm; irradiating one nucleolus of 2 in a single nucleus produces an intermediate effect. These results indicate that a large proportion (approximately 50%) of nucleoplasmic labelling is dependent on nucleolar activity.

Fig. 3 shows the effect of irradiation of parts of the nucleus on cytoplasmic RNA labelling. When a nucleoplasmic area, or one nucleolus of 2, is irradiated, there is a
Fig. 2. Effect of increasing the dose of irradiation to different parts of the nucleus on nucleoplasmic RNA labelling. •, cells in which a nucleoplasmic area was irradiated; ○, cells in which one nucleolus of 2 in the same nucleus was irradiated; ▲, cells in which a solitary nucleolus was irradiated. (Error bars are omitted for clarity, but the errors were of the same order as those shown in Fig. 1.)

Fig. 3. Effect of increasing the dose of irradiation to different parts of the nucleus on cytoplasmic RNA labelling. •, cells in which a nucleoplasmic area was irradiated; ○, cells in which one nucleolus of 2 in the same nucleus was irradiated; ▲, cells in which a solitary nucleolus was irradiated.
Role of nucleolus

Fig. 4. Effect of increasing the dose of irradiation to different parts of the nucleus on A, cytoplasmic RNA labelling, and B, nucleoplasmic RNA labelling. Irradiation dose was derived by comparing the inhibition of nucleolar labelling after irradiation of the solitary nucleolus with the inhibition of nucleolar labelling after irradiation of the whole nucleus; the ratio of these 2 measurements is expressed as a percentage. It is assumed that cells irradiated in a nucleoplasmic area or in one nucleolus of 2 within the same nucleus receive the same dose of irradiation when treated in the same way in the same preparations. ○, cells in which a nucleoplasmic area was irradiated; □, cells in which one nucleolus of 2 in the same nucleus was irradiated; ▲, cells in which a solitary nucleolus was irradiated. (Error bars are omitted in Fig. 4B for clarity, but errors were of the same order as those shown in Fig. 4A.)

reduction in cytoplasmic RNA labelling; a maximal effect is reached at a dose of 6 s. When a solitary nucleolus is irradiated, however, cytoplasmic RNA labelling falls progressively until it is almost eliminated. The effect is progressive beyond a dose of 6 s which produces maximal inhibition of nucleolar RNA labelling. These results show that nucleolar irradiation inhibits the transfer of RNA from the nucleus to the cytoplasm. This effect is produced partly by the inhibition of RNA synthesis in the nucleolus, partly by reducing the net accumulation of labelled RNA in the nucleoplasm and partly by the destruction of a nucleolar mechanism that is not dependent on nucleolar RNA synthesis.

The effects of irradiating different sites in the nucleus on cytoplasmic and nucleoplasmic RNA labelling are summarized in Fig. 4. Inhibition of nucleolar RNA labelling by more than 95% causes almost complete elimination of cytoplasmic RNA labelling (Fig. 4A), even though nucleoplasmic labelling at a reduced level continues (Fig. 4B).
When a comparable area of nucleoplasm is irradiated the reduction in cytoplasmic RNA labelling (Fig. 4A) is a direct reflection of the reduction in nucleoplasmic labelling (Fig. 4B). Irradiation of one nucleolus of 2 within the same nucleus produces intermediate levels of inhibition of both cytoplasmic and nucleoplasmic labelling.

**Chemical analysis of cytoplasmic RNA**

Fig. 5 shows the distribution of radioactivity in cytoplasmic RNA extracted from cells labelled under the same conditions as those used in the irradiation experiments. Ribosomal 18s and 28s RNA contains approximately 55% of the radioactivity and transfer RNA approximately 40%. Other RNAs contain less than 5% of the total radioactivity found in cytoplasmic RNA, as estimated from the distribution of counts in the gel.

**DISCUSSION**

These experiments permit the conclusion that the nucleolus is involved in the transfer to the cytoplasm not only of ribosomal RNA, but also of other kinds of nuclear RNA. Nucleolar irradiation clearly inhibits the flow of transfer RNA to the cytoplasm; but the limits of measurement allow no conclusion about the transfer of informational RNA, even if this could be identified by its labelling characteristics. Previous experiments (Deák et al. 1972) on the synthesis of specific proteins determined by reactivated erythrocyte nuclei in heterokaryons suggest, however, that the passage of informational RNA to the cytoplasm is also dependent on some nucleolar function.

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REFERENCES


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