EFFECT OF pH AND DIVALENT CATIONS ON THE RELEASE AND RE-ENTRY OF RNA IN ISOLATED NUCLEI

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1. Introduction

Release of prelabeled RNA from isolated nuclei is inhibited by divalent cations and low pH [1–3]. This phenomenon is still poorly understood. It is believed that divalent cations act through binding nucleoside triphosphates which supply energy for RNA transport [4–6]. Divalent cations may also interact with components of nuclear envelope and alter its structure. As for incubation medium pH, the mechanism of its influence on RNA release from isolated nuclei has not been considered before.

We demonstrate here that low pH of incubation buffer causes re-entry of in vitro released RNA into nuclei. Divalent cations do not exhibit such effect.

2. Materials and methods

Nuclei were isolated from 1 h pre-labeled ([^{3}H]-uridine, 25 Ci/ml) Djungarian hamster embryonic cells growing in monolayer after transformation by SV-40 virus [7]. All isolation procedures were performed at 4°C. The isolation medium contained solution A (0.25 M sucrose, 5 mM $MgCl_2$, 25 mM KCl, 20 mM 2-mercaptoethanol, 50 mM Tris–HCl (pH 7.6)) and 0.15% Triton X-100. After cell homogenization and centrifugation of homogenate at 26 000 rev./min (Beckman ultracentrifuge, rotor SW 27) for 1 h through 1.5 M sucrose in solution A, the nuclei forming the pellet were twice washed in solution A and incubated for RNA release at 30°C for 15 min with agitation. Final concentrations of the incubation medium components were: 10^7 nuclei/ml, 2 mM ATP, 5 mM phosphocreatine, 0.03 mg creatine phosphokinase/ml, all in 1 ml solution A. In the cases when $MgCl_2$ ($CaCl_2$) concentration or pH varied their values are indicated on the figures. At the end of incubation the samples were cooled and, as soon as possible, the incubation medium containing released RNA was separated from the nuclei by centrifugation at 3000 × *g* for 15 min. Samples were then precipitated with trichloroacetic acid (up to 5%), collected on HUFS filters (CSSR), and the amount of radioactivity determined by liquid scintillation counting. The RNA release from nuclei was estimated as a ratio (%) between released acid-insoluble radioactivity and a total acid-insoluble radioactivity of nuclei and supernatant.

To study RNA re-entry, nuclei were isolated from unlabeled Djungarian hamster cells. Procedures of isolation and incubation were as above. The incubation mixture contained unlabeled nuclei and postnuclear supernatant obtained after RNA release from an equivalent amount of labeled nuclei. In some cases labeled deproteinized RNA (30 cpm/μg RNA; 40 μg/ml incubation medium) was substituted for postnuclear supernatant. RNA re-entry into nuclei was estimated as % ratio between nuclear acid-insoluble radioactivity and total acid-insoluble radioactivity of nuclei and postnuclear supernatant.

3. Results and discussion

Standard pH for RNA release system is 7.6–8.0 [8]. RNA release from isolated Djungarian hamster cell nuclei has been shown to depend strongly on the incubation medium pH (fig.1a). Over pH 8.0–5.0 the release of acid-insoluble radioactivity decreased as pH of the reaction mixture became lower. At pH 6.0 the RNA release was very low: 0.8% as against 9% release at pH 7.6.

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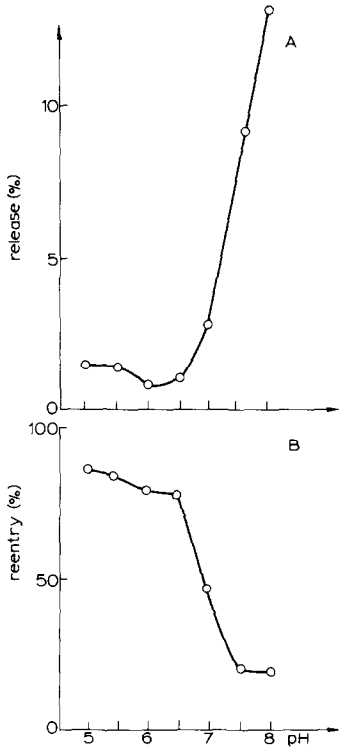

Fig.1. The dependence of the RNA release from (a) and RNA re-entry into (b) isolated nuclei on pH of the incubation medium.

There can be various causes for the observed decrease of RNA release from nuclei:

(i) Activation of a certain form of ribonuclease at low pH leading to reduction of acid-insoluble radioactive material in the incubation medium;

(ii) Reverse translocation of just released RNA into nuclei;

(iii) Changes in the nuclear envelope preventing RNA release.

Possibility (i) is excluded because the summed radioactivity of nuclei and postnuclear supernatant even slightly increased at lower pH (not shown).

To examine the possibility of released RNA re-entry into nuclei (ii), the system of reverse RNA transport was used (section 2). Unlabeled nuclei were incubated in the presence of pre-released [^{3}H]uridine-labeled RNA at different pH-values. Fig.1b shows that RNA re-entry into nuclei increases against lowering pH values of incubation medium. At pH 5.0–6.0 the level of RNA uptake by isolated nuclei was over 80% of all radioactivity added. When labeled deproteinized RNA was added into the incubation mixture instead of pre-

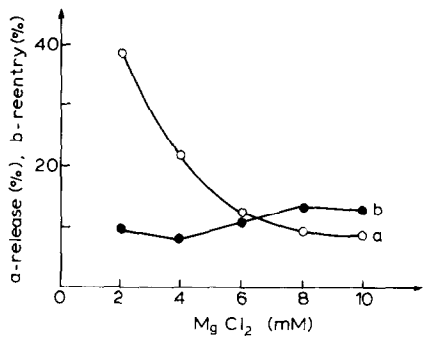

Fig.2. The dependence of the RNA release from (a) and RNA re-entry into (b) isolated nuclei on [$MgCl_2$].

released RNA, 55% of added radioactivity was taken up by isolated nuclei at pH 5.0–6.0. Therefore, we suppose that the reduction of RNA release observed upon decrease of pH is the result of RNA re-entry into nuclei (ii) rather than of its retaining inside the nuclei (iii).

A light-microscopic examination of nuclei indicated no effect of pH on their agglutination. Additional washings (2–3) of nuclei with solution A after incubation did not remove the radioactive material from the nuclei. Moreover, post-incubation washing of nuclei with 0.5% Triton X-100 in solution A removed only 7.8% of nuclear radioactivity. This allows us to rule out the possibility of RNA accumulation in nucleus clusters or on the surface of nuclei at low pH.

Therefore, a correlation between medium pH and RNA re-entry has been established.

As mentioned earlier, divalent cations inhibit RNA release from isolated nuclei. We explored the possibility of RNA uptake by nuclei in a medium with such Mg^{2+} (or Ca^{2+}) concentrations that markedly reduce RNA release (fig.2, fig.3). We have found that

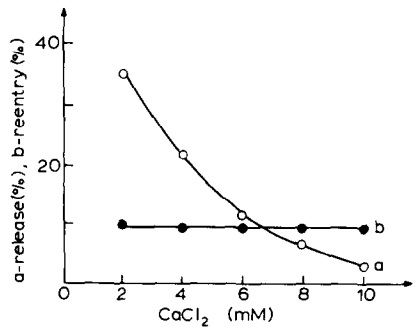

Fig.3. The dependence of the RNA release from (a) and RNA re-entry into (b) isolated nuclei on [$CaCl_2$].

an increase of Mg^{2+} (or Ca^{2+}) from 2–10 mM did not change appreciably the level of RNA re-entry into nuclei (fig.2, fig.3).

We conclude that inhibition of RNA release by low pH and by divalent cations may be the result of two different processes. The concentrations of divalent cations used prevent RNA release from nuclei while low pH stimulates the re-entry of released RNA.

RNA re-entry into isolated rat liver nuclei was reported in [9]. However, pH-dependent character of the process was not demonstrated.

It is not easy to envisage RNA entry from the cytoplasm into the nucleus in the living animal cell. But some evidence is available in favour of this process [10]. The phenomonon of reverse entry of RNA into nucleus, probably, plays an important role in cellular physiology, for instance, in nuclear reformation after the completion of mitosis. We suggest that this question is worth further investigation.

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