A GENERAL METHOD FOR ISOLATION OF INDIVIDUAL POLY-RIBOSOMES AND PURE mRNAs BY IMMUNOSORPTION TECHNIQUE

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ABSTRACT. A highly specific procedure has been developed for the isolation of individual polyribosomes by immunosorbents containing immobilized antigen–antibody complexes. Polyribosomes synthesizing immunoglobulin IgG1 and serum albumin were quantificated and isolated in the native state from MOPC 21A plasmacytoma and rat liver cells. For albumin polyribosomes, the presumptive messenger RNA was demonstrated; it contained three components: 20S, 16S and 9S.

ABBREVIATIONS. IgG, murine immunoglobulin; RSA, rat serum albumin; RGG, rat gammaglobulin; EDTA, ethylene diamine tetraacetate; SDS, sodium dodecyl sulphate.

I. INTRODUCTION

Up to now, for the isolation of individual polyribosomes and, consequently, pure mRNAs, immunological methods have mainly been used. These methods are based on the interaction of an antibody with nascent polypeptides on ribosomes (for a recent review see refs. 1, 2). In some cases, pure or enriched mRNA preparations were obtained by some other approaches [3, 4]. Presence of RNAse in antibody preparations as well as the necessity to provide the specificity of immunochemical precipitation of polyribosomes by using Fab fragments instead of whole antibodies [5] sharply restricts the possibilities of this approach, particularly on preparative scale.

Previously we have shown [6] that immunosorbents of the ‘sandwich’ type containing antigen–antibody complex covalently bound to an insoluble matrix may be used for the isolation of individual polyribosomes being free from the shortcomings mentioned above. However, conditions providing the sorption specificity have not been determined completely.

In the present paper, conditions providing high specificity of polyribosomal immunosorption are described. As an example, the quantification of albumin and γ-globulin polyribosomes was performed, and these polyribosomal species were separated from other cellular polyribosomes. In addition, sedimentation characteristics of the presumptive albumin mRNA have been obtained.

II. MATERIALS AND METHODS

Animals, tumors, treatments. White male rats of 180–200 g weight and BALB/c female mice of 14–16 g weight with the transplantable solid MOPC 21A plasmacytoma producing IgG1 (k-type) were used. Polyribosomes were isolated from rat liver and murine plasmacytoma.
To label rRNA, 40–50 μCi of $^{14}$C orotic acid (10 mCi/mmole, ‘Isotop’, U.S.S.R.) or $^{14}$C uridine (300 mCi/mmole, ‘UVVVR’, Czechoslovakia) per 100 g body weight was injected intraperitoneally for 24 hr.

For selective labelling of mRNA, the same labels were introduced to rats for 4 hr in an hour after the intraperitoneal injection of actinomycin D (25 μg per 100 g body weight).

To label nascent polypeptides, 10 μCi of $^{14}$C leucine (185 mCi/mmole, Amersham, England) were injected either intraperitoneally to rats or directly into the tumor to mice for 5 min.

**Preparation of antigens and antibodies.** IgG was isolated from the sera of tumor bearing mice as described in [7], rat serum albumin was prepared from rat sera [8].

The anti-IgG and anti-RSA antisera were raised in rabbits injected by 300–500 μg of IgG or RSA in complete Freund adjuvant (Difco) into popliteal lymph nodes and reinjected with a soluble protein (1 mg) into three sites a month later.

RGG was purified from anti-sera by (NH$_4$)$_2$SO$_4$ precipitation (40% of saturation) and fractionation on DEAE-cellulose. The content of anti-IgG and anti-RSA antibodies was usually about 10% of immuno-electrophoretically pure RGG preparation.

**Preparation of sandwich-sorbents.** Immunosorbents containing IgG or RSA covalently bound to diazotized aminocellulose were saturated by corresponding antibodies [9], washed by 0.14 M NaCl and standard buffer (0.01 M K$^+$-phosphate, pH 7.4; 0.15 M KCl; 0.015 M MgCl$_2$) and stored for 1–2 weeks in this buffer at the concentration of 5 mg ml$^{-1}$. Usually, ‘sandwich’-sorbents contained about 300 μg of antibodies per mg of dry weight.

**Preparation of polyribosomes.** Polyribosomes from rat liver and plasmacytomas were obtained by the modified technique of Blobel [10] using a Ti60 Spinco rotor (55000 rpm, 2 hr). To the post-mitochondrial supernatant 3 M KCl and 20% Triton X-100 were added up to 0.15 M and 2%, respectively. Polyribosomes were pelleted through a discontinuous sucrose gradient (0.5/2 M), dissolved in the standard buffer (see above) and cleared by low speed centrifugation (15000 rpm, 10 min).

**Binding of polyribosomes to the immunosorbent.** Polyribosomes were mixed with the immunosorbent in the standard buffer (usually, in 2 ml volume) and incubated for about 30 min at 0° upon continuous stirring. Thereafter the sorbent was separated by low speed centrifugation (3000 rpm, 3 min) and washed by the standard buffer. Immunosorbent pellets with specifically bound polyribosomes were used for the analysis of the nascent polypeptides or polyribosomal RNA and for the estimation of the binding degree. In the latter case, washed sorbent was transferred onto the millipore filter and dried to measure the radioactivity.

**Polyacrylamide gel electrophoresis of nascent polypeptides.** Immunosorbent carrying polyribosomes with labelled nascent polypeptides was suspended in the solution containing 0.02 M EDTA, 0.01 M Tris-HCl pH 7.4, 0.1 M NaCl (usually, 10–15 mg in 0.8 ml) and incubated for 5 min at 0°. Then 20% SDS was added up to 2% and the mixture was incubated for 1–2 min at 100°. The sorbent freed from nascent polypeptides was separated by low speed centrifugation (5000 rpm, 5 min). The eluate was 10-fold concentrated with polyethylene glycol (MW 40000) and subjected to SDS-polyacrylamide electrophoresis according to Schimke et al. [1].

**Analysis of polyribosomal mRNA in sucrose gradients.** Total and specifically bound polyribosomes with labelled mRNA were treated as described above for the polyribosomes with labelled...