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HEPARIN RELEASES MONOSOMES AND POLYSOMES FROM ROUGH ENDOPLASMIC RETICULUM

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SUMMARY

Incubation of membrane-bound polyribosomes isolated from murine myeloma cells with heparin caused release of material which sedimented in the polysome, monosome and ribosomal subunit regions of linear sucrose gradients. The released material corresponded to approximately one half that which could be released by treatment with heparin plus Triton X-100. The action of heparin appeared to be related to its polyanionic nature. The use of heparin as a ribonuclease inhibitor in the separation and isolation of free and membrane-bound polysomes could cause artificial accumulation of detached polysomes in the free polysome fraction.

INTRODUCTION

Polyribosome attachment to the membrane of the endoplasmic reticulum is mediated, in part, by the nascent polypeptide chain (2,22). However, using several procedures to disassemble membrane-bound polysomes *in vivo* and *in vitro*, a direct association of eukaryotic mRNA (1,4,6,11,14,20,23) and large ribosomal subunit (8,9) with rough endoplasmic reticulum have been demonstrated. Binding of the 60S subunits occurs to two membrane glycoproteins (ribophoryins, 8,9) whereas the mRNA-membrane association was found to involve the 3' terminus of the mRNA. Sabatini and colleagues (1,10,11) have presented contrasting data on the attachment of mRNA to membrane and Kruppa and Sabatini (10) dispute the finding of a rat liver mRNA-membrane attachment presented by Cardelli et al. (4). Following isolation of rat liver microsomes, Kruppa and Sabatini (10) added heparin as an RNase inhibitor to all buffers used for *in vitro* disassembly of polysomes from membranes. In Sabatini's previous reports on the association of mRNA with membranes (1,11), heparin was not added. In this communication,

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we show that heparin (a polyanion, see ref 3) causes the release of polysomes, monosomes and ribosomal subunits from rough endoplasmic reticulum isolated from the P3 myeloma cell line.

MATERIALS AND METHODS

Cell Maintenance and Isotopic Labeling: The IgG1 secreting mouse myeloma tissue culture line P3 (kindly provided by Dr. Matthew D. Scharff, Albert Einstein College of Medicine) was maintained in Dulbecco's modified medium (Grand Island Biological Co.) supplemented with 10% fetal calf serum, and 74.0 µg streptomycin, 100 units penicillin, 40 units mycostatin per ml. P3 cells at 7-8.5 x 10^5 cells/ml were diluted with one volume of fresh medium and incubated for 4 hours with 1 µCi/ml [5,6-^{3}H] uridine (Amersham, 49 Ci/mmol) immediately before isolation of polysomes. Identically treated unlabeled cells (2.4 x 10^8) were added to 8 x 10^7 labeled cells before polysome isolation to obtain sufficient polysomes for absorbance profiles.

Isolation of Polysome Fractions: The cells were rapidly cooled by pouring over crushed, frozen saline. All subsequent procedures were performed at 0-4°C. The cells were pelleted by centrifugation for 8 min at 500 x g max, washed once with RSB (20 mM HEPES, pH 7.5, 10 mM Na^+-NaCl plus NaOH used to pH the HEPES, 3 mM $MgCl_2$), resuspended in RSB and allowed to swell for 7 min. The cells were then pelleted, resuspended in 15% w/w sucrose (ribonuclease-free, Schwarz/Mann)-RSB and immediately homogenized in a Dounce homogenizer (Kontes Co.) with ten strokes of the B (loose) pestle. Nuclei were pelleted from the homogenate by centrifugation for 5 min at 900 x g max and washed once with 15% w/w sucrose-RSB. The wash was added to the first postnuclear supernatant which was then used as the source of polysomes. The postnuclear supernatant was layered over 2 ml of a 15% x 32% w/w sucrose-RSB linear gradient (17) which was centrifuged in an SW 50.1 rotor (Beckman) for 45 min at 27,000 x g max. The supernatant was stored at -80°C and used as a source of postmicrosomal polysomes. The pellet was resuspended and centrifuged as before. The resulting pellet was resuspended in 15% w/w sucrose-RSB and stored at -80°C for use as a source of membrane-bound polysomes.

Sucrose Gradients: Polysomes were analyzed on 15% x 40% w/w sucrose-RSB linear gradients consisting of 4.4 ml of gradient formed over a 0.5 ml cushion of 62% w/w sucrose-RSB. The gradients were centrifuged at 4°C for 40 min at 243,000 x g max in the SW 50.1 rotor. Fractions of 0.4 ml were collected from the top using an ISCO Model 640 density gradient fractionator. Absorbance at 254 nm was monitored continuously. Fractions were collected into scintillation vials and counted in 5 ml of toluene, Triton X-100, water (6:3:1) plus Omnifluor (New England Nuclear).

RESULTS

Membrane-bound polysomes were isolated from myeloma cells by differential centrifugation of a postnuclear supernatant. Polysome profiles were displayed on linear sucrose gradients. The membrane-bound polysomes did not exhibit an absorbance profile in the polysome region of the gradient (Fig. 1). Membrane-bound polysomes that had been incubated with heparin (1 mg/ml) released material which migrated in the polysome and monosome-subunit region of the gradient. The

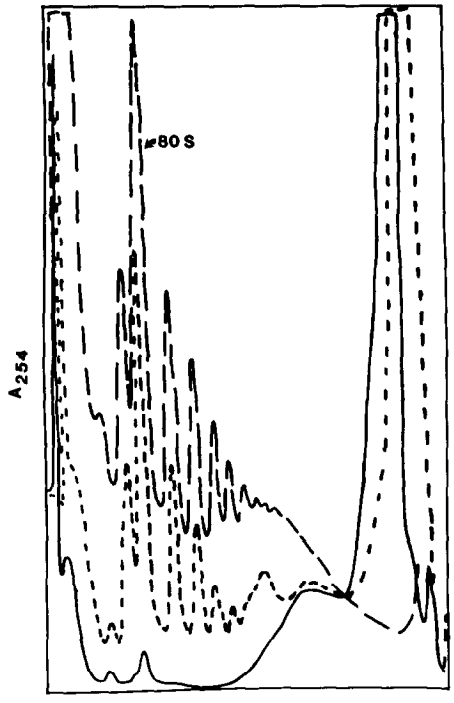

FIGURE 1. Linear-sucrose gradient absorbance profiles of P3 membrane-bound polysome fractions incubated for 10 min at 4°C with RSB (Control, ———), 1.0 mg/ml heparin (-----), or 1.0 mg/ml heparin plus 1% v/v Triton X-100 (— — —). Direction of sedimentation is from left to right.

released material corresponded to approximately one half of the material which could be released in the presence of heparin plus 1% v/v Triton X-100 (Table I). Similar results were obtained when membrane-bound polysomes were incubated with

TABLE I

RELATIVE DISTRIBUTION OF ^{3}H-URIDINE LABELED MATERIAL RELEASED FROM P3 ROUGH MICROSOMES

Gradient Region	Relative Distribution (% radioactivity) Following Treatment with RSB (Control)	Heparin	Heparin + TX-100
Subunits-Monosomes	19.5	39.1	60.8
Polysomes	6.8	20.0	34.4
Membrane-Bound Polysomes	73.7	40.9	4.8

Samples of the membrane-bound polysome preparation were incubated (at final concentrations of 22.5 A_{260}/ml) for 10 min at 4°C with RSB (control), 1.0 mg/ml heparin (sodium salt), or 1.0 mg/ml heparin plus 1% v/v Triton X-100. The preparations were analyzed on linear sucrose gradients (3.1 A_{260}/gradient). The gradient regions were determined by correlating the radioisotope distribution with the absorbance profiles shown in Fig. 1.

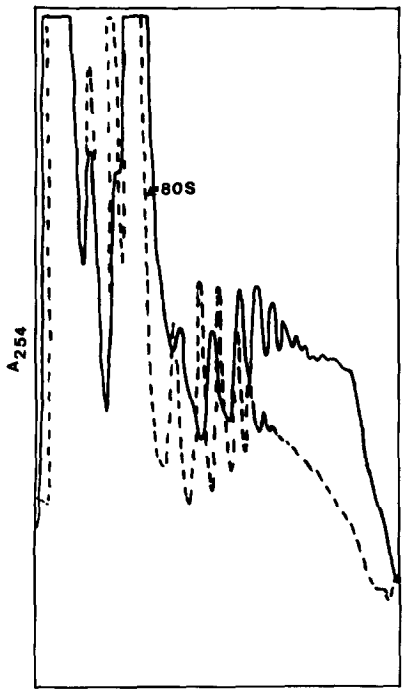

FIGURE 2. Linear sucrose-gradient absorbance profiles of P3 postmicrosomal polysome fraction (free polysomes) incubated for 10 min at 4°C with RSB (control, ———) or 1.0 mg/ml heparin (-----). 1.5 A_{260} units (control sample) and 1.1 A_{260} units (heparin-treated sample) were applied per gradient. Direction of sedimentation is from left to right.

the polyanion dextran sulfate while no material was released following incubation with the polycation spermine (data not shown).

The absorbance profile of free polysomes also was affected by incubation with heparin (Fig. 2). Two changes were observed: i) the apparent size of the large polysomes or aggregates was reduced and ii) shoulders merging with the monosome peak on the polysome and subunit sides of the gradient were absent. Incubation of free polysomes with Triton X-100 alone had no effect on the polysome profile (data not shown).

DISCUSSION

The interaction of protein synthetic machinery with endoplasmic reticulum may occur via three components acting alone or in various combinations. Linkage of the large ribosomal subunit to the ribophoryins of the membrane is well-documented (8,9) as is nascent polypeptide chain-membrane association (2,22).

Direct binding of mRNA to membrane is controversial. Two factors must be considered in the conflicting evidence for mRNA-membrane binding. First, the cell type and its growth characteristics may be of fundamental importance in the nature of polysome-membrane association (see refs 13,16,21). Second, conditions and reagents used for disassembly of membrane-bound polysomes must be compared.

Rapidly dividing cells, whether or not specialized for glycoprotein secretion, may have too few ribophoryins and/or receptors (?) for the protein's signal sequence and require an mRNA-membrane linkage as an initial step in protein synthesis. P3 myeloma cells growing in tissue culture are an example. Evidence to support this contention includes: i) a large proportion of P3 membrane-bound polysomes are sensitive to RNase-catalyzed release from membrane (13, our unpublished data); ii) P3 membrane-bound polysomes released from membrane by detergent treatment do not produce detectable authentic L chains in addition to expected precursor L chains in a readout system for cell-free protein synthesis (7,15, Freidlin and Patterson, manuscript submitted), iii) heparin mediates release of ribosomes and polysomes from membrane under conditions that should not disrupt nascent polypeptide chain or 60S subunit-membrane associations. Thus, disassembly of membrane-bound polysomes from these cells would show a proportion of mRNA directly associated with membrane even following removal of ribosomal subunits and/or nascent chains.

In contrast, cells dividing more slowly (i.e., myeloma cells grown as subcutaneous or ascites tumors, normal liver and pancreas cells) would be expected to have sufficient nascent chain signal receptors and/or ribophoryins to accomodate most secretory or membrane protein mRNA. In readout systems for cell-free protein synthesis, membrane-bound polysomes from these cells would produce both authentic and precursor polypeptides (2) and polysome disassembly would result in nearly complete release of mRNA (10,19).

Our finding that heparin releases polysomes and ribosomes from P3 microsomes may provide the basis for reconciliation between contrasting results on

membrane-binding of mRNA. The inclusion of heparin in buffers before in vitro disassembly of rough membranes by Kruppa and Sabatini (10) may have disrupted mRNA-membrane linkages, allowing complete mRNA release by treatments that disrupt nascent chain and ribosomal subunit-membrane linkages. Cardelli et al. (4,5), however, would not have observed complete mRNA release since heparin was not included prior to microsome disassembly. The experiments by Sabatini and coworkers (1,11) showing direct mRNA-membrane attachment in human diploid fibroblasts were conducted without heparin.

Heparin treatment appears to allow better resolution of the ribosome and subunit composition of free polysomes and results in a reduction in the apparent sedimentation rate of large polysomes. We speculate that heparin may be releasing polysomes and ribosomes that associate with a cytoskeleton structure similar to that observed by Lenk et al. (12). These authors showed that HeLa cells treated with detergent so as to remove greater than 90% of cellular membranes retain nearly all polysomes and rapidly labeled RNA and release only inactive ribosomes.

Heparin may merely release contaminating free or "loosely bound" polysomes from the microsomal fraction. This is unlikely since the membrane-bound polysomes were sedimented two times through linear sucrose gradients before treatment and any contaminating free or "loosely bound" polysomes probably were separted from the membrane-bound fraction (17). Thus, our results suggest that, at least for P3 myeloma cell homogenates, the presence of polyanion nuclease inhibitors such as heparin or dextran sulfate could cause artifactual accumulation of detached polysomes in the free polysome fraction.

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