RNA ASSOCIATED WITH PLASMA MEMBRANES OF EHRLICH ASCITES CARCINOMA CELLS

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1. Introduction

RNA has been detected biochemically in plasma membrane preparations from a number of sources [1]. The peripheries of several cell types, including Ehrlich ascites cells, have been shown to contain ribonuclease susceptible ionogenic groups, presumably RNA, which contribute to cellular electrophoretic mobility [2, 3]. There have been few attempts to elucidate the physiochemical nature of this "membrane RNA". Warren et al. [4] have demonstrated that isolated plasma membranes of "L" cells can carry out protein synthesis, indicating that the plasma membrane fraction must contain ribosomes, and thus ribosomal RNA. However, Shapot and Davidova have claimed that the bulk of the RNA isolated from plasma membranes has a molecular size distribution different from ribosomal RNA. These authors found that "membrane RNA" molecules sedimented in a density gradient with a bimodal distribution; the major peaks corresponded to 12 S and 3–5 S RNA [5]. By contrast, ribosomal RNA is composed of 30 S and 18 S components.

The present study concerns the characterization of the RNA derived from the plasma membranes of Ehrlich ascites tumor cells. Using biochemical methods, we have sought evidence for nonribosomal as well as ribosomal RNA species in plasma membrane preparations. We have also attempted to identify the RNAase-susceptible species which contributes to cellular electrophoretic mobility. In addition, this study delineates the possibility of artefacts due to degradation of RNA during membrane preparation.

2. Methods

Ehrlich ascites tumor cells were maintained in antibiotic-free suspension culture, as previously described [6]. Cellular RNA was labelled by incubating $5 \times 10^8 - 1 \times 10^9$ exponentially growing cells with 10–50 μCi of [^{14}C] or [^{3}H] uridine (Schwarz-Mann) for 16–20 hr. The plasma membranes of the Ehrlich ascites cells were isolated using a modification of the method of Warren et al. [7], in which approx. 3×10^8 cells were washed in normal saline and then fragmented in a loose-fitting Dounce homogenizer in the presence of zinc chloride and Tween 20 at 0–5° [7]. The homogenate was made up to 25% in sucrose, layered over a 50% sucrose solution, and centrifuged in a swinging bucket rotor for 1 hr at 100 *g*. The upper layer was removed and the interfacial layer was diluted with 25% sucrose, layered over 50% sucrose and recentrifuged. The resulting upper layer was pooled with the first, then diluted to 5% in sucrose and centrifuged for 10 min at 3,000 *g* in an angle head rotor. The pellet was recovered and layered over 20% sucrose; this was centrifuged for 30 min at 3,500 *g* in a swinging bucket rotor. The resulting pellet was the plasma membrane fraction (PMI). A further purification was achieved using a sucrose step gradient. The plasma membranes (PMII) banded at the 55/60% interface. The sucrose stock solutions used in this procedure were freshly made up, autoclaved (to inhibit RNAase activity) and then allowed to cool. The procedure was routinely monitored by phase contrast microscopy and the final product consisted almost entirely of large membranous fragments.

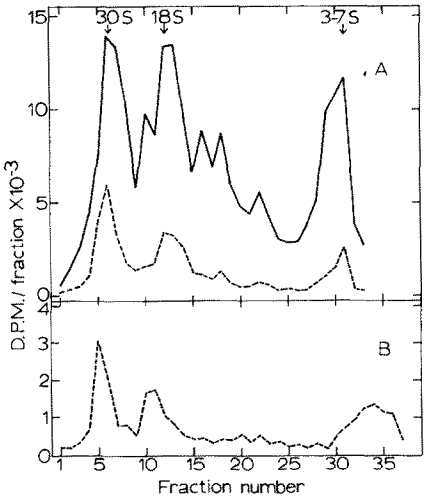

Fig. 1. Gel patterns of whole cell RNA and plasma membrane RNA. Radioactive RNA was electrophoresed on 2.5% acrylamide gels at 65 V for 45 min. Electrophoresis was performed at 5° ambient temperature. The gels were analysed as described in the methods section. Fig. 1A illustrates a gel containing both whole cell [^{3}H] RNA, and [^{14}C] RNA prepared from plasma membranes (PMI). Fig. 1B shows a gel containing [^{14}C] RNA prepared from repurified plasma membranes (PMII). Whole cell RNA: (——), plasma membrane RNA: (- - -).

RNA was extracted from cells, homogenates and plasma membranes by the method of Scherer and Darnell [8]. The RNA was characterized by acrylamide gel electrophoresis according to the method of Peacock and Dingman [9]. Gels containing radioactive RNA were sliced into uniform 2 mm sections on a Joyce Loebel gel slicer, digested overnight in 1 ml of 30% H_2O_2 and then dissolved in 10 ml of Bray's solution and counted. Gels containing both [^{14}C] and [^{3}H] RNA were analyzed by means of a double label program.

The following approach was used in seeking evidence for the RNAase susceptible ionogenic groups of Ehrlich ascites cell plasma membranes. Cells were labelled with either [^{3}H] or [^{14}C] uridine. The washed [^{14}C] labelled cells were incubated briefly with RNAase under the conditions used to demonstrate RNAase induced changes in cellular electrophoretic mobility [9]. The RNAase treated cells were washed 3 times in phosphate buffered saline and the [^{14}C] and [^{3}H] labelled cells were pooled. An aliquot was removed and used as a source of whole cell RNA, and the remainder of the cells were fractionated and used to prepare "membrane RNA". Samples containing [^{14}C] and [^{3}H] whole cell RNA, or "membrane RNA" were run on acrylamide gels, sliced and counted using the double label procedure.

3. Results and discussion

Whole cell RNA typically gave a trimodal pattern when run on acrylamide gels (see fig. 1A) [8]. The two slowly migrating peaks were 30 S and 18 S ribosomal RNA while the rapidly migrating peak probably consisted of t-RNA, 5 S ribosomal RNA and degradation products. When [^{3}H] labelled whole cell RNA, and [^{14}C] labelled plasma membrane RNA (PMI) were run in the same gel, they showed similar patterns, except that the plasma membrane (PMI) fraction contained relatively little rapidly migrating RNA (fug. 1A). The [^{3}H] RNA from repurified plasma membranes (PMII) gave essentially the same pattern as the plasma membrane (PMI) RNA when run in parallel (see fig. 1B). Thus most of the plasma membrane RNA of Ehrlich ascites tumor cells seems to be ribosomal RNA. Some low molecular weight RNA is also present, but there is no evidence for a 12 S component in the RNA from Ehrich ascites tumor plasma membranes.

Shapot and Davidova have claimed that liver cell plasma membrane 12 S and 3–5 S RNA is not ribosomal RNA [5], and have presented convincing evidence that the plasma membrane-associated RNA is not simply a contaminant [5]. However, they have not ruled out the possibility of artefacts due to degradation of RNA during preparation of the membranes.

We have attempted to assess the possibility of RNA degradation in our experiments by the following means. Radioactive RNA was isolated from plasma membranes, and from whole cell homogenates which had been exposed to the same concentrations of $ZnCl_2$, sucrose and detergent at the same temperature and for the same length of time as used in membrane preparation. This type of experiment was performed in two ways. In the first case the sucrose was not autoclaved and the sample was allowed to warm up during handling. In the second case autoclaved sucrose was used and the sample was constantly at 0–5°.

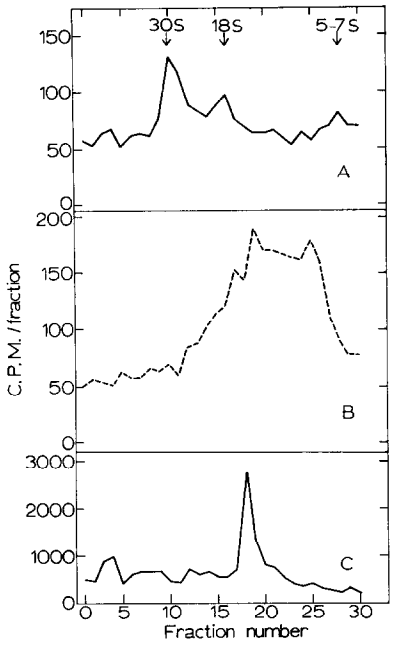

Fig. 2. Gel pattern of whole cell RNA and degraded whole cell RNA and plasma membrane RNA. [^{3}H] labelled RNA was electrophoresed as in fig. 1. The samples are A) untreated whole cell RNA, B) plasma membrane RNA, C) RNA of whole cells treated with detergent, zinc and sucrose. The conditions used to prepare the samples for B) and C) favored degradation of high molecular weight RNA.

Fig. 2 illustrates the electrophoretic patterns of the labelled RNA resulting from the first treatment. Untreated whole cells yielded RNA with the usual trimodal distribution (see fig. 2A). The RNA from plasma membranes (PMI) had a bimodal distribution with the two major peaks corresponding to 15 S and 3–7 S RNA (see fig. 2B). The whole cells which had been treated with zinc and non-autoclaved sucrose yielded material which lacked the usual 30 S, 18 S and 3–7 S peaks and instead contained a large 15 S peak. In some other experiments of this type only the 3–7 S component appeared in the plasma membrane RNA fraction.

Fig. 3 shows the electrophoretic pattern of labelled RNA resulting from the second treatment. The plasma membrane RNA is mainly of high molecular weight, from 18 S to more than 30 S. The zinc and sucrose treated whole cell homogenate also contains mainly high molecular weight RNA, but distinct 18 S and 30

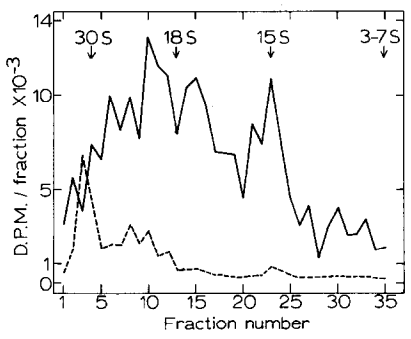

Fig. 3. Gel patterns of plasma membrane RNA and RNA of a whole cell homogenate. Electrophoresis was conducted as in fig. 1. The gel illustrated contained both [^{14}C] plasma membrane RNA (- - -) and [^{3}H] RNA from whole cells treated with detergent, zinc and sucrose (——). The conditions used to prepare the samples represented an attempt to minimize degradation of high molecular weight RNA.

S peaks are absent and a considerable 15 S peak is present.

Our interpretation of the above observations is that high molecular weight plasma membrane RNA can be degraded during membrane preparation. The high molecular weight RNA of whole cells (mainly ribosomal RNA) and of plasma membranes is degraded first to a 15 S component and then to smaller products if conditions are unfavorable. This degradative process can be minimized by uniformly maintaining samples at low temperatures and by inhibiting or removing the RNAase activity of the solutions used in cell fractionation.

The effect of RNAase treatment on the gel electrophoretic pattern of whole cell RNA and "membrane RNA" can be seen in fig. 4. In the case of whole cell RNA (A), the distribution of [^{3}H] label (untreated cells) and [^{14}C] label (RNAase treated cells) are essentially the same. In the case of "membrane RNA" (B), there is a slight reduction in the amount of [^{14}C] RNA migrating just ahead of the major peak but otherwise the distribution of [^{3}H] RNA and [^{14}C] RNA are the same. The small difference noted was not easily reproducible. These results indicate that the bulk of the plasma membrane associated RNA is not affected by a ribonuclease treatment of whole cells sufficient to alter cellular electrophoretic mobility.

The plasma membranes of cultured mammalian

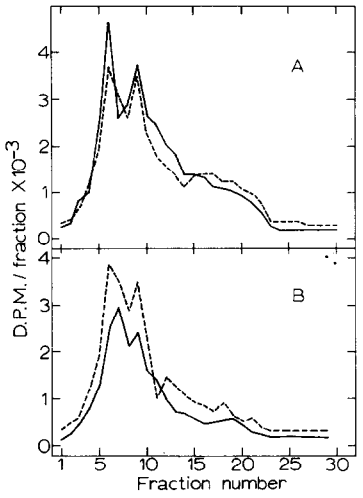

Fig. 4. Gel patterns of whole cell RNA and plasma membrane RNA from cells treated with ribonuclease. Electrophoresis was conducted as in fig. 1. Fig. 4A illustrates a gel containing whole cell RNA from both [^{14}C] labelled cells treated with ribonuclease (——) and [^{3}H] labelled control cells (- - -). Fig. 4B illustrates a gel containing plasma membrane (PMI) RNA from both [^{14}C] labelled cells treated with ribonuclease (——) and [^{3}H] labelled control cells (- - -).

cells prepared by the present method usually contain about 5% weight of RNA [10]. We have shown that this consists largely of ribosomal RNA. Shapot and Davidova [5] have found 2–3% by weight of RNA in liver and hepatoma plasma membranes and claim that this material represents a unique, non-ribosomal type of RNA. The possibility exists that plasma membranes prepared by the present method are contaminated with cytoplasmic ribosomes, while the membranes prepared by Shapot and Davidova are free of ribosomal contamination but contain a compensating amount of a unique type of RNA. It seems more likely however, that ribosomes are a usual component of plasma membranes, and that the RNA investigated by Shapot and Davidova represents degraded ribosomal RNA.

It has been estimated that the RNA associated with plasma membranes prepared by the zinc method represents 1–2% of the total cell RNA [11]. Very approximate calculations based on electrophoretic data suggest that the RNAase susceptible ionogenic groups represent 0.06–0.6% of total cell RNA [2]. Thus the ionogenic, cell surface RNA may only comprise 3–30% of the total plasma membrane-associated RNA. A species of RNA which was sensitive to RNAase treatment of whole cells, and which comprised 30% of the plasma membrane associated RNA would be easily detected in an experiment such as that shown in fig. 4. It is possible therefore that the RNA detected at the cell surface by electrophoretic mobility measurements represents only a small fraction of total plasma membrane-associated RNA. As an RNAase treatment which reduces cellular electrophoretic mobility is not sufficient to degrade membrane ribosomal RNA it is unlikely that the electrophoretic RNA is ribosomal RNA.

It is quite possible that the role of the plasma membrane associated ribosomal RNA is the synthesis of membrane proteins [4]. The role of the RNAase susceptible ionogenic groups remains to be elucidated.

Acknowledgements

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