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Isolation and Characterization of hnRNA-snRNA-Protein Complexes from Morris Hepatoma Cells

Angela PRÜSSE, Christos LOUIS, Angel ALONSO, and Constantin E. SEKERIS

Institutes of Cell Research and Experimental Pathology, German Cancer Research Center, Heidelberg; and National Hellenic Research Foundation, Biological Research Center, Athens

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Of the RNA labelled after incubation of hepatoma cells with radioactive precursors for 20 and 150 min, 35% and 70%, respectively, can be isolated from nuclei by two consecutive extractions with 0.14 M NaCl at pH 8. The isolated RNA is complexed with nuclear proteins forming structures with sedimentation coefficients of < 30 S to > 100 S. Similar complexes from rat liver isolated under the same experimental conditions show coefficients of 30–40 S. The RNA-associated proteins are similar, on the basis of sodium dodecyl sulphate/polyacrylamide gel electrophoresis, to the respective proteins of other cell types. The presence on these RNP complexes of six discrete small nuclear RNAs (snRNA) has been established. Experiments with a reversible inhibitor of RNA synthesis, D-galactosamine, demonstrated differences in the turnover of hnRNA and snRNA. The half-lives of the six snRNA species has been determined, varying from 32 h for snRNA species a, b and d, to 22 h for snRNA species e and f and to 13 h for snRNA species c.

Treatment of the nuclear extracts with 0.7 M and 1 M NaCl results in dissociation of hnRNA from the 'core' and other polypeptides, whereas snRNA remains complexed with polypeptides of M_r 54000–59000. Incubation of the nuclear extracts at 0 °C with low doses of pancreatic RNase (up to 1.5 μg/ml), which renders approximately 80% of the hnRNA acid-soluble and cleaves most of the snRNA, results in conversion of the high-molecular-weight hnRNPs to 30-S structures, without disrupting the 30-S RNP. Treatment of the nuclear extracts with higher doses of RNase (3 μg/ml) leads to disruption of the 30-S RNP and release of the hnRNA-associated proteins, underlining the importance of hnRNA-protein interaction for the retainment of the hnRNP structures.

Hormone-regulated biological systems are favored experimental models for the study of control of gene expression. Glucocorticoids induce the *de novo* synthesis of enzymes [1–3] in hepatocytes by increasing the amount of translatable mRNA [4–6]. Current evidence suggests an effect of the hormones on transcrption of the respective mRNAs [1–3, 7], although additional post-transcriptional sites of action cannot be ruled out [8, 9]. More information is needed on the nature of the primary transcripts, their association with nuclear structures, the site(s) of processing, the nature of the processing enzymes and the movement of the message out of the nucleus, before a detailed picture of the action of glucocorticoids on RNA metabolism can be obtained.

Most, if not all, of newly synthesized heterogeneous RNA (hnRNA) is found complexed to a discrete set of non-histone nuclear proteins [10–16] in the form of high-molecular-weight ribonucleoprotein complexes. In addition, small nuclear RNAs (snRNA), known for a long time to be present in nuclei of animal cells [17–24], have also been identified as part of the ribonucleoprotein complex [25–32] and have been implicated as participating in the splicing of hnRNA [32–35]. The presence of various enzymic activities associated with processing of hnRNA on these RNP structures [36–38] makes them likely sites of post-transcriptional modification of hnRNA.

In the present paper we report on the isolation of nuclear RNPs containing hnRNA and snRNA from hepatoma cells in culture. We have initiated a study of their protein and RNA components as a first step in gaining more information on the role of these structures on the formation of mRNA for the glucocorticoid-inducible enzymes tyrosine aminotransferase and tryptophan oxygenase.

Abbreviations. RNP, ribonucleoprotein; snRNA, small nuclear RNA.

MATERIALS AND METHODS

Reagents

[^{3}H]Uridine (spec. act. 40 Ci/mmol), [^{14}C]uridine (spec. act. 56 Ci/mmol), [^{3}H]orotic acid (spec. act. 26 Ci/mmol), [^{14}C]leucine (spec. act. 49 Ci/mmol) and [*Me*-^{3}H]methionine (spec. act. 14 Ci/mmol) were obtained from Amersham-Buchler (Braunschweig), 5-S tRNA was purchased from Miles Laboratories (England) yeast tRNA, pancreatic RNase (five times crystallized, 70 Kunitz units/mg) from Boehringer (Mannheim), sucrose (RNase-free) from Schwartz-Mann (Orangeburg, NY), RP-Royal X-Omat films from Eastman-Kodak (Rochester, NY) and 3MM filters from Whatman (London). All other chemicals were of analytical grade obtained commercially.

Animals and Cells

Male Wistar rats, weighing 120–180 g, were obtained commercially. Morris hepatoma cells, strain HS 22 were grown on Swim's S-77 medium supplemented with 10% newborn calf serum [39]. For most experiments cells were centrifuged from growth media, usually at a cell density of 6×10^5 cells/ml.

Isolation of hnRNP Particles from Hepatoma Cells

The cells were harvested with a Christ-Heraeus cryofuge by centrifugation at 1000 rev./min for 5 min. All subsequent steps were performed at 0–4 °C. The cells were then suspended in 20 vol. buffer A, left standing for 10–15 min and then homogenized in a tight-fitting Dounce homogenizer (10–20 strokes). Cell lysis was monitored by phase-contrast microscopy. Subsequently, the suspension was centrifuged at 3000 rev./min for 5 min in a SS-34 Sorvall head. The pellet was taken up in 20 vol. buffer B, evenly suspended by light homogenization in a glass Potter with a Teflon pestle, overlayed on 8 ml buffer B followed by centrifugation at 27000 rev./min for 2 h in a Beckman SW 27 head.

The nuclear pelled obtained was utilized for the extraction of hnRNP by the method used for the extraction of similar complexes from rat liver [26]. Briefly, nuclei washed in buffer C to eliminate ribosomes of the outer nuclear membrane and suspended in buffer D were left standing for 60 min. Subsequently, the suspendion was subjected to ultrasonic treatment (Branson sonifier, six 10-s bursts at 40 W) and centrifuged 10 min at 10000 rev./min in an SS34 Sorvall head. The sediment was subjected to another round of ultrasonic treatment. Either the first or the combined supernatants were submitted to sucrose gradient centrifugation (15–30% linear gradients in buffer B, overlayered on 4 ml of a 50% sucrose in buffer B cushion) in a Beckman SW 27 head at 26000 rev./min for 5 h or 16 h. Fractions were collected with an Isco gradient collector.

Radioactive Labelling of RNA and Determination of the Incorporated Radioactivity

Cells were incubated at 37 °C in the presence of the radioactively labelled precursor (1 μCi [^{3}H]uridine/ml or 0.05 μCi [^{14}C]uridine/ml) for various time periods, as indicated in the legends to the figures. For the determination of the radioactivity incorporated into RNA, aliquots of the material (usually fractions from the sucrose gradients) were pipetted on 3MM filter discs (25-mm diameter) and then gently immersed into ice-cold 5% trichloroacetic acid. The filter paper discs were washed three times with 5% trichloroacetic acid at 0–4 °C, then, at room temperature, twice with ethanol and twice with ether. The filter paper discs were dried and counted in a Nuclear Chicago mark III or a Packard 3385 scintillation counter in vials containing T-fluor. The data were processed by an IBM 370-158 computer and expressed in curve form. We thank Dr P. Mainzer (Institute for Documentation and Statistics, Deutsches Krebsforschungszentrum, Heidelberg) for his help in these measurements.

Sodium Dodecyl Sulphate/Polyacrylamide Gel Electrophoresis of Proteins

Electrophoresis was performed according to Laemmli [40]. The polypeptides bands were visualized by staining with 0.25% Coomassie blue in 10% acetic acid, 54% methanol and destaining with 7% acetic acid in 54% ethanol. The gels were scanned in a Vernon model PH J6 scanner.

Formamide/Polyacrylamide Gel Electrophoresis of RNA

Electrophoresis was performed according to Pinder and Gratzer [41]. Usually 10% gels or 4–10% gradient gels were used. Staining was accomplished with 0.5% toluidine.

Fluorography

Fluorography of the gels was performed according to Laskey and Mills [42].

Isolation of RNA from Nuclear Fractions [43]

The fractions of the sucrose gradient were combined, made 0.2% with respect to sodium dodecyl sulphate, 20 μg/ml proteinase K was added and the fractions incubated at 37 °C for 30 min. Equal volumes of phenol/chloroform (1 : 1) were then added, the suspension shaken at 0 °C for 5 min, centrifuged at 8000 rev./min for 10 min and to the aqueous layer 2 vol. ethanol were added and kept at −20 °C overnight. The RNA was collected by centrifugation, desalted over a Sephadex G-25 column and the RNA-containing fractions lyophilized.

Buffers

Buffer A: 5 mM Tris/HCl, pH 7.55, 2.5 mM KCl, 1 mM $MgCl_2$, 25 mM sucrose and 5 mM EDTA; buffer B: 50 mM Tris/HCl, pH 7.55, 25 mM KCl, 10 mM $MgCl_2$ and 1.8 M sucrose; buffer C: 50 mM Tris/HCl, pH 7.55, 25 mM KCl, 10 mM $MgCl_2$, 250 mM sucrose and 50 mM EDTA; buffer D: 10 mM Tris/HCl, pH 8.0, 140 mM NaCl and 1 mM $MgCl_2$.

RESULTS

Isolation of hnRNA-snRNA-Protein Complex from Hepatoma Cells

hnRNA-snRNA-protein complexes were isolated by a method previously used to obtain similar complexes from rat liver [26]. Nuclei were first isolated from hepatoma cells by hypotonic lysis of the cells and ultracentrifugation through hypertonic sucrose, then treated with 50 mM EDTA to remove ribosomal material attached to the nuclear membrane and subsequently subjected to ultrasonic treatment in buffered 0.14 M NaCl. To evaluate the recovery of synthesized RNA the cells were previously labelled with [^{14}C]uridine for 20 min and [^{3}H]uridine and 150 min. Approximately 35% of the rapidly labelled RNA and 75% of the RNA labelled with ^{3}H were recovered by two consecutive salt extractions (see Table 1).

The combined salt extracts were subjected to sucrose gradient centrifugation (Fig. 1) and the incorporated radioactivity evaluated in the various fractions. The rapidly labelled RNA ([^{14}C]RNA) is broadly distributed throughout the gradient, with a peak in the 30–60-S region and in the lower part of the gradient, at the interphase with the sucrose cushion. Similarly, the RNA labelled with ^{3}H is distributed throughout the gradient, with a prominent peak at the gradient-cushion interphase.

Extraction of hepatoma cell nuclei was also performed in the presence of rat liver RNase inhibitor. Under these conditions, only a small shift in the sedimentation of the hnRNP complexes was observed (results not shown), denoting a low level of nuclear RNase activity of hepatoma cells.

The Protein and RNA Moiety of the RNP Complexes

The RNA extracted from hepatoma nuclei is complexed with proteins, as is the case for RNA obtained from nuclei of other cell types [10–16]. This can be demonstrated by

Table 1. *Recovery of synthesized RNA from hepatoma cell nuclei by two consecutive 0.14 M salt extractions*
Hepatoma cells were incubated in the presence of [^{14}C]uridine for 20 min and [^{3}H]uridine for 150 min. Nuclei were then prepared, washed with 50 mM EDTA-containing buffer (buffer C, see Materials and Methods) and subjected to two consecutive extractions with 0.14 M salt (buffer D). ^{3}H and ^{14}C radioactivity incorporated into material insoluble in 5% trichloroacetic acid was measured

Sample	Atom	Total radioactivity	Radioactivity incorporated
		counts/min	% total
Nuclei	^{3}H	1139980	100
	^{14}C	580060	100
EDTA wash	^{3}H	270475	23.7
	^{14}C	68275	11.8
First 0.14 M	^{3}H	234460	20.5
NaCl extraction	^{14}C	60860	10.4
Second 0.14 M	^{3}H	596640	52.3
NaCl extraction	^{14}C	135280	23.3

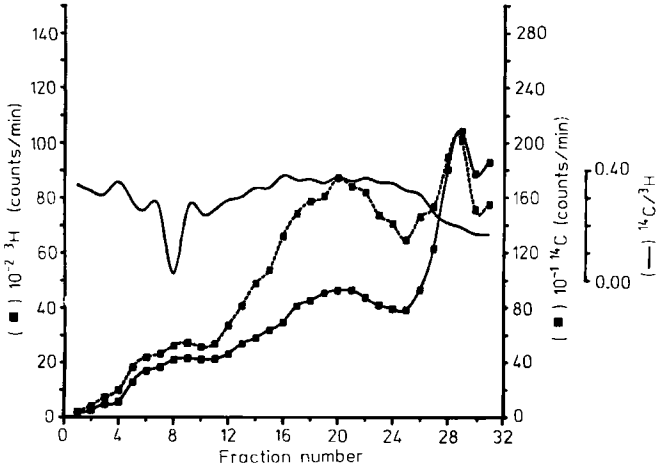

Fig. 1. *Sucrose gradient centrifugation of the 0.14 M salt extract of hepatoma cell nuclei.* Hepatoma cells were incubated with [^{3}H]uridine for 150 min and [^{14}C]uridine for 20 min, before preparation of the nuclei. 15–30% sucrose gradients, centrifugation time 16 h at 26000 rev./min. (■——■) ^{3}H radioactivity incorporated into material insoluble in 5% trichloroacetic acid; (■---■) ^{14}C radioactivity incorporated into material insoluble in 5% trichloroacetic acid; (——) ^{14}C/^{3}H ratio

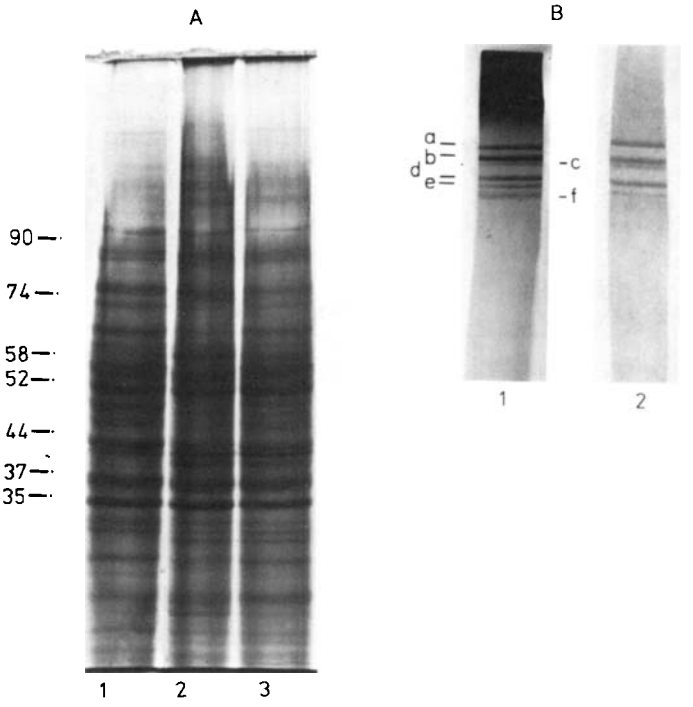

Fig. 2. *Electrophoresis of proteins and RNA of nuclear RNP.* (A) Sodium dodecyl sulphate gel electrophoresis of proteins of fractions of the sucrose gradient depicted in Fig. 1: 1 = fractions 13–16, 2 = fractions 17–19, 3 = fractions 20–24. (B) Formamide/polyacrylamide gel electrophoresis of RNA of fractions of the sucrose gradient depicted in Fig. 1: 1 = fractions 13–16, 2 = fractions 20–24. Numbers on the left represent $M_r \times 10^{-3}$; a–f, represent snRNA species

Table 2. *Comparison of the nomenclature used in this paper for snRNA association with nuclear RNP to the snRNA nomenclature of Busch [23] and Penman [20]*

snRNA	Nomenclature according to	
	Busch	Penman
a	U_2	C
b	U_{1B}	D
c	U_{1A}	F
d	5 S	G
e	–	–
f	4.5 S	H

formaldehyde fixation and CsCl isopycnic centrifugation of the material sedimenting at the 30-S and higher-molecular-weight regions of the gradient (fractions 12–28, Fig. 1). The fixed material bands at 1.40–1.44 g/cm^3, demonstrating a protein/RNA ratio of 4–6/1. Both ^{3}H-labelled and ^{14}C-labelled RNA is found in the banded ribonucleoprotein.

The RNA-associated proteins have been analyzed by SDS/acrylamide gel electrophoresis. A heterogeneous polypeptide pattern is obtained (Fig. 2A) with prominent bands having M_r of 35000–45000 and 50000–60000. The polypeptide pattern of the 30-S and of the higher-molecular-weight complexes is very similar (compare lanes 1–3 of Fig. 2A) and is qualitatively similar to the pattern of the proteins associated with hnRNA of rat liver and of other cell types [10–16].

The double-labelling experiments with RNA precursor described above, in conjunction with the CsCl isopycnic centrifugation data and the experiments with D-galactosamine, see below, demonstrate the presence of two populations of RNA in the RNP structures. We submitted to formamide/acrylamide gel electrophoresis RNA isolated from fractions 13–16 and 20–24 of the gradient depicted in Fig. 1. In addition to the smear of RNA at the top of the gels, which corresponds to degraded hnRNA (Fig. 2B), distinct bands of small nuclear RNA are detected, corresponding to the snRNA isolated previously from RNP of rat liver nuclei and of other cells [25–32]. In this paper we are using the nomenclature of Seifert et al. [44] (see also [27]) for the hnRNA-associated snRNA. In Table 2 we compare this nomenclature to that of Busch [23] and Penman [20].

Turnover of snRNA

Hepatoma cells were incubated in the presence of [^{3}H]-uridine for 90 min, washed and further incubated 120 min

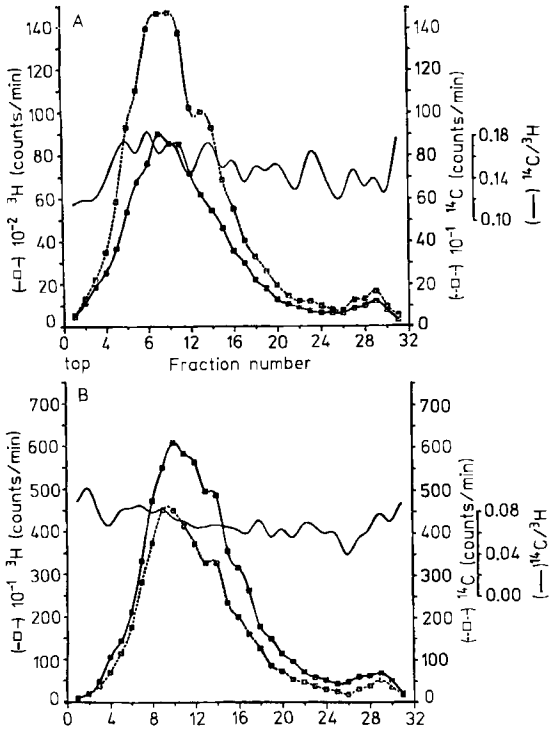

Fig. 3. *Effects of D-galactosamine on the incorporation of radioactive precursor into nuclear RNA.* Hepatoma cells were labelled with [^{3}H]-uridine for 90 min, washed and further incubated for 120 min with 50 mM D-galactosamine. The cells were washed again and then incubated 20 min with [^{14}C]uridine. Subsequently nuclei were prepared and the 0.14 M NaCl nuclear extract was submitted to a 10-min centrifugation at 10000 rev./min and subsequently to sucrose gradient centrifugation (15–30% gradient) for 5 h at 26000 rev./min. Radioactivity insoluble in 5% trichloroacetic acid was then determined. (A) Cells not treated with D-galactosamine; (B) D-galactosamine-treated cells. (□——□) ^{3}H; (□ - -□) ^{14}C; (——) ^{14}C/3H

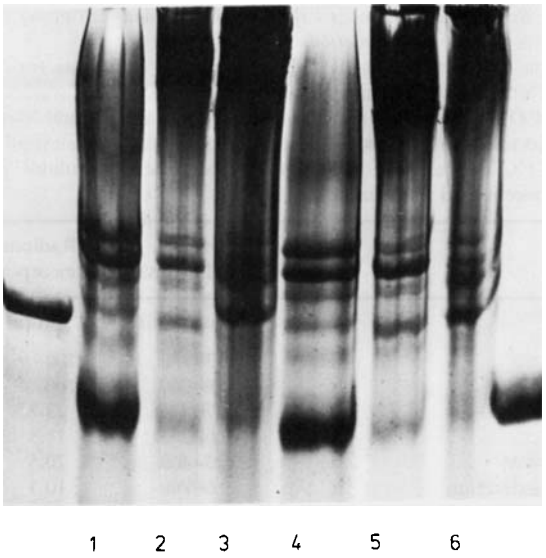

Fig. 4. *Formamide/polyacrylamide gel electrophoresis of RNA from fractions of the gradient depicted in Fig. 3.* Outside lanes: marker 5-S and 4-S RNA. Lanes 1–3, fractions 8–15, 16–23 and 26–30 of Fig. 3A, respectively; lanes 4–6, fractions 8–15, 16–23 and 24–30 of Fig. 3B, respectively

with 50 mM D-galactosamine. D-Galactosamine is an inhibitor of RNA synthesis by virtue of its effect on the nucleotide pool and its action is reversible upon its removal [45]. The cells were washed again and incubated 20 min with [^{14}C]-uridine. Subsequently nuclei were prepared, extracted with buffer D and the extracts, after a 10-min centrifugation at 10000 rev./min, submitted to sucrose gradient centrifugation. Preparations from cells not treated with D-galactosamine were similarly centrifuged. In this experiment the centrifugation period was reduced to 5 h instead of 16 h so that even very large hnRNP complexes could be retained in the gradient. As shown in Fig. 3, incorporation of [^{14}C]uridine into RNA is inhibited approximately 70% by D-galactosamine, whereas labelling of RNA with [^{3}H]uridine is only 20% reduced. Formamide/acrylamide gel electrophoresis of the RNA isolated from the various RNP fractions of the sucrose gradient of Fig. 3 reveals that the yield (see Fig. 4, compare lanes 1–3 with 4–6) and labelling (results not shown) of snRNA from control and galactosamine-treated cells is not markedly affected, leading to the conclusion that the turnover of the snRNAs is considerably slower than that of hnRNA.

We proceeded to determine the half-lives of the individual snRNA species. Cells were incubated in the presence of [^{3}H]-uridine for 20 h, washed with medium and further incubated for various time periods (2, 6, 10 and 24 h) in the absence of labelled precursors and in the absence of inhibitors of RNA synthesis. Nuclear extracts were then prepared from aliquots of the cells and submitted to sucrose gradient centrifugation (Fig. 5). RNA was isolated from the 30–60-S region of the gradient (fractions 10–24), submitted for formamide/acrylamide gel electrophoresis (Fig. 6A) and fluorography (Fig. 6B) and the fluorograms scanned. The absorbances of the individual snRNA species at time 0, which are directly related to radioactivity incorporated into snRNA, were taken as 100%. The values of the absorbances of the individual snRNA species obtained at the different time periods were expressed as a percentage of the 0-times values and plotted (Fig. 7). The half-lives of the various snRNAs were derived from the plots. A $t_{1/2}$ of 32 h has been determined for snRNA species a, b and d, 22 h for snRNAs e and f and 13 h for snRNA c. The values obtained are approximations due to the difficulty in discriminating the separated snRNA, in the variations in the yield of RNA extracted and to the background activity of hnRNA. The activity due to the presence of hnRNA can be significantly reduced if [^{3}H]methionine is used as precursor, as the snRNAs are highly methylated. Furthermore, the measurements were done in the absence of inhibitors of RNA synthesis, which may overestimate the values of half-life.

The Effect of NaCl and RNase on the Association of Proteins and RNA in Nuclear RNP [45–48]

To gain information on the nature of the association of hnRNA, snRNA and proteins within the RNP structure nuclear extracts were prepared from cells previously incubated with [^{3}H]uridine for 18 h and [^{14}C]uridine for 1 h and then subjected to treatment with 0.7 M and 1 M NaCl or with pancreatic RNase. The extracts were submitted to sucrose gradient centrifugation (Fig. 8) and the protein and RNA components of the material either sedimenting at 30–60 S or released in the top fractions of the gradient analyzed by acrylamide gel electrophoresis (Fig. 9 and 10).

In the presence of 0.7 M or 1 M NaCl a decrease both in the A_{254} and in the labelling, particularly of rapidly labelled RNA, of the material sedimenting at > 30 S is seen (Fig. 8A

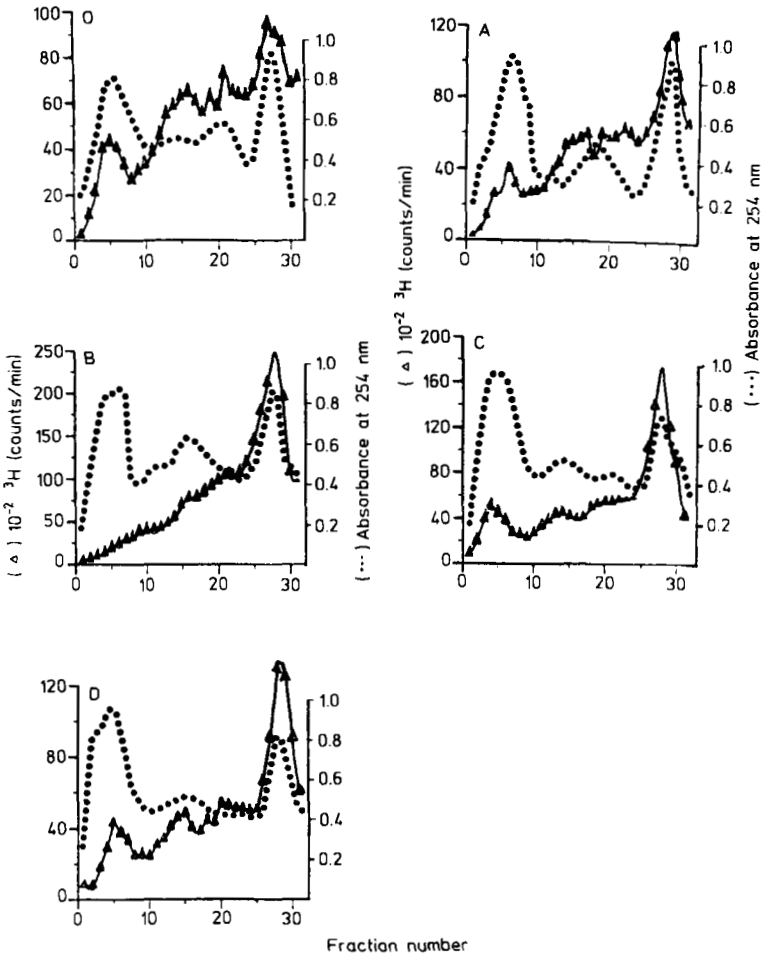

Fig. 5. *Kinetics of degradation of radioactively labelled nuclear RNA from hepatoma cells.* Hepatoma cells were incubated for 20 h in the presence of [^{3}H]uridine. The cells were then washed and further incubated in the absence of the labelled precursor. At certain time periods 0.14 M salt extracts were prepared from isolated nuclei and submitted to sucrose gradient centrifugation (15–30% gradient). Centrifugation time 16 h at 26000 rev./min. Direction of centrifugation from left to right. Incorporation of precursor into material insoluble in 5% trichloroacetic acid was determined. (O) Zero time (immetiately after washing the cells); (A) 2 h; (B) 6 h; (C) 10 h; (D) 24 h. (·····) A_{254}; (Δ——Δ) ^{3}H

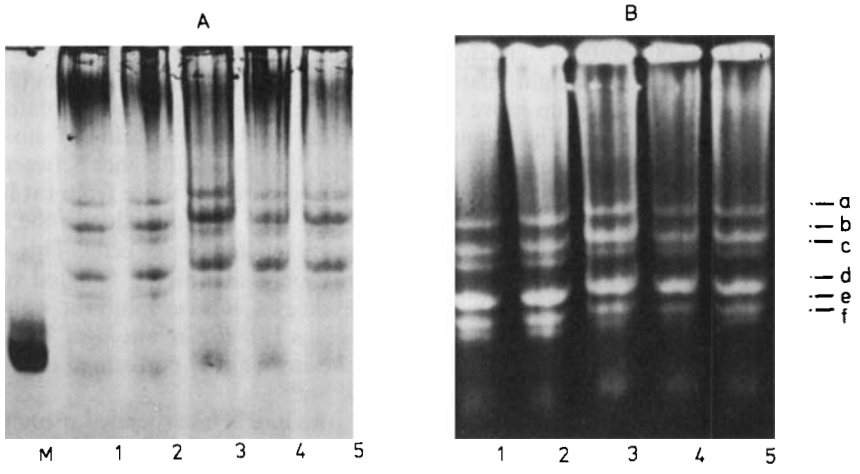

Fig. 6. *Formamide/polyacrylamide gel electrophoresis and fluorography of RNA isolated from fractions 10–24 of Fig. 5.* The RNA was isolated from the pooled fractions and submitted to electrophoresis and fluorography. (A) Methylene blue staining of the electropherogram; (B) fluorography. Lane M, 4-S marker RNA; lane 1, 0 time; lane 2, 2 h; lane 3, 6 h; lane 4, 10 h; lane 5, 24 h

and B). The released RNA is not quantitatively recovered in the top fractions of the gradient; we assume that it is either degraded after release or it aggregates, sedimenting at the bottom of the tube.

The material sedimenting at 30–60 S after salt treatment of the nuclear extract was further analyzed as regards its protein composition. Many of the polypeptides previously present in the 30–60-S RNP structures had been released as

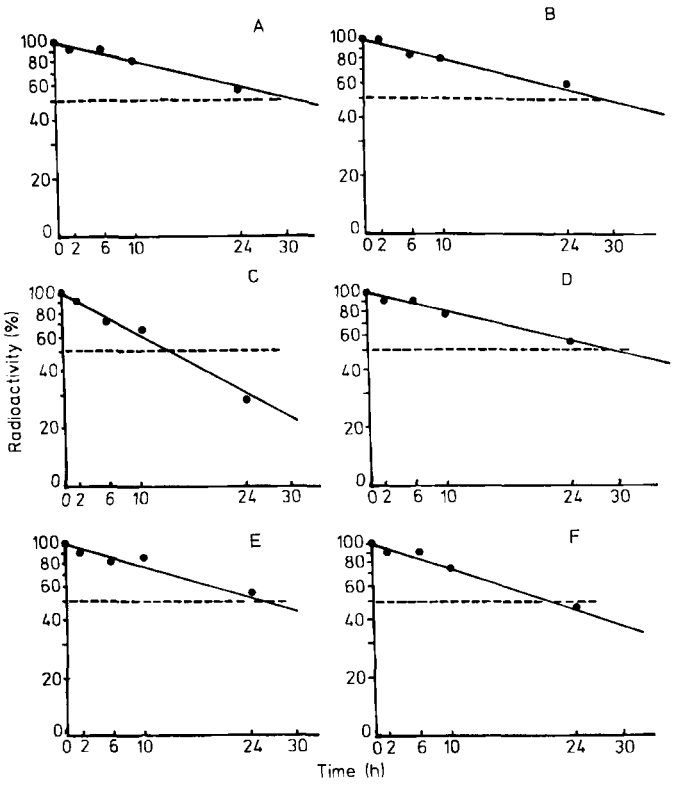

Fig. 7. *Half-life of snRNA of hepatoma cell RNP.* The fluorograms depicted in Fig. 6B were scanned and the absorbance of the individual bands at 0 time were taken as 100%. The values at the other time periods were expressed as a percentage of the zero-time values. A = snRNA a, B = snRNA b, C = snRNA c, D = snRNA d, E = snRNA e, F = snRNA f

a result of the salt treatment and were detectable at the top of the gradient (Fig. 9, compare lanes 1, 4 and 7). Prominent among the released proteins are the 30000–45000-M_r polypeptides which represent mainly the 'core' proteins [49,50] (Fig. 9, compare lanes 2, 5 and 8).

One group of proteins with M_r 54000–59000 is tenaciously retained in the 30-S and higher-molecular-weight material after 0.7 M and 1.0 M salt treatment, making up more than 80% of the protein recovered in these fractions (Fig. 9, lanes 5, 6 and 8, 9). These proteins are recovered in these fractions even after 2 M salt treatment (results not shown). Labelling experiments with [^{3}H]leucine revealed that the turnover of these proteins is considerably higher than that of the polypeptides released after salt treatment (results not shown, see also [51]).

Analysis by formamide/acrylamide gel electrophoresis of the RNA moiety of the material sedimenting at 30–60 S after 0.7 M and 1 M salt treatment (Fig. 10A and B) revealed that all snRNA species present in the untreated RNP are retained in these fractions (see also [46]).

Treatment of the nuclear extracts with 1.5 μg pancreatic RNase/ml at 0 °C for 30 min leads to a decrease in the A_{254} and of labelling with radioactive precursors (Fig. 8C) of the material sedimenting at 30–60 S. The effect is more prominent at higher doses of the enzyme (3 μg/ml, Fig. 8D).

After digestion with low RNase concentrations, the A_{254} is drastically reduced in the 30–60-S region of the gradient. As is known, mild RNase digestion cleaves high-molecular-weight hnRNP releasing the 30-S monoparticles [10,36]. Accordingly, the bulk of the hnRNA-associated proteins, with the exception of some higher-molecular-weight (> 60000) polypeptides, is still present in the 30-S RNP, not in the regions of the gradient corresponding to structures with high sedimentation coefficient (Fig. 9, compare lanes 2 and 3 to lanes 11 and 12).

Treatment with the higher doses of RNase results in disappearance of the 30-S hnRNP as well, the proteins being released in the top fractions of the gradient (Fig. 9, lane 13). This underlines the role of RNA-protein interactions in the retainment of the 30-S hnRNP structure.

One major group of proteins still appears in the 30–60-S region of the gradient even after treatment with the high doses of RNase. These are polypeptides of M_r 50000–60000 (Fig. 9, lanes 14 and in particular lane 15). Formamide acrylamide gel electrophoresis and fluorography of the RNA isolated from the 30 S structures after RNA digestion (Fig. 11) reveals, that in addition to hnRNA, most of the snRNAa, with the possible exception of snRNA d, have been degraded or released as a result of nuclease action.

DISCUSSION

The elucidation of the molecular mechanisms involved in post-transcriptional modification of hnRNA necessitates a detailed knowledge of both the site(s) and the enzymes participating in these processes. Biochemical studies have demonstrated that hnRNA is present in cell nuclei in association with a heterogeneous group of nuclear proteins in the form of hnRNP structures, the morphological equivalent of which are the perichromatin fibres [52]. The association of enzymes implicated in the processing of hnRNA, such as endonucleases [36,38] and poly(A) polymerases [37], with hnRNP, the presence of snRNA in these complexes [25–32] currently discussed in connection with splicing [32–35] of hnRNA and the heterogeneity of the hnRNA-associated proteins [11–16] suggesting multiple functions [11] for these polypeptides, suggests the need for a more detailed study of their structure and function. With this in mind, we have began a study of hnRNP complexes from hepatoma cells, which respond to the action of glucocorticoids with well-studied biochemical parameters [3].

hnRNP complexes were isolated using methods previously applied for the preparation of similar structures from rat liver nuclei. One main difference between complexes from hepatoma cells compared to those from rat liver is the larger size of the former, very probably due to the lower endogenous nuclease activity of hepatoma cells. This is documented by the observation that hnRNP isolated from hepatoma cells in the presence of RNase inhibitor only slightly increases in size, whereas the size of rat liver hnRNP increases dramatically if the isolation is performed in the presence of the inhibitor [53,54].

The hnRNA-associated proteins from hepatoma cells are heterogeneous, as is the case for similar proteins from a variety of cells and tissues [10–16], most prominent being polypeptides with M_r 35000–45000, which are considered as the 'core' and 'packaging' proteins [16,48,49]. The polypeptide patterns of the hepatoma cell preparations are very similar to those from rat liver and solid Morris hepatoma [11–13] with minor qualitative differences. Examination of the RNA moiety of the nuclear RNPs revealed, in addition to hnRNA, a series of small nuclear RNAs. These small RNA species have been previously detected in hnRNP complexes from a variety of cells [25–32] and have also been found in

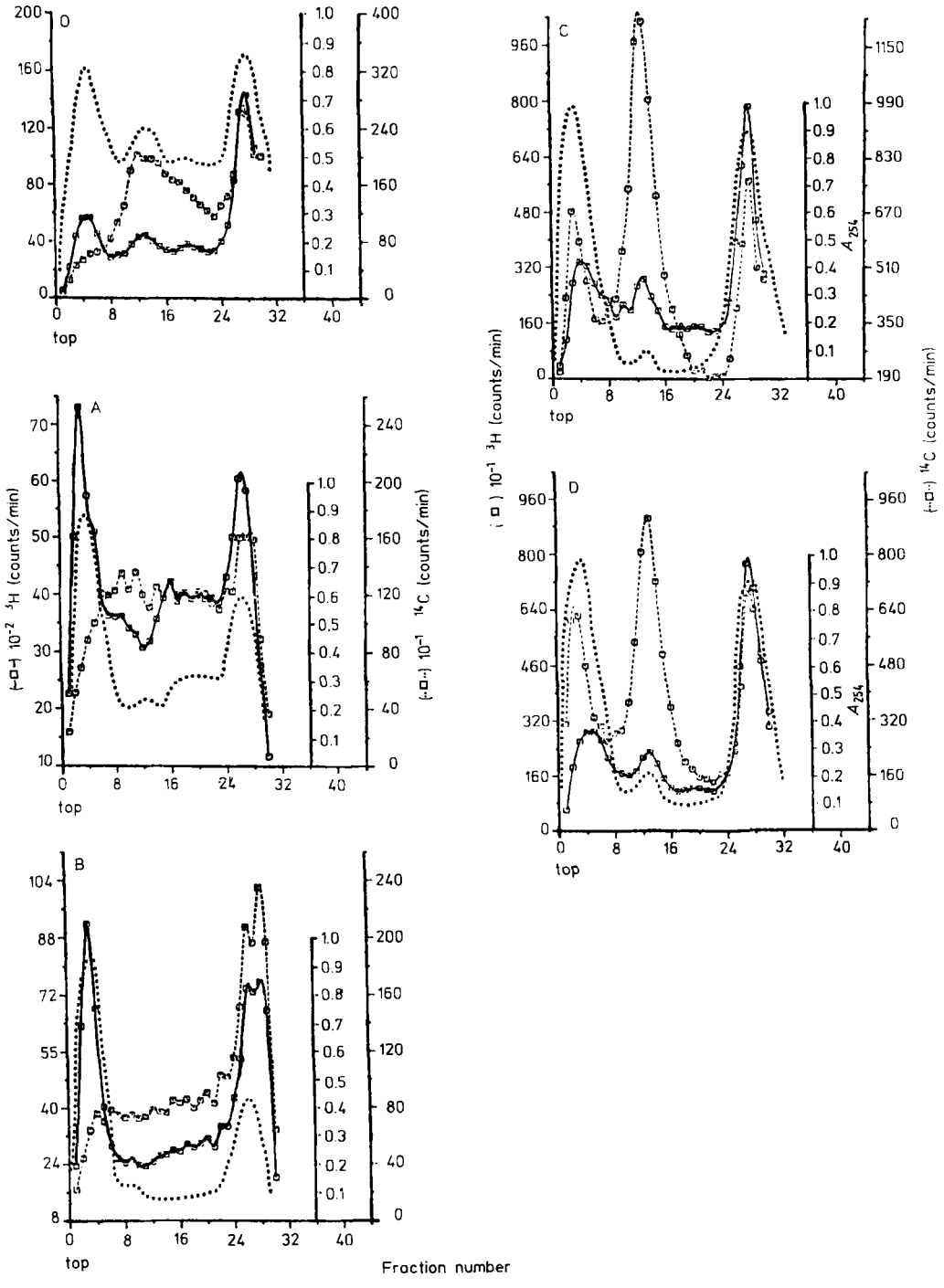

Fig. 8. *Effect of NaCl and RNase on nuclear RNP.* Hepatoma cells were incubated with [^{3}H]uridine for 18 h, washed and further incubated 1 h in the presence of [^{14}C]uridine. Nuclear 0.14 M salt extracts were then prepared and subjected to sucrose gradient centrifugation (15–30% gradient) for 18 h at 26000 rev./min either as such (O) or after treatment at 0 °C for 30 min with: (A) 0.7 M NaCl, (B) 1 M NaCl, (C) 1.5 μg pancreatic RNase and (D) 3 μg pancreatic RNase. ^{3}H and ^{14}C radioactivity incorporated into material insoluble in 5% trichloroacetic acid was measured in aliquots of the fractions. (·····) A_{254}; (□·–·–·□) ^{14}C incorporation; (□——□) ^{3}H incorporation

the form of 10–20-S RNP complexes [55–58]. Their half-life is considerably longer than that of hnRNA and varies among the various species from 32 h (snRNA a, b and d) to 22 h (snRNA e and f) and 13 h for snRNA c. Such a variation in the half-lives of snRNA has been recently documented regarding snRNA from rat cerebral cortex [59]. It is now known that these snRNAs are hydrogen-bonded to hnRNA [32,60] and show base complementarity to the consensus sequence of the exon-intron boundaries of hnRNA [34], findings which have led to their implication in the splicing of hnRNA [33–35].

Previous experiments with hnRNP from rat liver suggested the presence of two types of 30-S RNP structures, one composed mainly of hnRNA and of proteins easily dissociable by salt treatment but resistant to mild nuclease digestion and another composed mainly of snRNA and proteins resistant to salt treatment but sensitive to nuclease digestion [61–64]. Our present findings with the RNPs from hepatoma cells do

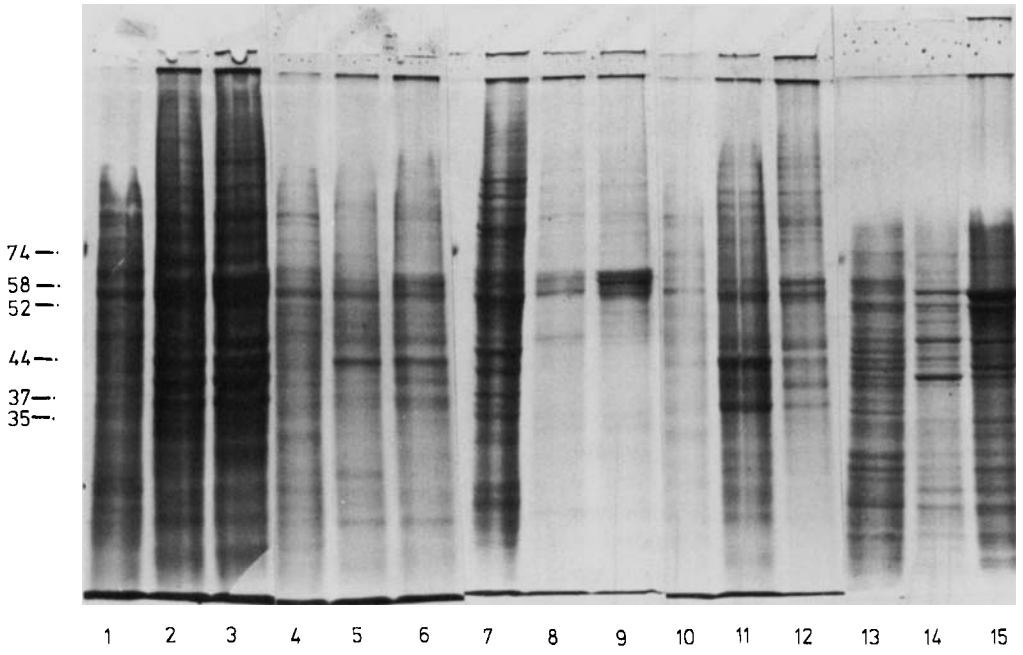

Fig. 9. *Sodium dodecyl sulphate/polyacrylamide gel electrophoresis of proteins of the fractions of the sucrose gradient depicted in Fig. 8.* Lanes 1–3, fractions 1–8, 9–17 and 18–24, respectively, of Fig. 8O; lanes 4–6, fractions 1–8, 9–17 and 18–24, respectively, of Fig. 8A; lanes 7–9, fractions 1–8, 9–17 and 18–24, respectively, of Fig. 8B; lanes 10–12, fractions 1–8, 9–17 and 18–24, respectively, of Fig. 8C; lanes 13–15, fractions 1–8, 9–17 and 18–24, respectively, of Fig. 8D. Numbers on the left represent $M_r \times 10^{-3}$

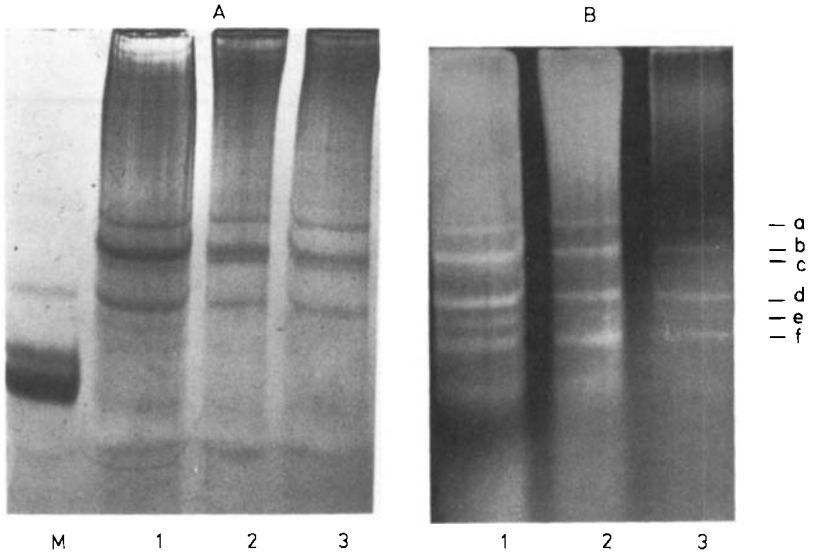

Fig. 10. *Formamide/polyacrylamide gel electrophoresis and fluorography of RNA from NaCl-treated RNP of the experiment depicted in Fig. 8.* The fractions were pooled, RNA isolated as described in Materials and Methods and submitted to electrophoresis. (A) RNA visualized by methylene blue staining; (B) fluorogram. M = marker 4-S RNA; lane 1 = fractions 9–22 of Fig. 8O; lane 2 = fractions 9–22 of Fig. 8A; lane 3 = fractions 9–22 of Fig. 8B. a–f are snRNA species

not disagree with the previous results. Thus, treatment with 0.7–1 M NaCl leads to dissociation of hnRNA-protein complexes (see also [46–50]). Polypeptides of M_r 54000–59000, however, remain in association with snRNA, even in the presence of 2 M NaCl [46, 51]. Treatment of the nuclear extracts with low concentrations of RNase under conditions degrading up to 80% of hnRNA results in conversion of the high-molecular-weight hnRNP structures to 30-S hnRNP. Disruption of the particle structure with release of the hnRNA-associated polypeptides is accomplished, however, with more drastic nuclease treatment, stressing the significance of RNA-protein interactions for the retainment of the RNP structure [25, 48]. RNA-protein crosslinking experiments will surely shed light on the interaction sites and will help in elucidating the existence of multiple RNP structures [65, 66].

The existence of two basic 30-S RNP complexes, one containing mainly snRNA, the other mainly hnRNA, as its nucleic acid moiety, has also been suggested by previous experiments involving metrizamide isopycnic centrifugation [61, 63] and recent experiments involving electrophoresis in agarose (A. Guialis and M. Patrinou-Georgoula, unpublished). It is interesting to note that the proteins attached to snRNA

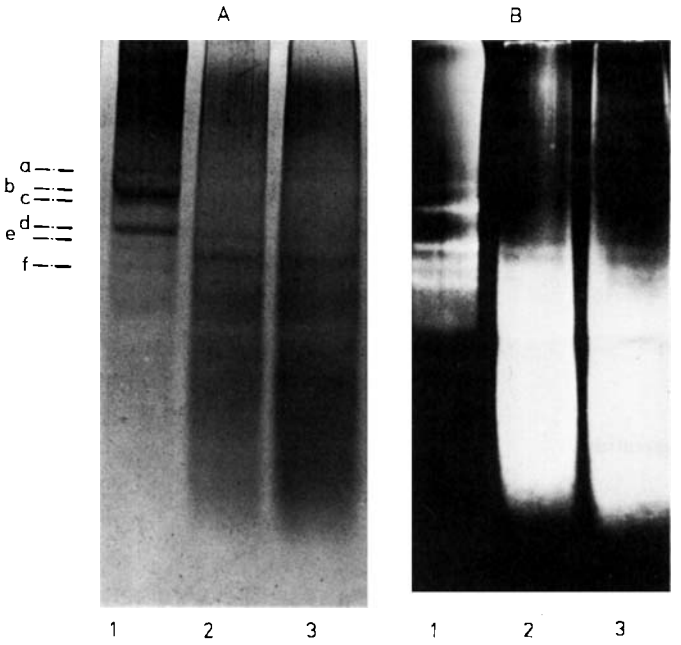

Fig. 11. *Formamide/polyacrylamide gel electrophoresis and fluorography of RNA from RNase-treated RNP of the experiment depicted in Fig. 8.* The fractions were pooled, RNA isolated and submitted to electrophoresis. Lattering of gels as in Fig. 10. Lane 1 = fractions 9–22 of Fig. 8O; lane 2 = fractions 9–22 of Fig. 8C; lane 3 = fractions 9–22 of Fig. 8D

are the ones with the higher turnover (unpublished experiments). As already mentioned, snRNA has been found in RNP complexes sedimenting at 10–20 S [55–58]. The relation of these complexes to the 30-snRNPs is currently under investigation.

The demonstration of snRNA-hnRNA hybrids [32, 60, 67] suggests that the snRNP and hnRNP complexes are associated in the higher-molecular-weight structures by virtue of snRNA-hnRNA base-pairing. It is tempting to speculate that enzymes involved in splicing are associated with the snRNP complexes and correctly placed on hnRNA by such interactions [68].

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A. Prüsse, Institut für Tropenhygiene,
Im Neuenheimer Feld 324, D-6900 Heidelberg, Federal Republic of Germany

C. Louis, Department of Biology, Princeton University,
Princeton, New Jersey, USA 08540

A. Alonso, Institut für Experimentelle Pathologie, Deutsches Krebsforschungszentrum,
Im Neuenheimer Feld, D-6900 Heidelberg, Federal Republic of Germany

C. E. Sekeris, Biological Research Center, National Hellenic Research Foundation,
Vassileos Konstantinou 48, Athens 501/1, Greece