MOLECULAR AND CELLULAR BIOLOGY, Jan. 1981, p. 21-34
0270-7306/81/01-0021/14$02.00/0

Vol. 1, No. 1

Arrangement of 30S Heterogeneous Nuclear Ribonucleoprotein on Polyoma Virus Late Nuclear Transcripts

JOAN ARGETSINGER STEITZ†* and ROBERT KAMEN
Imperial Cancer Research Fund, London, WC2A 3PX, England

Heterogeneous nuclear ribonucleic acid (hnRNA) molecules in eucaryotic cell nuclei associate with a well-defined group of abundant, highly conserved proteins to form heterogeneous nuclear ribonucleoproteins (hnRNP). The exact manner in which these 30S complexes assemble on nuclear transcripts, however, has not been well documented. To determine whether any site selectivity in the formation of hnRNP can be detected (e.g., preferential recognition of intervening sequences or of premessage regions), we investigated the distribution of 30S hnRNP on a particular nuclear RNA, the polyoma virus late transcript. Hybridization studies showed not only that the majority of polyoma late nuclear RNA sequences can be isolated in the form of 30S complexes, but that the RNP were located equally on intervening sequences and premessage portions of the transcript. The latter conclusion was confirmed by ribonuclease T_1 oligonucleotide fingerprint analysis of polyoma virus-specific RNA recovered from native 30S complexes. However, fingerprint analysis of the small segments of viral RNA in the 30S fraction that survived extensive ribonuclease treatment revealed that oligonucleotides corresponding to intervening sequences were preferentially lost. We discuss these findings in relation to the structure of 30S hnRNP and their function in RNA biogenesis.

The nascent transcripts of eucaryotic cells become rapidly associated with protein and appear, in the electron microscope, as beaded structures consisting of globular units several hundred Ångstroms in diameter connected by ribonuclease (RNase)-sensitive strands. Superb examples of such micrographs were recently published by McKnight and Miller (29) and Lamb and Daneholt (22). Most of the rapidly labeled ribonucleic acid (RNA) (heterogeneous nuclear RNA [hnRNA]) in mammalian or avian cell nuclei can, moreover, be isolated in the form of RNA-protein complexes sedimenting at about 30S (5, 19, 25, 37). If special care is taken to avoid RNase action during preparation, polyparticles ranging in size up to 200S can be obtained. The basic 30S heterogeneous nuclear ribonucleoprotein (hnRNP) unit contains 2×10^5 daltons of RNA (about 600 nucleotides) and about threefold as much protein. There are approximately six major or "core" protein components with molecular weights of 30,000 to 40,000. These are prominent nuclear proteins, constituting about 10% of the nonchromatin protein in actively growing cells (19). The six polypeptides, moreover, are highly conserved among higher eucaryotic species in both size and amino acid composition; an unusual modified amino acid, N^G,N^G-dimethylarginine, is frequently present (5, 19, 31). Nuclease digestion studies suggest that the hnRNA segment of each hnRNP is arranged in a relatively accessible way on the surface of the particle (see reference 26). Virus-specific nuclear transcripts are likewise recovered in the form of 30S hnRNP from adenovirus-infected (24) or simian virus 40-infected (3, 30) cells.

The ubiquity of the RNA binding proteins found in the 30S hnRNP suggests that these complexes play a central role in the biogenesis of eucaryotic messenger RNA (mRNA). For instance, they may organize hnRNA sequences for accurate splicing, or they may participate in the selection of certain portions of an hnRNA molecule for export from the nucleus; alternatively, they may have a less specific topological function, such as the minimization of undesirable RNA strand interactions or tangling. To assign these or other possible functions to the 30S hnRNP, detailed information concerning the specificity of interaction of the proteins with nuclear RNA molecules is clearly required. Previous hybridization studies (20) with total mouse liver hnRNP suggested that, whereas the bulk of the particle-bound RNA turns over with the half-life of hnRNA and includes sequences not

† Permanent address: Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

found in the cytoplasm, the majority of sequences represented in cytoplasmic RNA are also present. Also, Maundrell and Scherrer (28) showed that the RNA extracted from purified RNP of duck erythroblast nuclei contains globin mRNA sequences at a concentration comparable to that in total nuclear RNA. On the other hand, some selectivity in the interaction of 30S particles with hnRNA is indicated by the observation that most nuclear polyadenylate tracts are associated with another type of RNP sedimenting at about 15S (34–36). Likewise, double-stranded hnRNA regions are reported to be readily released from 30S hnRNP (9, 26; J. Karn and W. Jelinek, personal communication). Finally, Beyer et al. (6) have observed a nonrandom distribution of RNP on nascent hnRNA of *Drosophila* embryos.

Here, for the first time, the actual distribution of 30S hnRNP along a specific nuclear RNA has been investigated. We chose the polyoma virus late transcript for several reasons. Not only has the polyoma virus genome been completely sequenced, but its expression in the form of nuclear transcripts, cytoplasmic mRNA's, and proteins is well understood (11, 15, 39). The late region of the viral deoxyribonucleic acid (DNA), corresponding approximately to the bottom half of the 5.29-kilobase circular genome (Fig. 1), determines three related mRNA's which share common 5′ and 3′ termini but differ in the size of intervening sequences that are removed by RNA splicing (18; see Fig. 1). These mRNA's separately encode the three virion capsid proteins VP3, VP2, and VP1. It has been known for some time (2) that the nuclear precursors of the late

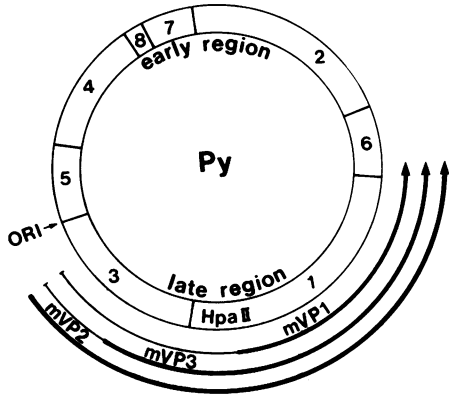


FIG. 1. *Schematic map of the polyoma virus genome. The regions represented in the three cytoplasmic late mRNA's are shown relative to the fragments produced (16) by HpaII digestion of polyoma virus. The bold lines indicate the colinear segments of the mRNA's. Intervening sequences are indicated by the thinner connecting lines.*

mRNA's consist of giant molecules up to four times the length of the viral DNA. Such transcripts are complementary to the DNA L-strand of the entire genome, as demonstrated by miniblot hybridization (7), electron microscopy of RNA-DNA hybrids (1), and most recently by S1 nuclease gel mapping (R. Treisman, personal communication). The last study indicated that the large nuclear RNA includes a series of molecules with structures appropriate for intermediates involved in the processing of giant transcripts to yield mature spliced and polyadenylated mRNA's. Legon et al. (23) discovered that the common 5′-terminal segment of the three late mRNA's which is spliced onto the different 3′ segments (see Fig. 1) contains multiple copies of a sequence present only once in the viral DNA. Direct electron micrographic evidence for the reiterated nature of these "leader" sequences has recently been obtained (42). This unusual structure is best explained by a processing mechanism whereby the giant late nuclear transcripts undergo a series of "leader to leader" splices, generating a tandem repeat of the leader sequence in the product mRNA's (18, 23). Consistent with this interpretation are the observations that the leader sequences are not reiterated with respect to other sequences in large nuclear RNA (23) and that approximately one copy of mRNA-like sequences is exported to the cytoplasm per giant nuclear molecule (N. Acheson, submitted for publication). The processing model implies that the sequences corresponding to the entire upper half of the circular genome, as shown in Fig. 1 (the "early region"), can be considered as intervening sequences in large polyoma virus late nuclear transcripts. Therefore, the division of such transcripts into large blocks of sequences destined for either degradation or possible export, in addition to the relative abundance of these transcripts in the nuclei of infected cells, makes the polyoma virus late nuclear RNA a favorable subject for detailed studies of the specificity of RNA-protein interactions involved in 30S hnRNP structure and function.

MATERIALS AND METHODS

Preparation of hnRNP from polyoma virus-infected cells. Polyoma virus was grown on secondary mouse embryo cells and labeled, and the nuclei were prepared as described by Flavell and Kamen (13) up to the step at which the infected cells are suspended in the Nonidet P-40 buffer. Normally, about 20 dishes of infected cells were exposed to a total of 25 mCi of $^{32}PO_4$ for 4 to 5 h, beginning 21 to 25 h after infection. To obtain cleaner nuclei, the cells in 5 ml of buffer A [140 mM NaCl, 1.5 mM $MgCl_2$, 10 mM tris(hydroxyethyl)aminomethane (Tris)hydrochloride (pH 7.5), 0.5% (wt/vol) Nonidet P-40 (BDH)] were subjected to 10 strokes in a Dounce homogenizer, incubated for 10

min at 0°C, and then given another 10 strokes. Nuclei were isolated by sedimentation through an equal volume of 10 mM Tris-hydrochloride (pH 7.5)-5 mM $MgCl_2$-1% Nonidet P-40-24% (wt/vol) sucrose (Schwarz/Mann; RNase-free) for 15 min at 8,000 rpm in a Sorvall HB-4 rotor. The nuclei were then washed through the two following solutions, spinning for 3 min at 2,000 rpm in the HB-4 rotor between steps: (i) 5 ml of 0.3 M sucrose-8 mM KCl-1.5 mM $MgCl_2$-1 mM dithiothreitol-15 mM Tris-hydrochloride (pH 7.5); (ii) 5 ml of 10 mM Tris-hydrochloride (pH 7.5)-1 mM $MgCl_2$-0.1 M NaCl-1 mM dithiothreitol. The washed nuclei were then resuspended in 0.5 ml of 10 mM Tris-hydrochloride (pH 8.0)-1 mM $MgCl_2$-0.1 M NaCl-1 mM dithiothreitol and extracted by incubation with gentle shaking at room temperature for 75 min. Every 15 min during this period the nuclei were precipitated by centrifugation for 2 min at 2,000 rpm and room temperature, and the supernatants (total volume, 2 ml) were pooled and kept on ice as the nuclear extract. At each step during the washing and extracting of nuclei, aliquots were removed, and RNA was prepared (13) and used to assess the proportion of polyoma virus RNA (by hybridization to polyoma virus DNA cellulose) and the viral sequence composition by miniblot analysis. Results comparable to those shown in Fig. 2 were obtained when a nuclear extract was prepared by sonication rather than by extraction at high pH. Likewise, fingerprints comparable to those in Fig. 7 were produced by analysis of polyoma-specific 30S particle-bound RNA from late infected 3T6, rather than mouse embryo, cells.

Nuclear extracts were further treated as follows. Per 2 ml, 0.1 ml of deoxyribonuclease I (pretreated as described in reference 12) at 150 μg/ml was added and allowed to incubate for at least 1 h at 0°C. Portions of the extract were next exposed to the indicated concentrations of T_1 or pancreatic RNase for 30 min at 0°C. A total of 0.5 to 1 ml of the extract was then applied to a 10 to 30% sucrose gradient (13 ml) in 0.1 M NaCl-10 mM Tris-hydrochloride (pH 7.5)-1 mM $MgCl_2$-1 mM dithiothreitol; fractionation was for 13 h at 33,000 rpm and 3°C in a Spinco SW41 rotor.

After collection, sucrose gradient fractions were assayed for optical density or radioactivity or both, and various regions were pooled. Appropriate portions were then treated with proteinase K followed by phenol extraction (as in reference 13) to obtain the RNA for further analysis. Alternatively, proteins were precipitated by making the pooled fractions 10% in trichloroacetic acid, incubating for 1 h at 0°C, sedimenting, and washing the precipitate with ether. The samples were then resuspended directly in sodium dodecyl sulfate sample buffer and fractionated on 10% sodium dodecyl sulfate-polyacrylamide gels (21).

Analysis of polyoma-specific sequences. Before being used in miniblot hybridization analysis, RNA fractions were redigested with deoxyribonuclease (100 μg/ml in 10 mM Tris-hydrochloride-10 mM $MgCl_2$ [pH 7.5] for 120 min at 37°C) to remove any remaining traces of polyoma DNA. Miniblot hybridization was performed as described previously (7) on pooled sucrose gradient fractions and on aliquots removed at various steps in the preparation. Not only were *Hpa*II digests of polyoma DNA used for blots, but *Hha*, *Hsu*I/*Sst*I, *Sst*I/*Bam*HI, and *Mbo*I digests likewise yielded patterns indicating equal representation of all polyoma sequences in native 30S particles. In each blot experiment, a fivefold-lower concentration of the most radioactive RNA sample was tested in parallel to insure that the hybridizations were being done in DNA excess (see Fig. 2). Also, results from samples treated with alkali before blotting revealed that DNA had been completely removed. Finally, miniblots containing separated strands of viral restriction fragments demonstrated that the amount of early strand transcript present in our preparations was only about 5%, as previously reported by Flavell and Kamen (13) for nuclear RNA from late polyoma virus-infected cells.

Selection of polyoma virus-specific RNA was accomplished by hybridization to DNA immobilized on cellulose as described by Flavell et al. (14). This technique was utilized both to quantitate the proportion of polyoma virus sequences in RNA fractions and to prepare RNA to be analyzed on polyacrylamide gels and by fingerprinting. Gels for RNA fractionation were 0.3 mm thick and contained 20:1 acrylamide-bisacrylamide, 0.4 g of urea per ml, and 89 mM Tris-borate buffer (pH 8.3), which was also used as the running buffer. RNA samples were suspended in 10 M urea and heated to 70°C for 3 min before loading; DNA restriction fragment markers were denatured by heating to 95°C in 0.1 M NaOH and 10 M urea. RNase T_1 fingerprints were prepared as described by Barrell (4), using diethylaminoethyl cellulose thin layers (Macherey-Nagel & Co., Polygram Cel 300/DEAE/HR-2115) and homomixture II (17) for the second dimension. Spots were eluted and analyzed by standard procedures (4).

RESULTS

Polyoma late transcripts are associated with 30S hnRNP. We first asked whether the majority of polyoma virus-specific RNA sequences in cell nuclei at late times during productive infection could be isolated in the form of 30S hnRNP. Previous experiments (13) had established that at such times 90 to 95% of the viral RNA synthesized in nuclei are the DNA L-strand transcripts which are the putative late mRNA precursors; the remainder of the viral RNA sequences are DNA E-strand transcripts, precursors of the mRNA's for the "early" viral proteins, and will not be discussed further here.

Late RNAs in polyoma virus-infected secondary mouse embryo cells were labeled with $^{32}PO_4$ for 4 to 5 h beginning 21 to 25 h postinfection. Nuclei were prepared, washed, and then extracted at room temperature, using a pH 8.0 buffer (25), one of the standard procedures for obtaining high yields of 30S hnRNP from cultured cells (24). Before analysis, the extracts were treated with pancreatic deoxyribonuclease to destroy the 21S progeny viral DNA molecules.

The ^{32}P-labeled nuclear extracts were then fractionated by sucrose gradient centrifugation,

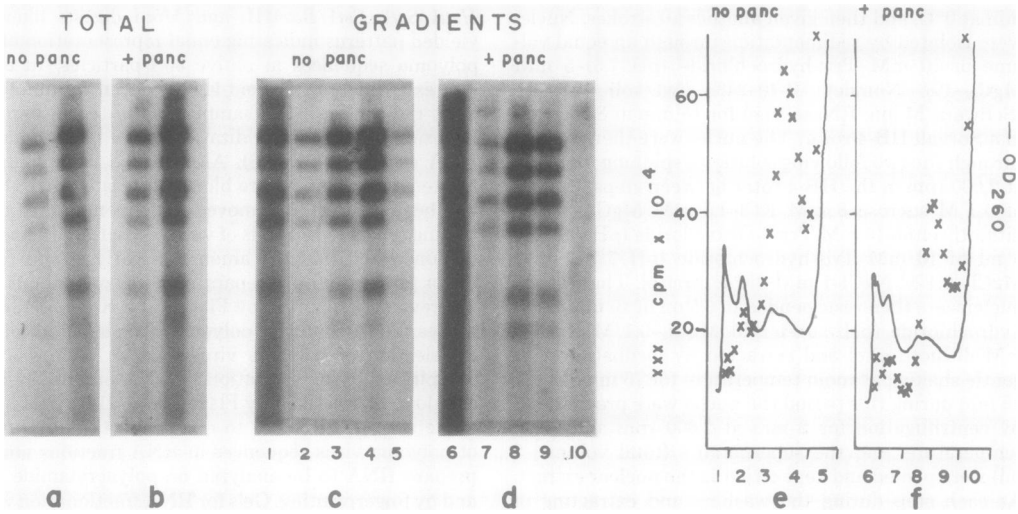


FIG. 2. *Miniblot hybridization analysis of RNA from polyoma virus-infected nuclear extracts fractionated on sucrose gradients.* ^{32}*P-labeled extracts were prepared, and the RNA was recovered from various fractions and analyzed as described in the text. The miniblots were prepared from an HpaII digest of polyoma virus DNA. (a, c, and e) Untreated nuclear extract; (b, d, and f) other half of the same extract exposed to 0.1 μg of pancreatic RNase (panc) per ml for 30 min at 0°C before gradient fractionation. In (a) and (b), an aliquot from each of the two extracts was hybridized using two RNA concentrations (fivefold different); the linear response of the band intensities demonstrates that the hybridization was performed in DNA excess. The numbers in (c) and (d) correspond to the gradient fractions in (e) and (f); equal portions of each fraction were analyzed by hybridization to blots. The direction of sedimentation in (e) and (f) was from right to left. Comparison with markers run in parallel gradients showed that the optical density peaks in fractions 2 and 7 correspond to 60S ribosomal subunits and fractions 3 and 8 correspond to 40S ribosomal subunits, whereas fractions 4 and 9 contain 30S hnRNP.* OD_{260}*, Optical density at 260 nm.*

either before (Fig. 2e) or after (Fig. 2f) mild digestion with pancreatic RNase. The optical density profiles revealed a distribution of RNP particles of different sizes. The two faster-sedimenting peaks were contaminating 60S and 40S ribosomal subunits; their level was calculated to be less than 3% of the total cellular ribosome population. The broad peaks sedimenting at approximately 30S in Fig. 2e and f were identified as hnRNP complexes by sodium dodecyl sulfate-polyacrylamide gel analysis (Fig. 3); the characteristic pattern of the 30,000- to 40,000-dalton core proteins of 30S hnRNP (see lanes labeled "30S" in Fig. 3) predominated in these regions of the sucrose gradients (Fig. 3, lanes 3, 4, 8, and 9). The profiles of total ^{32}P radioactivity ("x" in Fig. 2e and f) showed the expected peaks of labeled RNA centered on the 30S optical density maxima. Such sucrose gradient patterns are typical of the distribution of rapidly labeled RNA in nuclear extracts from uninfected mammalian cells (5, 19).

The distribution of viral RNA in the fractionated nuclear extracts was monitored by hybridization to restriction fragments of polyoma virus DNA immobilized on nitrocellulose strips under conditions of viral DNA excess (miniblot hybridization; 7). Figure 2c and d shows that most of the virus-specific sequences sedimented in those gradient fractions corresponding to the 30S hnRNP (Fig. 2e and f, fractions 3, 4, 8, and 9). The polyoma virus RNA which pelleted to the bottom of the gradients may have been derived from hnRNP trapped in released chromatin (25). To exclude the possibility that the ^{32}P radioactivity hybridizing to the miniblots was viral DNA, each sample was exhaustively digested with deoxyribonuclease before hybridization; control experiments (not shown) confirmed the alkaline lability of the remaining viral nucleic acid. We standardly recovered in the nuclear extracts about 50% of the total ^{32}P radioactivity originally present in the washed nuclei. Since much of the added $^{32}PO_4$ is incorporated into molecules other than RNA (e.g., DNA, proteins, lipids), we conclude that most of the radioactive nuclear RNA was extracted. Analyses of the proportion of polyoma-specific sequences in total nuclear RNA and in 30S RNP gave the same result: 5 to 10%, depending on the experiment. We thus further conclude that the majority of the nuclear viral RNA cosedimented with 30S hnRNP.

The cosedimentation of viral RNA with 30S

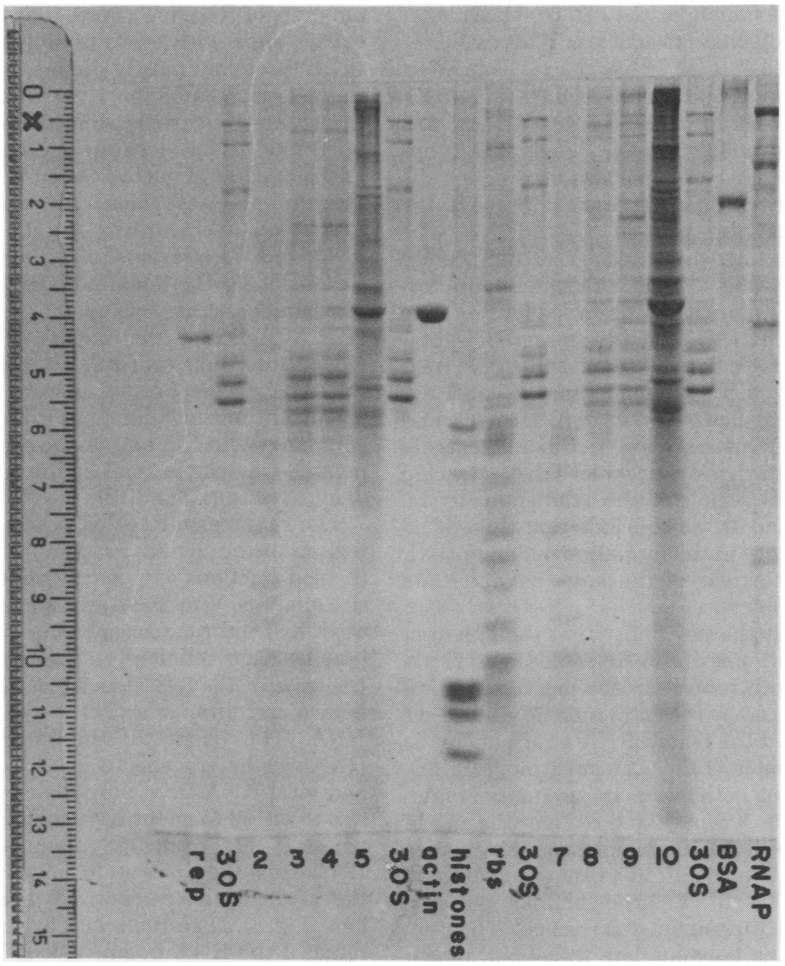


FIG. 3. *Sodium dodecyl sulfate-polyacrylamide gel analysis of proteins from polyoma virus-infected nuclear extracts fractionated on sucrose gradients. The numbers 2 through 5 and 7 through 10 correspond to the gradient fractions in Fig. 2e and f; aliquots of each fraction were analyzed. The following marker proteins were coelectrophoresed: RNAP, E. coli RNA polymerase (a gift of A. Travers), subunits 160K, 90K, 40K; BSA, bovine serum albumin, 68K; 30S, 30S hnRNP isolated from mouse liver as described by Martin et al. (25); rbs, mouse liver polyribosomes (prepared as described by Blobel and Sabatini [8]); histones, the chromatin fraction of mouse liver nuclei (prepared as described by Pederson [32]); actin, rabbit actin, (gift of K. Weber), 42K; rep, E. coli lac repressor (gift of N. Geissler), 40K. Note that the protein profiles for the 30S hnRNP from infected mouse embryo cells (fractions 3, 4, 9, and 10) show a few additional bands relative to 30S particles isolated from mouse liver; however, the characteristic 30K–40K core proteins dominate both patterns. Also note the prominent actin band in the top fraction of each gradient (fractions 5 and 10).*

hnRNP in nuclear extracts (Fig. 2) strongly suggested that the viral RNA was associated with protein. Although intact viral nuclear RNA molecules themselves would sediment as a broad peak centered at 30S, the extraction conditions used allow endogenous ribonucleolytic cleavage, and thus RNA molecules of this size would not be expected. The RNA associated with the 30S hnRNP, even without exogenous nuclease treatment (Fig. 2c and e), in fact averaged only 500 nucleotides in length (see below). Conversely, the relative resistance of the viral RNA at 30S to pancreatic RNase digestion (Fig. 2d and f) confirmed that the polyoma virus-specific sequences were protected, presumably by interactions with the proteins of the 30S hnRNP. When a comparable amount of deproteinized ^{32}P-labeled RNA from infected cell nuclei was mixed with an unlabeled extract and then treated with the same amount of pancreatic RNase, no radioactivity whatsoever sedimented at 30S (not shown); all counts were converted to small material sedimenting near the top of the gradient. As previously concluded for nuclear

transcripts of simian virus 40 (3, 30) and adenovirus (24), polyoma late nuclear RNA is therefore packaged into RNP complexes similar to those containing cellular hnRNA.

All portions of the late transcript are represented in 30S hnRNP. The miniblot hybridization studies of Fig. 2 also determined the relative distribution of different portions of the polyoma late transcript in 30S hnRNP. The RNA in the untreated nuclear extract before fractionation (Fig. 2a) had the same hybridization pattern as previously observed for total late viral nuclear RNA (7): nearly equal representation of sequences derived from each *Hpa*II fragment, with a slight preponderance of label hybridized to fragments 1 and 3 (which correspond to the late mRNA's; see Fig. 1). Since essentially the same pattern is observed for RNA extracted from the 30S region of the gradient (Fig. 2c, fractions 3 and 4), we conclude that there is no gross selectivity in the formation of 30S hnRNP on various portions of the large polyoma late transcript.

To rule out the possibility that the apparent non-selectivity was due to RNA tails not directly associated with protein in the native 30S complexes produced by the extraction procedure, we also examined the bound RNA after pancreatic RNase treatment of the nuclear extract (Fig. 2f). Such trimming both lowers the amount of radioactivity in the 30S peak and reduces the size of the associated RNA fragments from an average of about 500 to about 125 residues (data not shown; see Fig. 5). Yet the miniblot patterns (Fig. 2b and d) remained the same. Thus, all portions of the polyoma late transcript appear to make close contact with the proteins of the 30S hnRNP.

RNase T_1 oligonucleotide fingerprinting of 30S-associated viral RNA. Fingerprint analysis of polyoma virus nuclear RNA fragments involved in 30S hnRNP complexes progressively digested with RNase was next used to explore the selectivity of the protein-RNA interaction in more detail. RNase T_1 fingerprints of late viral nuclear RNA (23) show a complicated pattern consistent with the 5.29-kilobase complexity, but a number of large oligonucleotides are resolved, some of which have been localized on the physical map. Fingerprints of the fractioned cytoplasmic mRNA's are simpler and show distinct differences when compared with nuclear RNA or with one another (23). Thus, by fingerprinting we could in principle detect differences in the recovery of relatively short segments of the late nuclear transcript as protein-RNA complexes.

Figure 4 shows sucrose gradient profiles of ^{32}P-labeled nuclear extracts which had been treated before fractionation with different amounts of RNase T_1. Note that the undigested extract apparently contains polymers of 30S particles (probably mostly dimers, as indicated by the gel analysis of the RNA discussed below); these were converted to a much larger 30S peak upon mild RNase treatment (2.5 μg/ml). Then, as the amount of nuclease used was increased to 10 μg/ml, the 30S radioactivity peak diminished in size, in agreement with the RNase sensitivity of 30S hnRNP previously documented by others (5, 26, 33, 38, 41). Sodium dodecyl sulfate-polyacrylamide gel analysis of the pooled fractions (Fig. 5) indicated that some of the complexes indeed lose their integrity after digestion since the typical core protein pattern becomes less prominent in the 30S region (fractions 8 and 11 compared with 1, 2, and 5); the constituent polypeptides appear instead at the top of the gradient (fractions 9 and 12).

The ^{32}P-labeled RNA was extracted from 30S regions of the sucrose gradients shown in Fig. 4 (pooled fractions 5, 8, and 11) and subjected to size analysis. Viral RNA was purified by preparative hybridization to polyoma virus DNA immobilized on cellulose (14). Figure 6 compares the sizes of the 30S-associated RNA fragments before and after selection of the virus-specific RNA. The length of the hnRNP-associated RNA was heterogeneous in all cases. The total and viral RNA from polyparticles ranged from less than 100 to about 1,600 nucleotides (Fig. 6), whereas that from 30S monoparticles before RNase digestion was up to 800 nucleotides long, the average being about 500 nucleotides (30S lane, Fig. 6). Digestions with 2.5 μg of RNase T_1 reduced the RNA fragment length to just over 100 nucleotides (Fig. 6); this treatment yielded an increase in both the fraction of total ^{32}P radioactivity and core hnRNP proteins recovered in the 30S monoparticle peak (Fig. 4 and 5). Further digestion with 10 or 25 μg of RNase reduced the average length of the total hnRNP-associated RNA to about 60 and 30 nucleotides, respectively. Under these conditions, the complexes had begun to fall apart, as judged by the reduced recovery of 30S radioactivity and protein. The size distribution of RNP-associated viral RNA fragments after digestion of the extract with 2.5 or 10 μg of RNase was similar to that of the cellular RNA, which constituted more than 90% of the radioactivity. Note, however, that after more severe nuclease treatment (25 μg; see Fig. 6), the average size of viral RNA selected by preparative hybridization was considerably greater than that of the nonviral fragments. This could mean that the viral RNA was in fact larger than the average fragment size or that the hybridization efficiency of very small fragments was reduced; the percentage of polyoma-specific RNA recovered supports

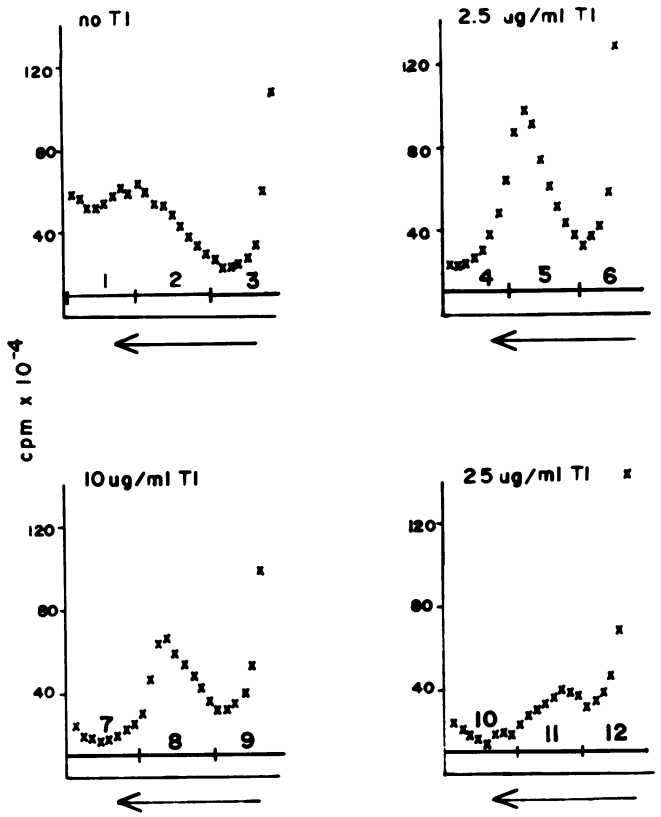


FIG. 4. *Gradient profiles of progressively digested nuclear extracts. Equal portions of an extract prepared from mouse embryo cells labeled with $^{32}PO_4$ late during polyoma virus infection were treated with various amounts of RNase T_1 for 30 min at 0°C. Sedimentation was in the direction indicated by the arrows.*

the latter explanation (see legend to Fig. 6). It is significant, however, that the size distribution of viral RNA fragments extracted from an aliquot of the unfractionated nuclear extract after digestion with 10 μg of RNase (track "Σ + 10 μg" in Fig. 6) was very similar to that of the corresponding 30S-associated RNA; this observation is consistent with our earlier conclusion that the majority of the labeled viral RNA in cell nuclei is protected from nuclease digestion by association with the proteins of the 30S hnRNP.

Figure 7 compares the RNase T_1 fingerprints of viral RNA in total extracts (Fig. 7a) with that of 30S hnRNP complexes containing RNA fragments of decreasing size (Fig. 7b, c, and d). The T_1 oligonucleotide fingerprint of substantially intact viral nuclear RNA (Fig. 7a) and that from 30S hnRNP obtained without exogenous nuclease treatment (Fig. 7b) are indistinguishable. These fingerprints, moreover, are the same as those of many other preparations of polyoma virus nuclear RNA prepared in this laboratory, such as that published by Legon et al. (23); they are also the same as those of uniform transcripts of the entire DNA L-strand synthesized in vitro with *Escherichia coli* RNA polymerase (Fig. 7h) or of the endogenous activity of viral transcription complexes (23). It is important to note that the fingerprints of total RNA and 30S-associated RNA are the same even though the endogenous RNase(s) active during particle extraction had reduced the RNA size from 2 to 15 kilobases to only about 500 nucleotides. The fingerprint (not shown) of viral RNA purified from polyparticles was likewise indistinguishable from that of total viral nuclear RNA.

Mild RNase T_1 trimming of the hnRNP complexes to an average RNA fragment size of 100 nucleotides (Fig. 6, lane labeled "2.5 μg after py-DNA cellulose") began to alter the relative intensities of certain oligonucleotides in the 30S-associated viral RNA (Fig. 7c). This effect was most pronounced in the fingerprints of the shortest viral RNA fragments from more highly digested 30S hnRNP (Fig. 4, fraction 11). Figure 7d clearly demonstrates that a specific set of oligonucleotides is drastically reduced in intensity relative to the complete patterns shown in Fig. 7a and b. The fingerprint is not simply that of a partial nuclease digest of polyoma nuclear

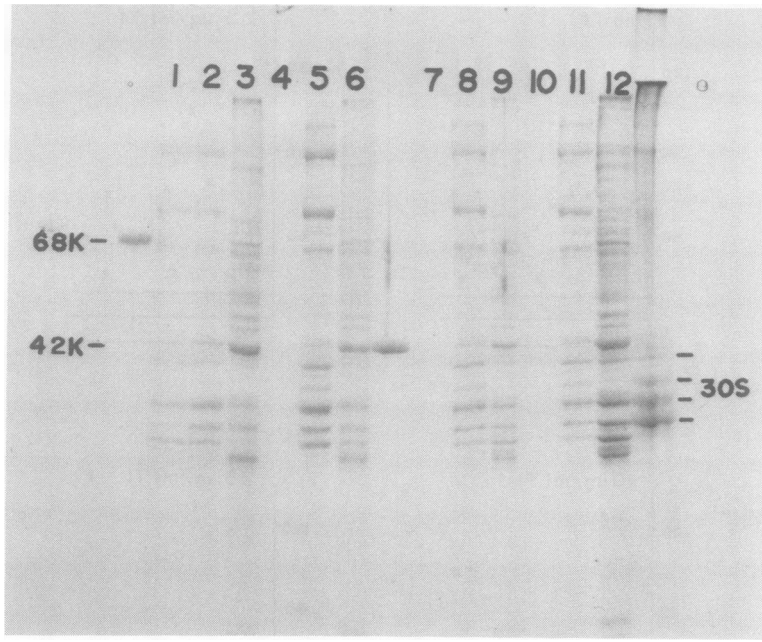


FIG. 5. *Sodium dodecyl sulfate-polyacrylamide gel analysis of gradient fractions from progressively digested nuclear extracts. Proteins recovered as described in the text from the regions numbered in Fig. 4 were examined. Markers were bovine serum albumin (68K), actin (42K) and isolated mouse liver 30S hnRNP.*

RNA: when deproteinized RNA was digested with far smaller amounts of RNase T_1 to yield fragments about 50 nucleotides long, the viral RNA purified by subsequent DNA cellulose hybridization had a distinctly different fingerprint (data not shown). This suggested that the oligonucleotide pattern of Fig. 7d was a characteristic of highly digested viral hnRNP complexes.

To evaluate the significance of the simplified T_1 oligonucleotide pattern which was specific to the viral RNA purified from highly digested 30S hnRNP, we compared it with the fingerprints of cytoplasmic mRNA's. To aid in these comparisons, Fig. 7e and f show previously published (23) fingerprints of size-fractionated mVP1 (16S, Fig. 7e) and mVP2 + mVP3 (18S + 19S, Fig. 7f) viral mRNA. It is immediately apparent, as indicated in the tracings shown in Fig. 7g, that essentially all of the T_1 oligonucleotides characteristic of the major species, mVP1, are still present in good yields in the viral RNA associated with highly digested 30S hnRNP. The majority of the oligonucleotides that have virtually disappeared from such fingerprints are derived from the "anti-early" region of the genome, whereas other oligonucleotides present with reduced intensity correspond specifically to the minor late mRNA species, mVP2 or mVP3. Many of the oligonucleotides resolved in fingerprints such as those shown in Fig. 7 were eluted and subjected to secondary analysis with pancreatic RNase. The compositions of these oligonucleotides were then compared with the catalog of all T_1 oligonucleotides predicted by the known DNA sequence of the genome (11, 15, 39). This analysis confirmed many of the identities indicated by visual comparisons of the fingerprints and verified the conclusion that the apparently missing spots derive from RNA sequences not represented in the mature mRNA's. It is important to note, however, that certain T_1 oligonucleotides which are not present in mRNA's survive in the highly digested hnRNP (indicated by open spots in Fig. 7g). Analysis of the same RNA samples by miniblot hybridization (not shown) also suggested that the viral RNA recovered from the highly digested complexes was enriched for mRNA-like sequences.

DISCUSSION

Our purpose was to examine the specificity of association of the RNP complexes called 30S hnRNP with a particular hnRNA, the polyoma virus late nuclear transcript. We wished to determine whether 30S complexes are found exclusively either on intervening sequences or on premessage portions of an hnRNA molecule; such complete specificity would have profound implications for the function of 30S hnRNP in mRNA biogenesis. Our studies with miniblot hybridization and RNase T_1 oligonucleotide analysis to examine polyoma virus-specific sequences associated with 30S hnRNP trimmed to

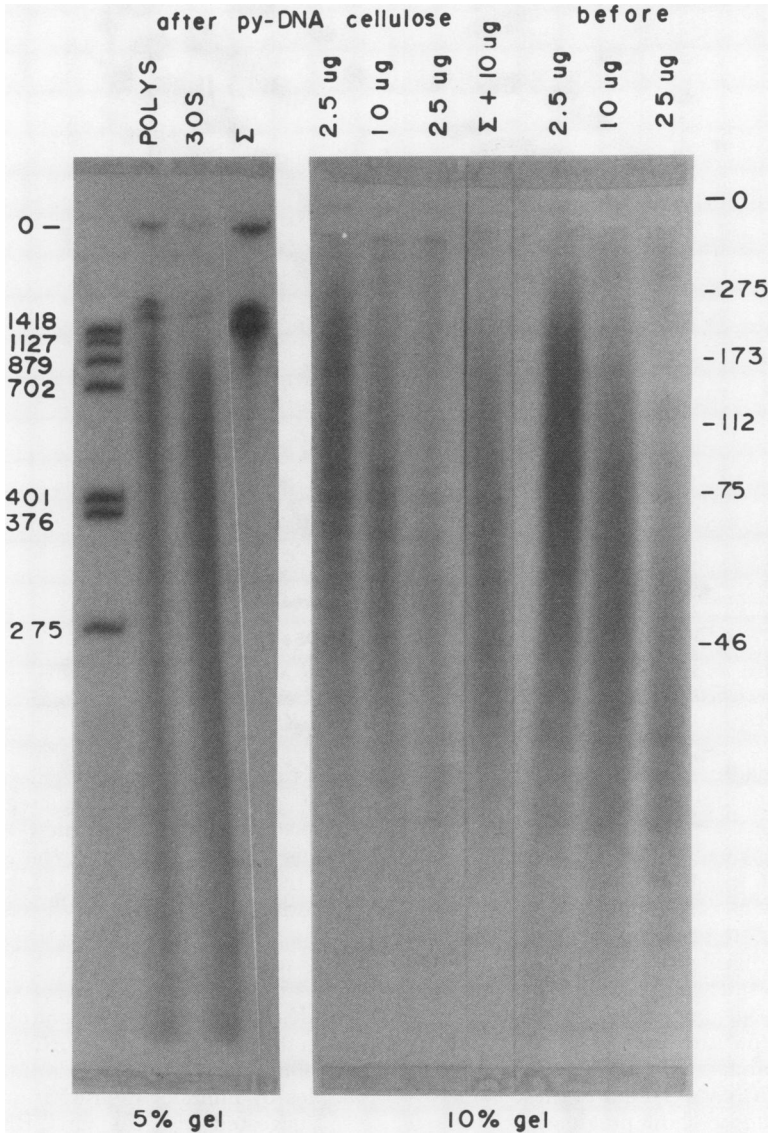


FIG. 6. *Polyacrylamide gel analysis of RNA recovered from progressively digested 30S particles. RNA isolated from the gradient regions indicated in Fig. 4 were examined on a 5 or 10% gel before and after selection on polyoma virus (py) DNA celulose. (See text.) The following RNAs are shown after selection for polyoma-specific molecules: POLYS, fraction 1 from the untreated extract of Fig. 4; 30S, fraction 2 from the same extract; Σ, total RNA prepared from the washed nuclei before extraction at high pH (note the large size of the polyoma virus late transcript; the doubling of the band is apparently a gel artifact); 2.5 μg, fraction 5, the 30S peak from the extract digested with 2.5 μg of RNase T_1 per ml; 10 μg, fraction 8 from the extract treated with 10 μg of T_1 per ml; 25 μg, fraction 11 from the extract treated with 25 μg of T_1 per ml; Σ + 10 μg, total RNA from the nuclear extract exposed to 10 μg of T_1 per ml before fractionation on the sucrose gradient. The percent counts per minute recovered from the cellulose (percent polyoma) for these fractions were as follows: POLYS, 11.3; 30S, 12.9; Σ, 6.6; 2.5 μg, 9.4; 10 μg, 8.0; 25 μg, 7.4; Σ + 10 μg, 6.0. In the lanes labeled 2.5, 10, and 25 μg are shown RNA fragments isolated from fractions 5, 8, and 11 in Fig. 4 before selection on polyoma DNA cellulose. The mobilities of various size markers (single-stranded DNA restriction fragments) are indicated.*

various extents with exogenous RNase showed that 30S complexes are located on all portions of the polyoma late nuclear transcript. However, when the RNA was digested to pieces much smaller than the unit length assigned to a 30S monoparticle, we observed an enrichment for coding sequences in the remaining protein-associated RNA fragments.

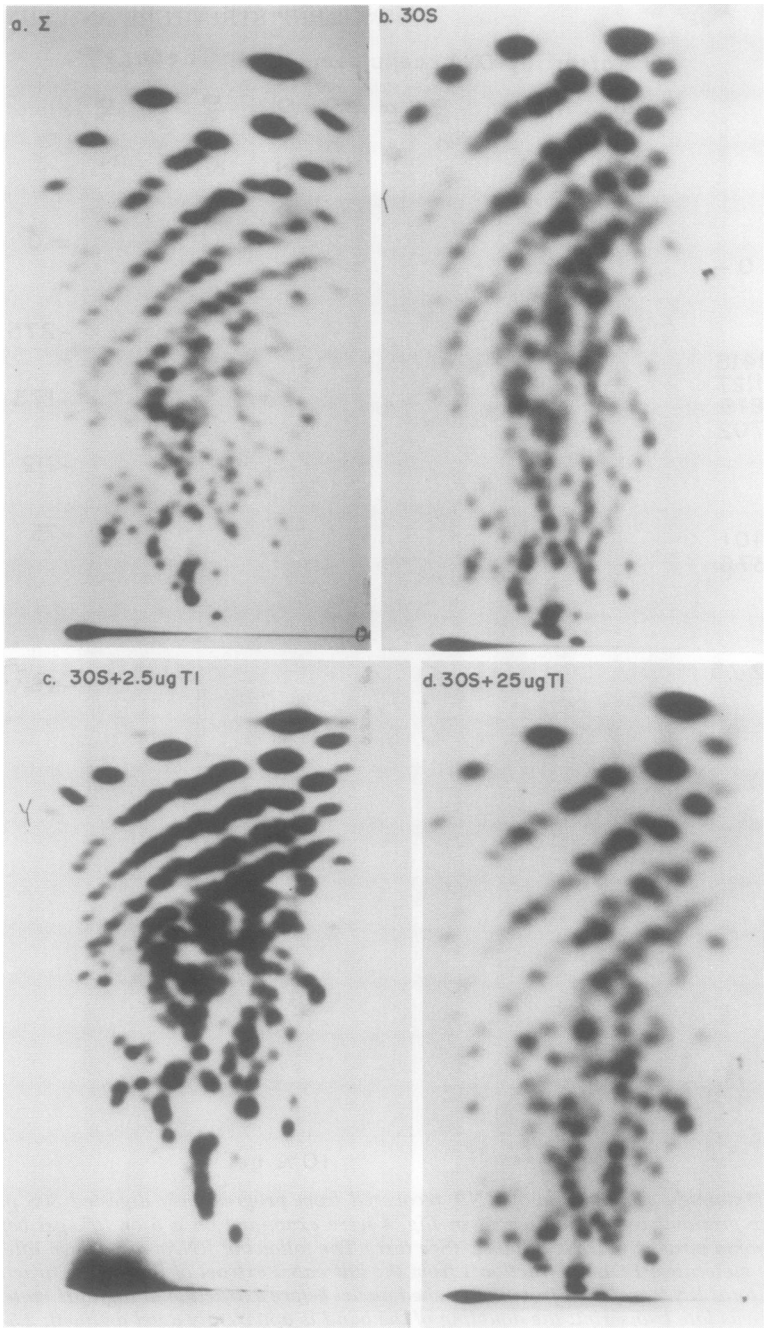
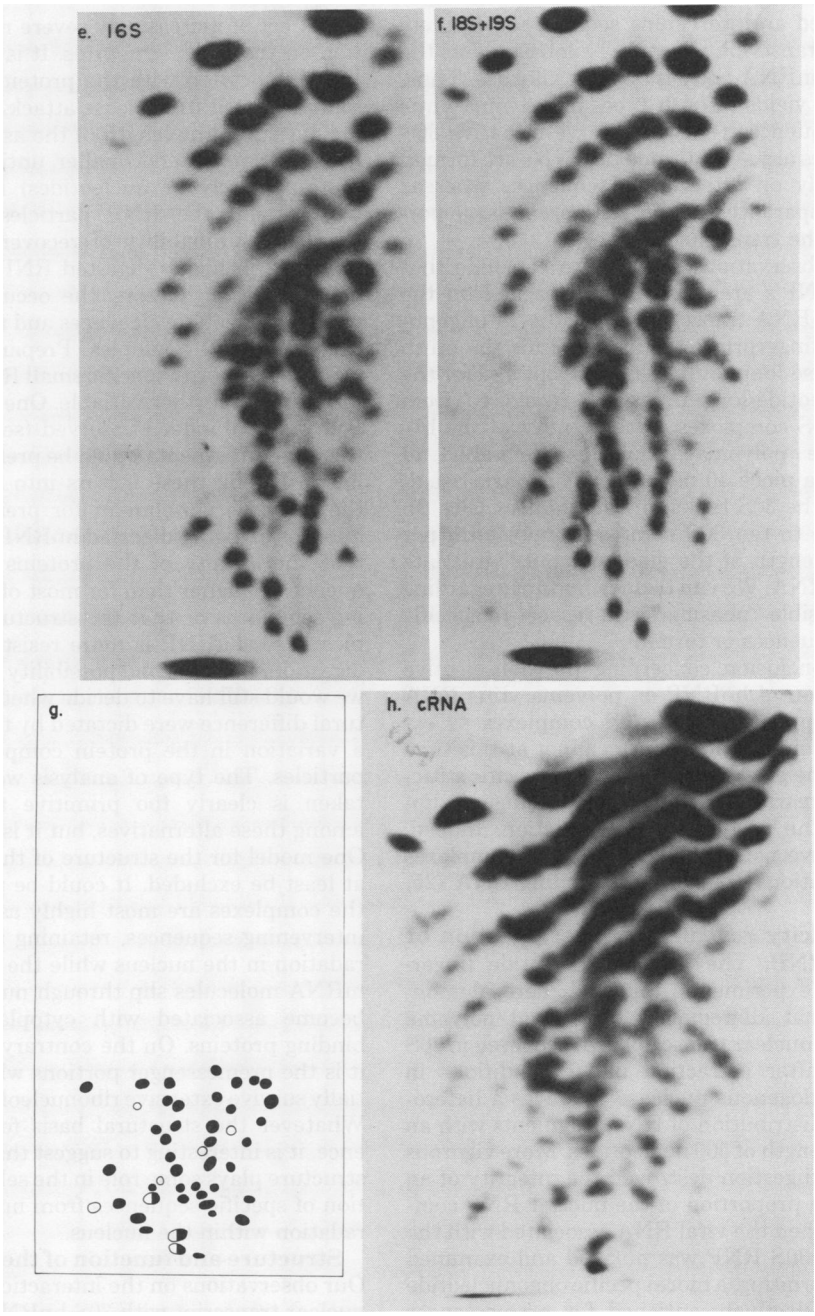


FIG. 7. *RNase T_1 fingerprints of polyoma virus-specific RNA recovered from progressively digested 30S particles. (a) Total virus-specific RNA from the nuclei of polyoma virus-infected cells before extraction at high pH (the large-sized RNA shown in Fig. 6, lane Σ). (b) Polyoma virus-specific RNA recovered from native 30S hnRNP (fraction 2, Fig. 4); average size, about 500 residues (Fig. 6). (c) Polyoma virus-specific RNA isolated from 30S RNP exposed to 2.5 μg of RNase T_1 per ml (fraction 5, Fig. 4); average size, about 125 residues (Fig. 6). (d) Polyoma virus-specific RNA from 30S RNP exposed to 25 μg of RNase T_1 per ml (fraction 11, Fig. 4); average size, about 50 residues (Fig. 6). (e) 16S mRNA fingerprint, from Legon et al. (23). (f) 18S + 19S fingerprint, from Legon et al. (23). (g) Tracing of lower portion of the fingerprint shown in (d): filled spots are oligonucleotides present in the 16S or 18S + 19S fingerprints of (e) and (f); open spots are not from the late region. The identities of most of these oligonucleotides, as well as those which have disappeared, were confirmed by secondary analyses; many of the spots are oligonucleotide mixtures. (h) Fingerprint of entire DNA L-strand transcript produced by E. coli RNA polymerase (23).*



Lack of selectivity of 30S hnRNP association. Our finding that both intervening sequences and premessage sequences from the polyoma virus late transcript are represented in native 30s hnRNP complexes (created by exposure to endogenous nuclease during extraction) is in agreement with the previous conclusions of Kinniburgh and Martin (20). Their studies of total ascites cell hnRNP indicated that exclusively nuclear sequences as well as sequences eventually found in the cytoplasm could be recovered from 30S complexes. However, in their system, the average sizes for intervening and premessage sequences were not known; thus, a possible explanation was that one type of sequence was selectively bound but the other sequence class appeared simply because it resided on the same 500-nucleotide-long pieces of RNA carried by the 30S monoparticles. By contrast, the polyoma virus early region, which can be

considered an intervening sequence in the late nuclear transcript, is nearly 3 kilobases long; the shortest mRNA body is about 1 kilobase. Thus, the good yields of both types of polyoma virus RNA sequences that we observe in native 30S complexes argue that some hnRNP's are formed exclusively on intervening sequences whereas others apparently include only premessage portions of the transcript.

Two observations allow us to conclude that the hnRNP's are quite closely packed on the polyoma RNA transcript. First, the T_1 oligonucleotide fingerprints are identical for the up to 50-kilobase-long total late transcript and for the 500-nucleotide-long fragments recovered from native 30S complexes. Second, most of the hybridizable polyoma sequences (probably all those in a piece 40 nucleotides or longer) sediment in the 30S region of the gradient (Fig. 2). Thus, up to ten 30S complexes may form per genome length of the giant polyoma virus late nuclear RNA. We can deduce nothing regarding their possible "phasing" with respect to specific RNA sequences or termini.

Our conclusion concerning the nonselective distribution of hnRNP on polyoma virus RNA strictly applies only to 30S complexes as extracted from the nuclei. We cannot at this time exclude the possibility that a more specific structure had rearranged because of exchange or slippage of the proteins during isolation. Indeed, others have noted that purified 30S complexes have additional capacity for binding RNA (25, 26).

Specificity revealed by overdigestion of 30S hnRNP. The T_1 oligonucleotide fingerprinting experiments reported here demonstrated that all regions of the giant polyoma virus late nuclear transcript are recovered in 30S hnRNP after extraction under conditions in which endogenous nuclease produces a heterogeneous distribution of RNA fragments with an average length of 500 nucleotides. More vigorous nuclease digestion destroyed the integrity of an increasing proportion of the nuclear RNP complexes. When the viral RNA associated with the surviving 30S RNP was purified and examined by fingerprinting, a more specific oligonucleotide pattern selectively enriched for premessenger sequences was observed. The fingerprints of viral RNA in highly digested complexes were not, however, identical to those of mature mRNA because certain oligonucleotides derived from intervening sequences were also present. Moreover, the pattern cannot be due to contaminating cytoplasmic mRNA since the oligonucleotides corresponding to the reiterated leader are not overrepresented (cf. Fig. 7d with 7e and f).

To interpret these results we must consider the effect of increasingly severe nuclease digestion on the RNP structures. It is clear that the RNA associated with the proteins is only partially resistant to nuclease attack. With increasing digestion, the length of the associated RNA becomes smaller and smaller, until a critical size (approximately 50 nucleotides) is reached, at which point the RNP particles disintegrate. Thus, the probability of recovering a specific sequence in highly digested RNP is a function of at least two factors: the occurrence of two nearby nucleolytic cleavages and the stability of the nicked RNP complex. Preparative hybridization of the virus-specific small RNA fragments introduces a further variable. One would expect, and we have indeed observed (see Fig. 6), that the larger fragments would be preferentially isolated. Taking these factors into consideration, the selective enrichment for premessenger sequences in highly digested hnRNP means either that the affinity of the proteins for these sequences is higher than for most of the intervening sequences or that the structure of the premessenger hnRNP is more resistant to RNase digestion. If the second possibility were the case, we would still have to decide whether this structural difference were dictated by the RNA or by a variation in the protein composition of the particles. The type of analysis we have undertaken is clearly too primitive to distinguish among these alternatives, but it is the first step. One model for the structure of the hnRNP can at least be excluded. It could be proposed that the complexes are most highly associated with intervening sequences, retaining these for degradation in the nucleus while the newly spliced mRNA molecules slip through nuclear pores to become associated with cytoplasmic mRNA binding proteins. On the contrary, we find that it is the premessenger portions which preferentially survive extensive ribonucleolytic digestion. Whatever the structural basis for this preference, it is interesting to suggest that the hnRNP structure plays some role in the selective protection of specific sequences from nucleolytic degradation within the nucleus.

Structure and function of the 30S hnRNP. Our observations on the interaction of a specific nuclear transcript with 30S hnRNP are consistent with previous conclusions that these RNP complexes form on all nonribosomal nuclear transcripts. It appears (though we have not rigorously proven) that the giant polyoma late nuclear transcript becomes associated with the same set of highly conserved core 30S hnRNP polypeptides as are found in the uninfected cell. Indeed, many additional proteins have been detected in the 30S hnRNP from various cell types, their yield dependent upon the exact isolation

procedure used. For instance, Pagoulatos and Yaniv (30) report finding new simian virus 40-induced cellular polypeptides in hnRNP complexes prepared from virus-infected cells. Figure 3 likewise reveals that certain polypeptides not found in mouse liver 30S structures (lanes labeled "30S") appear in hnRNP from polyoma-infected mouse embryo cells (lanes 3, 4, 8, and 9); however, our purification procedure was not as rigorous as that used in the simian virus 40 work, and it is quite possible that the additional proteins simply represent tissue-specific variation in hnRNP polypeptides (see references 5 and 25). It is certainly conceivable that different nuclear transcripts assemble hnRNP's with slightly different complements of minor proteins, perhaps related to the special processing needs of that particular hnRNA.

Our analyses of polyoma RNA recovered from progressively digested complexes also provide support for the 30S hnRNP substructure model proposed by Martin et al. (26). They suggest that the hnRNA coils within or around the hnRNP proteins in a manner quite available to the environment (perhaps similar to what we know of the arrangement of DNA on the nucleosome). Although the hnRNP-associated RNA is significantly shielded from nuclease action, the amount of RNase needed to introduce nicks into the 500-nucleotide-long RNA segment on each monoparticle is low (relative, for instance, to amounts used to produce ribosome-protected fragments of mRNA [40]). This supports the idea (26) that not every nucleotide makes equal contact with 30S proteins; rather, multiple interrupted interactions are made along the length of the 500-residue hnRNA segment. Moreover, when the RNA is reduced to pieces smaller than 1/10 the unit particle length (i.e., an average of 50 nucleotides in length), the 30S complex falls apart, confirming that some continuity of the RNA chain is required for particle integrity.

If, then, all portions of a nascent transcript are initially equally (and nearly completely) coated by hnRNP, what role do these protein-RNA complexes play in splicing and other processing events crucial to mRNA biogenesis? Since eucaryotic cell nuclei lack the ribosomes that rapidly attach to nascent transcripts in procaryotic organisms, the 30S hnRNP may function primarily to extend the hnRNA and reduce its tendency to tangle or knot. (That the latter phenomenon can be a problem for large protein-free RNA molecules is supported by the recent work of Cantor [10].) Avoiding such complications while affording some protection to the RNA certainly must facilitate nuclear processing. Since we found that sequences near polyoma splice junctions are selectively neither bound nor not bound by the hnRNP proteins, the involvement of the entire hnRNA in such complexes must be assumed when contemplating specific mechanisms for the splicing process.

Finally, our observation that premessage sequences seem to be preferentially protected in 30S hnRNP requires that there exist some active mechanism for removing the 30S polypeptides as the finished mRNA passes across the nuclear membrane. Recent indirect immunofluorescence studies on whole cells support the earlier biochemical conclusion that the major proteins of the 30S complexes are exclusively nuclear (27). How one set of RNA binding proteins is exchanged for another during export remains one of the most intriguing questions in eucaryotic mRNA biogenesis.

ACKNOWLEDGMENTS

We are grateful to J. Favoloro, A. Cowie, and J. Weliky for superb assistance. J. Karn, M. Bretscher, and R. Kornberg provided helpful criticisms on the experiments and the manuscript.

This work was conducted in part while J.A.S. was a sabbatical fellow of the Josiah Macy Jr. Foundation (and a guest in the laboratory of M. Bretscher, Medical Research Council Laboratory of Molecular Biology, Cambridge) and later with the support of a grant from the National Science Foundation.

LITERATURE CITED

1. **Acheson, N. H.** 1978. Polyoma giant RNAs contain tandem repeats of the nucleotide sequences of the entire viral genome. Proc. Natl. Acad. Sci. U.S.A. **75**:4754–4758.
2. **Acheson, N. H., E. Buetti, K. Scherrer, and R. Weil.** 1971. Transcription of the polyoma virus genome: synthesis and cleavage of giant late polyoma-specific RNA. Proc. Natl. Acad. Sci. U.S.A. **68**:2231–2235.
3. **Aloni, Y.** 1974. Biogenesis and characterization of SV40 and polyoma RNAs in productively infected cells. Cold Spring Harbor Symp. Quant. Biol. **39**:165–178.
4. **Barrell, B. G.** 1971. Fractionation and sequence analysis of radioactive nucleotides, p. 751–779. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acids research, vol. 2. Harper & Row, New York.
5. **Beyer, A. L., M. E. Christensen, B. W. Walker, and W. M. LeStourgeon.** 1977. Identification and characterization of the packaging proteins of core 40S hnRNP particles. Cell **11**:127–138.
6. **Beyer, A. L., O. L. Miller, and S. L. McKnight.** 1980. Ribonucleoprotein structure in nascent hnRNA is nonrandom and sequence-dependent. Cell **20**:75–84.
7. **Birg, F., J. Favaloro, and R. Kamen.** 1977. Analysis of polyoma virus nuclear RNA by mini-blot hybridization. Proc. Natl. Acad. Sci. U.S.A. **74**:3138–3142.
8. **Blobel, G., and D. Sabatini.** 1971. Dissociation of mammalian polyribosomes into subunits by puromycin. Proc. Natl. Acad. Sci. U.S.A. **68**:390–394.
9. **Calvet, J. P., and T. Pederson.** 1978. Nucleoprotein organization of inverted repeat DNA transcripts in heterogeneous nuclear RNA-ribonucleoprotein particles from HeLa cells. J. Mol. Biol. **122**:361–378.
10. **Cantor, C. R.** 1980. Physical and chemical techniques for the study of RNA structures on the ribosome, p. 23–49. *In* G. Chambliss, G. R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura (ed.), Ribosomes. University Park Press, Baltimore.
11. **Deininger, P., A. Esty, P. La Porte, and T. Friedman.**

1979. Nucleotide sequence and genetic organization of the polyoma late region: features common to the polyoma early region and SV40. Cell **18**:771-779.

12. **Favaloro, J., R. Treisman, and R. Kamen.** 1980. Transcription maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease S1-gel mapping. Methods Enzymol. **65**:718-749.
13. **Flavell, A., and R. Kamen.** 1977. Strand-specific transcription of polyoma virus DNA late during productive infection. J. Mol. Biol. **115**:237-242.
14. **Flavell, A. J., A. Cowie, S. Legon, and R. Kamen.** 1979. Multiple 5' terminal cap structures in late polyoma virus RNA. Cell **16**:357-371.
15. **Friedman, T., A. Esty, P. La Porte, and P. Deininger.** 1979. The nucleotide sequence and genome organization of the polyoma early region: extensive nucleotide and amino acid homology with SV40. Cell **17**:715-724.
16. **Griffin, B. E., M. Fried, and A. Cowie,** 1974. Polyoma DNA: a physical map. Proc. Natl. Acad. Sci. U.S.A. **71**: 2077-2081.
17. **Jay, E., R. Bambara, R. Padmanabhan, and R. Wu.** 1974. DNA sequence analysis: a general, simple and rapid method for sequencing large oligodeoxyribonucleotide fragments by mapping. Nucleic Acids Res. **1**: 331-353.
18. **Kamen, R., J. Favaloro, and J. Parker.** 1980. Topography of the three late mRNA's of polyoma virus which encode the virion proteins. J.Virol. **33**:637-651.
19. **Karn, J., G. Vidali, L. C. Boffa, and V. G. Allfrey.** 1977. Characterization of the non-histone nuclear proteins associated with rapidly labeled heterogeneous nuclear RNA. J. Biol. Chem. **252**:7307-7322.
20. **Kinniburgh, A. J., and T. E. Martin.** 1976. Detection of mRNA sequences in nuclear 30S ribonucleoprotein subcomplexes. Proc. Natl. Acad. Sci. U.S.A. **73**:2725-2729.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680-685.
22. **Lamb, M. M., and B. Daneholt.** 1979. Characterization of active transcription units in balbiani rings of Chironomus tentans. Cell **17**:835-848.
23. **Legon, S., A. J. Flavell, A. Cowie, and R. Kamen.** 1979. Amplification in the leader sequence of late polyoma virus mRNAs. Cell **16**:373-388.
24. **Lukanidin, E. M., E. S. Zalmonzon, L. Komaromi, O. P. Samarina, and G. P. Georgiev.** 1972. Structure and function of informofers. Nature (London) New Biol. **238**:193-197.
25. **Martin, T., P. Billings, A. Levey, S. J. Ozarslan, T. Quinlan, H. Swift, and L. Urbas.** 1974. Some properties of RNA:protein complexes from the nucleus of eukaryotic cells. Cold Spring Harbor Symp. Quant. Biol. **38**:921-932.
26. **Martin, T., P. Billings, J. Pullman, B. Stevens, and A. Kinniburgh.** 1978. Substructure of nuclear ribonucleoprotein complexes. Cold Spring Harbor Symp. Quant. Biol. **42**:899-909.
27. **Martin, T., R. Jones, and P. Billings.** 1979. hnRNP core proteins: synthesis, turnover and intracellular distribution. Mol. Biol. Rep. **5**:37-42.
28. **Maundrell, K., and K. Scherrer.** 1979. Characterization of pre-messenger-RNA-containing nuclear ribonucleoprotein particles from avian erythroblasts. Eur. J. Biochem. **99**:225-238.
29. **McKnight, S.L., and O. L. Miller, Jr.** 1979. Post-replicative nonribosomal transcription units in D. melanogaster embryos. Cell **17**:551-563.
30. **Pagoulatos, G. N., and M. Yaniv.** 1978. Proteins bound to heterogeneous nuclear RNA of simian-virus-40-infected cells. Eur. J. Biochem. **91**:1-10.
31. **Patel, N. T., A. Kurosky, and V. Holoubek.** 1978. Isolation and characterization of the predominant protein in nuclear ribonucleoprotein particles from rat liver. Biochim. Biophys. Acta **533**:282-286.
32. **Pederson, T.** 1974. Gene activation in eukaryotes: are nuclear acidic proteins the cause or the effect? Proc. Natl. Acad. Sci. U.S.A. **71**:617-621.
33. **Pederson, T.** 1974. Proteins associated with heterogeneous nuclear RNA in eukaryotic cells. J. Mol. Biol. **83**: 163-183.
34. **Quinlan, T. J., P. B. Billings, and T. E. Martin.** 1974. Nuclear ribonucleoprotein complexes containing polyadenylate from mouse ascites cells. Proc. Natl. Acad. Sci. U.S.A. **71**:2632-2636.
35. **Quinlan, T. J., A. J. Kinniburgh, and T. E. Martin.** 1977. Properties of a nuclear polyadenylate·protein complex from mouse ascites cells. J. Biol. Chem. **252**: 1156-1161.
36. **Samarina, O. P., N. A. Aitkhozhina, and J. Besson.** 1973. PolyA blocks in the nuclear ribonucleoprotein complexes, containing pre-mRNA. Mol. Biol. Rep. **1**: 193-199.
37. **Samarina, O. P., E. M. Lukanidin, J. Molnar, and G. P. Georgiev.** 1968. Structural organization of nuclear complexes containing DNA-like RNA. J. Mol. Biol. **33**: 251-263.
38. **Sekeris, C. E., and J. Niessing.** 1975. Evidence for the existence of a structural RNA component in the nuclear ribonucleoprotein particles containing heterogeneous RNA. Biochem. Biophys. Res. Commun. **62**:642-650.
39. **Soeda, E., J. R. Arrand, N. Smolar, J. E. Walsh, and B. E. Griffin.** 1980. Coding potential and regulatory signals of the polyoma virus genome. Nature (London) **283**:445-453.
40. **Steitz, J. A.** 1979. Prokaryotic ribosome binding sites. Methods Enzymol. **60**:311-321.
41. **Stevenin, J., H. Gallinaro-Matringe, R. Gattoni, and M. Jacob.** 1977. Complexity of the structure of particles containing heterogeneous nuclear RNA as demonstrated by ribonuclease treatment. Eur. J. Biochem. **74**: 589-601.
42. **Zuckerman, M., H. Manor, J. Parker, and R. Kamen.** 1980. Electron microscope demonstration of the presence of amplified sequences at the 5'-ends of the polyoma virus late mRNAs. Nucleic Acids Res. **8**:1505-1519.