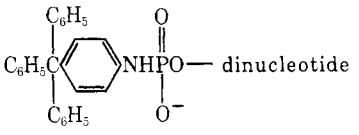
Both trityl- and naphthylcarbamoylcelluloses were, in general, satisfactory for the separation of the trityl from the non-trityl products. However, one may be preferred over the other depending upon the nature of the reaction mixtures. Trityl-containing compounds are absorbed to tritylcellulose more strongly than to naphthylcarbamoylcellulose and, consequently, with larger oligonucleotides, higher salt concentration was required for eluting from tritylcellulose. Elutions from naphthylcarbamoylcellulose require low salt and a further practical point is that the latter derivative is prepared more easily and inexpensively.

While the trityl group was the major source of the nonionic interaction, the groups used to protect the amino groups, such as benzoyl and anisoyl, also contributed in a supplementary way to the overall afinity of the trityl oligonucleotides for the tritylcellulose. Guanine nucleotides when protected with an aliphatic group, such as the isobutyryl group, showed less affinity for both cellulose derivatives.

Both tritylcellulose and naphthylcarbamoylcellulose columns show considerable affinity for other aromatic-type groups present within oligonucleotides such as TPM-phosphoramidates of the general structure



Oligonucleotides containing only the naphthylcarbamoyl grouping at their 3′ terminus such as d-TpTpTpA-CONHC$_{10}$H$_7$ were likewise absorbed strongly to tritylcellulose but showed weak binding to naphthylcarbamoylcellulose (Agarwal and Khorana, 1972). Recently these observations have been extended to a 3′-naphthylcarbamoyl-terminated octanucleotide (K. L. Agarwal, unpublished data). Finally the method developed in this paper may find applications in other areas such as in polypeptide or carbohydrate chemistry.

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Nuclear Double-Stranded Ribonucleic Acid of Mammalian Cells. Characteristics and Biosynthesis†

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ABSTRACT: Ribonuclease-resistant RNA synthesis was examined in Sarcoma-180 cells, Ehrlich ascites tumor cells, and L cells in the presence of high or low concentrations of actinomycin D. The RNase-resistant species were characterized by sucrose gradient analysis, Sepharose 2B column chromatography, and thermal denaturation studies. These species are predominantly of nuclear origin and their synthesis is sensitive to 5 μg/ml of actinomycin D. In all the cell lines, this RNA species is polydisperse on sucrose gradient and of low molecular weight. By thermal denaturation profile, it appears to be heterogeneous and not completely base paired. Sarcoma-180 cells not only contain the low molecular weight species, but also a high molecular weight double-stranded RNA that has characteristics of a completely base-paired molecule, when compared with the replicative form of a picornavirus.

Ribonuclease-resistant "presumptive" double-stranded species of RNAs have been detected recently in low amounts in uninfected animal cells (Montagnier, 1968; Stollar and Stollar, 1970; Kimball and Duesberg, 1971). A portion of this double-stranded RNA (ds-RNA)[1] has been reported to be synthesized in the presence of actinomycin D in Burkitt lymphoma cells (Stern and Friedman, 1971).

ds-RNA is also known to occur in the replicative cycle of

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[1] Abbreviations used are: ds-RNA, double-stranded ribonucleic acid; Hn-RNA, heterogeneous nuclear ribonucleic acid; PFU, plaque-forming units; MEM, minimum essential medium; RF, replicative form; SSC, standard saline citrate; ss-RNA, single-stranded ribonucleic acid.

RNA phage (Weissmann and Ochoa, 1967) and in a number of RNA viruses (Bishop *et al.*, 1965; Wall and Taylor, 1970). A similar species of RNA has been detected in low amounts in uninfected and potato spindle tuber virus-infected potato plants (Lewandowski *et al.*, 1971) and in the mycelium of *Penicillium chrysogenum* (Cox *et al.*, 1971).

In this article we describe the isolation, purification, and partial characterization of RNase-resistant RNA from Sarcoma-180, Ehrlich ascites tumor, and L cells labeled under conditions of high and low concentrations of actinomycin D. This ds-RNA was compared with the RF form of a picornavirus.

Our data suggest that the majority of the RNase-resistant RNA in uninfected cells is not truly base paired or in the ds-RNA form, but is possibly a loop structure, that forms a part of the heterogeneous nuclear RNA (Hn-RNA). However, a second class of high molecular weight, base-paired ds-RNA can be detected in both the nucleus and the cytoplasm of Sarcoma-180 cells.

Materials and Methods

Actinomycin D, RNase A, RNase T_1, and DNase were purchased from Sigma Chemical Co., St. Louis, Mo. Pronase was purchased from Calbiochem, San Diego, Calif. [^{3}H]-Uridine was purchased from New England Nuclear Corporation, Boston, Mass. BD-Cellulose was from Schwarz/Mann Labs., Orangeburg, N. Y., and Sepharose 2B was purchased from Pharmacia Chemicals, Newark, N. J. All chemicals used were of analytical grade.

Cell Lines. L cells were grown as confluent monolayers in Hank's minimal essential medium (MEM) supplemented with 5% calf serum, streptomycin (100 μg/ml), penicillin (100 units/ml), and fungizone (5 units/ml). Sarcoma-180 and Ehrlich ascites tumor cells were maintained by intraperitoneal passage every 8 days in adult Swiss Webster mice. For radioisotope labeling, cells were washed twice with saline and suspended in suspension medium containing calf serum and antibiotics as above.

Virus Growth and Infection. Bovine enterovirus was grown by infecting confluent monolayers of L cells ($\sim 10^7$ cells/32 oz bottle) at a multiplicity of infection of 10–20 plaque-forming units (PFU) per cell. Virus was absorbed to the monolayer for 1 hr after which minimal amounts of MEM containing antibiotics were added to the culture. The infected cells were incubated for 15–20 hr at 37° and virus was released by freezing and thawing. The lysate was clarified by centrifugation at 3000 rpm for 5 min, and the supernatant containing the virus was passed through a Millipore filter. Bovine enterovirus was assayed on L cells by the plaque technique as described by Taylor *et al.* (1970).

Radioisotope Labeling. Sarcoma-180 and Ehrlich ascites tumor cells (5×10^6 cells/ml), suspended in suspension medium (MEM), and L cells grown as confluent monolayers (10^7 cells/32 oz culture bottle), were preincubated with actinomycin D (5.0 μg/ml and 0.5 μg/ml) for 1 hr followed by incubation with [^{3}H]uridine (2 μCi/ml; specific activity, 26.4 Ci/mmol) for 6 hr at 37°. Cell viability was more than 70% under these conditions. The cells were harvested by centrifugation and washed twice with MEM. In order to enrich for the RNase-resistant species and to localize the site of its synthesis, cells were fractionated into nuclear and cytoplasmic fractions according to a modification of the procedure of Penman (1966).

Extraction of Nuclear and Cytoplasmic RNA. All extractions unless otherwise stated were carried out at 4°. Pelleted cells were treated with 10 ml of hypotonic buffer (0.01 M NaCl–0.01 M Tris–0.0015 M $MgCl_2$; pH 7.4) and subsequently broken in a Dounce homogenizer. The nuclei were deposited by centrifugation at 1600*g* and resuspended in hypotonic buffer. The purity of the nuclei was examined under a phase microscope. The nuclei were further purified from cytoplasmic contaminants and unbroken whole cells by treating them with a mixture of ionic detergent (sodium deoxycholate) and nonionic detergent (Tween 40) (1:2, v/v). The supernatant fractions pooled from all the centrifugations were designated as the cytoplasmic fractions.

The nuclear pellet was suspended in 5 ml of high salt buffer (0.5 M NaCl–0.01 M Tris–0.05 M $MgCl_2$; pH 7.4) and was vigorously shaken with electrophoretically pure DNase (50 μg/ml) to obtain a viscous liquid after bringing the suspension to room temperature. Subsequently, sodium dodecyl sulfate and EDTA (pH 7.4) were added to a final concentration of 0.5% and 0.1 M, respectively.

Nuclear RNA was extracted by the hot phenol–sodium dodecyl sulfate method described by Scherrer and Darnell (1962) with a modification by Penman (1966). Nuclear RNA was extracted first at 60° with phenol and then with chloroform containing 1% isoamyl alcohol. The RNA was precipitated in 2.5 vol of ethanol at −20° and dissolved in a suitable buffer. Cytoplasmic RNA was extracted by the phenol–sodium dodecyl sulfate extraction method.

Isolation and Purification of RNase-Resistant Species. The "presumptive" ds-RNA was separated from high molecular weight ss-RNA by the differential salt precipitation method of Bishop and Koch (1967). Nuclear RNA precipitated by 2.5 vol of ethanol was suspended in 0.02 M sodium phosphate–0.15 M NaCl buffer (pH 7.2) and the final salt concentration was raised to 1.0 M. The RNA solution was frozen at −20° and subsequently thawed at 4°. The precipitate was removed by centrifugation at 2000 rpm for 20 min. The supernatant was reprecipitated in 2.5 vol of ethanol at −20°, and redissolved in 5 ml of 1 × SSC buffer (0.15 M NaCl–0.015 M sodium citrate, pH 7.0). In other experiments, the differential salt precipitation procedure was eliminated and the RNA precipitate was directly dissolved in 1 × SSC buffer.

Further contamination by DNA and ss-RNA was eliminated by digesting the RNA extracts with DNase (20 μg/ml) in 1 × SSC for 30 min, and with RNase A (20 μg/ml) and RNase T_1 (2 μg/ml) in 0.3 M NaCl for 2 hr. The nuclease activity was stopped by digesting the mixture with Pronase (1 mg/ml) for 2 hr at 37° as described by Stern and Friedman (1971). The digested RNA was extracted with phenol and was precipitated three times in 2.5 vol of ethanol at −20°. Protein and DNA contaminations were found to be insignificant, as assayed by the procedures of Lowry *et al.* (1951) and Burton (1968), respectively.

Preparation of Viral ds-RNA. Infected Ehrlich ascites tumor cells (5×10^6 cells/ml) were harvested by centrifugation at 6.5–7 hr after infection with bovine enterovirus (multiplicity of infection of 20 PFU), the approximate time of maximum accumulation of viral ds-RNA (Cordell-Stewart and Taylor, 1971). The cell pellet was resuspended in PSM buffer (0.02 M sodium phosphate–0.15 M NaCl–0.001 M $MgCl_2$, pH 7.2). Sodium dodecyl sulfate was added to a final concentration of 0.5%. RNA was extracted with phenol saturated with PSM buffer, precipitated overnight with 2.5 vol of ethanol at −20°, and redissolved in PSM buffer. The viral ss-RNA species were separated from the viral ds-RNA by the differential salt precipitation method described earlier.

TABLE I: Amount of RNase-Resistant RNA in 1.0 M NaCl Supernatant and Precipitate Fractions of Sarcoma-180.

Fraction	Total cpm	RNase-Resistant cpm	%
Supernatant	182,730	2059	1.12
Precipitate	464,690	1438	0.30

The supernatant containing the viral ds-RNA was precipitated with 2.5 vol of ethanol and dissolved in 1 × SSC buffer (0.15 M NaCl–0.015 M sodium citrate, pH 7.0). Contamination by DNA and ss-RNAs was further eliminated by nuclease digestion with DNase and RNase A and T_1 as described for the cellular species. The digested RNA sample was precipitated with 2.5 vol of ethanol at −20° and redissolved in a suitable buffer.

The viral ds-RNA was further purified through BD-cellulose and Sepharose 2B columns as described in a later section. The physical-chemical characteristics of this viral ds-RNA were used as a standard for studying the nature of the cellular species.

Column Chromatography. The ds-RNA preparation was fractionated through a BD-cellulose column (20 × 1.5 cm i.d.) at room temperature, using a 0.2–1.8 M NaCl gradient as described by Stern and Friedman (1971). A 1-ml sample was loaded onto the column, which was washed with 50 ml of 0.2 M NaCl solution and was eluted with 100 ml of the gradient, and 2-ml fractions were collected. Sepharose 2B column (60 × 1.5 cm i.d.) chromatography of viral ds-RNA, previously fractionated through a BD-cellulose column, and of total nuclear RNA from Sarcoma-180 cells was performed using Tris buffer (0.05 M Tris-HCl–0.15 M NaCl–0.001 M EDTA, pH 7.2) as described by Erikson and Gordon (1966). The RNA preparation was placed on the column in 1 ml of the same buffer with fractions of 50 drops being collected at 4° with a reservoir head of 18 cm pressure.

Zonal Centrifugation in Density Gradient. Nuclease-digested nuclear and cytoplasmic RNA preparations (2 ml) were layered over preformed gradients of sucrose (5–30%, w/v) made in Tris buffer (0.005 M Tris-HCl–0.15 M NaCl, pH 7.4) as suggested by Bishop *et al.* (1965). The gradients were centrifuged at 25,000 rpm in a Spinco, Model L, with a SW-1 rotor, for 30 hr at 4°. After centrifugation 1-ml samples were collected from the top using ependorf pipets.

Measurement of Radioactivity. Samples of RNA were precipitated with 10% trichloroacetic acid with yeast tRNA as a carrier at 4° for 1 hr. The precipitates were collected on Millipore filters and washed three times with 5% trichloroacetic acid, and the dried filters were counted for 5 min in a toluene scintillation fluid in a Beckman liquid scintillation counter.

Measurement of Thermal Hyperchromicity. RNA samples were dialyzed against either 0.01 × SSC or 1 × SSC. The melting profiles of the RNA species sampled from the sucrose gradients were obtained as described by Mahler *et al.* (1966) using a Gilford spectrophotometer, Model 2000, coupled to a colora ultrathermostat and a thermosensor unit. The hyperchromicity parameters were calculated as per Mahler and Dutton (1964) and Mahler *et al.* (1968).

TABLE II: Effect of Actinomycin D and Localization of RNase-Resistant RNA (1.0 M NaCl Supernatant Fraction).

Cell Type	Concn of Act. D (μg/ml)	Fraction[a]	Total cpm	RNase-Resistant cpm	%
Sarcoma-180	0.5	N	208,000	7,912	3.8
		C	804,600	2,668	0.33
	5.0	N	72,400	7,088	9.79
		C	142,800	74	0.05
Ehrlich ascites tumor	0.5	N	346,000	62,518	18.06
		C	330,000	424	0.12
	5.0	N	56,000	8,878	15.85
		C	23,200	20	0.08
L	0.5	N	316,000	42,300	13.30
		C	36,300	315	0.87
	5.0	N	62,800	9,500	15.12
		C	10,750	24	0.22

[a] N, nuclear; C, cytoplasmic.

TABLE III: Localization and Amount of RNase-Resistant RNA in Total Cell RNA in Sarcoma-180.

RNA Fraction[a]	Concn of Act. D (μg/ml)	Total cpm	RNase-Resistant cpm	% Total
N	0	396,825	30,940	7.8
C	0	2,500,000	56,400	2.2
N	0.5	179,400	17,900	9.9
C	0.5	347,000	4,000	1.15
N	5.0	50,000	7,350	14.7
C	5.0	157,400	2,150	1.36

[a] N, nuclear; C, cytoplasmic.

Results

RNase-Resistant RNA Synthesis. The synthesis of RNase-resistant RNA was examined in Sarcoma-180, Ehrlich ascites cells, and in L cells in the presence of low concentrations (0.5 μg/ml) and high concentrations (5 μg/ml) of actinomycin D. Initially RNase-resistant RNA was isolated by the procedure of Bishop and Koch (1967) in the 1 M NaCl supernatant fraction of RNase-treated RNA. This method had been devised for the isolation of viral replicative form (RF). Table I shows that although the bulk of the ds-RNA is found in the supernatant fraction, a small amount (0.22% of total cellular, but 41% of total ds-RNA) is found in the high-salt precipitate. An analysis of the nuclear and cytoplasmic RNAs isolated as described in Materials and Methods, and found in the supernatant fraction, demonstrated that the bulk of the ds-RNA was of nuclear origin (Table II). The data in Table II also show that the synthesis of RNase-resistant RNA was not sensitive to a low concentration of actinomycin D, and only partially to a high concentration of actinomycin D (5 μg/ml) in Sarcoma-180. This is in contrast to Ehrlich ascites tumor

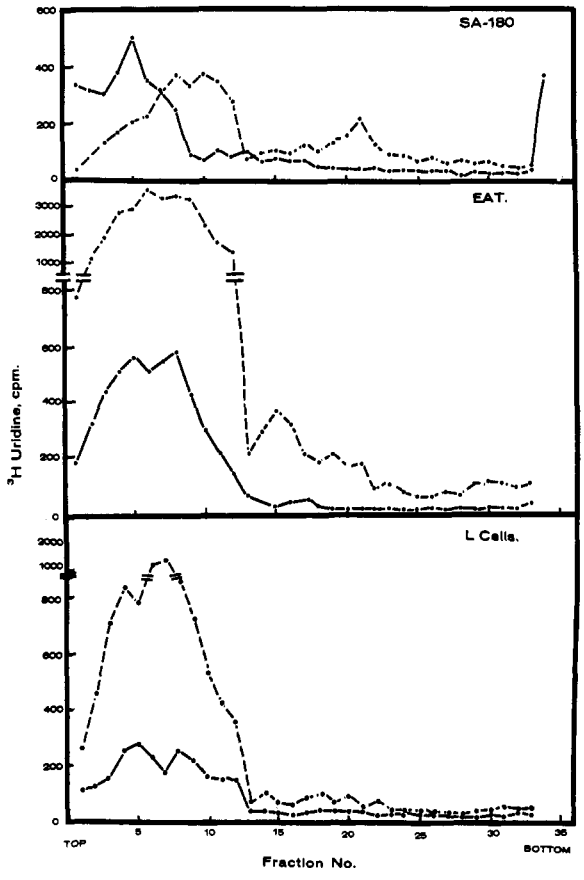

FIGURE 1: Sedimentation profiles of acid-precipitable RNase-resistant radioactivity of nuclear RNA from sarcoma-180 (SA-180), Ehrlich ascites tumor cells (EAT), and L cells: (·——) 5 μg/ml of actinomycin D; (·---) 0.5 μg/ml of actinomycin D. Centrifugation was in a 5–30% sucrose gradient in 5 mM Tris buffer–0.15 M NaCl, pH 7.4, for 30 hr at 25,000 rpm, at 4° in a Spinco SW-25.1 rotor.

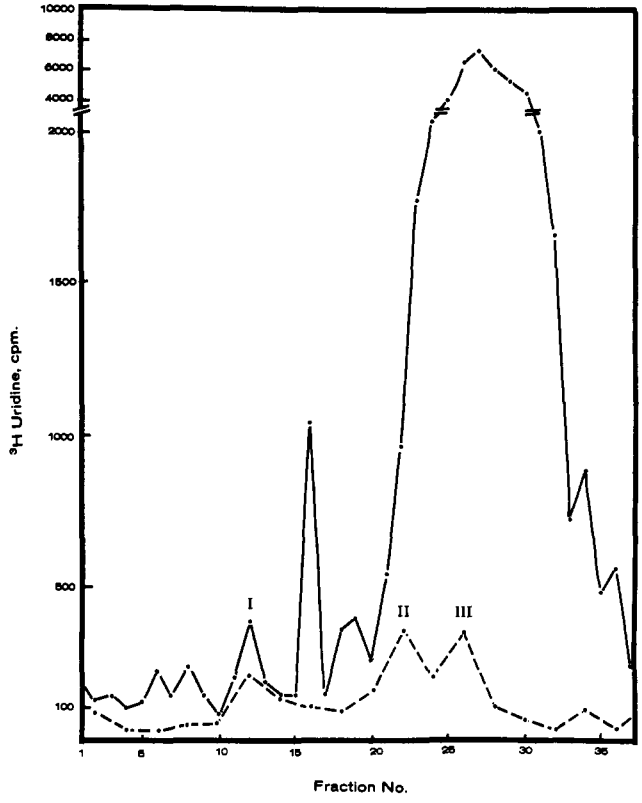

FIGURE 2: Sepharose 2B chromatography of total labeled nuclear RNA from Sarcoma-180 cells. Individual fractions were collected and acid-precipitable radioactivity was counted. Portions of alternate fractions were treated for RNase resistance as described under Materials and Methods: (·——) before RNase treatment; (·---) after RNase treatment.

cells and L cells in which the total amount of ds-RNA synthesized decreases although the relative percentage remains the same (Table II).

Since the above experiments were done with the 1 M NaCl fractions rather than total cell RNA, the localization of ds-RNA and effect of actinomycin D were examined in total RNA from Sarcoma-180. These data are presented in Table III, and confirm the observations reported in Table II, that the bulk of ds-RNA is of nuclear origin, and only trace amounts can be detected in the cytoplasm. Although the total amount of ds-RNA synthesized is depressed by 5 μg/ml of actinomycin D, there is still some escape synthesis.

Zonal Centrifugation in Sucrose Gradients. RNase-resistant species of RNA isolated from the nuclear fraction (1 M NaCl supernatant) of the three cell lines were layered over 5–30% preformed sucrose gradients. In all the cell lines the RNase-resistant species of RNA banded as polydisperse bands between 4 and 13 S (Figure 1). The low levels of RNase-resistant RNA detectable in Sarcoma-180, Ehrlich ascites tumor, and L cells, treated with 5 μg/ml of actinomycin D, were also banded and gave a similar profile.

It should be noted that almost equivalent amounts of RNase-resistant RNA could be detected in Sarcoma-180 labeled in both 0.5 and 5 μg/ml of actinomycin D. This escape synthesis is similar to that described by Stern and Friedman (1971) in Burkitt lymphoma cells. In Sarcoma-180 cells, a second distinct band of RNase-resistant RNA could be detected at approximately 20 S on the sucrose gradient. This RNA species was not found in the other cells.

Sepharose 2B Chromatography. The total nuclear RNA labeled for 6 hr with [^{3}H]uridine was isolated from Sarcoma-180 cells, and fractionated through a Sepharose 2B column (Figure 2). The Sepharose 2B chromatography demonstrated the presence of a small peak of high molecular weight RNA eluting before the bulk of the RNA. That this first peak is ds-RNA was confirmed by treating this RNA sample with ribonucleases. Figure 2 shows that the bulk of this high molecular weight RNA (peak I) was resistant to RNase and that there are species of RNA (peaks II and III) that are resistant to RNase and probably account for the polydisperse fractions detectable on sucrose gradient.

Thermal Hyperchromicity. One way of examining the degree of base pairing and the uniqueness of RNA species is by following the hyperchromic effect upon heat denaturation. Both the polydisperse fractions of Sarcoma-180, Ehrlich ascites tumor, and L cells and the high molecular weight species in Sarcoma-180 cells were examined by melting in 0.01 × SSC (0.15 M NaCl–0.015 M sodium citrate, pH 7.0) (Figure 3). The pooled fraction (2–13) from the polydisperse zone gave melting curves suggestive of a heterogeneous population of base-paired molecules. It is very unlikely that these samples contained tRNA and degraded ribosomal RNA, since they were derived from the 1 M NaCl fraction and treated with RNase and the specific activity was measured continuously. In all the cell lines, this polydisperse species showed higher $\sigma_{2/3}$ values in contrast to those of homogeneous

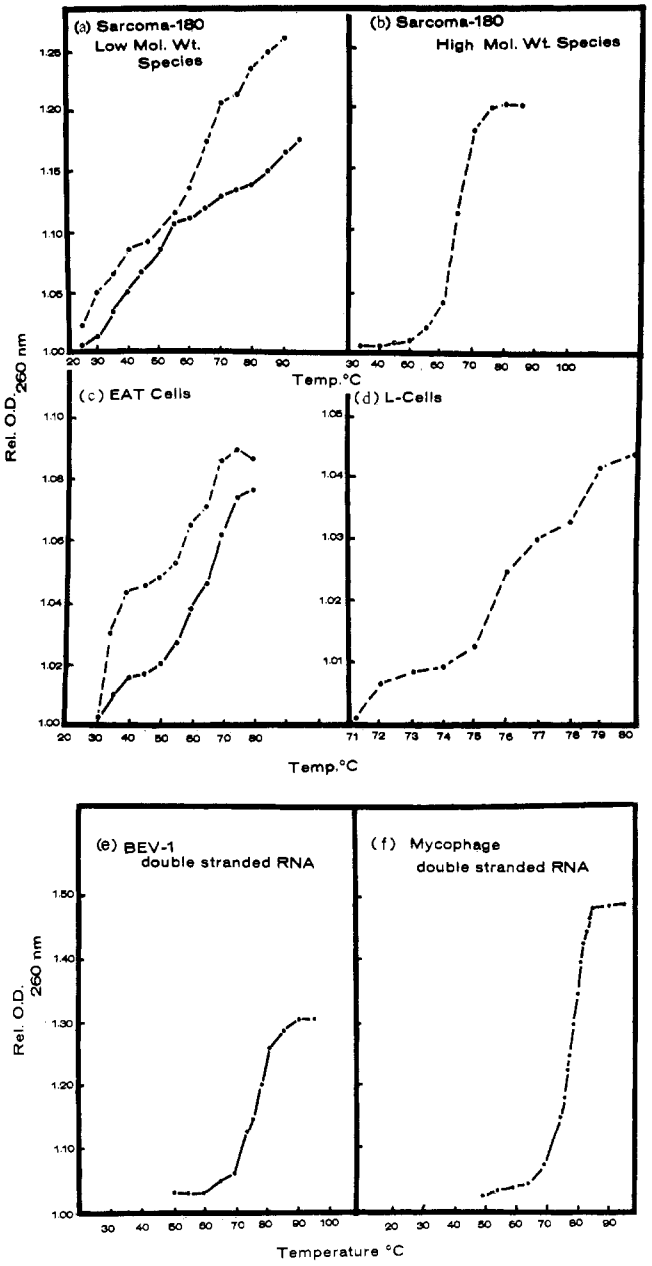

FIGURE 3: Thermal denaturation profiles of RNase-resistant species isolated from uninfected cells: (a) polydisperse species from Sarcoma-180; (b) high molecular weight species from Sarcoma-180; (c) polydisperse species from Ehrlich ascites tumor cells; (d) polydisperse species from L cells; 0.5 μg/ml of actinomycin D (- - -); 5.0 μg/ml of actinomycin D (·—) (all the above species were isolated from nuclear fractions); (e) bovine enterovirus ds-RNA; (f) mycophage ds-RNA. Thermal denaturation was done in 0.01 × SSC. At 20° initial values of E_{260nm} were between 0.3 and 0.5.

standard viral and mycophage ds-RNA, suggesting that these species are not completely base paired (Table IV). However, examination of the melting curves (Figure 3a, c) reveals that part of the curves between 40 and 80° show some degree of base pairing as seen from the $\sigma_{2/3}$ values. These have also comparatively higher T_m values, associated with lower hyperchromicity (Table IV). The lower hyperchromicity is due to the heterogeneity of the population. Although we cannot discern from these data whether the melting in this particular temperature region could be attributed to an individual species among the various species or a part of one of the species, it does show the presence of RNA having some amount of ordered secondary structure. However, in the nuclear fraction of L cells treated with lower concentrations of actinomycin D (0.5 μg/ml), a portion of the melting curve, at higher temperature, showed a comparatively sharp transition (lower $\sigma_{2/3}$ value) suggesting some base-paired sequences among the heterogeneous population (Figure 3d). However, the hyperchromicity of such transition seemed to be quite low (Table IV). The lower T_m values of these polydisperse species suggest that the G–C content of these RNAs is low compared to that of standard ds-RNA.

The heavier species of RNA from Sarcoma-180 (fractions 19–25) showed a sharp melting transition (lower $\sigma_{2/3}$), having a T_m value of 64.39° in 0.01 × SSC with about 20% hyperchromicity. The shape of the melting curve suggests the presence of a homogeneous population. By increasing the ionic strength to 1 × SSC, the T_m value was increased to 86°. Although the sharp transition (lower $\sigma_{2/3}$ value) and the shape of the curve suggested a base-paired double-stranded character, the lower T_m value as compared to the viral and mycophage ds-RNA again suggests that the G–C content is lower.

It may be noted that no label was found under this zone in the nuclear sample treated with a higher concentration of actinomycin D (5.0 μg/ml). But a similar melting profile was obtained from the pooled sample under this peak. This observation would suggest that this species was present prior to the incubation and is resistant to lower actinomycin D treatment (0.5 μg/ml) but is susceptible to higher doses of the antibiotic. Similar results for other cells have been reported by Montagnier (1968).

Discussion

These studies expand those of Stern and Friedman (1971) and of Montagnier (1968) on the characterization of RNase-resistant RNA present in mammalian cells. In the three cell lines used in the present study, Sarcoma-180, Ehrlich ascites tumor cells, and L cells, RNase-resistant RNA appears to be confined predominantly to the nuclear RNA. In agreement with this is the finding that the bulk synthesis of this RNA is inhibited by 5 μg/ml of actinomycin D, and not by 0.5 μg/ml. We cannot discern whether the low levels of RNase-resistant RNA detectable in the presence of actinomycin D are identical with those found in the absence of the antibiotic or whether they are the result of random breakdown of RNA and self-annealing.

It is obvious from these studies that the choice of cell lines is important in studying such macromolecules. In Sarcoma-180 there are at least two major classes of ds-RNA: (a) a low molecular weight species which on sucrose gradient forms a polydisperse fraction between 5 and 13 S, and which is probably of the same nature as that found in Ehrlich ascites tumor cells, L cells, and as recently reported by Jelinek and Darnnell (1972) in HeLa cells, and (b) high molecular weight ds-RNA discussed below.

The low molecular weight material could be a part of the Hn-RNA. On the basis of thermal denaturation studies (Table I), it does not appear to be truly based paired but rather to be an assortment of base-paired regions, perhaps forming loops that may reflect the secondary structure of mRNA (Min Jou *et al.*, 1972) or act as processing signals for Hn-RNA conversion to mRNA. The low G–C content of this material, as reflected by T_m, may be indicative of attached regions of poly(A). Binding studies on poly(U) filters would in fact agree

TABLE IV: Thermal Properties of RNase-Resistant RNA Species Isolated from Uninfected and Virus-Infected Cells.[a]

Sample[b]	Concn of Act. D (μg/ml)	T_m (°C)	$\sigma_{2/3}$ (°C)	h	Remarks
Nuclear fraction 4–13 S					
Sarcoma-180	0.5	58.88	47.58	0.256	Total melting
		62.13	15.11	0.109	Melting between 45 and 75°
	5.0	49.50	40.27	0.162	Total melting
Ehrlich ascites tumor	0.5	42.50	33.44	0.088	Total melting
		60.84	12.29	0.040	Melting between 45 and 80°
	5.0	61.88	29.25	0.082	Total melting
		65.00	17.15	0.065	Melting between 45 and 80°
L cells	0.5	52.70	29.00	0.125	Total melting
		76.50	6.30	0.042	Melting between 70 and 80°
Nuclear fraction ~20 S					
Sarcoma-180	0.5	64.39	9.85	0.203	
Bovine enterovirus ds-RNA		74.50	8.55	0.267	
Mycophage ds-RNA		75.00	8.68	0.439	

[a] All measurements performed in 0.01 × SSC (0.15 M NaCl–0.015 M sodium citrate, pH 7.0); T_m = melting temperature = $(T_{A_f} + A_i)/2$); h = hyperchromicity = $(A_f/A_i) - 1$; $\sigma_{2/3}$ = transition dispersion = $T_{0.83xh_{max}} - T_{0.17xh_{max}}$, where A_f = absorbance at 260 nm at final temperature, A_i = absorbance at 260 nm at initial temperature, and h_{max} = maximum hyperchromicity. [b] Uninfected actinomycin D treated cells.

with this hypothesis, since 25% of the RNase-resistant RNA of nuclear orgin is bound to poly(U) filters (Patnaik and Taylor 1973[2]).

The high molecular weight ds-RNA found in Sarcoma-180 cells (but not in other cell lines) appears to be of a different origin. It is much more sensitive to the presence of actinomycin D than the other ds-RNA, and has a $\sigma_{2/3}$ typical of a completely based-paired double-stranded structure, as compared to the bovine enterovirus replicative form and mycophage ds-RNA. These observations indicate that this material is probably of viral origin. It is well recognized that many murine sarcomas contain C type and other viral particles. It is possible that this RNA reflects the replicative form of an unknown class of DNA virus. Further experiments are progressing to determine whether this RNA bears any relationship to known murine virus RNAs.

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