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Studies on the 3′-Terminal Sequences of the Large Ribosomal Ribonucleic Acid of Different Eukaryotes and Those Associated with 'Hidden' Breaks in Heat-Dissociable Insect 26S Ribonucleic Acid

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The 3′-terminal sequences associated with the large rRNA complex from a range of eukaryotes were determined after pancreatic or T_1-ribonuclease digestion of RNA terminally labelled with [^{3}H]isoniazid. In all higher eukaryotes examined except *Drosophila melanogaster*, the 3′-terminal sequences Y-G-U_{OH} and G-C-U_{OH} were demonstrated for the large RNA component(s) and for 6S RNA respectively. The 3′-terminal sequence of *Saccharomyces cerevisiae* 26S RNA was Y-G-U_{OH} and that of 6S RNA Y-A-U-U-U_{OH}. Three 3′-terminal sequences were found in equimolar amounts in the heat-dissociable 26S rRNA characteristic of insect ribosomes. These were Y-G-U-G-U_{OH}, Y-C-G-U_{OH} and G-C-U_{OH} for cultured *Antheraea eucalypti* cells, Y-G-U_{OH}, Y-G-U_{OH} and G-C-U_{OH} for *Galleria mellonella* larvae and Y-C-G-A_{OH}, Y-G-U-A_{OH} and G-Y-U-G_{OH} for *Drosophila melanogaster* flies. Thus the introduction of the central scission in insect 26S rRNA results in the generation of a unique 3′-terminus and does not arise from random cleavage of the polynucleotide chain.

The large ribosomal RNA isolated from certain of the lower eukaryotes dissociates into a number of fragments when subjected to treatments (e.g. heat, dimethyl sulphoxide) which disrupt complementary base-pairing. This property is characteristic of the large rRNA found in some Protozoa (Loening, 1968; Rawson & Stutz, 1968; Bostock *et al.*, 1971; Stevens & Pachler, 1972), in the marine mud snail (Koser & Collier, 1971), in most insects (Shine & Dalgarno, 1973) and in plant chloroplast ribosomes (Leaver & Ingle, 1971). Where this feature has been more closely examined, evidence suggests that the breaks in the molecule are present *in vivo* and are not introduced during extraction (Bostock *et al.*, 1971; Stevens & Pachler, 1972; Shine & Dalgarno, 1973).

The cleavages introduced during processing of the high-molecular-weight precursor of rabbit reticulocyte rRNA result in unique 3′-terminal sequences in the mature 28S RNA, 18S RNA and 6S RNA species (Hunt, 1970). However, there are no published terminal-sequence data for any RNA species that contain 'hidden' breaks, and it is therefore not known whether introduction of a particular break gives rise to a unique terminal sequence. Such data would provide information on the specificity of the nuclease(s) involved, which could be related to the specificity of other nucleases concerned in processing the precursor molecule.

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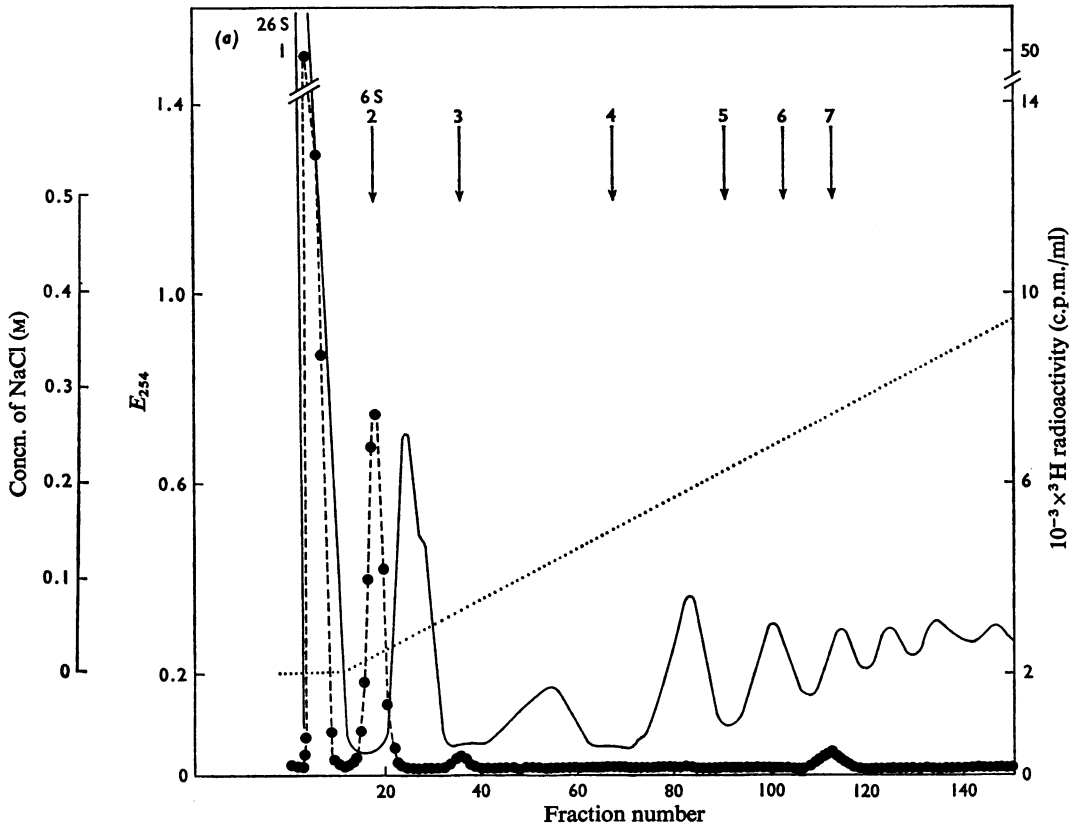
In this study we have used insect 26S RNA as an example of heat-dissociable RNA containing 'hidden' breaks. It has previously been shown that 26S RNA from cultured mosquito (*Aedes aegypti*) cells is a complex of three polynucleotide chains (Shine & Dalgarno, 1973). A small RNA of 130–160 nucleotides (6S RNA) is hydrogen-bonded to the larger molecule, which contains a central scission; 26S RNA dissociates on heating into 6S RNA and two polynucleotides of approximate molecular weight 0.7×10^6 ('18S' RNA).

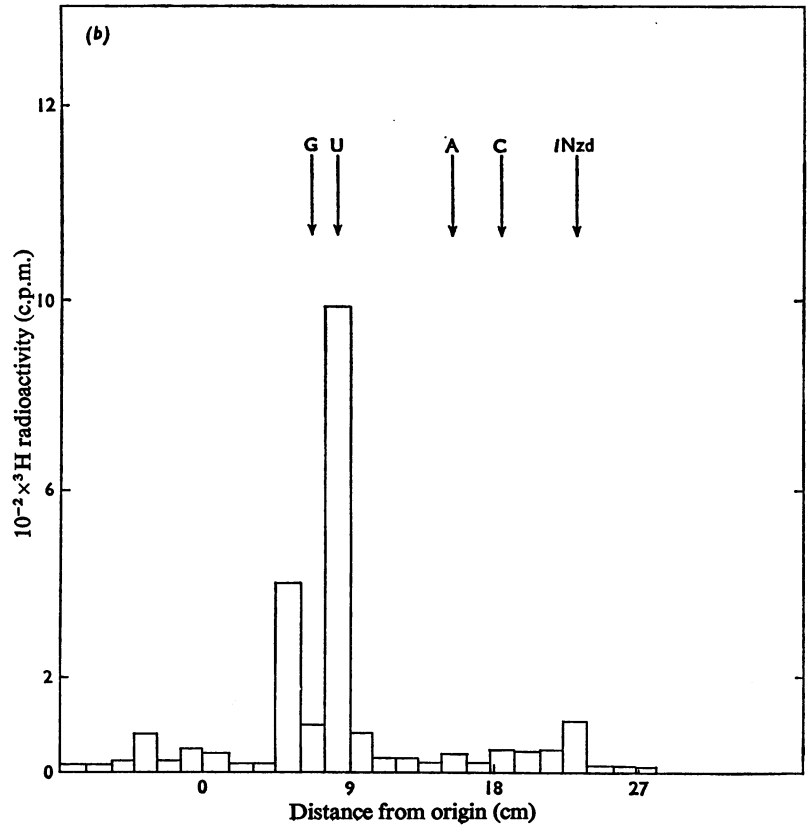
In the present paper we show sequence data for the three 3′-termini of 26S rRNA from a number of insects. These sequences demonstrate that the introduction of the characteristic mid-point scission gives rise to a specific 3′-terminus in this RNA species. We have also sequenced the two 3′-termini in the 28S RNA–6S RNA complex from other eukaryotes. The data obtained are discussed in relation to the types of homologies that exist between the 3′-termini of rRNA from such organisms.

Experimental

Media and materials

Drosophila melanogaster flies (wild-type) were obtained from Dr. A. J. Howells (Australian National University, Canberra, Australia). Propagation of *Galleria mellonella* larvae, *Antheraea eucalypti* cells,


(a)
26 S
1
6 S
2
3
4
5
6
7
Concn. of NaCl (M)
E_{254}
$10^{-3} \times {}^3$H radioactivity (c.p.m./ml)
Fraction number


(b)
G
U
A
C
INzd
$10^{-2} \times {}^3$H radioactivity (c.p.m.)
Distance from origin (cm)

baby-hamster kidney (BHK) cells, African green-monkey kidney (Vero) cells and *Saccharomyces cerevisiae* were as described previously (Shine & Dalgarno, 1974*b*). Media and materials used have been described (Shine & Dalgarno, 1974*b*).

Preparation of rRNA

Extraction of rRNA was as described previously (Shine & Dalgarno, 1974*b*).

*Stepwise degradation of rRNA and labelling with [^{3}H]isoniazid (iNzd)**

Periodate-oxidized rRNA species from *Saccharomyces cerevisiae* and *Drosophila melanogaster* were subjected to several cycles of stepwise degradation and labelled with [^{3}H]*i*Nzd as previously described (Shine & Dalgarno, 1974*b*). The 26S rRNA from cultured *Antheraea eucalypti* cells was similarly treated and labelled except that residual *i*Nzd was removed by four cycles of precipitation of the labelled RNA with 2vol. of ethanol and resuspension in 0.1M-NaCl–0.01M-sodium acetate (pH5). The labelled RNA was further purified by sucrose-gradient centrifugation before ribonuclease digestion.

3'-Terminal sequence determination

Specific ribonuclease digestion of terminally labelled RNA, previously subjected to no, one or two stepwise degradations, was used to determine the 3'-terminal sequences present in the larger rRNA. This method permits determination of two 3'-terminal sequences simultaneously, provided that they can be readily associated with each original sequence (Hunt, 1970). The labelled 3'-oligonucleotides released by ribonuclease digestion were characterized according to size by chromatography on DEAE-Sephadex (Shine & Dalgarno, 1974*a*; Hunt, 1973). The identity of mononucleoside hydrazones and dinucleoside monophosphate hydrazones was determined by paper electrophoresis (Hunt, 1965; Shine & Dalgarno, 1974*a*). Conditions for enzyme digestion, DEAE-Sephadex chromatography and paper electrophoresis have been described previously (Shine & Dalgarno, 1974*b*).

* Abbreviations: *i*Nzd, isonicotinic acid hydrazide (isoniazid); *i*NicHz, isonicotinoylhydrazone.

Results

Sequences of the three 3'-termini of insect 26S rRNA

26S rRNA from cultured emperor gum moth (Antheraea eucalypti) cells. Labelling of *Antheraea eucalypti* 26S rRNA with [^{3}H]*i*Nzd demonstrates the presence of three 3'-termini in this RNA species (cf. Shine & Dalgarno, 1973). Digestion of the labelled RNA with T_1 ribonuclease releases, in equimolar amounts, a mononucleoside isonicotinoylhydrazone from two of the 3'-ends and a dinucleoside monophosphate hydrazone from the third (Fig. 1*a*, Table 1). Paper electrophoresis of the digest shows the presence of 2mol of U-*i*NicHz and 1mol of a dinucleoside monophosphate hydrazone (Fig. 1*b*). Thus the 3'-terminal sequence at two of the termini is G-U_{OH}. The dinucleoside monophosphate hydrazone, which runs behind G-*i*NicHz, is derived from the 3'-terminus of the associated 6S RNA. This was shown by heat-dissociating 26S RNA labelled with [^{3}H]*i*Nzd and isolating 6S RNA by sucrose-gradient fractionation (Shine & Dalgarno, 1973). T_1-ribonuclease digestion of the isolated 6S RNA confirmed that the dinucleoside monophosphate hydrazone was derived from this species, demonstrating the 3'-terminal sequence G-Z-N_{OH} (results not shown), where N is any nucleoside and Z any nucleoside except G.

Pancreatic-ribonuclease digestion of terminally labelled *Antheraea eucalypti* 26S RNA gives a dinucleoside monophosphate hydrazone from the

Fig. 1. *DEAE-Sephadex chromatography (a) and paper electrophoresis (b) of a T_1-ribonuclease digest of terminally labelled Antheraea eucalypti 26S rRNA*

(*a*) Approx. 1mg of [^{3}H]*i*Nzd-labelled 26S rRNA and 3mg of carrier *Galleria mellonella* rRNA were digested with T_1 ribonuclease and applied to a column (1cm×25cm) of DEAE-Sephadex A-25. A mixture of unlabelled mononucleoside hydrazones (1mg) was added to the digest before chromatography to act as a marker. These appear as the E_{254} peak in the void volume; they were not added in the experiments described in Figs. 2(*a*) and 3(*a*). Elution was with a 1-litre linear gradient of 0–0.5M-NaCl in 7M-urea–0.01M-phosphate (pH7.4), at a flow rate of 40ml/h; 5ml fractions were collected and 1ml portions assayed for absorbance and radioactivity. No significant radioactivity was eluted when the column was treated with 100ml of 1M-NaCl in the same buffer. Peaks of oligonucleotide hydrazones are designated according to the number of constituent nucleoside residues (Hunt, 1973; Shine & Dalgarno, 1974*a*). Some free [^{3}H]*i*Nzd is released during digestion and is eluted with the mononucleoside hydrazones (see also Table 1 and Figs. 2*a* and 3*a*). Radioactivity peaks marked 26S and 6S refer to the oligonucleotide hydrazones derived from the 3'-termini of 26S RNA and 6S RNA respectively (see the text). ●, ^{3}H; ——, E_{254}; ····, [NaCl]. (*b*) Electrophoresis was as described previously (Shine & Dalgarno, 1974*b*). The preparation of unlabelled mononucleoside hydrazones was described by Hunt (1965). The electrophoretogram was cut into strips (approx. 1.5cm) and assayed for ^{3}H (Shine & Dalgarno, 1974*b*). The arrows give the positions of the marker isonicotinoylhydrazones of guanosine, uridine, adenosine and cytidine.

Table 1. *Radioactivity associated with 3'-terminal oligonucleotides in ribonuclease digests of terminally labelled Antheraea eucalypti RNA*

The oligonucleotides were separated by column chromatography on DEAE-Sephadex. For further details see the text.

Treatment of RNA	No. of nucleoside residues and 10^{-3} × associated radioactivity (c.p.m.)						
	1 (N-*i*Nzd+*i*Nzd)*	2 (N_2-*i*Nzd)	3 (N_3-*i*Nzd)	4 (N_4-*i*Nzd)	5 (N_5-*i*Nzd)	6 (N_6-*i*Nzd)	7 (N_7-*i*Nzd)
T_1-ribonuclease digestion of 26S RNA labelled with [^{3}H]*i*Nzd (from Fig. 1*a*)	61.0 (73.6)	28.3	0.82	0.15	0.11	0.22	1.06
Pancreatic ribonuclease digestion of 26S RNA labelled with [^{3}H]-*i*Nzd (from Fig. 2*a*)	7.5 (10.8)	15.8	0.05	0.05	0.07	0.04	0.04
T_1-ribonuclease digestion of 26S† and 18S RNA after one stepwise degradation and labelling with [^{3}H]*i*Nzd (from Fig. 3*a*)	0.18 (6.6)	15.2	0.93	0.61	14.6	15.3	0.36

* A constant percentage (12%) of the radioactivity was released from the terminally labelled RNA during ribonuclease digestion; this is eluted in the void volume with the mononucleoside hydrazones and probably arises from the small amount of non-specifically bound [^{3}H]*i*Nzd previously found in unoxidized controls (Shine & Dalgarno, 1973). The radioactivity associated with mononucleoside hydrazones is corrected for the presence of free [^{3}H]*i*Nzd. The values in parentheses are the uncorrected values.

† 6S RNA is absent from this preparation (see the text).

3'-ends of the two major polynucleotides (Fig. 2*a*, Table 1). A mononucleoside hydrazone is released from the 3'-end of 6S RNA (Fig. 2*a*, Table 1) and was identified as U-*i*NicHz by paper electrophoresis (Fig. 2*b*). The 3'-sequence of both major polynucleotides in 26S rRNA is thus Y-G-U_{OH} and that of 6S RNA is G-Y-U_{OH}.

Further sequence data were obtained by subjecting *Antheraea eucalypti* 26S RNA to one stepwise degradation. Owing to incubation of the RNA in aniline, base-pairing is disrupted during the stepwise-degradation procedure and the 26S complex dissociates into 6S RNA and two molecules of '18S' RNA. 6S RNA is thus absent from the absorption peak of RNA ('18S' RNA) isolated from sucrose gradients (Shine & Dalgarno, 1973) (see Fig. 3*a*). The two 3'-terminal oligonucleotides produced by T_1-ribonuclease digestion of the stepwise-degraded [^{3}H]*i*Nzd-labelled 26S rRNA are different (Fig. 3*a*, Table 1). One is a dinucleoside monophosphate hydrazone, establishing the sequence G-Y-G-U_{OH}; the other is a pentanucleoside tetraphosphate hydrazone, establishing the sequence G(Z)$_3$Y-G-U_{OH}. Terminally labelled 18S rRNA was similarly treated as a control. T_1-ribonuclease digestion of [^{3}H]*i*Nzd-labelled 18S rRNA releases a heptanucleoside hexaphosphate hydrazone (Shine & Dalgarno, 1974*b*). As only a hexanucleoside pentaphosphate hydrazone was released from the 3'-terminus of 18S rRNA under the conditions described in Fig. 3(*a*) (see also Table 1), the stepwise-degradation procedure was complete. Moreover, pancreatic-ribonuclease digestion of the stepwise-degraded 26S RNA gave only G-*i*NicHz; no dinucleoside monophosphate hydrazone was present (compare Fig. 2*a*). After one stepwise degradation and labelling with [^{3}H]*i*Nzd, 6S RNA was isolated as described above and digested with T_1-ribonuclease. Fig. 3(*b*) shows that only C-*i*NicHz is released, establishing the sequence G-C-U_{OH} for 6S RNA (Table 2).

After two cycles of stepwise degradation, 26S RNA (previously freed of 6S RNA) was labelled with [^{3}H]*i*Nzd and treated with T_1-ribonuclease. The expected tetranucleoside triphosphate hydrazone was released from one 3'-end and U-*i*NicHz from the other. Pancreatic ribonuclease digestion gave C-*i*NicHz and a dinucleoside monophosphate hydrazone. The two 3'-ends associated with 26S rRNA after removal of 6S RNA are therefore Y-G-U-G-U_{OH} and G(Z)$_2$Y-C-G-U_{OH} (Table 2).

Although we have not been able to determine which of the two 3'-terminal sequences results from the introduction of the mid-point scission it is clear that a unique 3'-terminal sequence is generated. This result also demonstrates that the two '18S' halves of insect 26S RNA are not identical.

26S rRNA from Galleria mellonella (wax moth) larvae. 3'-Terminal labelling of 26S RNA from the larvae of another lepidopteran, *Galleria mellonella*, also showed the existence of three 3'-termini in this RNA species (Shine & Dalgarno, 1973). The sequences at these termini were determined as for *Antheraea*. Two sequences, Y-G-U_{OH} and Y-G-U_{OH}, were associated with the two '18S' components of 26S RNA; G-C-U_{OH}

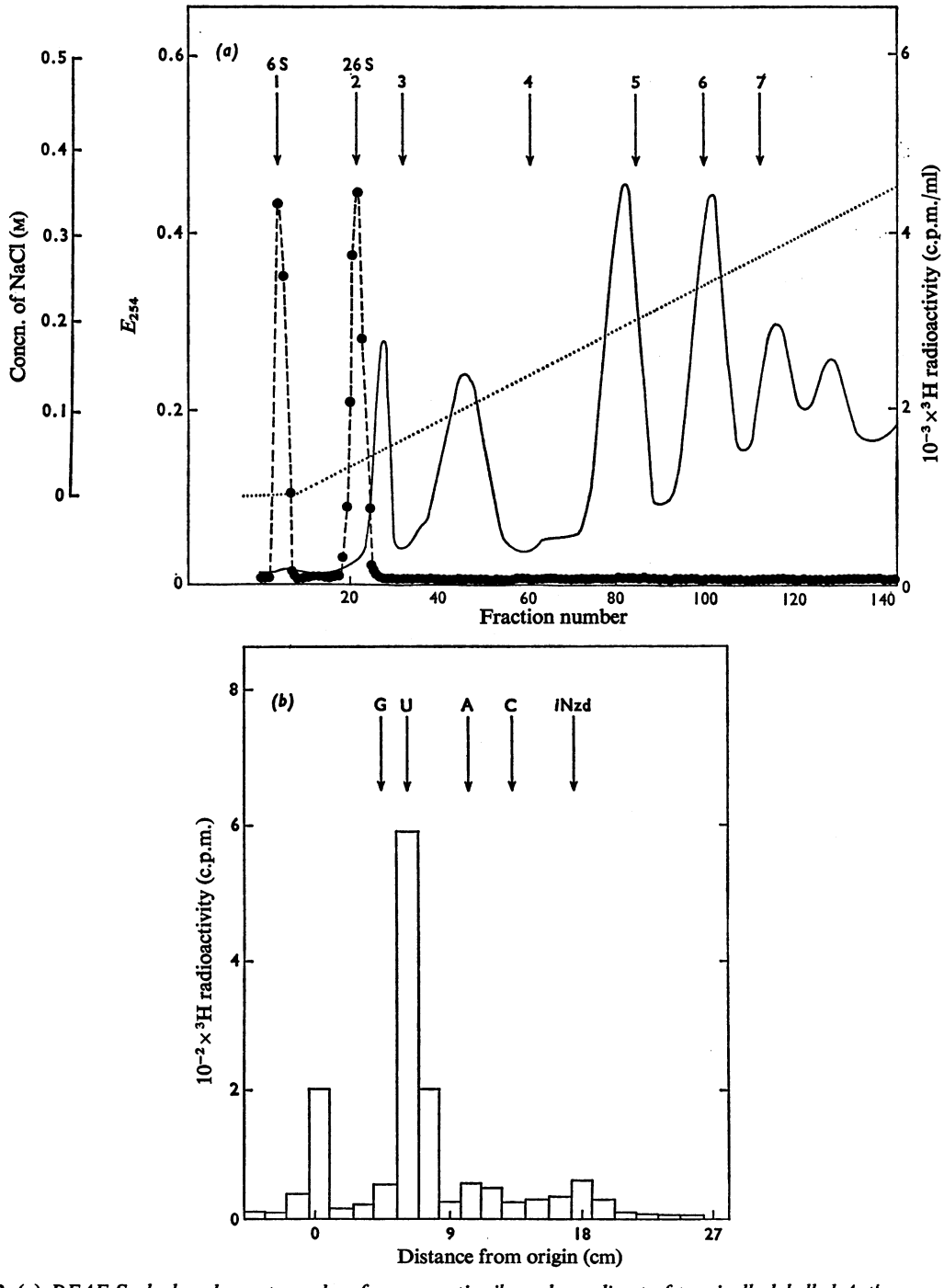
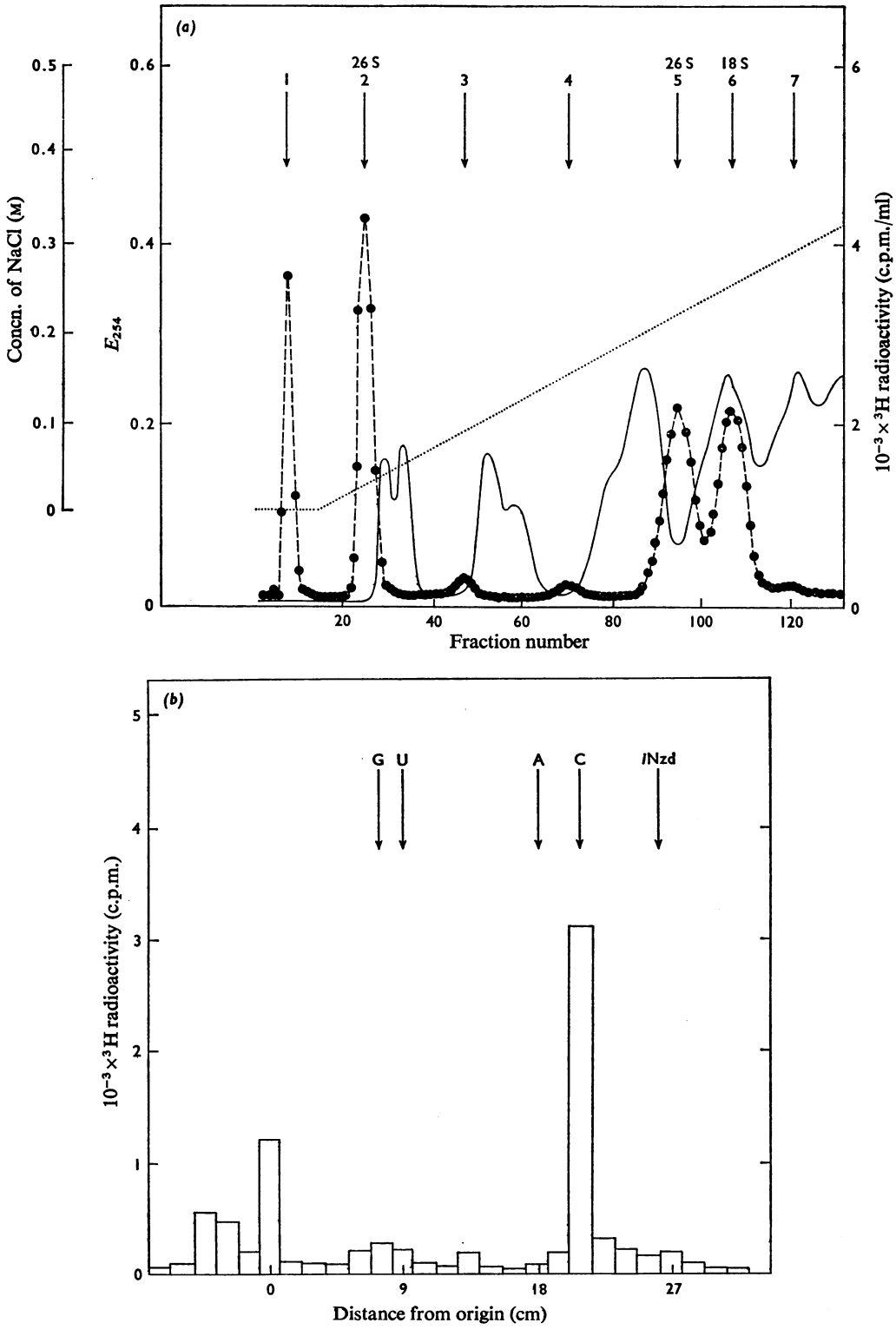


Fig. 2. (*a*) *DEAE-Sephadex chromatography of a pancreatic ribonuclease digest of terminally labelled Antheraea eucalypti 26S rRNA and* (*b*) *paper electrophoresis of a pancreatic ribonuclease digest of terminally labelled Antheraea eucalypti 6S RNA*

(*a*) Elution and numbering of peaks is as described for Fig. 1(*a*). Carrier RNA was added as in Fig. 1(*a*). ●, ^3H; ——, E_{254}; ····, [NaCl]. (*b*) [^3H]*i*Nzd-labelled 6S RNA was isolated as described in the text. Conditions are as for Fig. 1(*b*). The radioactivity remaining at the origin is not associated with RNA and is removed by chromatography on DEAE-cellulose (Shine & Dalgarno, 1974*a*).


(a)
26 S
18 S
1
2
3
4
5
6
7
Concn. of NaCl (M)
E_{254}
$10^{-3} \times {}^3$H radioactivity (c.p.m./ml)
Fraction number
(b)
G
U
A
C
INzd
$10^{-3} \times {}^3$H radioactivity (c.p.m.)
Distance from origin (cm)

was obtained for the associated 6S RNA (Table 2). Thus, as in *Antheraea*, the 3′-terminal dinucleotide of both 'halves' of 26S RNA is G-U_{OH} and a more extensive homology is not excluded. The 3′-dinucleotide of rabbit reticulocyte 28S rRNA is also G-U_{OH} (Table 2). It is also noteworthy that the 3′-terminal trinucleotide of 6S RNA from *Antheraea* cells, *Galleria* larvae and rabbit reticulocytes is, in each instance, G-C-U_{OH} (Table 2).

26S rRNA from Drosophila melanogaster. To test the possibility that such homologies may be universal we have determined the 3′-sequences associated with 26S rRNA from *Drosophila* (Table 3) and with the 28S RNA–6S RNA complex from a number of

Table 2. *3′-Terminal sequences in the large rRNA from various eukaryotes*

Source of RNA	26S–28S RNA	6S RNA	Reference
Rabbit reticulocytes	G-U-U-U-G-U_{OH}	G-U-C-G-C-U_{OH}	Hunt (1970)
Cultured *Antheraea eucalypti* cells	Y-G-U-G-U_{OH} $G(Z)_2$Y-C-G-U_{OH}	G-C-U_{OH}	This study
Galleria mellonella	Y-G-U_{OH} Y-G-U_{OH}	G-C-U_{OH}	This study
Drosophila melanogaster	Y-C-G-A_{OH} Y-G-U-A_{OH}	G-Y-U-G_{OH}	This study
Cultured African green-monkey kidney (Vero) cells	Y-G-U_{OH}	Y-U_{OH}	This study
Cultured baby-hamster kidney (BHK) cells	Y-G-U_{OH}	G-C-U_{OH}	This study
Avian myeloblasts	Y-A-G-G-U_{OH}	Not done	Ahmad *et al.* (1972)
Saccharomyces cerevisiae	Y-G-U_{OH}	G(Z)Y-A-U-U-U_{OH}	This study; Rubin (1973)

Table 3. *Major labelled digestion products from the 3′-termini of* [^{3}H]*iNzd-labelled Drosophila melanogaster 26S RNA*

For details see the text. Values in parentheses represent the percentage of the total radioactivity associated with the particular *i*Nzd derivative. The radioactivity associated with mononucleoside hydrazones eluted from DEAE-Sephadex is corrected for the presence of free [^{3}H]*i*Nzd (see Table 1).

Treatment of RNA before labelling with [^{3}H]*i*Nzd	Ribonuclease used	DEAE-Sephadex column	Paper electrophoresis		Sequence
None	T_1	N-*i*Nzd (31.2)	A-*i*Nzd (30.3)	26S	G-A_{OH}
		N_2-*i*Nzd (25.6)	N_2-*i*Nzd (29.6)		G(Z)N_{OH}
		N_3-*i*Nzd (29.4)	$N_{>2}$-*i*Nzd (32.3)	6S RNA	$G(Z)_2N_{OH}$
	Pancreatic	Not run	N_2-*i*Nzd (31.6)	26S	Y-G-A_{OH}
			A-*i*Nzd (24.3)		G-Y-A_{OH}
			G-*i*Nzd (35.0)	6S RNA	G(Z)Y-G_{OH}
One stepwise degradation	T_1	Not run	$N_{>2}$-*i*Nzd (28.9)	26S	$G(Z)_nY$-G-A_{OH}
			U-*i*Nzd (27.8)		G-U-A_{OH}
			N_2-*i*Nzd (30.0)	6S RNA	G(Z)Y-G_{OH}
	Pancreatic	Not run	G-*i*Nzd (28.1)	26S	$G(Z)_nY$-G-A_{OH}
			N_2-*i*Nzd (27.9)		Y-G-U-A_{OH}
			U-*i*Nzd (32.3)	6S RNA	G-Y-U-G_{OH}
Two stepwise degradations*	Pancreatic	Not run	C-*i*Nzd (43.0)	26S	$G(Z)_nY$-C-G-A_{OH}
			G-*i*Nzd (45.6)		Y-G-U-A_{OH}

* Purified by sucrose-gradient fractionation; 6S RNA is absent.

Fig. 3. (*a*) *DEAE-Sephadex chromatography of a T_1-ribonuclease digest of Antheraea eucalypti 26S rRNA after one stepwise degradation and labelling with* [^{3}H]*iNzd and* (*b*) *paper electrophoresis of a T_1-ribonuclease digest of Antheraea eucalypti 6S RNA after one stepwise degradation and labelling with* [^{3}H]*iNzd*

(*a*) Elution and numbering of peaks is as described for Fig. 1(*a*). [^{3}H]*i*Nzd-labelled 18S rRNA, subjected to one stepwise degradation, was added before enzyme digestion as a control; 6S RNA is absent (see the text). Carrier RNA was added as in Fig. 1(*a*). ●, ^{3}H; ——, E_{254}; · · · ·, [NaCl]. (*b*) Conditions are as for Fig. 1(*b*).

Table 4. *Major labelled digestion products from the 3'-termini of [^{3}H]iNzd-labelled Saccharomyces cerevisiae 26S RNA*

For details see the text and Table 3.

Treatment of RNA before labelling with [^{3}H]*i*Nzd	Ribonuclease used	DEAE-Sephadex column	Paper electrophoresis		Sequence
None	T_1	N-*i*Nzd (50.1)	U-*i*Nzd (43.6)	26S	G-U_{OH}
		N_6-*i*Nzd (46.3)	$N_{>2}$-*i*Nzd (47.0)	6S RNA	G(Z)$_5N_{OH}$
	Pancreatic	Not run	N_2-*i*Nzd (42.3)	26S	Y-G-U_{OH}
			U-*i*Nzd (46.5)	6S RNA	G(Z)$_4$Y-U_{OH}
One stepwise degradation*	T_1	N-*i*Nzd (9.0)	Not run		
		N_2-*i*Nzd (1.1)			
		N_3-*i*Nzd (5.6)			
		N_4-*i*Nzd (1.4)		6S RNA	G(Z)$_4$Y-U_{OH}
		N_5-*i*Nzd (82.9)			
	Pancreatic	Not run	G-*i*Nzd (11.7)		
			U-*i*Nzd (72.6)		
			A-*i*Nzd (5.6)		
			C-*i*Nzd (10.1)	6S RNA	G(Z)$_3$Y-U-U_{OH}
Two stepwise degradations*	Pancreatic	Not run	G-*i*Nzd (8.1)		
			U-*i*Nzd (16.6)		
			A-*i*Nzd (3.8)		
			C-*i*Nzd (5.2)		
			N_2-*i*Nzd(AU)(66.4)	6S RNA	G(Z)Y-A-U-U-U_{OH}

* 6S RNA only.

other eukaryotes (see below). *Drosophila* RNA was chosen since its base composition differs substantially from that of rabbit reticulocyte RNA (42% G+C compared with 56% G+C respectively; Hastings & Kirby, 1966; Gould *et al.*, 1966).

Two sequences, Y-C-G-A_{OH} and Y-G-U-A_{OH}, were found in equimolar amounts at the 3'-termini of the two '18S' dissociation products of *Drosophila* 26S RNA (Table 2). The sequence G-Y-U-G_{OH} is present at the 3'-terminus of 6S RNA. None of these sequences shows any similarity with the corresponding sequences of *Antheraea eucalypti* 26S RNA, *Galleria mellonella* 26S RNA or rabbit reticulocyte 28S RNA.

3'-*Terminal sequences of* 26*S*–28*S RNA and* 6*S RNA from other eukaryotes*

The results of less extensive 3'-terminal sequence studies on the larger rRNA species from cultured African green-monkey kidney (Vero) cells and cultured baby-hamster kidney (BHK) cells together with other published data for the 3'-termini of eukaryotic 26S–28S rRNA are listed in Table 2. Except for *Drosophila* 26S rRNA, the larger rRNA from all the higher eukaryotes examined share a common 3'-terminal dinucleotide (G-U_{OH}), whereas the 6S RNA from the same organisms terminates in the same trinucleotide, G-C-U_{OH}.

The 3'-terminal sequences of the 26S RNA–6S RNA complex from a lower eukaryote, *Saccharomyces cerevisiae*, were also determined (Table 4). The sequence Y-G-U_{OH} was found for the large 26S component and G-Z-Y-A-U-U-U_{OH} for the 6S RNA. The 3'-terminal sequence of 6S RNA shows no homology with the corresponding sequence in higher eukaryotes.

Discussion

The absence of any significant heterogeneity at the 3'-termini of eukaryotic rRNA is demonstrated by the presence of unique sequences at the 3'-termini of the different RNA molecules comprising the 26S rRNA–28S rRNA complex. Thus insect 26S rRNA, which consists of three polynucleotide chains (Shine & Dalgarno, 1973), contains three unique 3'-terminal sequences. The introduction of the 'hidden' mid-point scission (Shine & Dalgarno, 1973) therefore results from the action of a nuclease of defined specificity. The production of the 3'-terminus could result from either a single endonucleolytic cleavage or from an initial scission followed by exonucleolytic degradation to the sequence concerned.

The 3'-terminal dinucleotide from both '18S' dissociation products of 26S RNA from *Antheraea* cells is G-U_{OH}. This suggests that the enzyme concerned in generating the mid-point break may also be involved in cleaving 26S RNA from the 30S precursor (Dalgarno *et al.*, 1972). This is noteworthy since generation of the mid-point break occurs after the formation of 26S RNA from 30S RNA (L. Dalgarno, unpublished work).

It is also significant that the same 3'-dinucleotide (G-U_{OH}) is found at the 3'-terminus of both 'halves' of *Galleria* 26S rRNA, in 26S rRNA from *Saccharomyces*, in 28S rRNA from hamster and monkey kidney cell lines and in 28S rRNA from both rabbit reticulocytes (Hunt, 1970) and avian myeloblasts (Ahmad *et al.*, 1972). The nucleases that process 28S rRNA from the high-molecular-weight precursor, and which generate the mid-point break in 26S rRNA from insects, therefore show some conservation of speci-

ficity between a number of unrelated eukaryotes. This conservation of specificity is not absolute, however, since the 3′-termini of the two '18S' dissociation products of *Drosophila* 26S rRNA are G-A_{OH} and U-A_{OH}.

Similarly, the 3′-terminal trinucleotide, G-C-U_{OH}, is found in 6S RNA from *Antheraea*, *Galleria*, cultured BHK cells and rabbit reticulocytes, whereas a different 3′-terminal sequence is found in 6S RNA from both *Drosophila* (G-Y-U-G_{OH}) and yeast (Y-A-U-U-U_{OH}). In some cases therefore similarities in 3′-terminal sequence between the large component of 26S–28S rRNA appear to parallel similarities in the 3′-terminal sequence of the associated 6S RNA molecule.

In all eukaryotes examined, including yeast and *Drosophila*, the 3′-terminal sequence of 18S rRNA from the small ribosome subunit is G-A-U-C-A-U-U-A_{OH} (Shine & Dalgarno, 1974*b*). It has been suggested that this apparently universal homology for at least the 3′-terminal eight nucleotides reflects the involvement of this region of 18S rRNA in a critical role in protein synthesis (Dalgarno & Shine, 1973). Such homology contrasts with the more limited and clearly non-universal identities in 3′-terminal sequence found in 26S–28S RNA and in 6S RNA.

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