The Nature of the 5'-Linked 5' Nucleotide Sequence at the 5' End of Rabbit Globin Messenger Ribonucleic Acid

By JOHN A. HUNT and GARY N. OAKES

Department of Genetics, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822, U.S.A.

(Received 15 January 1976)

Poly(A)-containing messenger RNA isolated from rabbit reticulocytes as estimated by periodate oxidation and condensation with [³H]isoniazid has two oxidizable end groups per molecule of mol.wt. 220000. When the mRNA is subjected to stepwise degradation by β -elimination, only one oxidizable end-group is found. This indicates that one of the 2',3' hydroxyl end-groups is linked through the normal 3'–5' phosphodiester bond, but that the other is linked in such a way that after stepwise degradation no new 2',3' hydroxyl group is revealed. This structure could be a 5'-linked 5'-phospho di- or tri-ester. On digestion with ribonuclease the isoniazid-labelled RNA produced oligonucleotide hydrazones consistent with a poly(A) sequence at the 3' end plus fragments that are not found after stepwise degradation. These fragments have a charge of –6 and –8 from pancreatic ribonuclease or –7 from ribonuclease T₁ digestion. These charges are changed to –3.4 and –4.1 after pancreatic ribonuclease, ribonuclease T₂ and alkaline phosphatase digestion. methyl-³H-labelled-poly(A)-containing RNA isolated from late erythroid cells contain a methyl-labelled fragment resistant to endonuclease and phosphodiesterase II digestion. After digestion with phosphodiesterase I this fragment produces methyl-³H-labelled nucleotides with the electrophoretic mobility of pm⁷G and pAm. It is concluded that globin mRNA has the 5' sequences m⁷G(5')ppp'AmpYpGp... and m⁷G(5')ppp-AmpApGpYp...

Reports of the discovery of a 5'-linked methylated 5' nucleotide triphosphate at the 5' end of reo, vaccinia and polyhedrosis virus messenger RNA (mRNA) synthesized *in vitro* (Furiuchi *et al.*, 1975a; Wei & Moss, 1975; Furiuchi & Miura, 1975) led us to re-investigate previous results obtained by condensation of labelled [³H]isoniazid with periodate-oxidized rabbit globin mRNA (Hunt 1973a). In that previous work (Hunt, 1973a), analysis of oligonucleotide fragments produced by degradation with pancreatic ribonuclease and ribonuclease T₁ led to the conclusion that rabbit globin mRNA had a mol.wt. close to 160000, and had 3' terminal sequences GpY(pA)_{6–7}OH* and G(pN)_{9–16}pY(pA)_{2–3}. However, because these estimates were made on mRNA isolated from sucrose gradients, and it was assumed that there was contamination with lower-molecular-weight RNA molecules, further purification of the mRNA by absorption to oligo-(dT)-cellulose columns was made. The results described in the present paper were essentially the same as before in that approximately two molecules of isoniazid are bound per molecule of mol.wt. 220000. However, after stepwise degradation by

β -elimination after periodate oxidation (Steinschneider & Fraenkel-Conrat, 1966), one of the end-groups is removed in such a way that a new oxidizable end is no longer available, whereas the other end-group is removed and uncovers a new periodate-oxidizable end. This behaviour, and the properties of the terminal fragments that are removed by enzyme digestion, are all in agreement with the postulation of a 5'-linked 5' nucleotide at the 5' end of globin mRNA as found in the viral mRNA species.

This conclusion was also reached by several other workers by examination of methyl-³H- or ³²P-labelled-poly(A)-containing RNA from HeLa, L and myeloma cells (Furiuchi *et al.*, 1975b; Wei *et al.*, 1975; Perry *et al.*, 1975; Adams & Cory, 1975). We examined the structure of methyl-³H-labelled-poly(A)-containing RNA from rabbit erythroid cells and found a structure that has the properties of the sequence m⁷G(5')pppAmpN. This, together with the results from isoniazid-labelled globin mRNA, would indicate that two sequences, namely m⁷G(5')pppAmpYpGp and m⁷G(5')pppAmpApGpYp, are present at the 5' termini of globin mRNA.

Materials and Methods

[G-³H]Isonicotinic acid hydrazide (sp. activity 1 Ci/mmol) was obtained from Amersham/Searle

* For an explanation of CBN recommended symbols for nucleotides and their substituents, see *Biochem. J.* (1970) 120, 449–454.

(Des Plaines, IL, U.S.A.). Sodium metaperiodate was from Matheson, Coleman and Bell (East Rutherford, NJ, U.S.A.). Sephadex G-75 and DEAE Sephadex A-25 were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Oligo(dT)-cellulose T-2 was from Collaborative Research Inc. (Waltham, MA, U.S.A.). Triethylamine, from Eastman Kodak Co. (Rochester, NY, U.S.A.) was redistilled before use. Other chemicals were Reagent-Grade from Mallinckrodt (St. Louis, MO, U.S.A.). Ribonuclease T₁ (EC 2.7.7.26) from Sankyo (Tokyo, Japan) was obtained through Calbiochem (Los Angeles, CA, U.S.A.). Pancreatic ribonuclease (EC 3.1.4.22) and ribonuclease-free bacterial alkaline phosphatase (EC 3.1.3.1) were from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.). Phosphodiesterase I from *Crotalus adamanteus* venom (EC 3.1.4.1), phosphodiesterase II from bovine spleen (EC 3.1.4.18) and ribonuclease T₂ (EC 3.1.4.23) were from Sigma.

Purification of globin mRNA

(a) *From reticulocytes.* Globin mRNA was isolated by phenol extraction from the 20S sub-ribosomal particle isolated from EDTA-treated polyribosomes on a sucrose gradient and the 10S region isolated from the RNA by further sucrose-gradient fractionation (Hunt, 1973a). This RNA was fractionated by chromatography on an oligo(dT)-cellulose column by the method of Aviv & Leder (1972).

(b) *From fractionated bone-marrow erythroid cells.* methyl-³H-labelled-poly(A)-containing RNA from rabbit bone-marrow erythroid cells was isolated from the main fraction of bone-marrow cells separated on a bovine serum albumin gradient (density 1.06–1.09 g/cm³) by the method of Borsook *et al.* (1969). These cells were incubated (concn. 2×10^7 cells/ml) in a medium containing 70% Eagles' minimal-essential-mineral (MEM) medium (without methionine), 20% foetal calf serum, 10% dialysed anaemic rabbit plasma, ferrous ammonium sulphate (30 µg/ml), 20 mM-sodium formate, 20 µM-adenosine and 20 µM-guanosine with 20 µCi of [methyl-³H]methionine (6.4 Ci/mmol)/ml at 38°C in spinner culture in an atmosphere of air+CO₂ (95:5) for 7 h. The methyl-³H-labelled RNA was prepared from polysomes extracted from 1×10^9 cells by lysis with 5 ml of rat liver supernatant (Grau & Favelukes, 1968), to inhibit ribonuclease activity, and centrifugation at 4°C at 165 000g for 60 min in the Spinco Ti 50 rotor. The extraction was by the pH9-phenol procedure described by Brawerman *et al.* (1972). Poly(A)-containing-RNA was obtained by oligo(dT)-cellulose chromatography by the method of Aviv & Leder (1972). RNA that was not bound to the oligo(dT)-cellulose column was further fractionated in a 5–20% sucrose gradient in 0.1 M NaCl to obtain methyl-³H-labelled 18S and 28S rRNA.

Purity of the mRNA. The poly(A)-containing mRNA isolated from reticulocytes and bone marrow contained only one component with a mobility corresponding to 10S when examined by sucrose gradients or polyacrylamide-gel electrophoresis. In either the wheat-embryo or the duck reticulocyte lysate cell-free system, the RNA from the bone-marrow cells and reticulocytes was equally active. The synthesis of globin chains stimulated by reticulocyte mRNA was 86% of the total and for the bone-marrow cells 71% (J. A. Hunt, E. S. Kawasaki & G. N. Oakes, unpublished work). Additionally, ³H-labelled poly(A)-containing mRNA from bone-marrow cells was completely inhibited from hybridization with total rabbit DNA by poly(A)-containing mRNA isolated from reticulocytes.

Condensation of isoniazid with periodate oxidised RNA

Reaction of globin mRNA with [³H]isoniazid was as described by Hunt (1973a). Stepwise degradation of the globin mRNA by the technique of Steinschneider & Fraenkel-Conrat (1966) was as described by Hunt (1970).

Enzyme digestion

Digestion of isoniazid-labelled RNA with pancreatic ribonuclease, ribonuclease T₁ and ribonuclease T₂ were as described by Hunt (1973a). When bacterial alkaline phosphatase was added to the digest after pancreatic ribonuclease and ribonuclease T₂ digestion, the pH was adjusted to 7.5 with 0.2 ml of 0.05 M-triethylammonium bicarbonate, pH9, and 100 µg of alkaline phosphatase/100 µg RNA was added, and incubation continued for a further 90 min at 25°C.

methyl-³H-labelled-poly(A)-containing RNA and 28S rRNA were digested with 11.2 units/ml of phosphodiesterase II, 100 µg of pancreatic ribonuclease/ml and 100 µg of alkaline phosphatase/ml in 10 mM-Tris/HCl, pH7.5, for 40 h at 37°C, 12–30 µg of RNA (sp. activity 3000 d.p.m./µg) was digested in a volume of 50 µl.

Enzyme-resistant fractions eluted with water from Whatman 3MM paper after electrophoresis were digested with 1 mg of phosphodiesterase I/ml in 20 mM-Tris/HCl, pH8, and 5 mM-MgCl₂ at 37°C for 150 min in a volume of 100 µl.

Column chromatography of digests of isoniazid-labelled RNA on DEAE Sephadex in 7 M-urea with a 1280 ml gradient of sodium acetate (0.1–2 M), pH7.5, was carried out as described by Hunt (1973a). The flow rate was 40 ml/h, and 2 ml/h was mixed with 30 ml/h of liquid-scintillation mixture, containing 5 parts of 0.5% PPO (2,5-diphenyloxazole) and 0.05% dimethyl-POP (1,4-bis-2-(4-methyl-5-phenyloxazol-2-yl)benzene) in toluene, to 1 part of Beckman BBS 3 solubilizer, to which 7% (v/v) of water had been added. The radioactivity of this

mixture was measured in a 3 ml flow-cell at a counting efficiency of 12% for ³H.

Paper electrophoresis was performed on Whatman 3MM paper on a flat-bed water-cooled apparatus by

Table 1. Binding of [³H]isoniazid to oxidized globin mRNA (Adapted from Hunt, 1973a)

For details of periodate oxidation and condensation of globin mRNA with isoniazid, see the text. For the purpose of estimation of RNA the E₂₆₀ of 1 mg of RNA was taken as 23 in 0.1 M-sodium phosphate, pH 7.0.

Preparation no.	Isoniazid bound (mol/mol of globin mRNA of mol.wt. 220000)	
	Uncorrected	Corrected*
165	2.6	2.1
172	1.7	1.3
180	2.3	2.0

* Correction was made for approximately 20% nonspecific binding of isoniazid.

using either 0.05M-sodium citrate, pH 5.0, or 0.1M-triethylammonium formate/5mM-EDTA, pH 3.5, as buffer, with a voltage gradient of 32 V/cm for 40 min. Radioactivity was determined on 1 cm strips cut from the electrophoretogram, either by direct counting in 0.5% PPO/0.05% dimethyl POPOP in toluene in a liquid-scintillation spectrometer, after which the paper could be washed with ether and the material eluted for further studies; alternatively elution was with 0.2ml of M-NaOH, and after neutralization with 0.1 ml of 0.2M-HCl, radioactivity was counted in a liquid-scintillation spectrometer after the addition of 3 ml of 0.5% PPO/0.05% dimethyl-POPOP (5:1) in toluene with Beckman BBS 3 solubilizer. The efficiency of ³H counting was approx. 3% in the former case and 20% in the latter.

Results and Discussion

Rabbit globin mRNA has a mol.wt. of 200000–240000 as measured by gel electrophoresis (Labrie, 1969; Gaskill & Kabat, 1971) and is similar to that of

Fig. 1. Column chromatography of pancreatic ribonuclease digests of isoniazid-labelled globin mRNA

Globin mRNA labelled with [³H]isoniazid was dissolved in water and digested by pancreatic ribonuclease, and the digest was mixed with pancreatic ribonuclease, alkaline phosphatase-digested carrier RNA in 7M-urea, absorbed on to a column (1 cm × 50 cm) of DEAE-Sephadex and eluted with a linear gradient (0.05–2.0M) of sodium acetate, pH 7.5, in 7M-urea in a total volume of 1280 ml at 4°C. Radioactivity and absorbance were monitored automatically (Hunt, 1970). Only the pattern of radioactivity is shown. The flow rate was 40 ml/h. (a) Pattern of oligonucleotide hydrazones obtained by pancreatic-ribonuclease digestion of [³H]isoniazid-labelled periodate-oxidized globin mRNA; (b) pattern of oligonucleotide hydrazones obtained by pancreatic ribonuclease digestion of [³H]isoniazid-labelled periodate-oxidized globin mRNA that had been subjected to two cycles of stepwise degradation.

other species of globin mRNA (Williamson *et al.*, 1971; Bishop *et al.*, 1972). The number of isoniazid residues that condense with globin mRNA, calculated by using a mol.wt. of 220000, are shown for preparations of globin mRNA that were prepared by sucrose-gradient sedimentation of RNA extracted from the 20S particle isolated by EDTA treatment of polyribosomes (Table 1). In previous work (Hunt, 1973*a*) it was estimated that the high number of isoniazid molecules that condensed with the globin mRNA was caused by contamination of the mRNA with 18S rRNA and tRNA, and that, after corrections were made for these impurities, a mol.wt. of 160000 for the mRNA as the acid could be estimated. However, when the only correction made is for an estimated maximum of 20% non-specific binding of isoniazid (as determined from controls with unoxidized mRNA, and from column chromatography), it is clear that the number of isoniazid molecules bound per molecule of mRNA is close to two (Table 1). Further studies on these preparations were made by digestion of the isoniazid-labelled mRNA with pancreatic ribonuclease or ribonuclease T₁, followed by chromatography on DEAE-Sephadex by eluting with a salt gradient at pH 7.5 in 7M-urea, to separate oligonucleotide hydrazones by the charge of their phosphate residues. Results identical with those

shown in Fig. 1(*a*) and Fig. 2(*a*) were obtained. The peaks in area I in Fig. 1(*a*) contain ApA-iNicHz and ApApA-iNicHz, when iNicHz is used to denote the isonicotinoyl residue, and are most probably caused by pancreatic ribonuclease digestion in low salt of an oligo(A) sequence (pA)_n-iNicHz (Mansbridge *et al.*, 1974). Further, when the peak in area IV, obtained by ribonuclease T₁ digestion, was digested with pancreatic ribonuclease in low salt, the di- and tri-oligo(A) sequences which could be obtained by digestion of isoniazid-labelled mRNA with pancreatic ribonuclease alone were also found. After digestion with ribonuclease T₂ the peak in area IV produced A-iNicHz (Hunt, 1973*a*). All of these results now indicate that there is a (pA)_nOH sequence at the 3' end of globin mRNA and that the value of *n* is greater than 20, as shown by the charge of the ribonuclease T₁ fragment (IV) and the amount of poly(U) that can be bound to globin mRNA (Mansbridge *et al.*, 1974; Hunt, 1973*b*).

The peak in area III (Fig. 2*a*) was determined to have the sequence GpY(pA)₆₋₇OH, where Y denotes a pyrimidine. Digestion of this oligonucleotide with pancreatic ribonuclease or ribonuclease T₂ would be expected to release ApAiNicHz and ApApAiNicHz, or AiNicHz respectively. This result was not found, and was ignored in the original sequence assignment.

Fig. 2. Column chromatography of ribonuclease T₁ digests of isoniazid-labelled globin mRNA

Globin mRNA labelled with [³H]isoniazid was digested by ribonuclease T₁. The conditions of chromatography were exactly as described in Fig. 1, except that a ribonuclease T₁ digest of carrier RNA was added. (*a*) Pattern of oligonucleotide hydrazones obtained by ribonuclease T₁ digestion of [³H]isoniazid-labelled periodate-oxidized globin mRNA; (*b*) pattern of oligonucleotide hydrazones obtained by ribonuclease T₁ digestion of [³H]isoniazid-labelled periodate-oxidized globin mRNA that had been subjected to two cycles of stepwise degradation.

To further characterize the sequence of the oligonucleotide hydrazones in areas III and II, two and three steps of stepwise degradation using periodate oxidation followed by β -elimination with aniline (Steinschneider & Fraenkel-Conrat, 1966; Hunt, 1970) were performed on globin mRNA that had been further purified by chromatography on an oligo(dT)-cellulose column by the method of Aviv & Leder (1972) to eliminate any possible rRNA or tRNA contamination. Two and three stepwise degradations were used to decrease the size of the oligonucleotides in areas II and III such that their sequence could be determined.

The first oddity noticed was that the amount of isoniazid that could be condensed with periodate-oxidized RNA was decreased by half after stepwise degradation (Table 2). This could have been explained by a failure of alkaline phosphatase to remove all of the 3'-phosphate left after stepwise degradation, which would result in an overall lower yield of the various oligonucleotide hydrazones. However, when pancreatic ribonuclease or ribonuclease T_1 digests of the isoniazid-labelled RNA after two or three steps of stepwise degradation were chromatographed on DEAE-Sephadex, it was found that the peaks in

areas II and III were quantitatively removed, and that the position of the oligonucleotides in areas I and IV were essentially unchanged, which indicates that the alkaline phosphatase had removed all of the available phosphates from the RNA. In fact, a very small shift of the peak in area IV can be detected after two or three stepwise degradations. Again the result, that the peaks in areas I and IV remain after stepwise degradation of mRNA, is quite consistent with the presence of an $(Ap)_nOH$ sequence, where $n > 20$, at the 3' end of the globin mRNA, and confirms that the results of enzyme digestion, which produces the peaks in area I, may be an artifact since the charge on the peak components is not changed after two or three stepwise degradations.

The quantitative decrease of isoniazid binding and elimination of the peaks in areas II and III can only be explained by the presence of a 5'-terminal structure in the mRNA which, if it is a nucleotide, has a 5'-linked phosphate and free 2',3' OH group to make it available for periodate oxidation and condensation with isoniazid and β -elimination, but which is not linked in the normal 3'-5' phosphodiester linkage, since no new 2',3' OH group is found after one or two stepwise degradation procedures.

Table 2. Binding of [3H]isoniazid to oxidized globin mRNA before and after stepwise degradation

Globin mRNA was further purified by oligo(dT)-cellulose before periodate oxidation and β -elimination. Other conditions were as described in the text and in Table 1.

Preparation no.	[3H]Isoniazid bound (mol/mol of globin mRNA of mol.wt. 220000)		
	Before stepwise degradation	After two stepwise degradations	After three stepwise degradations
216	2.2	1.1	1.1
218	1.9	1.3	—

Fig. 3. Column chromatography of a pancreatic ribonuclease, ribonuclease T_2 and alkaline phosphatase digest of isoniazid-labelled globin mRNA

Conditions of enzyme hydrolysis are given in the text. Conditions of chromatography were exactly as described in Figs. 1 and 2.

To further characterize the terminal-labelled sequences, isoniazid-labelled mRNA was digested with a mixture of pancreatic ribonuclease and ribonuclease T₂, followed by bacterial alkaline phosphatase. The digest was chromatographed on DEAE-Sephadex as before, and the results are shown in Fig. 3. As would be expected for [³H]isoniazid-labelled poly(A) from the 3' end, the peaks in area I are removed, and an increase in the amount of nucleoside isonicotinoyl hydrazones is found, but two peaks remain which chromatograph at a position corresponding to a charge of -3.4 and -4.1 as compared with the charge of -6 and -8 for the two peaks in area II. It is noteworthy that the relative charge of the oligonucleotides is calculated from plotting log (charge) against the position of elution relative to known oligonucleotide isonicotinoyl hydrazones and oligonucleotides, and is subject to some error. However, even if the charge of the oligonucleotides in area II were -5 and -7, the charge of the oligonucleotides found after pancreatic ribonuclease, ribonuclease T₂ and alkaline phosphatase digestion would only decrease to -3.2 and -3.7.

Further attempts to characterize the isonicotinoyl hydrazones resistant to enzyme digestion were not successful; however, it was possible to isolate

*methyl*³H-labelled-poly(A)-containing RNA from the polyribosomes of a fraction of rabbit erythroid cells which synthesize 80–90% of their protein as haemoglobin. This RNA is at least 70% globin mRNA, as determined by assay in the duck reticulocyte cell-free system. The [*methyl*³H]mRNA was digested with a mixture of pancreatic ribonuclease and phosphodiesterase II followed by bacterial alkaline phosphatase and fractionated by paper electrophoresis at pH 5.0. Fig. 4 shows that a fraction corresponding to 21% of the total label was resistant to digestion to nucleosides and that this fraction was not found in *methyl*³H-labelled 28S rRNA. This fraction was eluted from the paper and further digested with phosphodiesterase I and yielded two fractions after electrophoresis in 0.1M-triethylammonium formate, pH 3.5, as shown in Fig. 5. The position of the undigested fragment, between pA and pG and the position of the two peaks after digestion, one with the mobility of pA which, because of its methyl label, is most likely 2'-O-methyl pA, and the other, with a positive charge greater than pC at a position where pm⁷G is found by other investigators, indicate that the sequence containing the methylated bases is m⁷G(5')p(p)pAmpN or Ampm⁷G. The negative charge of the fragment isolated at pH 5.0 is

Fig. 4. Paper electrophoresis of *methyl*³H-labelled RNA from erythroid cells after digestion with pancreatic ribonuclease, phosphodiesterase II and alkaline phosphatase

Electrophoresis was in 0.05M-sodium citrate, pH 5.0, as described in the text. Each 1 cm strip was counted directly for radioactivity in a toluene scintillator without the elution procedures described in the text. (a) *methyl*³H-labelled 28S rRNA; (b) *methyl*³H-labelled poly(A) containing RNA.

Fig. 5. Paper electrophoresis of methyl- ^3H -labelled-resistant oligonucleotide from poly(A)-containing RNA at pH 3.5

The enzyme resistant fraction from Fig. 4(b) was eluted with water, and digested with phosphodiesterase I as described in the text. (a) Undigested control. The 1 cm strips were eluted with 0.1 M-NaOH for liquid-scintillation counting at an efficiency of 20%; (b) resistant oligonucleotide after digestion with phosphodiesterase I. The 1 cm strips were counted for radioactivity (at an efficiency of 3%) in the scintillator without the elution procedure.

greater than UpU or ApA which would eliminate the sequence Ampm ^7G . It is not possible from these experiments to determine whether the structure of the methyl-labelled fragment contains a 5'-linked di- or tri-phosphate, but its electrophoretic mobility at pH 5 and 3.5 is not inconsistent with the triphosphate structure determined in viral mRNA.

It is assumed that this 5'-terminal sequence is from α - and β -globin mRNA, and hence it is possible to combine these results with those obtained from isoniazid-labelled reticulocyte mRNA. In this way the two 5'-terminal sequences must be m $^7\text{G}(5')$ -pppAmpYpGp..., which would have a charge of -7 after ribonuclease T_1 digestion and -6 after pancreatic ribonuclease digestion and -4 after pancreatic ribonuclease, ribonuclease T_2 and alkaline phosphatase digestion; and m $^7\text{G}(5')$ -pppAmpApGpYp... to give charges of -7, -8 and -4 after digestion as above. Since the former sequence is present in 1.5 times the amount of the latter sequence (Hunt, 1973a) and reticulocyte poly-ribosomes contain α - and β -chain mRNA in the ratio 1.5:1 (Lodish, 1974) it is tempting to assign the sequence m $^7\text{G}(5')$ -pppAmpYpGp to the α -chain mRNA and the m $^7\text{G}(5')$ -pppAmpApGpYp sequence to the β -chain mRNA. However, only further sequence

analysis of globin mRNA will be able to confirm this assignment.

In all the other 5'-linked sequences so far determined, m ^7G has been found as the triphosphate-linked nucleotide, and our results also confirm the determination of m ^7G trialcohol from oxidized globin mRNA after borohydride reduction (Muthukrishnan *et al.*, 1975). However, in all other mammalian cells so far used, the ^3H -methylated bases adjacent to the m ^7G triphosphate have been heterogeneous with all four of the 2'-O-methylated bases being represented. It is encouraging to note that in erythroid cells only Am is found, which might indicate either a specificity in the mRNA terminal-addition site or a homogeneity in the original globin mRNA sequence at that point.

The determination of sequences adjacent to the 5' termini and of the extent of methylation of globin mRNA outside the 5' terminal region requires further study.

We acknowledge the expert technical assistance of Barbara E. Johnson and Otto F. Peter. This work was supported by grant GM 19076 from the National Institutes of Health and the General Research Support of the Medical School.

References

- Adams, J. M. & Cory, S. (1975) *Nature (London)* **255**, 28–33
- Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1408–1412
- Bishop, J. O., Pemberton, R. E. & Baglioni, C. (1972) *Nature (London) New Biol.* **235**, 231–234
- Borsook, H., Ratner, K. & Tattre, B. (1969) *Blood* **34**, 32–41
- Brawerman, G., Mendecki, J. & Lee, S. Y. (1972) *Biochemistry* **11**, 637–641
- Furiuchi, Y. & Miura, K. (1975) *Nature (London)* **253**, 374–375
- Furiuchi, Y., Morgan, M., Muthukrishnan, S. & Shatkin, A. J. (1975a) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 362–366
- Furiuchi, Y., Morgan, M., Shatkin, A. J., Jelinek, W., Salditt-Georgieff, M. & Darnell, J. (1975b) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1904–1908
- Gaskill, P. & Kabat, D. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 72–75
- Grau, O. & Favelukes, G. (1968) *Arch. Biochem. Biophys.* **125**, 647–657
- Hunt, J. A. (1970) *Biochem. J.* **120**, 353–363
- Hunt, J. A. (1973a) *Biochem. J.* **131**, 315–325
- Hunt, J. A. (1973b) *Biochem. J.* **131**, 327–333
- Labrie, F. (1969) *Nature (London)* **221**, 1217–1222
- Lodish, H. F. (1974) *Nature (London)* **251**, 385–388
- Mansbridge, J. N., Crossley, J. A., Lanyon, W. G. & Williamson, R. (1974) *Eur. J. Biochem.* **44**, 261–269
- Muthukrishnan, S., Both, G. W., Furiuchi, Y. & Shatkin, A. J. (1975) *Nature (London)* **255**, 33–37
- Perry, R. P., Kelley, D. E., Friderici, K. & Rottman, F. (1975) *Cell* **4**, 387–394
- Steinschneider, A. & Fraenkel-Conrat, H. (1966) *Biochemistry* **5**, 2735–2743
- Wei, C. M. & Moss, B. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 318–323
- Wei, C. M., Gershowitz, A. & Moss, B. (1975) *Cell* **4**, 379–386
- Williamson, R., Morrison, M., Lanyon, G., Eason, R. & Paul, J. (1971) *Biochemistry* **10**, 3014–3021