Identical 3'-Terminal Octanucleotide Sequence in 18S Ribosomal Ribonucleic Acid from Different Eukaryotes

A PROPOSED ROLE FOR THIS SEQUENCE IN THE RECOGNITION OF TERMINATOR CODONS

By JOHN SHINE and LYNN DALGARNO

Department of Biochemistry, School of General Studies, Australian National University, Canberra, A.C.T. 2600, Australia

(Received 7 January 1974)

The 3'-terminal sequence of 18S ribosomal RNA from *Drosophila melanogaster* and *Saccharomyces cerevisiae* was determined by stepwise degradation from the 3'-terminus and labelling with [³H]isoniazid. The sequence G-A-U-C-A-U-U-A_{OH} was found at the 3'-terminus of both 18S rRNA species. Less extensive data for 18S RNA from a number of other eukaryotes are consistent with the same 3'-terminal sequence, and an identical sequence has previously been reported for the 3'-end of rabbit reticulocyte 18S rRNA (Hunt, 1970). These results suggest that the base sequence in this region is strongly conserved and may be identical in all eukaryotes. As the 3'-terminal hexanucleotide is complementary to eukaryotic terminator codons we discuss the possibility that the 3'-end of 18S rRNA may have a direct base-pairing role in the termination of protein synthesis.

Termination of protein synthesis in prokaryotes occurs in response to the presence of one of the terminator codons (UAA, UAG or UGA) in the ribosomal 'A' site (Haselkorn & Rothman-Denes, 1973). The terminator triplet is specifically recognized since certain other triplet sequences which do not code for any amino acid do not lead to polypeptide release (Bretscher *et al.*, 1965). There is good evidence that tRNA does not recognize terminator codons (Capecchi, 1967; Bretscher, 1968; Fox & Ganoza, 1968) and this function has been assigned to two proteins, or release factors (Scolnick & Caskey, 1969), which are necessary for the release of nascent polypeptides in response to terminator codons (Capecchi, 1967; Scolnick *et al.*, 1968). However, attempts to demonstrate specific binding of terminator codons by release factors have been inconclusive (Capecchi & Klein, 1969). Although not as well understood, the process of polypeptide chain termination in eukaryotes appears to be fundamentally similar to that in prokaryotes. Thus UAA, UAG and UGA have been implicated as terminator codons (Hawthorne, 1969; Beaudet & Caskey, 1971; Stewart & Sherman, 1972) and a single release factor has been isolated with similar activity to the two prokaryotic factors (Beaudet & Caskey, 1971).

In the present paper we provide sequence data which suggest the possibility that the 3'-terminus of eukaryotic 18S rRNA plays a direct base-pairing role in the termination of protein synthesis in eukaryotes through specific recognition of the terminator codons.

A preliminary account of this work has been published (Dalgarno & Shine, 1973).

Experimental

Media and materials

Saccharomyces cerevisiae were grown aerobically in the medium described by Gordon & Stewart (1971). A clone of *Antheraea eucalypti* (emperor gum moth) cells was supplied by Dr. T. D. C. Grace (Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia) and grown in Grace's (1962) medium at 25°C. Propagation of African green-monkey kidney (Vero) and baby-hamster kidney (BHK) cells was as described by Raghow *et al.* (1973). *Galleria mellonella* (wax moth) larvae were reared on honeycomb at 25°C. *Drosophila melanogaster* (larvae and adults) were obtained from Dr. A. J. Howells (Australian National University, Canberra, Australia).

[G-³H]isonicotinic acid hydrazide (iNzd*) (1 Ci/mmol) and [carbonyl-¹⁴C]iNzd (11.1 mCi/mmol) were from The Radiochemical Centre (Amersham, Bucks., U.K.). Unlabelled iNzd was from Calbiochem (Los Angeles, Calif., U.S.A.). DEAE-Sephadex A-25 was from Pharmacia (Uppsala, Sweden). Sucrose was ribonuclease-free (Schwarz/Mann, Orangeburg, N.Y., U.S.A.). Aniline (May and Baker Ltd., Dagenham,

* Abbreviations: iNzd, isonicotinic acid hydrazide (isoniazid); iNicHz, isonicotinoylhydrazine.

U.K.) was redistilled before use. All other chemicals were reagent grade. Ribonuclease T₁ (grade III, from *Aspergillus oryzae*) and pancreatic ribonuclease were from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Bacterial alkaline phosphatase (BAPF), ribonuclease-free, was from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.). Nucleosides and dinucleoside monophosphates were from Sigma Chemical Co. PPO (2,5-diphenyloxazole) and POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] (scintillation grade) were from Packard Instrument Co., Downers Grove, Ill., U.S.A. BBS3 solubilizer was from Beckman Instruments Inc., Fullerton, Calif., U.S.A., and Triton X-100 from Ajax Chemicals Ltd., Sydney, Australia.

Methods

Preparation of rRNA. Ribosomal RNA was extracted from *Drosophila melanogaster* and *Galleria mellonella* by homogenization of larvae or adult insects in 20 vol. of phenol-cresol-sodium aminosalicylate (Shine & Dalgarno, 1973) at 4°C in a Teflon-glass homogenizer. The same method was used for *Saccharomyces cerevisiae* except that disruption was in a Braun homogenizer for 30 s. Pellets of cultured cells were disrupted by mixing directly with phenol-cresol-aminosalicylate (Shine & Dalgarno, 1973). The RNA was precipitated with 2 vol. of ethanol at -20°C and extracted twice with 3 M-sodium acetate (pH 6) to remove DNA and low-molecular-weight RNA species. Polysaccharide was removed by centrifugation at 40000 g for 30 min in 0.1 M-NaCl-0.01 M-sodium acetate (pH 5). Individual rRNA species were separated on gradients (34 ml) of 5-20% (w/v) sucrose in 0.1 M-NaCl-0.01 M-sodium acetate (pH 5) in the SW 27 rotor of a Spinco ultracentrifuge for 16 h at 25000 rev./min at 4°C. The 18S rRNA was isolated by pooling peak fractions and precipitating with 2 vol. of ethanol at -20°C. Less than 2% contamination of 18S rRNA with other rRNA species (26S, 5S and 4S RNA) was found on resedimentation. The sedimentation profile showed no evidence of any degradation of the RNA.

Stepwise degradation and reaction of iNzd with periodate-oxidized rRNA. Periodate oxidation of rRNA was as previously described (Shine & Dalgarno, 1973). Stepwise removal of the 3'-nucleotides from rRNA was essentially as described by Hunt (1970). Periodate-oxidized RNA was dissolved at 4 mg/ml in 0.1 M-NaCl-0.01 M-sodium acetate, pH 5. After addition of 3 vol. of 0.4 M-aniline (adjusted to pH 5 with conc. HCl), the RNA was incubated at 20°C for 4 h to remove the terminal nucleoside. Then 0.1 vol. of 1 M-NaCl-0.01 M-sodium acetate, pH 5, was added and the RNA was precipitated with 2 vol. of ethanol, resuspended in 0.1 M-NaCl-0.01 M-sodium acetate (pH 5) and reprecipitated with ethanol. To remove

the 3'-terminal phosphate, RNA (2 mg/ml) was dissolved in 20 mM-ammonium acetate (pH 6.9), made 1 M in MgCl₂ and 20 µg of alkaline phosphatase added/ml. The mixture was incubated for 45 min at 37°C. After addition of 3 vol. of 0.1 M-NaCl-0.01 M-sodium acetate (pH 5) the RNA was precipitated with ethanol and stored at -20°C. The whole procedure was repeated five times; after each cycle approx. 250 µg of oxidized RNA was removed and incubated with 50 µl of [³H]iNzd (50 µCi) in 1 ml of 0.1 M-NaCl-0.01 M-sodium acetate (pH 5) at 20°C for 20 h. Residual iNzd and the small amounts of contaminating polysaccharide were removed by DEAE-cellulose chromatography (Shine & Dalgarno, 1974). Labelled RNA was precipitated with 2 vol. of ethanol and stored at -20°C. The completeness of both the stepwise degradation procedure and the condensation of iNzd with periodate-oxidized rRNA have been previously demonstrated (Hunt, 1965, 1970; Shine & Dalgarno, 1973).

Enzymic digestion. Digestion of carrier RNA was at 37°C for 2 h in 0.01 M-sodium-potassium phosphate buffer (pH 7.4) with 10 µg of ribonuclease T₁/mg of RNA. [³H]iNzd-labelled RNA (0.5-1 mg/ml) in 0.01 M-phosphate (pH 7.4) was digested with 10 µg of ribonuclease T₁ or 50 µg of pancreatic ribonuclease/mg of RNA at 20°C for 3 h.

Column chromatography. Chromatography on columns (1 cm × 25 cm) of DEAE-Sephadex A-25 was used to determine the chain length of oligonucleotide hydrazones released by digestion of [³H]iNzd-labelled RNA. Elution was with a 1-litre linear gradient of 0-0.5 M-NaCl in 7 M-urea-0.01 M-phosphate, pH 7.4. Under these conditions oligonucleotide hydrazones containing from one to eight nucleoside residues exhibit a characteristic and reproducible elution profile which bears a fixed relationship to that of the corresponding unmodified oligonucleotides (Hunt, 1973; J. Shine, unpublished work). For the determination of radioactivity, 1 ml of each column fraction (5 ml) was added to 10 ml of scintillator solution containing 0.5% PPO and 0.05% POPOP dissolved in BBS3 solubilizer (1 vol.) and toluene (5 vol.). Fractions containing both ³H- and ¹⁴C-labelled oligonucleotides were counted for radioactivity on the restricted channels of a Packard liquid-scintillation spectrometer model 3375 and corrected for a 30% spill-over of ¹⁴C into the ³H channel.

Identification of mononucleoside hydrazones and dinucleoside monophosphate hydrazones. Ribonuclease digests of terminally labelled RNA were electrophoresed on Whatman 3 MM paper in 0.1 M-sodium formate buffer (pH 3). Electrophoresis was at 40 V/cm for 2 h. Under these conditions the two mononucleoside derivatives with most similar migration rates (G-iNicHz and U-iNicHz) are separated by about 1 cm. Marker mononucleoside hydrazones

and dinucleoside monophosphate hydrazones were prepared as described by Hunt (1965) and mixed with the digest of labelled RNA before electrophoresis. Their positions were determined under u.v. light; the electrophoretogram was cut into approx. 1.5cm strips, each strip wetted with 0.4ml of water and the radioactivity determined by liquid-scintillation counting. The scintillation solution contained Triton X-100 (1vol.) and toluene (2.5vol.) with 0.5% PPO and 0.05% POPOP.

Results

Preliminary studies showed that when 18S rRNA from a number of eukaryotes was condensed with [^3H]iNzd, the labelled 3'-terminal oligonucleotide released by T_1 -ribonuclease digestion was very similar in chain length to that released from rabbit reticulocyte 18S rRNA (Hunt, 1970). To determine whether the 3'-termini of 18S rRNA from different eukaryotes are in fact identical, we have examined in more detail the 3'-terminal sequence of 18S rRNA from yeast and *Drosophila*, two eukaryotes that

are markedly different phylogenetically both from each other and from rabbits.

Saccharomyces cerevisiae 18S rRNA was labelled with [^{14}C]iNzd and *Drosophila melanogaster* 18S rRNA with [^3H]iNzd. The two preparations were combined and digested with T_1 -ribonuclease. Chromatography of the digest on DEAE-Sephadex demonstrates that a single 3'-terminal heptanucleoside hexophosphate hydrazone is released from both 18S rRNA species by T_1 ribonuclease (Fig. 1). This establishes the 3'-terminal sequence of 18S rRNA as $\text{G}(\text{Z})_6\text{N}_{\text{OH}}$ in both instances (where N is any nucleoside and Z any nucleotide other than G). An identical result was obtained with 18S rRNA from cultured moth (*Antheraea eucalypti*) cells and has previously been reported for rabbit reticulocyte 18S rRNA (Hunt, 1970).

The sequence of the 3'-terminal octanucleotide was determined by pancreatic or T_1 -ribonuclease digestion of [^3H]iNzd-labelled 18S rRNA previously subjected to a series of stepwise degradations. The mononucleoside hydrazone or dinucleoside monophosphate hydrazone released by ribonuclease digestion

Fig. 1. Co-chromatography of a T_1 -ribonuclease digest of terminally labelled *Saccharomyces* and *Drosophila* 18S rRNA

[^3H]iNzd-labelled *Drosophila* 18S rRNA (100 μg) and [^{14}C]iNzd-labelled yeast 18S rRNA (1 mg) were digested with T_1 ribonuclease; 5 mg of a T_1 ribonuclease digest of unlabelled carrier RNA was added and the mixture applied to a column (1 cm $\times$ 25 cm) of DEAE-Sephadex A-25 in 10 ml of 7M-urea-0.01 M sodium-potassium phosphate buffer (pH 7.4). Elution was with a linear gradient of 0-0.5M-NaCl in 7M-urea-0.01 M-phosphate (pH 7.4) with a flow rate of 30 ml/h. The absorbance of the effluent was recorded with an ISCO u.v. monitor. $\circ$ — $\circ$, E_{254} ; $\circ$ — $\circ$, ^3H ; $\cdots$, ^{14}C ; —, NaCl gradient. Arrows labelled 1-12 refer to the elution positions of oligonucleotide hydrazones containing 1-12 nucleoside residues (Hunt, 1973; Shine & Dalgarno, 1974). Above 8 the numbering is somewhat arbitrary. The radioactivity eluted with the void volume is free [^3H]iNzd (determined by paper electrophoresis).

Fig. 2. Paper electrophoresis of ribonuclease digests of terminally labelled, stepwise-degraded *Drosophila* 18S rRNA

Drosophila 18S rRNA was subjected to six stepwise degradations. After the removal of successive 3'-nucleotides, a sample was removed, labelled with [³H]Nzd and digested with either pancreatic or T₁ ribonuclease. The digest (10 or 20 μl) was mixed with the appropriate unlabelled marker hydrazones and electrophoresed for 2 h at 40 V/cm. The arrows labelled U, G, A, C, AU, AC and N refer to the positions of the corresponding marker hydrazones and free iNzd. 0-6 refer to the number of stepwise degradations before labelling with [³H]Nzd. 0, 1, 2, 3 and 4 were digested with pancreatic ribonuclease, 5 and 6 with T₁ ribonuclease.

after each stepwise degradation was identified by paper electrophoresis. This procedure allows the determination of the sequence with double checks as the sequence progresses. The results obtained for *Drosophila melanogaster* 18S RNA are illustrated in Fig. 2 and the sequence determination is summarized in Table 1. These data indicate that the 3'-terminal sequence of *Drosophila melanogaster* 18S rRNA is G-A-U-C-A-U-U-A_{OH}. An identical result was obtained for *Saccharomyces cerevisiae* 18S rRNA.

The 18S RNA from both *Drosophila* and *Saccharomyces* was subjected to five stepwise degradations and labelled with [³H]iNzd. Pancreatic-ribonuclease

digestion released a pentanucleoside tetraphosphate hydrazone as the major labelled product in both instances. This establishes the 3'-terminal sequence as Y(R)₂G-A-U-C-A-U-U-A_{OH} for both organisms (where Y is a pyrimidine nucleoside and R a purine nucleoside). Sequence identity at the 3'-terminus may therefore extend for more than eight nucleotides, although the corresponding sequence for rabbit reticulocyte 18S rRNA is reported as Y(R)_{5or6}G-A-U-C-A-U-U-A_{OH} (Hunt, 1970).

In certain other eukaryotes examined the available data (Table 2) are consistent with a similar sequence to that obtained for yeast, *Drosophila* and rabbit reticulocyte 18S rRNA.

Table 1. Determination of the 3'-terminal sequence of *Drosophila melanogaster* 18S rRNA

For details see the text. Identical results were obtained with 18S rRNA from *Saccharomyces cerevisiae* (Dalgarno & Shine, 1973).

Number of stepwise degradations before labelling with [³ H]iNzd	Ribonuclease used	Major labelled digestion product*	Sequence
Nil	Pancreatic	A-iNicHz	Y-A _{OH}
1	Pancreatic	U-iNicHz	Y-U-A _{OH}
2	Pancreatic	A-U-iNicHz	Y-A-U-U-A _{OH}
3	Pancreatic	A-iNicHz	Y-A-U-U-A _{OH}
4	Pancreatic	C-iNicHz	Y-C-A-U-U-A _{OH}
5	T ₁	A-U-iNicHz	G-A-U-C-A-U-U-A _{OH}
6	T ₁	A-iNicHz	G-A-U-C-A-U-U-A _{OH}

* From Fig. 2.

Table 2. 3'-Terminal sequences of eukaryotic 18S rRNA

Organism	Sequence	Reference	Base composition (%) of 18S rRNA				Reference
			C	A	U	G	
<i>Drosophila melanogaster</i> flies*	G-A-U-C-A-U-U-A _{OH}	This study	20.3	28.8	27.4	23.5	Hastings & Kirby (1966)
<i>Saccharomyces cerevisiae</i>	G-A-U-C-A-U-U-A _{OH}	This study	19.1	26.6	28.1	26.1	Fauman <i>et al.</i> (1969)
Rabbit reticulocytes	G-A-U-C-A-U-U-A _{OH}	Hunt (1970)	24.3	22.2	21.2	32.1	Gould <i>et al.</i> (1966)
<i>Antheraea eucalypti</i> cells	G(Z) ₂ Y-A-U-U-A _{OH}	This study	21.7	25.4	23.2	29.7	J. Shine (unpublished work)
<i>Galleria mellonella</i> larvae	G(Z) ₅ Y-A _{OH}	This study	21.9	25.9	23.9	28.7	Ishikawa & Newburgh (1972)
African green-monkey kidney (Vero) cells	G(Z) ₅ Y-A _{OH}	This study	—	—	—	—	—
Baby-hamster kidney (BHK) cells	G(Z) ₅ Y-A _{OH}	This study	—	—	—	—	—
Avian myeloblasts	G-C[(AU) ₂ (C) ₂ (U) ₂]A _{OH}	Ahmad <i>et al.</i> (1972)	—	—	—	—	—

* No difference in the 3'-terminal sequence was found in 18S rRNA extracted from third-instar larvae of *Drosophila melanogaster*.

Discussion

The presence of the same sequence, G-A-U-C-A-U-U-A_{OH}, at the 3'-terminus of 18S rRNA from such phylogenetically different eukaryotes as yeast, *Drosophila* and rabbits contrasts with the less marked homology between the 3'-terminal sequences of the large rRNA from eukaryotes (Shine *et al.*, 1974). Thus the 3'-terminal sequence of 18S rRNA appears to have been conserved during eukaryotic evolution. The different base compositions of 18S rRNA from these organisms (Table 2) and the observation of only 10-13% homology between rRNA sequences of eukaryotes such as sea urchins and man (Birnstiel & Grunstein, 1972) support this view. The conservation of such a sequence suggests that it may have an important role in some common cellular function. As the complement of the 3'-terminal hexanucleotide is U-A-A-U-G-A-U-C, the 3'-terminal U-U-A_{OH} could therefore recognize UAA by standard pairing mechanisms and UAG, assuming 'wobble' in the third position (Crick, 1966); the adjacent U-C-A could recognize UGA. This suggests the possibility that 18S rRNA plays a direct role in the termination of protein synthesis through base-pairing with the terminator codons on mRNA. Such an interaction could signal the activation or attachment of the appropriate release factor(s) (Dalgarno & Shine, 1973).

The finding of the same 3'-trinucleotide (U-U-A_{OH}) in *Escherichia coli* 16S rRNA has prompted us to propose a similar role for the 3'-terminus of 16S rRNA (Shine & Dalgarno, 1974). We have suggested that, in this case, U-U-A_{OH} could recognize UAA and UAG by a mechanism similar to that proposed here for 18S rRNA and that UGA could pair with U-U-A_{OH} by utilizing two A-U pairs but with no net contribution to stability from the intervening G-U pair. Therefore interaction with UGA may be less efficient in *E. coli* owing to the presence of the G-U pair in the second position (Shine & Dalgarno, 1974). The evolution of an extra terminator anticodon (U-C-A) could permit eukaryotes to recognize UGA more efficiently.

The overall similarity of protein synthesis in prokaryotes and eukaryotes, and a number of observations indicating that the 3'-terminus of prokaryotic 16S rRNA is indispensable for protein synthesis, lend support to our proposed role for eukaryotic 18S rRNA. Thus treatment of susceptible *E. coli* with colicin E3 results in the rapid inhibition of protein synthesis; the sole lesion introduced is the removal of about 40 nucleotides from the 3'-terminus of 16S rRNA (Konisky & Nomura, 1967; Senior & Holland, 1971; Bowman *et al.*, 1971). It is significant that ascites-cell ribosomes are also inactivated by colicin E3 *in vitro* (Turnowsky *et al.*, 1973). Another antibiotic, kasugamycin, which also specifi-

cally inhibits protein synthesis, binds to the 30S ribosome subunit close to the 3'-terminus of 16S rRNA (Sparling, 1970; Helser *et al.*, 1971). A unique methylated sequence (m⁵A-m⁵A-C-C-U-G) near the 3'-terminus of 16S rRNA (Fellner *et al.*, 1972) is implicated in this binding (Helser *et al.*, 1971). A similar sequence is found in 18S rRNA from HeLa cells (Salim & Maden, 1973) and yeast (Klootwijk *et al.*, 1972).

We have recently proposed a dual role for the 3'-terminus of *E. coli* 16S rRNA in the recognition of ribosome-binding sites and terminator codons on prokaryotic mRNA (Shine & Dalgarno, 1974). It is possible that the 3'-terminus of eukaryotic 18S rRNA also has this dual function, although no information is available on ribosome-binding sites in eukaryotic mRNA and evidence also suggests that events occurring during formation of the initiation complex in eukaryotes may differ from those in prokaryotes (Schreier & Staehelin, 1973). However, it may be significant that the 3'-terminus of 18S rRNA contains the trinucleotide C-A-U which is complementary to the initiation triplet AUG.

This work was supported by a grant from the Australian Research Grants Committee.

References

- Ahmad, M. S., Markham, P. D. & Glitz, D. G. (1972) *Biochim. Biophys. Acta* **281**, 554-563
- Beaudet, A. L. & Caskey, C. T. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 619-624
- Birnstiel, M. L. & Grunstein, M. (1972) in *Functional Units in Protein Biosynthesis* (Cox, R. A. & Hadjiolov, A. A., eds.), Academic Press, London and New York
- Bowman, C. M., Dahlberg, J. E., Ikemura, T., Konisky, J. & Nomura, M. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 964-968
- Bretscher, M. S. (1968) *J. Mol. Biol.* **34**, 131-136
- Bretscher, M. S., Goodman, H. M., Menninger, J. R. & Smith, J. D. (1965) *J. Mol. Biol.* **14**, 634-639
- Capecchi, M. R. (1967) *Proc. Nat. Acad. Sci. U.S.* **58**, 1144-1151
- Capecchi, M. R. & Klein, H. A. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 469-477
- Crick, F. H. C. (1966) *J. Mol. Biol.* **19**, 548-555
- Dalgarno, L. & Shine, J. (1973) *Nature (London) New Biol.* **245**, 261-262
- Fauman, M., Rabinowitz, M. & Getz, G. S. (1969) *Biochim. Biophys. Acta* **182**, 355-360
- Fellner, P., Ehresmann, C., Stiegler, P. & Ebel, J.-P. (1972) *Nature (London) New Biol.* **239**, 1-5
- Fox, J. L. & Ganoza, M. C. (1968) *Biochem. Biophys. Res. Commun.* **32**, 1064-1070
- Gordon, P. A. & Stewart, P. R. (1971) *Microbios* **4**, 115-132
- Gould, H. J., Arnstein, H. R. V. & Cox, R. A. (1966) *J. Mol. Biol.* **15**, 600-618
- Grace, T. D. C. (1962) *Nature (London)* **195**, 788-789
- Haselkorn, R. & Rothman-Denes, L. B. (1973) *Annu. Rev. Biochem.* **42**, 397-438

- Hastings, J. R. B. & Kirby, K. S. (1966) *Biochem. J.* **100**, 532-539
- Hawthorne, D. C. (1969) *J. Mol. Biol.* **43**, 71-75
- Helser, T. L., Davies, J. E. & Dahlberg, J. E. (1971) *Nature (London) New Biol.* **233**, 12-14
- Hunt, J. A. (1965) *Biochem. J.* **95**, 541-551
- Hunt, J. A. (1970) *Biochem. J.* **120**, 353-363
- Hunt, J. A. (1973) *Biochem. J.* **131**, 315-325
- Ishikawa, H. & Newburgh, R. W. (1972) *J. Mol. Biol.* **64**, 135-144
- Klootwijk, J., van den Bos, R. C. & Planta, R. J. (1972) *FEBS Lett.* **27**, 102-106
- Konisky, J. & Nomura, M. (1967) *J. Mol. Biol.* **26**, 181-195
- Raghow, R. S., Grace, T. D. C., Filshie, B. K., Bartley, W. & Dalgarno, L. (1973) *J. Gen. Virol.* **21**, 109-122
- Salim, M. & Maden, B. E. H. (1973) *Nature (London)* **244**, 334-336
- Schreier, M. H. & Staehelin, T. (1973) *Nature (London) New Biol.* **242**, 35-38
- Scolnick, E. M. & Caskey, C. T. (1969) *Proc. Nat. Acad. Sci. U.S.* **64**, 1235-1241
- Scolnick, E., Tompkins, R., Caskey, T. & Nirenberg, M. (1968) *Proc. Nat. Acad. Sci. U.S.* **61**, 768-774
- Senior, B. W. & Holland, I. B. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 959-963
- Shine, J. & Dalgarno, L. (1973) *J. Mol. Biol.* **75**, 57-72
- Shine, J. & Dalgarno, L. (1974) *Proc. Nat. Acad. Sci. U.S.* **71**, 1342-1346
- Shine, J., Hunt, J. A. & Dalgarno, L. (1974) *Biochem. J.* **141**, 617-625
- Sparling, P. F. (1970) *Science* **167**, 56-58
- Stewart, J. W. & Sherman, F. (1972) *J. Mol. Biol.* **68**, 429-443
- Turnowsky, F., Drews, J., Eich, F. & Hogenauer, G. (1973) *Biochem. Biophys. Res. Commun.* **52**, 327-334