# THE NUCLEOTIDE SEQUENCES ADJACENT TO THE 5'-TERMINI OF YEAST SOLUBLE RIBONUCLEIC ACIDS

David Bell, R. V. Tomlinson and G. M. Tener*
Department of Biochemistry
University of British Columbia, Vancouver, British Columbia

Received January 21, 1963

It is well established that soluble ribonucleic acid (s-RNA) is a mixture of short chain polyribonucleotides, of molecular weight 20,000 to 30,000 (Osawa, 1960; Luborsky and Cantoni, 1962) having predominantly pGp... at the 5'-termini (Zillig et al., 1960; Singer and Cantoni, 1960) and ...pCpCpA sequences at the 3'-hydroxyl ends (Zachau et al., 1958; Hecht et al., 1958).

Using a technique which involved labelling of the terminal 5'-phosphate groups of s-RNA with radioactive aniline, Ralph et al. (1962) recently confirmed the predominance of pGp... as the 5'-terminal moiety, and at the same time obtained evidence for the probable existence of pAp... and pUp... ends also.

This report describes experiments which demonstrated the existence of chains terminating in pGp..., pUp..., pAp... and pCp..., and also an investigation of the adjacent nucleotide sequences.

EXPERIMENTAL. s-RNA was isolated from growing yeast by the direct phenol extraction technique of Monier et al. (1960). The crude s-RNA was incubated at 37° for 1 hr. in ammonium hydroxide at pH 9.0, then further purified by column chromatography on DEAE-cellulose (chloride) followed by ethanol precipitation.

---

* Medical Research Associate, Medical Research Council of Canada.
This work was supported by the United States Public Health Service Grant C-5342.

Digestion of s-RNA with alkali: A sample (235 mg.) of purified s-RNA was treated with 0.3 N sodium hydroxide for 18 hrs. at 37°. These conditions are known (Markham and Smith, 1952; Singer and Cantoni, 1960) to degrade s-RNA to give ribonucleosides from the 3'-hydroxyl ends of the chains, ribonucleoside-(2') 3',5' diphosphates from the 5'-terminus and ribonucleoside-(2') 3' phosphates plus a few alkali resistant dinucleotides (Lane and Butler, 1959) from the non-terminal positions.

The digest was neutralised with Dowex-50 (hydrogen) resin, applied to a DEAE-cellulose column (2 x 25 cm.) and eluted with a linear gradient of ammonium carbonate, 0.0 to 0.3 M (total volume, 5 litres), pH 8.7. The ammonium carbonate was subsequently removed by repeated evaporation under vacuum. The ultra violet absorbing material was eluted in three distinct regions. The first, which contained a total of 64 optical density (O.D.) units*, was shown (by paper chromatography of an aliquot, with authentic nucleoside markers, in water saturated butanol with ammonia in the vapour phase) to contain adenosine - 80% and cytidine - 20%. The second region contained ribonucleoside monophosphates (4,800 O.D. units) and the third contained alkali resistant dinucleotides and the nucleoside diphosphates, totalling 108 O.D. units. This last region was subdivided into five peaks, each of which was pooled as a separate fraction. After the removal of salt, two aliquots were taken from each of the five fractions. One was treated with E. coli phosphomonoesterase (PME) and then both were subjected to paper electrophoresis in 0.05 M citrate buffer, pH 5.0. The ultra violet absorbing areas were cut out, eluted with water and the spectra of the eluates recorded under acid, neutral and alkaline conditions. The dinucleotides were readily distinguished as those components which migrated on electrophoresis both before and

* An optical density unit is that amount of material per ml. which, in a 1 cm. light path, at 271 mμ, gives a spectrophotometric reading of one.

after PME treatment. Many of these had spectra which clearly indicated the presence of more than one base. The nucleoside diphosphates, which moved well ahead of the dinucleotides on electrophoresis before PME treatment, were converted to nucleosides by the enzyme and consequently remained at the origin on electrophoresis after PME treatment. The spectra of the fast moving nucleoside diphosphates corresponded to those of the non-migrating nucleosides and to the spectra of standard compounds. All five peaks were shown to contain dinucleotides. The first peak also contained pCp, the second pUp, the fourth pAp and the fifth pGp. The total nucleoside diphosphate (59 O.D. units) and the total nucleoside released from the 3'-termini, were compatible with an average chain length for this s-RNA of 75-85 nucleotide units. Of the s-RNA chains, 78% terminated in pGp... 10% in pUp..., 7% in pAp... and 5% in pCp.

Digestion of s-RNA with pancreatic ribonuclease (RNase): Purified s-RNA (380 mg) was dissolved in 30 ml. of 0.1 M Tris-hydrochloride buffer, pH 7.8, 10 mg of RNase were added and the mixture incubated for 22 hrs. at 37°. It was then diluted with 120 ml. of 7 M urea/ 0.005 M Tris-hydrochloride, pH 7.8 and applied to a DEAE-cellulose (chloride) column (2 x 100 cm.) previously equilibrated against the same solution. The column was washed with 250 ml. of the equilibration solution and then subjected to a gradient of sodium chloride, 0.0 to 0.3 M (total volume, 8 l), both solutions being 7 M in urea and 0.005 M in Tris-hydrochloride, pH 7.8.

In this system the digestion products were eluted as a series of peaks (see Figure 1) in which each major peak contained oligonucleotides bearing the same net negative charge (Tomlinson and Tener, 1962). Thus the fragments from the 5'-termini [i.e. of general form $p(Pup)_nPyp$] were eluted along with oligonucleotides, from inside the chain, which had no phosphate on the 5'-hydroxyl position but contained two more purine nucleotides (Tomlinson and

Tener, submitted to Biochemistry). That is to say, peak 3 (Figure 1) contained pPyp plus PupPupPyp; peak 4 contained pPupPyp plus PupPupPupPyp, and so on.

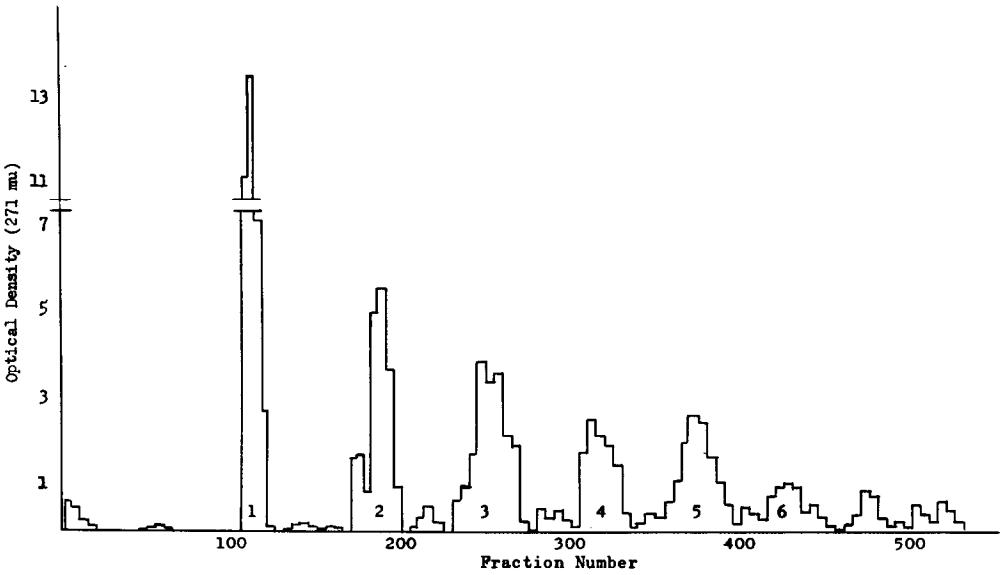


Figure 1. Elution pattern for products of RNase digestion of s-RNA (see text). Fraction volume 14 ml.

---

Urea and sodium chloride were removed from peaks 3, 4, 5 and 6 (which contained about 90% of the 5'-termini) by re-adsorption of the diluted pools on DEAE-cellulose (carbonate) followed by washing with 0.01 M ammonium carbonate (Rushizky and Sober, 1962). The nucleotide material was then eluted with 2 M ammonium carbonate and the ammonium carbonate removed by repeated evaporation to dryness in vacuo. The material from each of the peaks was incubated with PME to remove the primary phosphate groups. This treatment reduced the charge of the end-sequences by four (at pH 7.8), whereas the non-terminal material lost only two charges. As the terminal sequences in each peak then differed from the non-terminal sequences of that peak by two charges, they could be readily isolated by chromatography on DEAE-cellulose in the presence of 7 M urea.

The nucleosides isolated in this way from peak 3 were identified by paper chromatography with authentic nucleoside markers, in water saturated butanol with ammonia in the vapour phase and also in isobutyric acid : water : concentrated ammonia : : 66 : 33 : 1, by volume. The appropriate UV-absorbing areas were cut out, eluted with water and the spectra of the eluates taken. Only uridine and cytidine were found. The quantitative data obtained are shown in Table I.

TABLE I

Analysis of end-sequences isolated after RNase digestion

| Type of end-sequence obtained by RNase digestion | | pPyp | pPupPyp* | pPupPupPyp | pPupPupPupPyp |
|---|---|---|---|---|---|
| per cent of total end recovered | | 20.5 | 30.0 | 42.0 | 7.0 |
| Nucleosides isolated after SVD and PME treatment, per cent | A | - | 0.5 | 19.5 | 12.5 |
| | C | 36.0 | 48.0 | 13.5 | 21.0 |
| | G | - | 50.0 | 54.0 | 65.0 |
| | U | 64.0 | 1.5 | 13.0 | 1.5 |
| Ratio Pu/Py | | 0 | 1.02 | 2.8 | 3.4 |

* This material was shown (see text) to consist of pGpCp - 95.5%, pApUp - 2.5%, pGpUp - 1.5% and perhaps a trace of pApCp.

Samples of the PupPy from peak 4, PupPupPy from peak 5 and PupPupPupPy from peak 6 were incubated with a mixture of snake venom phosphodiesterase (SVD) and PME and the resulting nucleosides identified by paper chromatography in water saturated butanol with ammonia in the vapour phase, as above. The UV-absorbing areas were cut out, eluted with water and the spectra of the eluates taken. The percentage composition of each peak was calculated on the basis of the micromoles of each nucleoside recovered (see Table I).

The dinucleoside phosphates isolated from peak 4 were readily separated by paper chromatography in the ammonium isobutyrate system and also by electrophoresis in ammonium formate, pH 3.5. The individual components were identified by comparison with known dinucleoside phosphates in these systems and where possible from spectral data. The results of this analysis are shown as a footnote to Table I.

The complex results obtained in the same systems with the trinucleoside diphosphates, isolated from peak 5, precluded identification of the individual sequences present. This complexity, together with the high purine to pyrimidine ratio obtained for this fraction, seemed to indicate contamination with non-terminal material. This might have arisen through breakdown of the pentanucleotides which constituted the major part of peak 5 (perhaps during the removal of ammonium carbonate, after DEAE-chromatography in the presence of urea) to give compounds of the form PupPupPyp and PupPu-cyclic-p. Unlike any other breakdown products these compounds would not be separated from the terminal sequences by the subsequent treatment with PME and re-chromatography and would complicate the problem of isolating the individual sequences present by paper chromatography or electrophoresis. Some indication that this had, in fact, occurred was obtained when adenosine-2',3' cyclic phosphate was tentatively identified among the products formed by digestion of a sample of the supposed trinucleoside diphosphates with purified SVD. The purified form of this enzyme has negligible activity against ribonucleoside-2',3' cyclic phosphates (Khorana, 1961).

In the case of the PupPupPupPy, obtained from peak 6, the degree of contamination appeared to be much less, but lack of material prevented further investigation. It is clear, however, that very mild conditions must be used in the isolation of materials of this type.

## REFERENCES

Hecht, L. I., Stephenson, M. L., Zamecnik, P. C. and Scott, J. F., J. Biol. Chem. (1958) 233, 954.
Khorana, H. G., The Enzymes, Ed. Boyer, P. D., Lardy, H. and Myrback, K., Academic Press, New York & London. 1961.
Lane, B. G. and Butler, G. C., Can. J. Biochem. and Physiol. (1959) 37, 1329.
Luborsky, S. and Cantoni, G. L., Biochem. et Biophys. Acta (1962) 61, 481.
Markham, R. and Smith, J. D., Biochem. J. (1952) 52, 565.
Monier, R., Stephenson, M. L. and Zamecnik, P. C., Biochem. et Biophys. Acta (1960) 49, 1.
Osawa, S., Biochem. et Biophys. Acta (1960) 43, 110.
Ralph, R. K., Young, R. J. and Khorana, H. G., J. Am. Chem. Soc. (1962) 84, 1490.
Rushizky, G. W. and Sober, H. A., Biochem. et Biophys. Acta (1962) 55, 217.
Singer, M. F. and Cantoni, G. L., Biochem. et Biophys. Acta (1960) 39, 182.
Tomlinson, R. V. and Tener, G. M., J. Am. Chem. Soc. (1962) 84, 2644.
Zachau, G. H., Acs, G. and Lipmann, F., Proc. Nat. Acad. Sci., U. S. (1958) 44, 885.
Zillig, W., Schachtschnabel, D. and Krone, W., Z. Physiol. Chem. (1960) 318, 100.