The authors acknowledge the good advice, valuable criticism, and pure α-hydroxy-β-carboxy-isocaproic acid kindly supplied by H. E. Umbarger.

* This work was supported in part by grant GB-2094 from the National Science Foundation.

[1] Reviewed by Simpson, M. V., in *Ann. Rev. Biochem.*, **31**, 333 (1962).

[2] Fangman, W. L., and F. C. Neidhardt, *J. Biol. Chem.*, **239**, 1844 (1964).

[3] Eidlic, L., and F. C. Neidhardt, *J. Bacteriol.*, in press.

[4] Schlesinger, S., and B. Magasanik, *J. Mol. Biol.*, **9**, 670 (1964).

[5] Coles, R. S., and P. Rogers, *Bacteriol. Proc.*, 87 (1964).

[6] Fraenkel, D. G. and F. C. Neidhardt, *Biochim. Biophys. Acta*, **53**, 96 (1961).

[7] Gorini, L., *Bull. Soc. Chim. Biol.*, **40**, 1939 (1958).

[8] Umbarger, H. E., and B. Brown, *J. Biol. Chem.*, **233**, 415 (1958).

[9] Bauerle, R. H., M. Freundlich, F. C. Stormer, and H. E. Umbarger, *Biochim. Biophys. Acta*, **92**, 142 (1964).

[10] Freundlich, M., R. O. Burns, and H. E. Umbarger, these PROCEEDINGS, **48**, 1804 (1962).

[11] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[12] Reviewed by Gorini, L., *Bacteriol. Rev.*, **27**, 182 (1963).

[13] Strain I-9 grows in rich or minimal media at 30° and fails to grow at 37° in any medium, no matter how rich. Extracts prepared from cultures incubated at either 30 or 37° possess normal levels of aminoacyl-sRNA synthetase activity for 17 amino acids tested, but exhibit virtually no valyl sRNA synthetase activity, as measured by C^{14}-amino acid attachment to sRNA. When strain I-9 (F^-) is mated with K10 (Hfr), recombinants selected for growth at 37° possess normal valyl-sRNA synthetase activity. Apparently the valyl-sRNA synthetase of I-9 is unstable *in vivo* at 37°, and is unstable *in vitro* at any temperature. These results are all reported in ref. 3.

---

*SPECIFIC CLEAVAGE OF THE YEAST ALANINE RNA INTO TWO LARGE FRAGMENTS**

BY JOHN ROBERT PENSWICK AND ROBERT W. HOLLEY

DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY, AND U.S. PLANT, SOIL AND NUTRITION LABORATORY, U.S. DEPARTMENT OF AGRICULTURE, ITHACA, NEW YORK

Communicated by A. T. Blomquist, January 4, 1965

Nucleotide sequence determination of the alanine RNA has now reached the stage where the structures of the fragments produced by the action of pancreatic RNase and takadiastase RNase T1 are largely known. The remaining problem is to arrange the fragments, obtained from the two RNase digests, into a single sequence. Minor nucleotides and unique small sequences furnish overlaps between the two digests and establish sequences as long as 10 nucleotides. The determination of longer sequences requires additional information. Since it was anticipated that the isolation of large fragments from the RNA would be extremely useful, the possibility of limited digestion of the alanine RNA with RNase T1 was investigated. The results, described in this communication, demonstrate that the RNA can be cleaved specifically into two large fragments. The separation and analysis of the fragments is described.

A similar approach by Litt and Ingram,[1] using pancreatic RNase, gave large fragments, but a specific cleavage was not observed.

Limited Digestion of the Alanine RNA with RNase T1 and Separation of the Two Large Fragments.—To a solution of 5 mg of the yeast alanine RNA[2] in 1.0 ml of

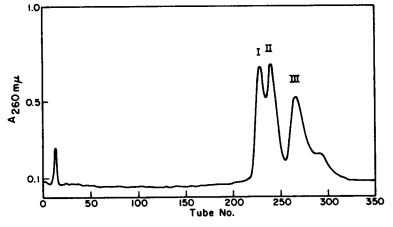

FIG. 1.—Chromatographic separation of two large fragments (I and II) obtained by limited digestion of the yeast alanine RNA with RNase T1.

0.02 *M* magnesium chloride in 0.02 *M* pH 7.5 Tris (chloride) buffer at 0° was added 0.10 ml (25 units) of an ice-cold solution of RNase T1 (Sankyo, Ltd., Tokyo) in the same buffer. After 4 min at 0°, the reaction was terminated by mixing the solution with 2 ml of phenol (previously equilibrated with an equal volume of 0.02 *M* magnesium chloride in 0.02 *M* pH 8.0 Tris buffer). The extraction was repeated twice with 2-ml portions of phenol, and the last traces of phenol were then removed by five extractions with ether. The aqueous solution was made 7 *M* with respect to urea, and the solution was diluted to 10 ml with 0.02 *M* pH 8.0 Tris in 7 *M* urea, for application to a DEAE-cellulose column.

DEAE-cellulose (Schleicher and Schuell, 70, Medium, 0.9 meq per gm, with fines removed by suspension in water) was washed with 0.2 *M* acetic acid, water, the maximum concentration of the elution gradient, and finally with water, and was stored in water. Before use, the DEAE-cellulose was washed thoroughly with 0.02 *M* pH 8.0 Tris (chloride) in 7 *M* urea, and a suspension of the DEAE-cellulose in this solution was used to pack a column 0.5 × 100 cm, allowing the DEAE-cellulose to settle by gravity. The solution of the limited RNase T1 digest described above was allowed to flow onto the column, and the last traces were washed into the column with 1 ml of the Tris-7 *M* urea solution. The column was eluted with a linear gradient of sodium chloride, formed from 200 ml each of 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 *M* sodium chloride in 7 *M* urea containing 0.02 *M* pH 8.0 Tris (cf. Tomlinson and Tener[3]) in six chambers of a Varigrad.[4] Fraction volumes were approximately 3.2 ml.

Peaks I and II were rechromatographed on DEAE-cellulose as above. (The contents of tubes comprising one peak in Fig. 1 were combined and diluted with an equal volume of water and the solution was applied directly to the column.) For analysis, the rechromatographed peaks were recovered by dialysis followed by evaporation in a rotary evaporator with a bath temperature of 40°C.

Analysis of Complete RNase T1 Digests.—Approximately 1 mg of rechromatographed peak I or peak II, or 2 mg of the alanine RNA, was dissolved in 2.0 ml of 0.1 *M* pH 7.5 Tris (chloride) buffer, and 0.4 ml (1000 units) of RNase T1 in 0.02 *M* pH 7.5 Tris, and 0.05 ml of chloroform was added. After incubation for 2 hr at 37°, 4.2 gm of urea were added, and the solution was diluted to 10 ml and applied to a 0.35 × 220-cm column of DEAE-cellulose packed in 7 *M* urea-0.02 *M* pH 8.0 Tris as above. (The column was packed in two 110-cm sections, which were then joined by a small piece of Tygon tubing.) The column was eluted with a linear gradient formed from 200 ml each of 0 and 0.3 *M* sodium chloride in 7 *M* urea–0.02 *M* pH 8.0 Tris. Fraction volumes were approximately 1.2 ml.

Results and Discussion.—Results of chromatography of the limited digest of the alanine RNA are shown in Figure 1. Assays of the peaks for alanine-acceptor activity[5] indicated that peak III was unchanged alanine RNA. Peaks I and II were inactive, and the positions of elution suggested that these peaks were large fragments of the RNA. Rechromatography of peak I or II at room temperature or

at 55° gave no evidence for the presence of more than one component in either peak.

Peaks I and II were analyzed by complete digestion with RNase T1 followed by chromatography of the products on DEAE-cellulose in 7 *M* urea. The chromatographic patterns were compared (Fig. 2) with the pattern obtained from a complete digest of the alanine RNA. As is shown in Figure 2, and summarized in Table 1, the analyses of peaks I and II were mutually exclusive and were complementary to each other. The sum of the analyses of I and II corresponds to the analysis of the original alanine RNA, indicating that the RNA molecule was cleaved into two large fragments of almost equal size. Each of the fragments was obtained over 90 per cent pure. The analyses, summarized in Table 1, divide the RNase T1 digestion products of the RNA into two groups corresponding to the two halves of the RNA molecule.

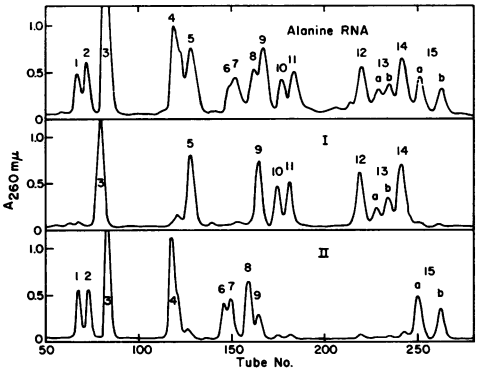

FIG. 2.—Chromatography of products of complete RNase T1 digestion of the alanine RNA, and of rechromatographed fragments I and II, on DEAE-cellulose in 7 *M* urea.

TABLE 1

ANALYSES OF RNASE T1 DIGESTS OF TWO LARGE FRAGMENTS OF THE ALANINE RNA

Peak no. (Fig. 2)	Identity*	Absorbancy Units at 260 mμ Fragment I	Fragment II	Alanine RNA
1	CpDiMeGp!		1.9	1.9
2	UpMeGp!		1.9	2.2
3	9 Gp	5.7	5.4	10.7
4	4 CpGp + UpGp		6.3	6.7
5	2 ApGp	4.3		5.1
6	DiHUpCpGp		1.4	3.3
7	DiHUpApGp		2.1	
8	UpApGp		2.7	2.8
9	UpCpCpApCpC + pGp	3.6	1.2	4.8
10	CpMeIpψpGp	2.1		2.0
11	TpψpCpGp	2.3		2.9
12	ApCpUpCpGp	3.4		3.4
13	Up(Cp,Up)CpCpGp	3.2		3.3
14	ApUpUpCpCpGp	4.1		4.3
15	Cp(Cp,Cp,Up)CpUpUpIp		4.2	4.3

* Identifications of the oligonucleotides in peaks 1–15 are described in detail in ref. 6. Also see ref. 7.

Studies of the chemical and physical properties of the two large fragments are under way.

The discovery that RNase T1 is highly selective in its action at 0° greatly simplifies approaches to the determination of the nucleotide sequences of nucleic acids.

* This work was supported in part by the National Science Foundation.

[1] Litt, M., and V. M. Ingram, *Biochemistry*, **3**, 560 (1964).

[2] Apgar, J., R. W. Holley, and S. H. Merrill, *J. Biol. Chem.*, **237**, 796 (1962).

[3] Tomlinson, R. V., and G. M. Tener, *Biochemistry*, **2**, 697 (1963).

[4] Peterson, E. A., and H. A. Sober, *Anal. Chem.*, **31**, 857 (1959).

[5] The authors wish to thank Susan H. Merrill for the determinations of amino acid-acceptor activity.

[6] Holley, R. W., G. A. Everett, J. T. Madison, and A. Zamir, *J. Biol. Chem.*, in press.

[7] Apgar, J., G. A. Everett, and R. W. Holley, these PROCEEDINGS, **53**, 546 (1965).

---

*ISOLATION OF LARGE OLIGONUCLEOTIDE FRAGMENTS FROM THE ALANINE RNA**

BY JEAN APGAR, GEORGE A. EVERETT, AND ROBERT W. HOLLEY

U.S. PLANT, SOIL AND NUTRITION LABORATORY, U.S. DEPARTMENT OF AGRICULTURE, AND DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY

Communicated by A. T. Blomquist, January 4, 1965

The discovery that very brief treatment of the alanine RNA with RNase T1 cleaves the RNA specifically into two large fragments[1] suggested that this enzyme might give additional specific cleavages if digestion conditions were a little more vigorous. The isolation of several large oligonucleotides resulting from such specific cleavages is the subject of this communication.

Yeast alanine RNA[2] was digested with 225 units of RNase T1 (Sankyo, Ltd., Tokyo) per mg of RNA in 0.2 *N* pH 7.5 Tris buffer for 1 hr at 0°C; and, after removal of the RNase T1 by phenol extraction, the digest was chromatographed on DEAE-cellulose in 7 *M* urea. The chromatographic pattern is shown in Figure 1. For comparison, a pattern obtained after complete digestion of the alanine RNA

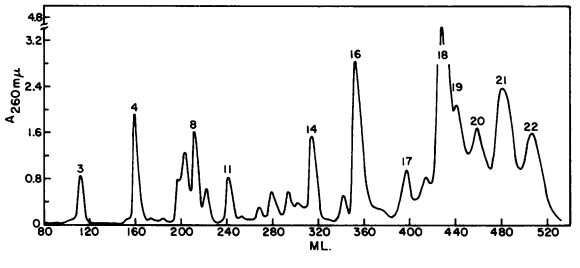

FIG. 1.—Chromatography of a partial RNase T1 digest (1 hr at 0°) of the alanine RNA on a 0.35 × 210-cm column of DEAE-cellulose in 7 *M* urea–0.02 *M* pH 8.0 Tris (chloride). Procedures were as described in ref. 1, except that the elution gradient was formed from 300 ml of 7 *M* urea–0.02 *M* pH 8.0 Tris and 300 ml of 0.4 *M* sodium chloride in 7 *M* urea–0.02 *M* pH 8.0 Tris.

with RNase T1 and chromatography under identical conditions is shown in Figure 2. Comparison of Figures 1 and 2 indicates that many small fragments present in Figure 2 are absent in the limited digest shown in Figure 1. In particular, peaks 1, 2, 5, 9, 10, 12, 13, and 15 are missing or greatly reduced in Figure 1. A number of large fragments, present in peaks *16–22*, are found instead. Analyses of four of these large fragments are summarized below.

Complete digestion of peak *16* with RNase T1, followed by chromatography, gave peaks *9* and *12*, as is shown in Figure 3. Peak *9* is UpCpCpApCpC, the amino