Cells that were examined shortly after being subjected to pressure had a significantly smaller diameter than they had prior to treatment, and no gas vacuoles were seen under the light microscope (Fig. 1*B*). The arrays of electron-transparent vesicles seen in electron micrographs of control material were conspicuously absent in cells subjected to pressure (Fig. 2*B*). Instead, many short membranous elements, each 6 mμ wide, 200 mμ or more in length, and resembling a typical "unit membrane," were seen in the regions of the treated cell where vesicles appeared in control cells.

Gas vacuoles were visible by 9 hours and were extensive after 24 hours of recovery from pressure treatment. Electron micrographs of cells allowed to recover for 9 hours showed some scattered electron-transparent vesicles in the locality of the 6-mμ membranous elements, and cells allowed to recover for 24 hours showed fairly extensive arrays of such vesicles (Fig. 2*C*).

We conclude that the vesicular aggregates we see with the electron microscope can be identified with the gas vacuoles observed by the light microscopist, and that individual gas vesicles can reversibly collapse and expand. The membrane of the collapsed gas vesicle has the dimensions and configuration of a unit membrane, and of a half-unit membrane when the vesicle is expanded by gas (Fig. 2*C*). However, unlike typical unit membranes, membranes of gas vesicles are not preserved by fixation with $KMnO_4$.

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5. Supported by USPHS grant HD 01248.

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Structure of a Ribonucleic Acid

Abstract. *The complete nucleotide sequence of an alanine transfer RNA, isolated from yeast, has been determined. This is the first nucleic acid for which the structure is known.*

Transfer RNA's are the smallest biologically active nucleic acids known. Their function is to carry activated amino acids to the site of protein synthesis. During protein synthesis, the amino acid sequence of the polypeptide chain is determined by the interaction of a messenger RNA with transfer RNA's specific for a given amino acid. The structures of transfer RNA's are crucial in this process.

Three transfer RNA's, obtained from yeast, and specific for alanine, tyrosine, and valine, respectively, have been purified in our laboratories (*1*). Studies of the compositions of pancreatic ribonuclease digests have established that the three RNA's have quite different structures, as indicated by oligonucleotide sequences (*2*). The identification of a number of oligonucleotides obtained from the three RNA's has been described (*3*). We now summarize the determination of the first complete nucleotide sequence, that of the alanine RNA.

The structure determination involved the identification of small fragments formed by complete digestion of the RNA with pancreatic ribonuclease and takadiastase ribonuclease T1, followed by the determination of the structures of successively larger fragments, until the complete sequence of the RNA was established.

Table 1. List of fragments obtained by complete digestion of the alanine RNA with pancreatic ribonuclease.

	COH†	MeG-G-C-
13	C-	A-G-C-
	ψ-	A-G-DiHU-
6	U-	G-A-U-
	A-C-	I-G-C-
	MeI-ψ-	G-G-T-
	DiMeG-C-	G-G-DiHU-
2	G-C-	G-G-A-C-
4	G-U-	pG-G-G-C-
	G-G-G-A-G-A-G-U*-	

† The presence of a free 3′-hydroxyl group on this fragment indicates that cytidine occupies the terminal position in the purified alanine RNA. This establishes that the terminal adenylic acid residue is missing, as it is from most transfer RNA's isolated from commercial baker's yeast. A terminal adenylic acid residue is replaced under assay conditions before the amino acid is attached.

Complete digestion of the alanine RNA with pancreatic ribonuclease, an enzyme that cleaves the RNA chain next to pyrimidine nucleotides, for example, C- and U- (*4*), gives the 19 products listed in Table 1 (*5*). Complete digestion of the RNA with takadiastase ribonuclease T1, a highly specific enzyme (*6*) that cleaves the RNA chain next to G- and I-, gives the 17 products listed in Table 2 (*5*). Proof of the structures of these products required the use of both classical and new methods of sequence determination, as well as the identification of certain new nucleotides (*5, 7*).

Combination of the results summarized in Tables 1 and 2 permits description of the structure of the alanine RNA in terms of 16 oligonucleotide sequences, shown in Table 3 (*5*). Except for the positions of the two end sequences, the arrangement of the 16 oligonucleotide sequences is not established by these data. The presence of a 5′-phosphate on the pG-G-G-C- sequence establishes that this is the left end of the RNA molecule as conventionally written, and the 3′-hydroxyl on the U-C-C-A-C-C-AOH sequence establishes that this is the right end of the molecule. The 16 oligonucleotide sequences account for a total of 77 nucleotide residues and give a calculated molecular weight for the RNA of 26,600 as the sodium salt.

Takadiastase ribonuclease T1 cleaves the alanine RNA selectively, under controlled conditions, and gives a number of large oligonucleotide fragments (*8, 9*). Analysis of these large fragments, in combination with the data in Tables 1 to 3, has furnished sufficient information to establish the complete nucleotide sequence of the RNA.

The structure of the alanine RNA is shown at the top of Fig. 1. Large fragments that were crucial in the proof of structure are shown in the lower part of Fig. 1.

Summary of proof of structure. To determine the structures of the large oligonucleotide fragments *a* to *k* (Fig. 1), a large fragment was digested with ribonuclease T1 giving certain of the previously identified fragments listed in Table 2, and additional information was then used to establish the arrangement of these complete digest fragments.

The isolation and proof of structure of fragments *a*, *b*, *c*, and *d* (Fig. 1) have been described (*8*). These frag-

ments were obtained from a limited ribonuclease T1 digest (1 hour at 0°C) of the RNA. Fragments *e* to *i* were isolated by rechromatography (at 55°C) of the largest fragments obtained from the same limited digest. The two halves of the RNA molecule, *j* and *k*, were obtained from a much more limited ribonuclease T1 digest (*9*).

Analyses of complete ribonuclease T1 digests of *j* and *k* indicated that the fragments listed in Table 2 fell into two groups, corresponding to the two halves of the molecule (*9*). Determination of the complete structure of *k*, the right half of the molecule, was simpler and will be considered first.

The analyses established that *k* contained the ribonuclease T1 fragments present in *c* and *d* plus three oligonucleotides, U*-C-U-C-C-G-, T-ψ-C-G, and A-U-U-C-C-G-, as well as additional G-'s. Fragment *g* gave, on ribonuclease T1 digestion, the components of *d* plus one of these oligonucleotides, A-U-U-C-C-G-, and one G-. The sequence shown for *g* in Fig. 1 is the only arrangement of these fragments that is consistent with the presence of a G-G-A-C- sequence in the RNA (Table 1).

Table 2. List of fragments obtained by complete digestion of the alanine RNA with taka-diastase ribonuclease Tl.

9	G-	DiHU-C-G-
	pG-	DiHU-A-G-
	C-DiMeGp!	C-MeI-ψ-G-
	U-MeGp!	T-ψ-C-G-
4	C-G-	A-C-U-C-G-
2	A-G-	U-C-C-A-C-COH†
	U-G-	U*-C-U-C-C-G-
	U-A-G-	A-U-U-C-C-G-
	C-U-C-C-C-U-U-I-	

† See Table 1.

Fragment *f* contained all the ribonuclease T1 fragments present in *k* that were not already accounted for in *g*. The structure of *f* was established by two pieces of information. First, the presence of G-G-G-A-G-A-G-U*- in a pancreatic ribonuclease digest of the RNA (Table 1) places the sequence U*-C-U-C-C-G- at the right end of *c* (Table 3). Second, pancreatic ribonuclease digestion of *f* gave G-G-T-, which could be obtained only if the sequence G-T-ψ-C-G is at the right end of the U*-C-U-C-C-G- sequence. The sequence of *f* must therefore be that shown in Fig. 1.

Fragment *i* confirmed the nature of the attachment of *f* to *g*. The sequence of *k*, the right half of the molecule, is therefore that shown in Fig. 1.

Determination of the structure of the left half of the molecule was more complicated, with the proof of structure of *e* being most difficult. Essential information was obtained from partial and complete enzymatic digestion of *e*, as well as from limitations imposed by the sequences shown in Table 1. Complete degradation of *e* with ribonuclease T1 gave four fragments, DiHU-C-G, G-, DiHU-A-G-, and C-G-, in addition to the components of *b*. Digestion of *e* with pancreatic ribonuclease gave Ip!, establishing that Ip! must be at the right end of *e*. All four of the small ribonuclease T1 fragments must therefore be to the left of *b*. Five

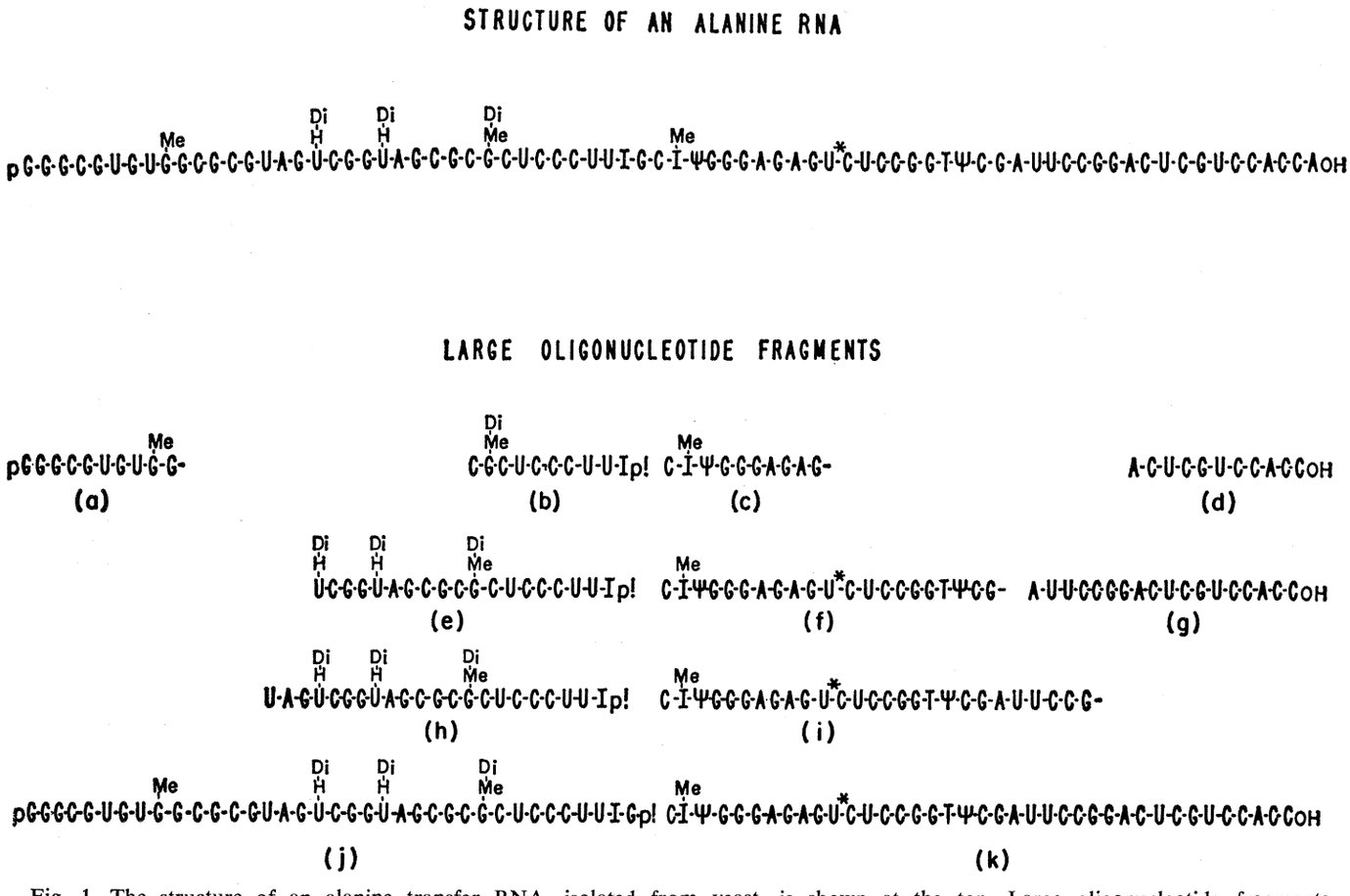

Fig. 1. The structure of an alanine transfer RNA, isolated from yeast, is shown at the top. Large oligonucleotide fragments that were crucial in the proof of structure are shown below.

arrangements of the four small fragments were consistent with the data of Table 1: DiHU-C-G-G-DiHU-A-G-C-G, DiHU-C-G-C-G-G-DiHU-A-G-, DiHU-A-G-C-G-G-DiHU-C-G-, C-G-G-DiHU-A-G-DiHU-C-G-, and G-DiHU-A-G-DiHU-C-G-C-G-. Since a pancreatic ribonuclease digest of *e* contained A-G-C-, the last two arrangements were eliminated. The third arrangement was eliminated because partial ribonuclease T1 digestion (½ hour at 0°C) removed a trinucleotide, DiHU-C-G, and left the remainder of *e* intact. Pancreatic ribonuclease digestion of the remainder of *e* gave only one trinucleotide, A-G-C, and a mixture of dinucleotides, eliminating the second arrangement. Therefore, the sequence DiHU-C-G-G-DiHU-A-G-C-G- must be attached at the left end of *b*; and fragment *e* has the structure shown in Fig. 1.

Fragment *h* differs from *e* only in the presence of an additional U-A-G- sequence. The presence of A-G-DiHU- in a pancreatic ribonuclease digest of the RNA (Table 1) is sufficient to establish the structure of *h* as that shown in Fig. 1.

Finally, the structure of the left half of the molecule, *j*, was established by the isolation of I-Gp! from a pancreatic ribonuclease digest of this fragment, an indication that -I-Gp! is the right end of *j*. With this terminal Gp! plus all the sequences known to be present in *a* and *h*, everything in *j* is accounted for except two C-G- sequences. Only one arrangement of these is possible, placing them between *a* and *h*. This gives the structure of *j* shown in Fig. 1. This sequence is consistent with the presence of MeG-G-C- and two G-C- sequences in Table 1.

Joining the two halves of the molecule, *j* and *k*, gives the I-G-C- sequence that is found in the pancreatic ribonuclease digest of the RNA (Table 1).

The entire RNA molecule is now accounted for except for one nucleotide. The purified alanine RNA, in common with most of the transfer RNA's isolated from commercial baker's yeast, lacks a 3′-terminal pA residue. Since this terminal pA residue is replaced before the amino acid becomes attached, the complete structure of the yeast alanine RNA is that shown at the top of Fig. 1.

Discussion. The structure shown in Fig. 1 is of interest in several respects:

There is no obvious pattern in the distribution of the "minor" or "unusual" nucleotide residues. They are scattered throughout most of the molecule, though none is present near the amino acid–acceptor end.

The pentanucleotide sequence G-T-ψ-C-G, which is believed to be a common feature of transfer RNA's (*10*), is located approximately 20 nucleotides from the amino acid–acceptor end of the molecule. It seems likely that this sequence is present at the same position in other transfer RNA's.

There are several possible trinucleotide sequences that might represent the coding triplet or "anticodon" for the transfer of alanine (*11*). If it is assumed that the coding triplet contains two G's and one C, though this has not been established for this alanine RNA, there are two particularly intriguing possibilities. One is that the coding triplet is between the two dihydrouridylic acid residues in the sequence DiHU-C-G-G-DiHU. The func-

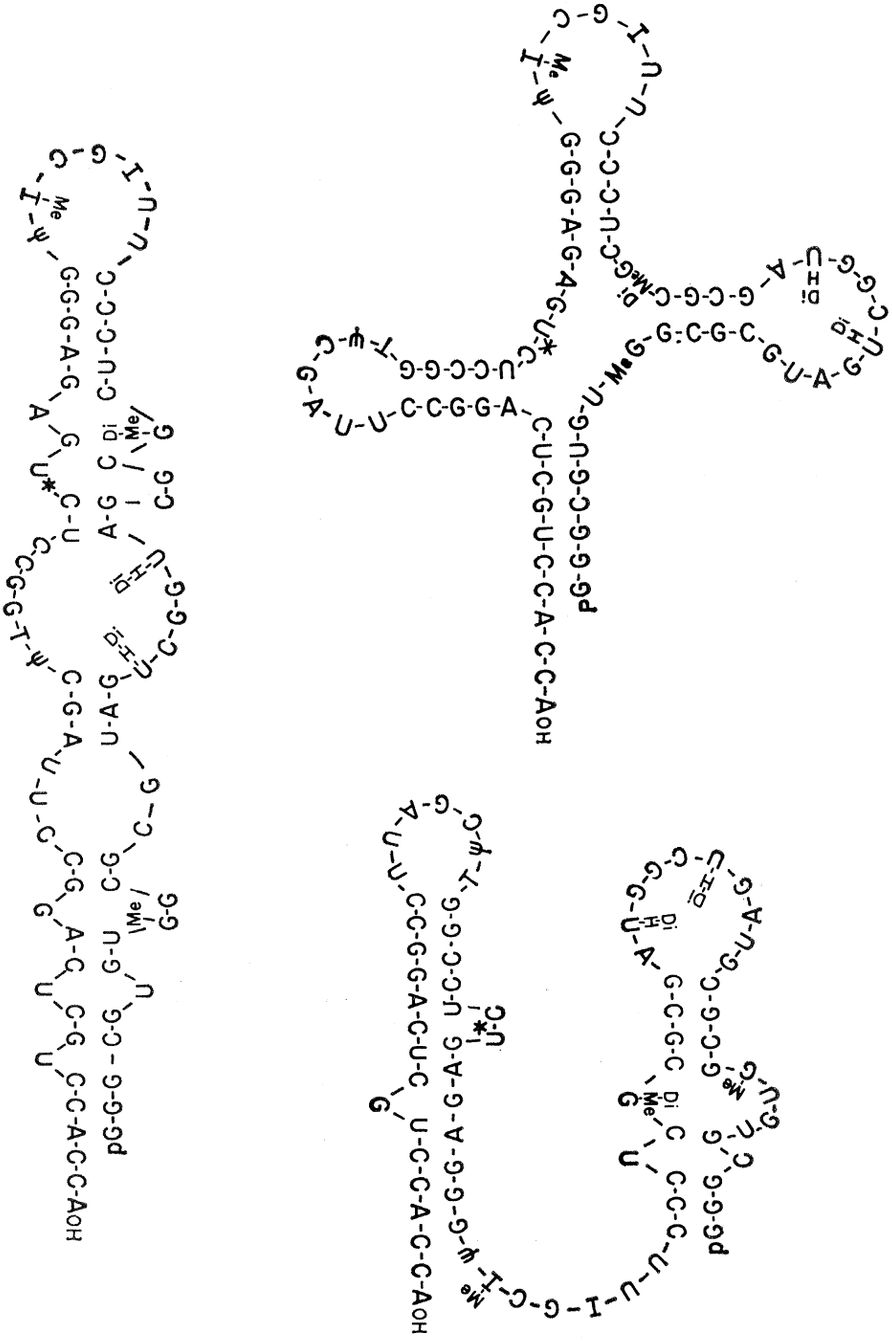

Fig. 2. Schematic representation of three conformations of the alanine RNA with short, double-stranded regions.

Table 3. Sequences that account for all of the nucleotide residues in the alanine RNA.

pG-G-G-C-, G-U-G-, U-MeG-G-C-, G-C-, G-U-A-G-, DiHU-C-G-, G-DiHU-A-G-, C-G-, C-DiMeG-, C-U-C-C-C-U-U-I-G-C-, MeI-ψ-, G-G-G-A-G-A-G-U*-C-U-C-C-G-, G-T-ψ-C-G-, A-U-U-C-C-G-, G-A-C-U-C-G-, U-C-C-A-C-C-AOH

tion of the dihydrouridylic acid residues may be to "insulate" the coding triplet from the influence of neighboring nucleotides in the polynucleotide chain. Another possibility for the coding triplet is the I-G-C sequence, which is in the middle of the chain and is particularly sensitive to ribonuclease T1. This sequence would be expected to be equivalent to G-G-C in coding properties. The sequence that is the actual coding triplet is still unknown.

It is of interest to consider the structure from the standpoint of the conformation of the RNA in solution. Examination of the nucleotide sequence shows that there are no long complementary sequences that could give G paired with C and A paired with U. In fact, the longest complementary sequences contain only five nucleotides. As a consequence, double-stranded regions must be relatively short or must contain many imperfections in base pairing. Three conformations that utilize different regions of the RNA chain in Watson-Crick type base pairing are shown schematically in Fig. 2. These should be considered only speculative, and in any case it is likely that the actual conformation of the RNA in solution varies with the conditions. The factors that might be expected to influence the conformation of an RNA have been discussed (*12*).

The determination of this structure of a nucleic acid represents the successful conclusion of a major undertaking—an attempt to isolate a biologically active nucleic acid and establish its structure. At the time that the isolation of the alanine RNA was undertaken, it was not known whether an individual nucleic acid could be isolated from a complex mixture of nucleic acids. Once individual transfer RNA's were isolated, it was not known whether a nucleic acid structure could be determined. In retrospect, the part of the structure determination that was completely uncharted, namely the determination of long nucleotide sequences, turned out to be easier than was anticipated. The time-consuming part of our work was the identification of new nucleotides and the proof of structure of the small fragments obtained by complete digestion of the RNA with pancreatic ribonuclease and ribonuclease T1. Once the analyses of the two digests were finished, the elucidation of the structure was greatly facilitated by two experimental developments. The first was the discovery that ribonuclease T1 shows a high selectivity of action at 0°C, making it possible to isolate large fragments from the RNA molecule. The second was the development of highly efficient chromatographic methods which make use of 7*M* urea solutions (*13*) with long, narrow DEAE-cellulose columns and which provide the resolution required to separate small amounts of many different oligonucleotides (*8, 9*). Together, these developments made it possible to isolate and analyze large fragments of the RNA, and the results furnished sufficient information to establish the sequence.

Determination of the structure of the alanine RNA indicates that the structures of other nucleic acids can also be determined and provides a basis for attempts to synthesize a biologically active nucleic acid.

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4. Abbreviations: p and - are used interchangeably to represent a phosphate residue; A-, adenosine 3′-phosphate; C-, cytidine 3′-phosphate; COH, cytidine (with the free 3′-hydroxyl group emphasized); DiHU-, 5,6-dihydrouridine 3′-phosphate; DiMeG-, N^2-dimethylguanosine 3′-phosphate; I-, inosine 3′-phosphate; MeG-, 1-methylguanosine 3′-phosphate; MeI-, 1-methylinosin 3′-phosphate; ψ-, pseudouridine 3′-phosphate; T-, ribothymidine 3′-phosphate; U-, uridine 3′-phosphate; U*-, a mixture of U-, and DiHU-; p!, 2′,3′-cyclic phosphate, for example: Ip!, inosine 2′,3′-cyclic phosphate; DEAE, diethylaminoethyl.
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Sweating: Direct Influence of Skin Temperature

Abstract. *The onset and magnitude of the sweating response is greatly influenced by the local skin temperature. This influence was evident with skin temperatures above 33°C and occurred in the physiological range for thermo-regulatory sweating. The sweating rates were recorded continuously and simultaneously from three areas of the skin.*

In spite of extensive study a universally accepted theory of the regulation of thermal sweating in man has not been developed. It has been suggested that the regulation of body temperature depends upon a proportional control system, in which the magnitude of the response is a function of an error signal. This signal is the difference between the actual hypothalamic temperature and a set point temperature (*1*). There is, however, a lack of agreement concerning the participation of skin temperature in this regulation. Interdependence of sweating rate and skin temperature had been suggested earlier by the findings of Kuno (*2*), Bazett (*3*), and Randall (*4*). From experiments in which rates of work and metabolism and rectal temperatures were maintained constant, Robinson (*5*) concluded that the rate of sweating was proportional to skin temperatures up to 35.5°C. Recently Belding and Hertig (*6*) in a