haploid chromosome number of this species of *Labyrinthula* is nine. Most of the bivalents have one differentiated area, presumably the centromere. Through length measurements and centromere position it is therefore possible to construct the pachytene karyotype of this protist.

The status of the synaptinemal complex as a concomitant of paired homologous chromosomes of meiotic prophase is now well established, and the evidence has been reviewed by Moses (*1*). Thus it is valid to interpret an observed synaptinemal complex as belonging to a bivalent at meiotic prophase. The presence of nine distinct and continuous complexes in four nuclei does not rule out the possible occurrence of chromosomal polymorphisms in this species of *Labyrinthula*. Minor nonhomologies may not be detectable in the fine structure because it seems that complexes can be formed between pairs of chromosomes that are less than fully homologous (*6*). Unusual chromosome associations at meiosis as in translocation or inversion heterozygotes are probably detectable by the unusual arrangement of chromosome cores in the synaptinemal complex (*7*). In a few *Labyrinthula* nuclei, not included in this report, irregular pairing forms were observed.

The complex has been used as a diagnostic of meiotic prophase in Myxomycetes and yeast (*8, 9*). In *Labyrinthula* sp. the synaptinemal complex has served (i) to establish the presence of a sexual cycle (*2*); (ii) to delineate meiotic prophase (*2*); and (iii) to determine the haploid chromosome number as nine. In general terms, the presence of the complex indicates where the reduction division occurs in the life cycle, and it alerts the investigator to the possibility of gamete formation or alternation of haploid and diploid generations. The synaptinemal complexes, and thereby the chromosome number and structure, can be an aid in determining the phylogenetic and taxonomic affinities of protists.

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Genetic Coding: Oligonucleotide Coding for First Six Amino Acid Residues of the Coat Protein of R17 Bacteriophage

Abstract. *Ribonucleic acids from the bacteriophage R17 and from R17 amber mutant AmB2 have been digested with ribonuclease T1. Of the products isolated, only one was different. It codes for the first six amino acid residues of the viral coat protein. The probable base sequence of the wild-type oligonucleotide is* CpUpUpCpUpApApCpUpUpUpApCpUpCpApGp.

RNA from the bacteriophage R17 contains about 3300 nucleotide residues (*1*). This sequence of bases is believed to specify the amino acid sequence of at least three proteins—viral coat protein, maturation protein, and RNA synthetase (*2*). Knowledge of the protein sequence (*3*) together with the genetic code (*4*) makes it possible to search for coat-protein encoding sequences within the viral RNA. This may be done by analysis of the fragments produced by a nuclease digestion.

Ribonuclease T1 cleaves RNA specifically at G (*5*) yielding Gp and oligonucleotides, all of which (except the 3′ end of the RNA) terminate in Gp. Such products may be fractionated according to their charge at neutral *p*H by chromatography on DEAE-cellulose or DEAE-Sephadex (resulting in a separation according to chain length) and refractionated at *p*H 3 (resulting in a separation according to U content and to a lesser extent according to A content) (*6*). In the case of R17 RNA such procedures result in the complete separation of all oligonucleotides of chain length greater than 13 (*7*). There are some two dozen such oligonucleotides which we have partially sequenced. At least three of them have base sequences consistent with the genetic code for specifying R17 coat protein.

Since the coat-protein encoding region is only about 9 percent of the total RNA, compatibility of relatively short RNA and coat protein sequences does not prove that they are congruent. Fortuitously, there exists a mutant of R17 whose comparison with wild type allows a positive identification of one of the T1 oligonucleotides. Phage R17 AmB2 is believed to have an amber mutation in the codon of the Gln^6 of coat protein (*2*). It thus has a sequence UAG corresponding to CAG in the wild-type counterpart and is easily identifiable. In principle, the fragment containing the mutational site could be 3, 12, 13, 15, or 17 nucleotides long (Fig. 1).

The large oligonucleotides of ^3H-labeled AmB2 were compared with those of ^{32}P-labeled R17 RNA by techniques similar to those used earlier to compare MS2 and a mutant of MS2 (*8*). Only one chromatographs differently. Oligonucleotide No. 14-6 of AmB2 elutes in the fraction which corresponds to oligonucleotides containing eight U's, while its R17 counterpart elutes with the seven-U fraction. We are determining its sequence by rigorous techniques as a part of our overall R17 RNA sequencing program. However, a variety of evidence (see below) indicates that No. 14-6 of R17 has the sequence CpUpUpCpUpApApCpUpUpUpApCpUpCpApGp.

Since the next base to the left must be G, this oligonucleotide encodes the first six amino acids of R17 coat protein.

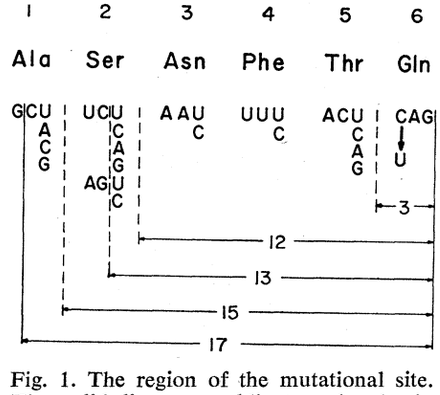

Fig. 1. The region of the mutational site. The solid lines are obligatory breaks in the RNA, occurring after guanyl residues which, according to the genetic code, must be present. The dotted lines are possible breaks.

Table 1. Paper electrophoresis of oligonucleotide No. 14-6 digested with ribonuclease.

	Radioactivity (count/min)	Relative amount	Nearest integral amounts
Up-cyclic	2750	1.1	
Up	14450	5.8	7
Cp	7850	3.1	3
ApCp	5300	2.1	2
ApGp	4700	1.9	2
ApApCp	7400	3.0	3
Total	42,450	17.0	17

^{32}P-Labeled R17 was grown according to the procedure of Roblin (*9*), with slight modification. Lysates were grown in two 200-ml cultures of *Escherichia coli* Q13, containing 5 mc of ^{32}P each. Phage AmB2 was grown on *E. coli* K37-6 (Su$^+$) in the presence of ^{3}H-uracil (1.5 mc per 100 ml of lysate); K37-6 was grown in Davis and Sinsheimer's TPG medium (*10*), slightly modified, containing 5 μg of uracil per milliliter. When the cell density was about 5×10^8 per milliliter, the culture was sedimented in a warm rotor, and the cells were resuspended in the same volume of TPG medium containing 1.5 mc of ^{3}H-uracil and unlabeled uracil to give a total uracil concentration of 5 μg/ml. After 5 minutes of growth, AmB2 was added at a multiplicity of 10. Infection was allowed to proceed for 5 hours, and reaction was terminated by freezing. RNA was extracted with phenol in the presence of bentonite and sodium dodocyl sulfate.

Ribonuclease T1 (90 μg/ml) and unlabeled R17 RNA (1.5 mg/ml) were added to a mixture of the labeled RNA's in 0.1*M* tris-Cl, *p*H 7.5. A few drops of toluene were added to discourage microbial growth. The mixture was incubated for 20 hours at room temperature and frozen. The reaction mixture was later thawed, made 7*M* in urea, and fractionated on a DEAE-Sephadex column at *p*H 7.5 (Fig. 2). Earlier experiments indicated that the fragments containing the mutational site were eluted in region 14.

Rechromatography of region 14 (Fig. 2) at *p*H 2.7 yields the chromatogram shown in Fig. 3. The ^{3}H and ^{32}P patterns are similar, except that one fragment from the wild type precedes the homologous amber fragment. This is expected, since the amber fragment should contain one additional uridyl residue which has no proton-accepting group and thus will have a correspondingly greater net negative charge. Rechromatography of other regions of Fig. 2 beyond region 12 revealed no other distinguishable fragments.

Enzymic digestions of R17 fragment 14-6 and subsequent analyses were carried out under several conditions. Buffer (50 μl of 0.4*M* KCl, 0.1 ionic strength in sodium potassium phosphate, *p*H 7.5) containing 10 μg of pancreatic ribonuclease (Worthington) were mixed with 40 μl of the ^{32}P-labeled wild-type fragment in water and with 10 μl of a complete T1 ribonuclease digest of R17 RNA (80 μg) in 0.02*M* tris-Cl, *p*H 7.5. The reaction mixture was incubated for 10 hours at 22°C and diluted tenfold with water. It was applied to DEAE-paper (Whatman DE81), and electrophoresis was carried out in 7 percent formic acid with a Savant flat-plate apparatus for 5.5 hours at 27.5 volt/cm (Table 1).

Table 2. Paper electrophoresis of oligonucleotide 14-6 partially digested with ribonuclease.

Product	Probable sequence	Radioactivity (count/min)	Percent of total
2A, 1C, 3U	ApApCpUpUpUp	1139	25.6
2C, 3U	CpUpUpCpUp	1061	23.9
1A, 2C, 1U	ApCpUpCp	729	16.4
1A, 1G	ApGp	632	14.2
1C, 2U	UpUpCp	201	4.5
1C, 1U	CpUpUpCp	140	3.2
5 Other	Unidentified	540	12.2

Very limited digestion of the fragment was carried out under conditions as above, except that the 50-μl portion of KCl phosphate buffer contained 10^{-2} μg of pancreatic ribonuclease and the incubation period was 1 hour. Under these conditions, cleavage occurs only at the most susceptible sites. Four major products were separated by electrophoresis: (i) 2A, 1C, 3U; (ii) 3U, 2C; (iii) 1A, 2C, 1U; and (iv) 1A, 1G. These accounted for 80.1 percent of the total radioactivity. There were seven minor products, the largest of which (composition 2U, 1C) accounted for about 4 percent of the total radioactivity (Table 2).

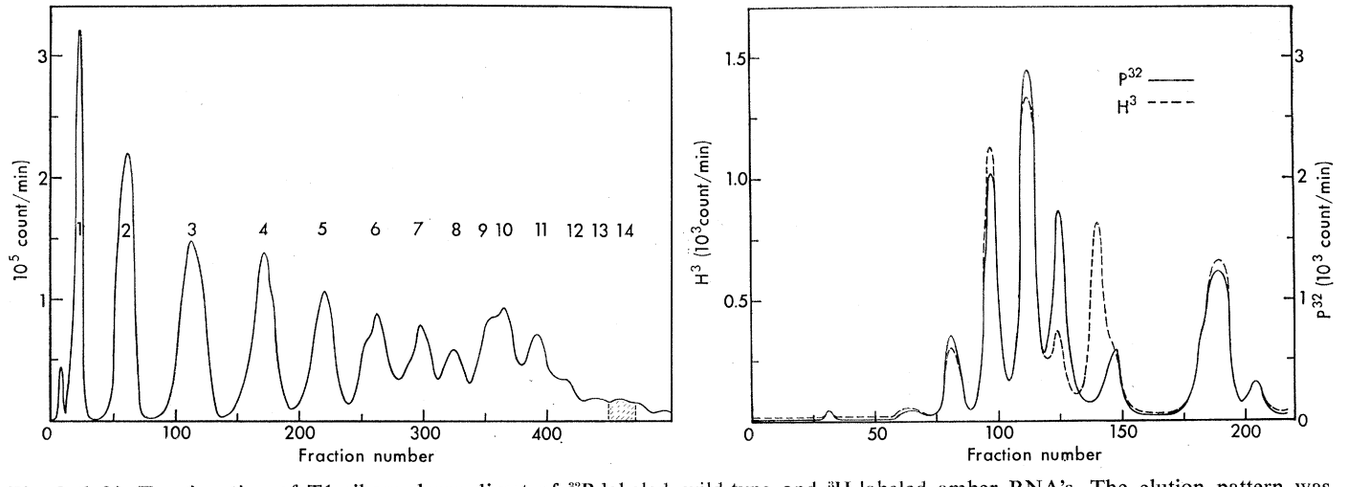

Fig. 2 (left). Fractionation of T1 ribonuclease digest of ^{32}P-labeled wild-type and ^{3}H-labeled amber RNA's. The elution pattern was obtained by chromatography at 22°C on a DEAE-Sephadex column (0.9 by 25 cm) with 2.8 liters of the following: 7*M* urea–0.05*M* tris-acetate solution (*p*H 7.5) containing a nonvarying gradient of 0.15 to 0.85*M* sodium acetate at a flow rate of 24 ml/hour. Fractions were collected every 10 minutes, and the Cherenkov radiation from the ^{32}P fragments was measured. Fig. 3 (right). Subfractionation of peak 14. The elution pattern was obtained by chromatography at room temperature on a DEAE-Sephadex column (0.5 by 25 cm) with 200 ml of 0.05*M* sodium citrate (*p*H 2.7) containing a nonvarying gradient of 0.25 to 0.80*M* sodium chloride at a flow rate of 4 ml/hour. Fractions were collected every 7.5 minutes, dissolved in scintillation fluid, and counted in a dual-channel scintillation counter. Beneath the ^{32}P peak associated with the wild-type fragment containing the mutational site is a small ^{3}H peak due to the registering of some ^{32}P radiation in the ^{3}H channel.

Analysis of products of other T1 oligonucleotides digested with ribonuclease under these conditions indicates that cleavage occurs almost exclusively at pyrimidines followed by A (or G). There is some cleavage between two C's and almost none between C and U and two U's. This is consistent with work of Witzel (*11*); he showed that the relative velocity constants for ribonuclease digestion were: CpA, 270; UpA, 110; CpG, 45; CpC, 22; UpC, 3.6; CpU, 2.5; and UpU, 1.

Partial digestion with spleen phosphodiesterase (Worthington) showed that the 5′ terminus of oligonucleotide 14-6 is Cp. The only sequence compatible with the analyses, above, the genetic code and the known coat protein sequence is:

CpUpUpCpUp ApApCpUpUpUp
ApCpUpCp ApGp

To our knowledge this is the first instance of the isolation of an RNA fragment which encompasses a site of mutation and a known protein sequence (*12*).

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5. Abbreviations used are Gln, glutaminyl residue; C, cytidine; U, uridine; A, adenosine; G, guanosine; p, 2′ or 3′ phosphates of the above four nucleosides; DEAE, diethylaminoethyl; tris, tris(hydroxymethyl)aminomethane.
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13. We thank Dr. J. J. Kelley for advice. This work was supported by NIH grants AI 01466 and AI 21,942.

4 August 1969

Physical and Chemical Studies on Ceruloplasmin: Crystallization of Desialized Human Ceruloplasmin Asialoceruloplasmin

Abstract. *Completely or partially desialized human ceruloplasmin crystallizes as blue hexagonal prisms terminating at both ends in shallow pyramids.*

When the terminal sialic acid residues from several of the carbohydrate chains of the plasma copper-protein, ceruloplasmin, are removed, there is an almost instantaneous disappearance of the infused desialized protein (*1*). Yet the blue color, copper content, oxidase activity, and immunochemical characteristics of asialoceruloplasmin and ceruloplasmin are identical, although such physical properties as electrophoretic mobility and solubility are altered by the loss of the sialic acid and its carboxyl groups. We now report that asialoceruloplasmin crystallizes as hexagonal prisms in contrast to the tetragonal prisms of ceruloplasmin (*2*).

Human ceruloplasmin (*2*) and neuraminidase from *Clostridium perfringens* (*3*), at respective concentrations of 7 percent and 0.06 unit/ml, or of 3 percent and 0.03 unit/ml, were incubated for 20 hours at 25°C and for 24 hours at 4°C, in an 0.10*M* sodium acetate buffer, *p*H 5.45, containing 1 percent NaCl (buffer A). The blue crystalline

Table 1. Quantitative comparison of some characteristics of ceruloplasmin and of asialoceruloplasmin. Analyses for protein concentration, sialic acid, copper, and enzymatic activity were carried out as in (*6*), except that the assay medium for enzymatic activity contained $1.5 \times 10^{-3}M$ $CaCl_2$ in addition to the other components. C, crystal. S, supernatant. Sialic acid is expressed as the percentage in protein. Purity is expressed as the ratios of absorbances (*A*) at 610 and 280 nm and of absorbance at 610 nm to copper (*6*).

Material analyzed	Incubation with neuraminidase (min)	Sialic acid (%)	A_{610}/A_{280}	$A_{610}/\mu g$ Cu	Enzymatic activity (ΔA_{530}/min × μg Cu)
		Pure crystalline ceruloplasmin			
Crystals	0	2.11	0.044	0.022	0.032
		Partially desialized ceruloplasmin			
Crystals	20	0.30	0.042	0.023	0.030
Supernatant	20	0.68	0.042	0.023	0.030
Crystals	60	0.40	0.040	0.021	0.031
Supernatant	60	0.43	0.043	0.022	0.032
		Asialoceruloplasmin			
Crystals	1200	0.00	0.042	0.022	0.034
Supernatant	1200		0.043	0.022	0.033

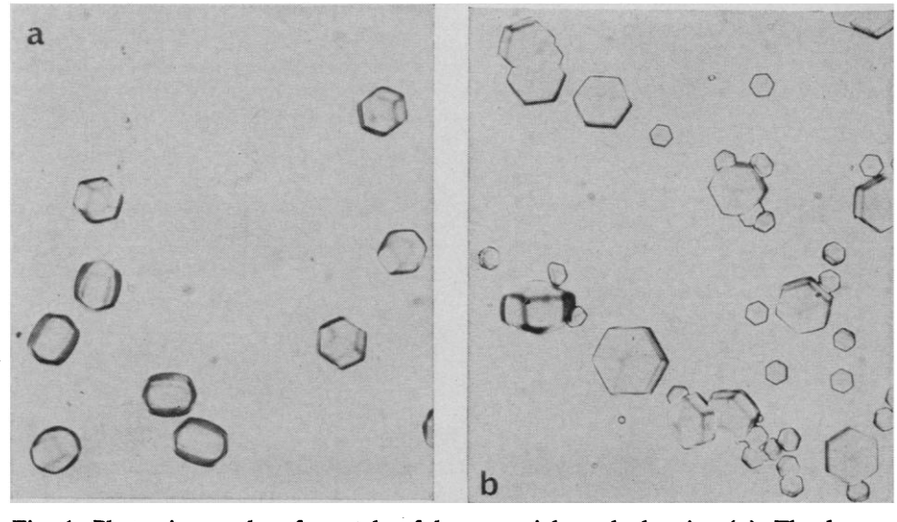

Fig. 1. Photomicrographs of crystals of human asialoceruloplasmin. (a) The longer prism sides of the crystals formed from 7 percent ceruloplasmin solution are evident when the crystals seen on edge at the left of the picture are compared to those seen on end at the right (× 280). (b) The short prism sides of the darkly outlined crystal at the left are characteristic of all crystals formed from 3 percent ceruloplasmin solution (× 250).