cupula in the chemically sensitive pit organ.

From the pit organ in the dorsal body surface, such sensitive mechanoresponses were hardly observed. This may be because the dorsal skin of the shark is very thick and tough and may prevent the deformation of skin from reaching the end organ, whereas the mandibular skin is rather thin and soft and much closer to the gill than the dorsal skin. We have observed the same phenomenon in the free neuromasts near the operculum of the mullet, one of the teleosts, which are sensitive to chemical stimulation.

Tasaki (*12*) once said that the giant axon membrane of squid develops higher mechanosensitivity in more concentrated potassium ionic medium (*11*). The pit organ of the shark, however, may provide more direct evidence of the enhanced activity of the receptor cell when it is immersed in high potassium medium.

We think the pit organ of the shark may be a better model of the inner ear of higher animals than the canal organ because the pit organ has both chemo- and mechanosensitivity.

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Chromosome Number of a Small Protist: Accurate Determination

Abstract. *Electron micrographs of serial sections through a meiotic prophase nucleus of the mycetozoan* Labyrinthula *sp. show that there are nine separate and distinct synaptinemal complexes. Since each complex represents a set of paired homologous chromosomes, it follows that the haploid chromosome number of this protist is nine.*

Unicellular organisms that have a nucleus of less than 4 μ, often have chromosomes that are difficult to resolve with the light microscope, because of their shortness, their small width, and their limited ability to bind stain. Electron microscopy is usually not helpful because the chromosomes are rather diffuse and because they have poorly defined boundaries. If a sexual cycle exists, however, each set of paired homologous chromosomes forms a synaptinemal complex during meiotic prophase (*1*), and the individual complexes are clearly distinguishable in electron micrographs. Where the nucleus is less than 4 μ in diameter, it is practical to make serial sections of a complete nucleus. The individual synaptinemal complexes can then be traced, and the chromosome number can be determined unambiguously.

Methods used to isolate and maintain the marine mycetozoan, *Labyrinthula* sp., as well as conditions for obtaining zoosporulation have been described (*2*). While still attached to agar blocks, sporulating cells are fixed for 1 hour in 2.5 percent glutaraldehyde buffered at *p*H 7.4 with 0.2*M* Millonig's phosphate buffer (*3*). The blocks are then rinsed in four changes of 0.2*M* phosphate buffer in 0.15*M* NaCl; they are then fixed in 1 percent

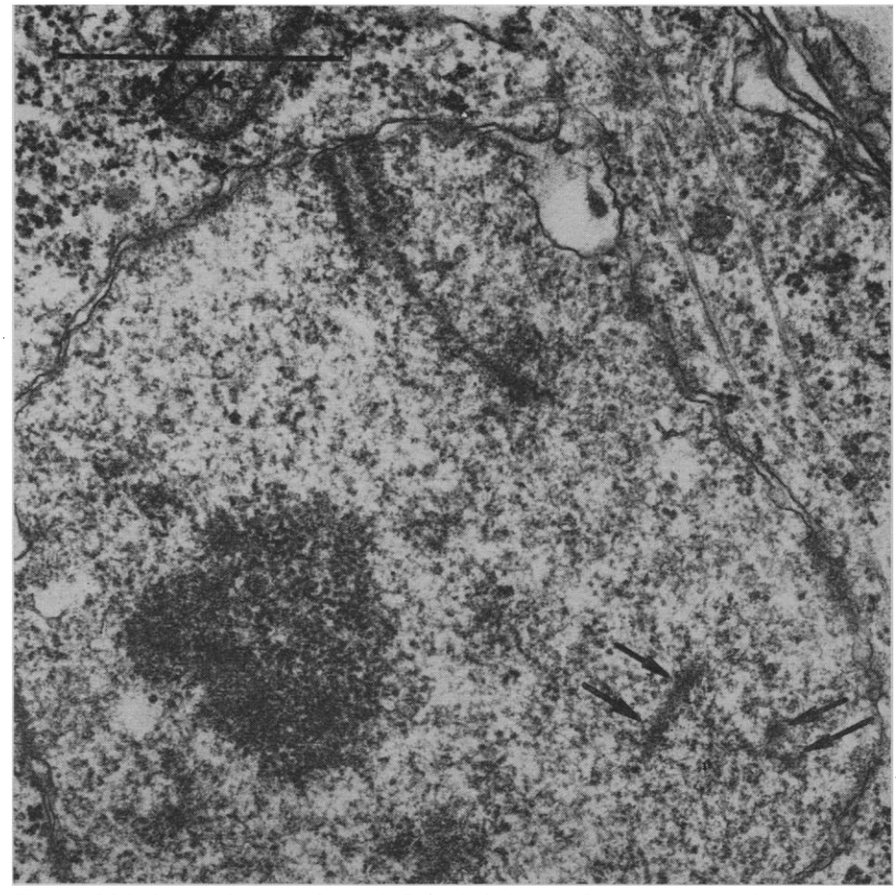

Fig. 1. Meiotic prophase nucleus of *Labyrinthula* sp. One synaptinemal complex in longitudinal section ends on the nuclear membrane. The two dense parallel bands are the lateral elements. In cross section the complex consists of two dark dots, marked by arrows.

OsO_4 at *p*H 7.4 in 0.2*M* Millonig's phosphate buffer. The tonicites of the glutaraldehyde solution, buffer rinse, and OsO_4 solutions are 700, 700, and 615 milliosmoles, respectively; thereby approximating the 615 to 700 milliosmole tonicity of the culture medium. All procedures are conducted at 23° to 24°C. The cells are embedded in Epon 812 which consists of a 0.60 anhydride : epoxy ratio.

Sections of about 800-Å thickness are cut on a Porter-Blum MTII microtome with a Dupont diamond knife; 50 to 60 sections are cut wtihout interruption. The ribbon is then broken up into rows of convenient lengths. The rows of sections are picked up with a single hole grid (1 by 2 mm) and deposited on a similar, Formvar-coated grid (*4*). If the order of sections is lost, it can later be reestablished through serial photographs of a single cell. The proper order of the sections was maintained for the nuclei reported here. A day after cutting, the sections are stained in a saturated, aqueous, uranyl acetate solution and in Reynold's lead acetate (*5*). The grids are rotary-carbon coated, by use of a screened carbon source. The direct heat of the carbon rods damages the large Formvar film. Coating is necessary to avoid drifting of the specimen in the beam and to avoid rupturing of the film. Electron micrographs were taken with a Philips EM 200 at 60 kv-peak, a low beam intensity, and with 35-mm Kodak fine-grain, positive film, about × 6500 at the negative.

In longitudinal section, the complexes have the conventional appearance of two densely staining lateral elements and a less dense central zone. In cross section, the two lateral elements appear as two dense dots. The complexes end on the nuclear membrane (Fig. 1). The serial sections of four complete nuclei were photographed. The average diameter per nucleus was 3.4 μ, and about 45 sections were necessary in each case to pass through the entire nucleus (somewhat thicker sections—1000 to 1200 Å—serve as well, and fewer are needed). The synaptinemal complexes in each subsequent photograph were traced on a single sheet of polyester drafting film [matte on one side, base thickness 0.008 cm (0.003 inch), Keuffel and Esser] (Fig. 2). The sections used to construct Fig. 2 are numbered 1 to 49. Complexes in cross section are indicated by two black squares (the lateral elements), joined by a line. The cross-sectioned complexes are given the number of the section in which they appear. Where a sequence of cross sections overlap, from a complex perpendicular to the plane of section, one to several cross sections have been omitted from the drawing. Occasionally a single section of a complex is lacking owing to the presence of a particle of stain or dust on that section. Complexes in the plane of section, or nearly so, show up mostly in longitudinal sections (near the top of Fig. 2). Segments of the nuclear membrane are indicated by solid lines where the complexes insert on the nuclear membrane. Each complex can be followed through its entire length to the insertions of both ends on the nuclear membrane. The complexes are well spaced in the nuclear volume. Intersecting complexes in Fig. 2 are separated by at least ten sections, and in no case is there doubt as to the continuity of the individual synaptinemal complexes. The structure of the complexes in another nucleus is summarized in Fig. 3.

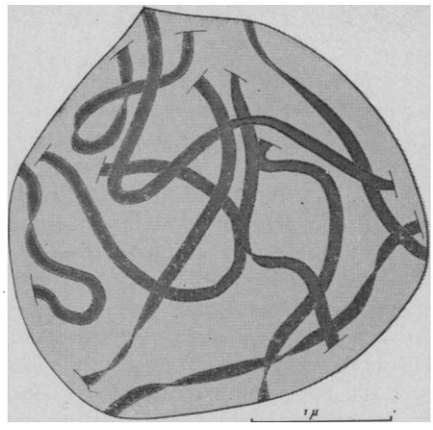

Fig. 3. Structure of the synaptinemal complexes in another nucleus, omitting the details shown in Fig. 2.

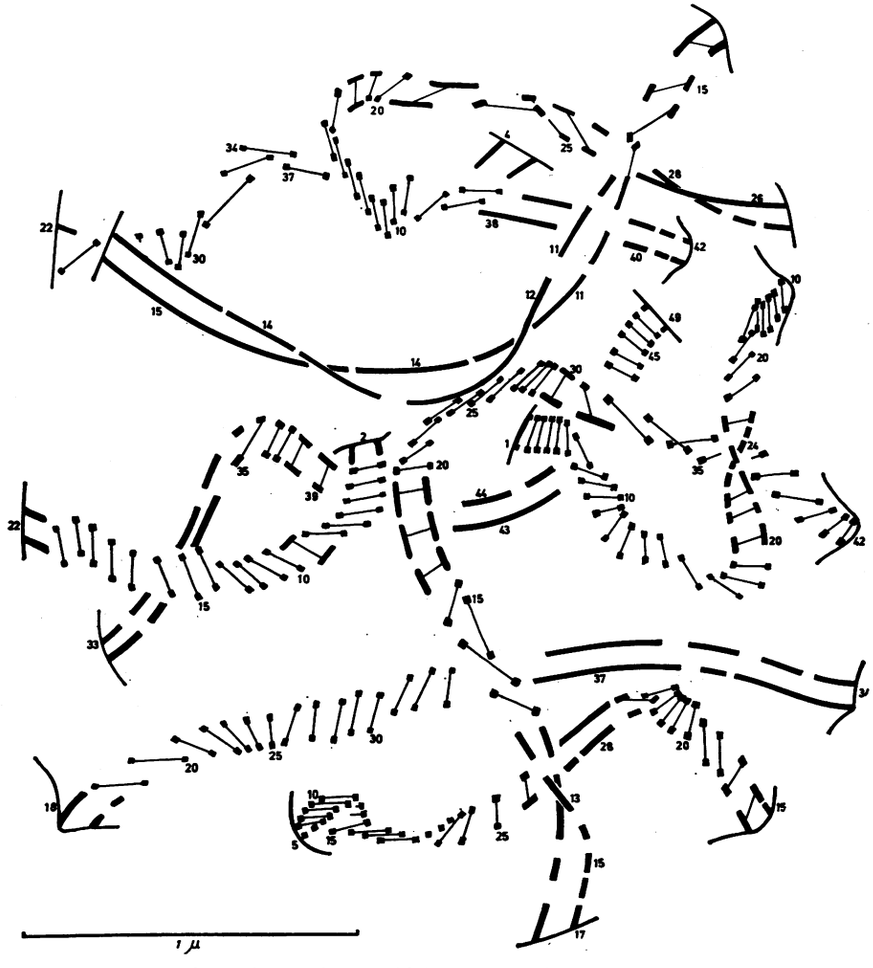

Fig. 2. The synaptinemal complexes which appeared in 49 consecutive sections of one nucleus are superimposed in this drawing. The lateral elements are represented by the fat black lines or squares. The numbers refer to the sections in which the segments of a complex were found. When the complex is attached to the nuclear membrane, the membrane is indicated by a solid line.

Each of the four nuclei examined has nine synaptinemal complexes (Figs. 2 and 3), and it is concluded that the

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.

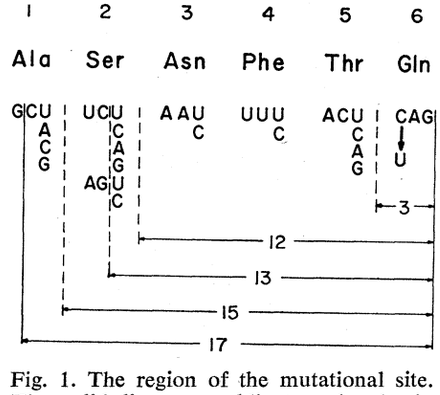

Fig. 1. The region of the mutational site. The solid lines are obligatory breaks in the RNA, occurring after guanyl residues which, according to the genetic code, must be present. The dotted lines are possible breaks.