THE SYNTHESIS OF MICROTUBULE AND OTHER PROTEINS OF THE ORAL APPARATUS IN TETRAHYMENA PYRIFORMIS

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ABSTRACT

Several proteins, including microtubule proteins, have been isolated from the oral apparatus of the ciliate Tetrahymena. The synthesis of these proteins has been studied in relation to formation of this organelle system by the cell. Electron microscopy has shown that the isolated oral apparatus consists primarily of basal bodies, pellicular membranes, and a system of subpellicular microtubules and filaments. Cilia were removed during the isolation; therefore none of the proteins studied was from these structures. Evidence was obtained from the study of total oral apparatus protein which indicates that at least some of the proteins involved in formation of this organelle system may be synthesized and stored in the cytoplasm for use over long periods. This pattern of regulation was found for three individual proteins isolated from the oral apparatus fraction after extraction with a phenol-acetic acid solvent. A different pattern of regulation was found for microtubule proteins isolated from the oral apparatus of Tetrahymena. The data suggest that microtubule proteins, at least in logarithmically growing cells, are not stored in a cytoplasmic pool but are synthesized in the same cell cycle in which they are assembled into oral structures.

INTRODUCTION

Information concerning the mechanisms regulating organelle formation is important for an understanding of cell development. An important problem concerning the control of organogenesis is the relationship between synthesis of organelar protein and morphogenesis of organelar structures. This problem has been investigated recently in a number of systems, including regenerating eukaryotic flagella (15, 16), regenerating bacterial flagella (3, 9), developing cytomembranes (12), and developing mitotic structures (22). The present study deals with the relationship between synthesis of organelar proteins and organelle formation in development of the oral apparatus of the ciliate, Tetrahymena pyriformis.

The oral apparatus (OA) of Tetrahymena is an organellar complex used in feeding which consists primarily of ciliated basal bodies, intracellular fibrillar material, and differentiated membrane (Nilsson and Williams [10], Williams and Luft [20]). This organellar complex, situated at the anterior end of the cell, is retained by the anterior product during cell division, whereas the posterior division product receives a newly formed OA which develops in the midequatorial region of the parent-cell cortex.

Studies with metabolic inhibitors by Frankel (5) have indicated that formation of the Tetrahymena oral apparatus requires de novo synthesis of protein. Additional information, however, was obtained in a radioautographic study of OA development in Tetrahymena by Williams et al. (21),
which suggests that this organelle system is constructed, at least in part, from proteins previously synthesized and stored within the cells. In this regard, development of the oral apparatus of *Tetrahymena* is like development of the mitotic apparatus of cleaving sea urchin eggs; the mitotic apparatus appears to be formed from proteins present within the egg, yet mitotic apparatus development depends upon *de novo* protein synthesis during cleavage (22). The suggestion that there may be different regulatory patterns associated with the synthesis of the various proteins involved in oral development in *Tetrahymena* has led to the present investigation, in which several individual proteins have been isolated from the oral apparatus and information has been obtained regarding patterns of synthesis.

A method of isolating oral apparatuses from *Tetrahymena* in quantities sufficient for biochemical studies has been developed by Zelnis (23). A macromolecular characterization of the OA in the study by Zelnis revealed that protein from the oral apparatus constitutes about 2.3% of the total cell protein. The OA was also found to contain 2% RNA and 0.6% DNA, although it was pointed out that the DNA values obtained were near the limit of resolution of the method used. With some modification, the isolation method of Zelnis was found to be reliable and has been used in the present investigation. The OA fraction has been characterized by electron microscopy in the present study, and several proteins, including microtubule proteins, have been isolated. The study of synthesis has shown that some OA proteins, not yet identified as to structural origin, may be stored in the cytoplasm for use over long periods, whereas microtubule proteins appear to be synthesized on demand in each cell cycle in logarithmically growing cells.

**MATERIALS AND METHODS**

**Experimental Design and Culture Methods**

*Tetrahymena pyriformis* strain GL stock cultures were maintained at 28°C in 5-ml quantities of Frankel's medium (4) contained in 25-ml screw-capped culture tubes. The cells were kept in logarithmic growth by subculturing daily. Oral apparatus isolations were carried out on cells grown in a special culture medium, which proved necessary for obtaining oral apparatus fractions uncontaminated with other particulate components. This medium consisted of 2% proteose peptone (Difco Laboratories, Detroit, Mich.) supplemented with 0.05% Liver Fraction L (Wilson and Co., Chicago, Ill.). The medium was heated to 90°C and filtered before autoclaving because this procedure prevented the formation of a precipitate which interfered with the isolation of oral apparatuses. It was found that this medium supported growth best when inoculated with cells grown in the stock medium (see above). All cultures used in the experiments were inoculated with stock cultures which were 48 hr old. Two 5-ml stock cultures were used as the inoculum for each 500 ml of proteose peptone medium used.

The basic experimental design used in all experiments consisted of growing 500-ml cultures, prepared as described above, to late logarithmic phase in the presence of labeled amino acids. At this time the cells were washed free of radioactive medium and resuspended in a quantity of unlabeled medium sufficient to permit resumption of logarithmic growth. Growth was followed subsequent to this "chase" by counting cell number in triplicate 1-ml samples, taken at frequent intervals, with a Coulter counter. Logarithmic growth always resumed after a 3 hr lag period. Specific activity was determined for proteins of interest at the time of resumption of logarithmic growth following the chase (the "predoubling" time) and again after completion of one doubling in cell number following the chase (the "postdoubling" time). Because each cell has a single OA, a doubling in cell number also means a doubling in the number of these organelles. Conclusions about the pattern of synthesis in relation to formation of oral apparatuses are drawn from comparisons of the predoubling and postdoubling specific activities.

The initial 500-ml cultures were inoculated as described above and permitted to grow for approximately 10 hr before labeled amino acids were added. Then labeled protein hydrolysate (Schwarz Bio Research Inc., Orangeburg, N. Y.) was added to attain a concentration of 2 \( \mu \text{Ci} \)/ml. The cells were permitted to grow for another 10 hr, at which time the cell density was approximately 2 \( \times \) \( 10^8 \) cells/ml. Then they were washed free of label and resuspended in fresh medium at a concentration of 8-9 \( \times \) \( 10^4 \) cells/ml, in order to permit further logarithmic growth. This adjustment of cell density required a considerable increase in culture volume, therefore the expanded cultures were placed in a large, covered, stainless steel pan which had previously been autoclaved. The cover contained many cotton-filled ventilation holes and the culture depth never exceeded 3.5 cm. In addition, 10 ml of Frankel's medium was added per 500 ml of expanded culture volume to insure proper cell growth.

**Oral Apparatus Isolation**

Oral apparatuses were isolated from 250–500-ml culture samples. The cells were washed free of medium
by low speed centrifugation with ice-cold distilled water. During the washing, cells were gently resuspended by swirling with a spatula; resuspending by any other means produced premature cell lysis, which produced contaminated oral apparatus fractions. The washed cells were combined in one centrifuge tube and pelleted. 20 ml of a 0.1% Triton X-100 solution (Sigma Chemical Co., St. Louis, Mo.) were added to the pellet (approximately $4 \times 10^7$ cells), and the mixture was swirled rapidly for 2 min with a spatula in order to lyse the cells. The lysate was combined with 180–190 ml of ice-cold distilled water and pumped through a Logeman homogenizer (Scientific Products, Evanston, Ill.). The homogenate was placed into four or more 50-ml polycarbonate conical centrifuge tubes, depending on the final volume of the homogenate. From this point on in the procedure, all solutions contained at least 0.01% Triton X-100. This was found necessary to insure reproducibility of OA isolation. A 2 ml, 1 M sucrose cushion made with 0.01% Triton was inserted into the bottom of each centrifuge tube below the homogenate. The centrifuge tubes containing the homogenate and sucrose cushion were centrifuged at 2000 g for 10–15 min or until a small pellet formed in each tube. The supernatant plus one-third of the sucrose was aspirated and the oral apparatus pellets were resuspended in 20 ml of 0.01% Triton per tube. A sucrose cushion was again added to each tube and the process was repeated. Finally, the OA pellets from all tubes were combined into one pellet for a final wash with 0.01% Triton. Fig. 1 is a micrograph showing a sample of oral apparatuses prepared as described. Protein yield from oral apparatuses prepared from 500-ml cultures was measured by the Lowry method and found to be between 300 and 500 µg.

**Preparation of OA Proteins**

Oral apparatuses were prepared for electrophoretic separation of microtubule proteins according to the methods of Renaud et al. (14) and Shelanski and Taylor (18). Best results were obtained with the former method, particularly when reduction was

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**Figure 1** Unstained phase-contrast photomicrograph of *Tetrahymena pyriformis* oral apparatuses isolated by the standard Triton procedure. The three membranelles (M) and the undulating membrane complex (UM) of one oral apparatus are indicated. X 2000.
carried out with dithiothreitol rather than mercaptoethanol. Identification of microtubule protein was made by co-electrophoresis with microtubule protein from purified ciliary axonemes (see below), and molecular weight determinations in sodium dodecyl sulfate (SDS) gels by the method of Shapiro et al. (17). Some samples of reduced and alkylated OA protein were dialyzed against distilled water, precipitated with 10% trichloroacetic acid, and protein was determined by the Lowry method (8) in order to determine the amount of protein obtained. Comparison of these values with protein measurements from equivalent total OA samples indicated that approximately 60% of the total OA protein was extracted by the procedure of Renaud et al. (14).

Solubilization of other proteins was accomplished with a solvent composed of phenol, acetic acid, and water (2:1:1, v/v/v) described by Takayama and Stoner (19). Estimates of the proportion of total OA protein extracted by this method ran close to 40%. The oral apparatus pellet from a 500 ml standard culture was extracted with 100 µl of Takayama solvent, and the insoluble material was removed by centrifugation at 2000 g for 10 min. Preparations of this type were made 2 M in urea before electrophoresis.

**Electrophoresis**

Final separation of microtubule proteins was done by electrophoresis on polyacrylamide gels after the manner of Davis (2). The electrophoretic gels were made 8 M in urea and 7% in acrylamide. A small sample of microtubule protein in 8 M urea and Tris buffer was placed on top of the spacer gel. Electrophoresis was carried out with a bromphenol-blue marker dye at 150-200 v until the dye was within 0.5 cm from the bottom of the gel. Following electrophoresis, the gels were stained for 0.5 hr with 1% fast green in 7% acetic acid. Destaining was done overnight with several changes of 7% acetic acid and constant shaking. Gorovsky et al. (7) have shown that fast green stains proteins from ciliary axonemes in accordance with Beer's law in amounts of up to 150-200 µg of protein on 6-mm gels. OA protein quantities per gel were within this range in all experiments. Identification of OA microtubule proteins was made by co-electrophoresis with microtubule proteins from purified ciliary axonemes prepared according to the method of Gibbons (9).

Gel electrophoresis of the acid-extracted OA proteins was carried out with the methods developed by Reisfeld et al. (13) and modified by Panyim and Chalkley (11). The gels were pre-electrophoresed until amperage was reduced to 3.5-4.0 ma/gel. The Takayama solvent containing OA proteins was made 2 M in urea and placed directly on top of the small-pore gel. No spacer or sample gels were used. The gels were 8 M in urea, 5% in acrylamide, and 1.2% in bis-acrylamide. The electrophoresis was carried out for 45 min at 120 v. The gels were stained with 1% amido-black in 7% acetic acid for 0.5 hr and destaining was done as previously mentioned for microtubule proteins. With the protein quantities used in all experiments, amido-black stained the OA proteins in accordance with Beer's law. This was determined by preparing a series of gels with a known range of OA protein concentrations and measuring the optical densities of the bands. A linear relationship existed between protein quantity placed on the gels and optical densities of the various bands. In any experiment the protein quantities per gel never exceeded those in the test series.

**Specific Activity Determinations**

Incorporation of radioactive amino acids into cell proteins was measured by scintillation counting. For total cell and total OA proteins, radioactivity was measured in hot trichloroacetic acid precipitates solubilized with NCS solubilizer (Nuclear Chicago, Des Plaines, Ill.). For proteins separated on polyacrylamide electrophoretic gels, incorporation was measured by counting the radioactivity in successive 1-mm gel slices. Each 1 mm gel slice was placed into a scintillation vial with 0.5 ml of NCS and shaken vigorously for 48 hr; scintillation fluid was then added and the samples were counted.

Total OA protein was measured by the Lowry method (8). To insure complete solubilization of protein samples, 1 N NaOH was added and, when the sample had liquified, normality was reduced to 0.1 N as required by the Lowry method. Densitometric measurements of OA proteins separated on polyacrylamide gels were obtained with a Gilford recording spectrophotometer modified for this purpose with a Gilford gel carriage, model number 2410 (Gilford Instrument Company, Oberlin, Ohio). Only relative protein concentrations of corresponding bands on different gels were measured.

Data derived by the previous methods were used to calculate specific activities of the various cell proteins. Total OA protein specific activities were calculated as counts per minute (cpm) per microgram protein. For the specific activity determinations of OA proteins separated on polyacrylamide gels, the du Pont curve analyser was used (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The curve analyser was used to convert the radioactivity data from its original histogram plot to a Gaussian curve plot (Fig. 10). Specific activities were calculated for the corresponding peaks by using the curve analyser to calculate areas under the peaks. Specific activities for the OA proteins separated electrophoretically were calculated by dividing the area of the cpm
curve by the area of the corresponding optical density curve (Fig. 1). Specific activity for total cell protein is expressed as the cpm per 80,000 cells and was calculated from the cpm in total cell protein in 1-ml culture samples taken every 0.5 hr during the experiments.

**Electron Microscopy**

Samples of the isolated oral apparatus fraction were examined by electron microscopy. It was found that a mixture of glutaraldehyde and osmium tetroxide preserved the structure in this material better than several other fixatives which were tried. The osmium tetroxide (0.5%) and glutaraldehyde (1%) were mixed in 0.1 M phosphate buffer at pH 7.0 and kept cold until use, which was within 0.5 hr after mixing. The isolated OAs were fixed for 20 min in this mixture, dehydrated, and embedded in Epon. Sections were cut and observations were made with an RCA EMU-3D electron microscope.

**RESULTS**

**Electron Microscopy**

The standard OA fraction was prepared for electron microscopy in order to characterize the isolated OA morphologically and to check for contamination of the preparation by other structural elements. All OA preparations examined appeared to be reasonably free of contamination, with only an occasional mitochondrion or cilium appearing in the sections.

The fine structure of the oral apparatus of *Tetrahymena* in intact cells has been described previously (10, 20). Examination of the fine structure of the OA isolated by the Triton method used in the present study has shown that nearly all structural elements are present, appear normal, and are in proper spatial relations with respect to each other (Figs. 2–5). The oral cilia, however, are not present, therefore the data in the present report essentially pertain to the composition and genesis of intracytoplasmic oral components rather than to cilia. The cilia break away at a characteristic point just above the central granule which marks the origin of the central ciliary axonemes (Fig. 2). This leaves a certain amount of ciliary substance attached to each basal body, although this amount is extremely small. The ciliary membranes appear to fuse across the top of the ciliary stubs. Sections of membranelar and undulating membrane basal bodies indicate that these structures are complete and normal, except that preliminary measurements suggest that these and other structural elements in the isolated and fixed OA may have undergone slightly more shrinkage than those seen in fixed and sectioned whole cells. The basal bodies show normal triplet microtubular structure (Fig. 3), and the internal dense rods and distal partitions can be seen in Fig. 2.

The pellicle in the oral region lines the buccal cavity and extends between the membranelles and the undulating membrane. The isolated OA shows the presence of the typical three unit membranes which define the pellicle. The oral basal bodies attach to the pellicle where the latter is differentiated into a “fused pellicular plate” (10), and this can be seen in Fig. 3. Pellicle-associated perisomal sacs and epiplasma (20) are also present; endoplasmic elements are completely absent, however.

Elements of the oral apparatus of *Tetrahymena* are interconnected by extensive collections of subpellicular microtubules. Many of these are grouped into gross “fibers” previously identified as (a) membranelar connectives, (b) anterior cross connective, (c) posterior cross connective, and (d) peripheral connective (10). These are all present in the isolated oral apparatus. In whole cells, microtubules of the right wall of the buccal cavity, the “ribbed wall,” extend below the cytopharynx as the “deep fiber bundle,” which may be as much as 15 μ long (10). This bundle of microtubules is lost in the isolated oral apparatus, presumably by breaking off at the cytopharyngeal region during isolation. The ribbed wall is intact, however, and includes the normal microtubular components (Fig. 4).

The *Tetrahymena* oral apparatus also includes structures composed of fine filaments which have been identified as the “coarse filamentous reticulum” and the “fine filamentous reticulum” (20). These are found in the isolated OA and are shown in Figs. 4 and 5, respectively. Study of sections of the isolated OA has revealed the presence of filamentous material in the membranelar regions as well, and has led to the understanding that this material pervades most of the oral apparatus. The filamentous material appears to be interconnected throughout the OA. The “filamentous reticulum” in *Tetrahymena* should therefore probably be regarded as a single pervasive network of filaments in the oral apparatus.
**Solubilization and Identification of Oral Apparatus Proteins**

Microtubule proteins extracted from oral apparatuses were identified in electrophoretic gels by co-electrophoresis with microtubule proteins isolated from purified ciliary axonemes. A typical result is shown in Fig. 6. The left gel contains OA protein only, the center gel contains microtubule protein from purified ciliary axonemes, and the right gel is a mixture of both. On the basis of electrophoretic mobility, it appears that the two prominent bands close together in the OA gel are identical (or very similar) to the two microtubule proteins found in the outer doublets of cilia. The two preparations were also compared on SDS gels (Fig. 8). Reduced and alkylated samples were treated according to the method of Shapiro et al. and compared with standards as shown in Fig. 8. The relative migrations of oral apparatus and ciliary axoneme proteins indicate a molecular weight of about 55,000 daltons for both, suggesting an identity with each other and with microtubule protein. The oral apparatus SDS gels also contained material at the 110,000 molecular weight position, suggesting the presence of dimers of microtubule protein in these preparations. The dark region near the top of the urea gels (Fig. 6) may also contain this material.

Acid-soluble proteins, solubilized in the phenol-acetic acid-water solvent, were electrophoretically separated as shown in Fig. 7. Since microscopical examination of the isolated OA fractions reveals that some contaminating material can be found even in the most pure preparations, a comparison of a relatively pure OA preparation with a sample of contaminants was desirable. The contaminant preparation was made from the supernatant remaining after the OAs had been centrifuged from the crude cell lysate. The microscopically visible particles in the supernatant appeared to be morphologically the same as those few contaminants found in the OA preparation. This supernatant was again centrifuged at 2000 g for 30 min over the customary 1 M sucrose cushion, producing a pellet. The electrophoretic patterns of proteins from the OA and contaminant preparations were compared in the acid gel system with the Takayama solvent, to identify those bands that represent solubilized OA material. This is seen in Fig. 7, where the left gel shows the banding pattern from a relatively clean OA preparation and the right gel shows the banding pattern produced by a contaminant preparation. The center gel shows the banding pattern produced when contaminants and OA preparations are mixed. The bands labeled 1, 2, and 3 are clearly associated with the presence of the oral apparatus and are thought to be proteins from this organelle.

It is desirable to know which structural elements in the OA give rise to each of the bands resolved electrophoretically. Protein from microtubules has been identified, although it must be pointed out that protein recovered might con-
Polyacrylamide gel electrophoresis comparison of microtubule proteins from oral apparatuses and ciliary axonemes. The gel on the left is microtubule protein prepared from isolated oral apparatuses according to the method of Renaud et al. (14). The gel in the center is microtubule protein isolated from a purified ciliary axoneme preparation by the method of Gibbons (6). The right gel contains a mixture of both preparations. See text for discussion.

It has been suggested in a previous study (21) that at least some of the oral apparatus proteins may be stored in the cytoplasm and used in organelle formation over long periods. This suggestion was based on radioautographic data. The first experiments in the present study were concerned with testing this possibility by using biochemical methods. A logarithmically growing culture of cells was first given an exposure to tritiated amino acids (2 μCi/ml) for 10 hr. Then the cells were washed and resuspended in fresh unlabeled medium at a concentration of 8–9 × 10⁴ cells/ml. Cell density was monitored subsequently by fre-

Synthesis of Oral Apparatus Proteins

Preliminary experiments with solutions at high and low pH and at various ionic strengths have not as yet given positive results. The morphological elements which give rise to the three bands in the acid gels following solubilization with the Takayama solvent are therefore unknown at present.

Oral apparatus proteins extracted with phenol-acetic acid-water (2:1:1). All oral apparatus preparations contain a small amount of microscopically visible contaminants. The proteins on the left gel are from a relatively pure oral apparatus preparation. The right gel contains proteins from a purified preparation of contaminants. The middle gel contains a mixture of both. Note that bands 1, 2, and 3 correlate with the presence of oral apparatus material.
Estimation of molecular weight of presumed microtubule proteins prepared from isolated oral apparatuses of *Tetrahymena* (OA-mt), and comparison with known microtubule proteins prepared from ciliary axonemes. The procedure was that of Shapiro et al. (17) and the standards were bovine serum albumin (BSA), deoxyribonuclease, and bovine hemoglobin. The determination was repeated many times. The same value is obtained for axoneme microtubule protein and oral apparatus protein. Discussion in text.

Subsequent sampling and determination of cell number with a Coulter counter. In this way, the length of the lag period could be predicted under standard conditions, and the course of logarithmic growth could be determined. It was found that a lag period of 3 hr always occurred, and that this was then followed by a period of logarithmic growth in which the doubling time was 3.5 hr.

Specific activities were determined in total cell protein and total oral apparatus protein at times subsequent to the chase until the end of the first doubling in cell number as indicated in Fig. 9. Virtually all of the OAs present until the resumption of growth (3 hr) were formed in the presence of labeled amino acids. In contrast, one-half of the OAs at the end of the doubling in cell number subsequent to resumption of growth (6.5 hr) were formed in the absence of labeled amino acids. Assuming an adequate chase (discussed below), a decline in specific activity in OA protein to one-half the initial level over this interval would indicate *de novo* synthesis, whereas a decline in specific activity significantly less than this would suggest the use of stored protein in OA formation under these conditions. Fig. 9 shows that the latter result was obtained.

The conclusion from this experiment regarding storage of OA protein by the cell depends upon the effectiveness of the chase, i.e. free labeled amino acids should not be available for new protein synthesis during the doubling in cell (and organelle) number subsequent to the chase. As a check on this, specific activity in total cell protein was determined from samples taken at 0.5 hr intervals during the experimental doubling. The results (Fig. 9) show that the specific activity of total cell protein fell to one-half the pre-doubling level by the time a doubling in cell number had occurred. This indicates that no net synthesis of radioactive proteins occurred after the chase. The chase, therefore, appears to have been completely effective.

The pattern of synthesis was next studied in individual OA proteins by using the same procedure as described in the previous experiment. Microtubule proteins were separated on polyacrylamide gels and specific activities were determined in predoubling and postdoubling samples, i.e. before (3 hr) and after (6.5 hr) the first doubling in cell number after the chase. The results are shown in Table I. It is seen that the specific activity of oral microtubule protein fell to one-half the initial value during the doubling in cell (and organelle) number. The oral microtubule proteins, therefore, appear to be regulated like the average cell protein (see Fig. 9). This
suggests that these oral proteins, unlike certain others, are not stored in a large cytoplasmic pool in logarithmically growing cells, but are assembled into OA structure in the same cell cycle in which they are synthesized.

The experiment was again repeated, and this

| Time of OA isolation | cpm | on | Specific activity |
|----------------------|-----|----|------------------|
| Predoubling exp 1    | 817 | 1230 | 0.66             |
| Postdoubling exp 1   | 169 | 510  | 0.33             |
| Predoubling exp 2    | 334 | 300  | 1.11             |
| Postdoubling exp 2   | 538 | 1000 | 0.538            |

Figure 10 Levels of radioactivity in the acid-extracted OA proteins before and after one doubling in cell number following a chase. Radioactivity was determined by scintillation counting of gel slices. Specific activity is expressed as counts per minute (cpm) per unit optical density (on).

Figure 11 Specific activities in the acid-extracted OA proteins before and after one doubling in cell number following a chase. The specific activities were determined from the curves in Fig. 10 and are expressed as the logarithm of the ratio of areas under corresponding peaks. A comparison is made with the changes in specific activity in oral microtubule proteins and in total cell protein. This is a graphic summary of the data in Fig. 10 and Table I. The limitation is that the curves imply linearity, which is not demonstrated except in the case of total cell protein (small circles). The over-all changes are accurately reflected, however, and the difference between the two types of proteins is clear.

The major objective in the present study has been to determine relations between synthesis of proteins of the oral apparatus of *Tetrahymena* and assembly of this organelle system. The data obtained with biochemical methods regarding total

**DISCUSSION**

The major objective in the present study has been to determine relations between synthesis of proteins of the oral apparatus of *Tetrahymena* and assembly of this organelle system. The data obtained with biochemical methods regarding total
found that not all oral proteins are regulated in the
experiments of the type described above, and it was
present study. These proteins were studied in ex-
tubule proteins, were isolated from the OA in the
cell. The simplest explanation at present,
therefore, would appear to be the storage of OA
cell proteins. The simplest explanation at present,

increase in radioactivity was found in oral ap-

tioned in OA development before the chase. This would mean that
some OA proteins are synthesized and stored in a
cytoplasmic pool for use over long periods. It is
 conceivable that the increase in labeled OA pro-
tins could be due to incorporation of amino acids provided by turnover of previously labeled cyto-
plasmic proteins. This seems unlikely, however,
because protein turnover is not demonstrated in
growing Tetrahymena cells (1). Even if some turn-
over were to occur, the data would require that
labeled amino acids made available for synthesis in this way would have to be preferentially di-
rected into the synthesis of oral apparatus proteins and kept from entering other proteins, i.e.,
the increase in radioactivity was found in oral apparatus protein but not in total cell protein during a period of active synthesis for, presumably, all cell proteins. The simplest explanation at present, therefore, would appear to be the storage of OA specific proteins by the cell.
Several individual proteins, including micro-
tubule proteins, were isolated from the OA in the
present study. These proteins were studied in ex-
periments of the type described above, and it was
found that not all oral proteins are regulated in the
same way. The data obtained from the study of
OA microtubule proteins indicate that they are
regulated like the average cell protein, i.e.,
the specific activity in microtubule proteins of the OA
debled to one-half the initial level as the number of

cells and OAs doubled. This decline in specific activity means that there was no net increase in labeled microtubule proteins incorporated into oral structures after the chase. This indicates that
there was no large reservoir of previously synthe-
sized microtubule proteins in these cells and that
only unlabeled amino acids were incorporated into
microtubule proteins synthesized after the chase.
The decline in specific activity in microtubule proteins of the OA to one-half the initial level following a doubling under chase conditions indi-
cates that the labeled microtubule proteins were
conserved in the population of OAs and that these
were diluted by synthesis of an equivalent amount of unlabeled microtubule proteins following the
chase.
The study of the acid-extracted OA proteins
revealed a regulatory pattern unlike that found for
microtubule proteins, but qualitatively similar
to that observed for total OA protein, i.e., specific
activity in these acid-extracted OA proteins de-
clined significantly less than 50% over one genera-
tion following the chase. This slight decline in
specific activity would be explained if it is assumed
that the proteins of this type used in formation of
the oral structures after the chase were synthesized before the chase when labeled amino acids were
present, then stored in the cell for a considerable
time before being assembled into structures. The
possibility of radioactivity incorporated into acid-
extractable OA proteins as a result of an inade-
quate chase or protein turnover seems unlikely, as
has been discussed previously for total OA protein.
Attempts to identify the structures in the oral
apparatus which contain the acid-extracted pro-
tins studied here have as yet been unsuccessful.
A possible clue to the source of these proteins is
found in their regulatory pattern. Evidence has
been obtained that free cytoplasmic pools of
certain membrane proteins may exist in some cells
(12), which suggests the possibility that the pooled
oral proteins of the oral apparatus of Tetrahymena
may be from the membranous components of this
organellar system.
Careful studies using inhibitors (5) have estab-
lished that de novo synthesis of protein is required
to support development of the oral structures in
Tetrahymena, at least in early and middle stages of stomatogenesis. The present studies demonstrate two classes of proteins in the oral apparatus with respect to patterns of synthesis: (a) some (microtubule proteins) which appear to be synthesized in rather close relation to development of the structure, and (b) others which may be stored over long periods before being assembled into the structure. The de novo requirement for synthesis indicated by the inhibitor studies might conceivably reflect the need for synthesis of microtubule proteins. This cannot be concluded, however, because the present data do not rule out the possibility that the synthesis of microtubule proteins may occur early in the cell cycle before OA development begins, with the requirement for synthesis during development reflecting the need for other components, either structural or catalytic, for assembly processes. Further studies now in progress using synchronous cultures should help to distinguish between these two alternatives.

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