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Relationship between the Flagellates and the Ciliates

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INTRODUCTION

The ciliates have long been recognized as a distinct group of organisms (26, 79, 114). The following classical features distinguish the ciliates from other organisms. (i) They exhibit nuclear dualism (the possession of two types of nuclei). They have a germ line diploid micronucleus, which is transcriptionally inactive, and a vegetative polyploid macronucleus, which is responsible for transcription in the cell. (ii) They possess cilia at some stage in their life history. Each cilium has a kinetosome (basal body) with characteristic fibrillar structures in the cytoplasm associated with it. (iii) They have alveoli in the cortical cytoplasm. An alveolus consists of a single flattened membrane cisternum that usually occurs beneath the plasma membrane and commonly has rows of microtubules under it. There are ciliates that lack one or more of the above characteristics. However, the vast majority of ciliates have these three features.

The flagellates have been considered to be the closest relatives of the ciliates, with their unicellular nature and the similarity in the structure of cilia and flagella providing the basis of this relationship (20, 22, 27, 80, 113, 115, 120, 121). In the following review, we will present first the morphological and cytological evidence, and then the molecular evidence, for the close relationship between the ciliates and the flagellates.

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COMPARISONS BASED ON MORPHOLOGICAL AND CYTOLOGICAL STRUCTURES

The dinoflagellates and two genera of uncertain taxonomic position, *Colponema* and *Katablepharis*, are the flagellates with morphological and cytological structures most similar to those of the ciliates. A comparison of each of these with the ciliates is presented.

Dinoflagellates and Ciliates

Historically, the dinoflagellates have generally been considered to be the most likely ancestors of the ciliates. In the 1800s, it was thought that the beating waves of the transverse flagellum encircling the cell in the cingulum of dinoflagellates was actually produced by the waves of closely packed cilia (23, 27, 68). This observation resulted in the inclusion of the dinoflagellates in the phylum Cilioflagellata with the ciliates.

More recently, Taylor (120, 121, 122) (Fig. 1) presented a phylogenetic tree with the ciliates arising from a branch just above the dinoflagellates. The resemblance between the cortical structures of the ciliates and dinoflagellates was presented as the strongest argument for the closeness of the

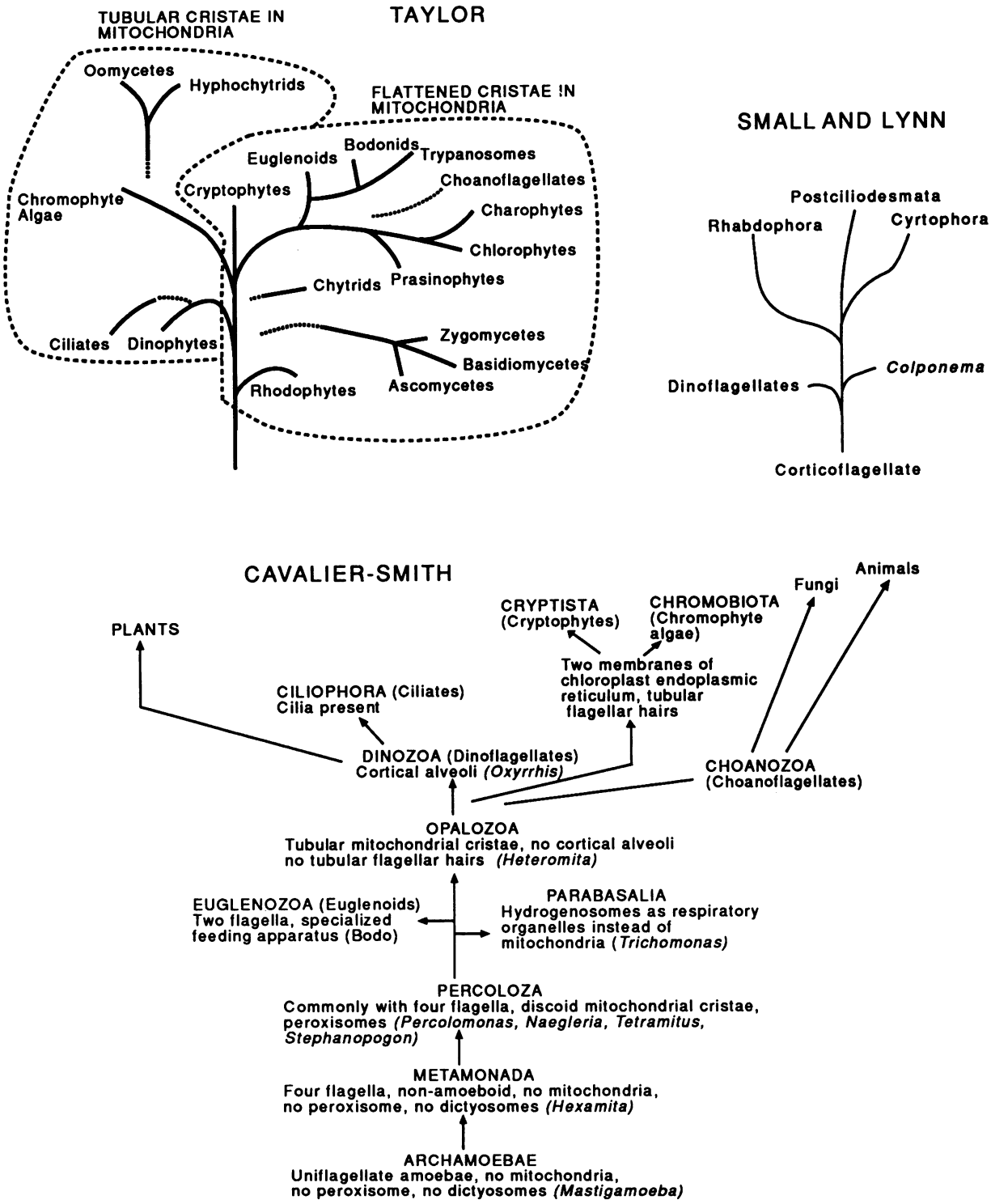

FIG. 1. Portions of evolutionary schemes that have involved the relationship between the dinoflagellates and the ciliates.

two groups. Other authors have derived the ciliates from the dinoflagellates through the dinoflagellate *Polykrikos* sp. (20, 92, 113). *Polykrikos* sp. is a multinucleate and multiflagellate dinoflagellate that consists basically of a number of dinoflagellate cells stacked one on top of another and fused to produce a single cell. This dinoflagellate probably evolved by mitosis followed by only partial cytokinesis. Such a multiplication of the nuclei and flagella within a single cell has been considered a first step toward the multinucleate and multiciliated condition in the ciliates.

Cavalier-Smith also believed that the ciliates evolved from the dinoflagellates (20–22) (Fig. 1) and placed both in the

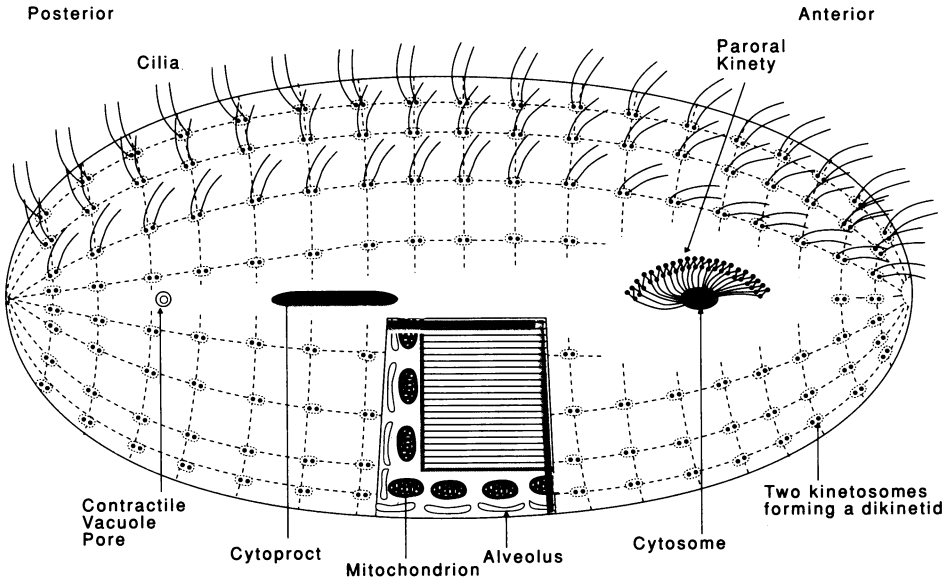

FIG. 2. Structure of a cell of a typical ciliate.

subphylum Corticoflagellata. The Corticoflagellata is characterized by a highly developed cortical microtubular system, a phagocytic mode of nutrition, a strong tendency to evolve repeated cortical structures and multiple nuclei, genomes or cells, and the absence of the transitional region star and of tubular mastigonemes. Similarly, Small and Lynn (78, 115) (Fig. 1) derived the ciliates from a corticoflagellate ancestor, with the dinoflagellates arising from a branch immediately under the ciliates. Corliss (24, 27) has reviewed the relationship of the ciliates with the flagellates and has also come to the conclusion that the dinoflagellates represent the most probable ancestor of the ciliates.

Comparison of dinoflagellates and ciliates. The dinoflagellates and ciliates have a number of cytological structures which are similar and some which are not. Their structures include the cortical alveoli, mitochondrial cristae, cilia and flagella, parasomal sacs, pusules, extrusive organelles, feeding apparatuses, and nuclei.

(i) Cortical alveoli. The cortical alveoli in the ciliates are flattened membrane sacs that lie just beneath the plasma membrane and above the epiplasm (Fig. 2; see also Fig. 4) (26, 79, 115). A row of microtubules frequently occurs beneath the alveoli. The dinoflagellates have flattened thecal vesicles under the plasma membrane (Fig. 3 and 4) (31, 32) that appear to be similar to the cortical alveoli of the ciliates. In the dinoflagellates, the thecal vesicles are commonly filled with thecal plates. Like the cortical alveoli of the ciliates, the thecal vesicles often lie above a row of microtubules. Cavalier-Smith (22) argues that the thecal vesicles are an evolutionary response to predation, with the thecal plates acting as a type of armor.

(ii) Mitochondrial cristae. In different organisms, the cristae of mitochondria can be flattened or tubular (120, 121). Only one type of mitochondrial crista occurs in a group of organisms, and it is possible to divide the protozoa into two different evolutionary lines on the basis of the shape of

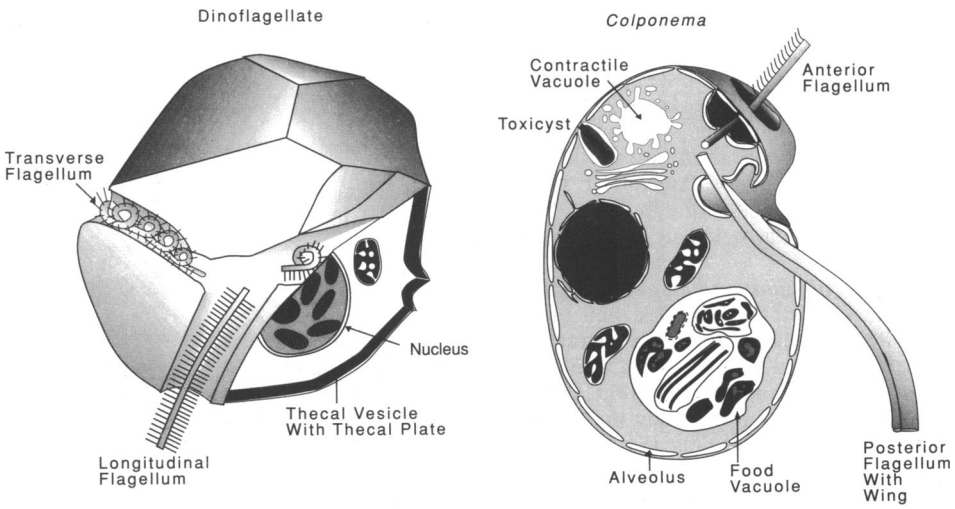

FIG. 3. Structures of a cell of a typical dinoflagellate and a *Colponema* cell.

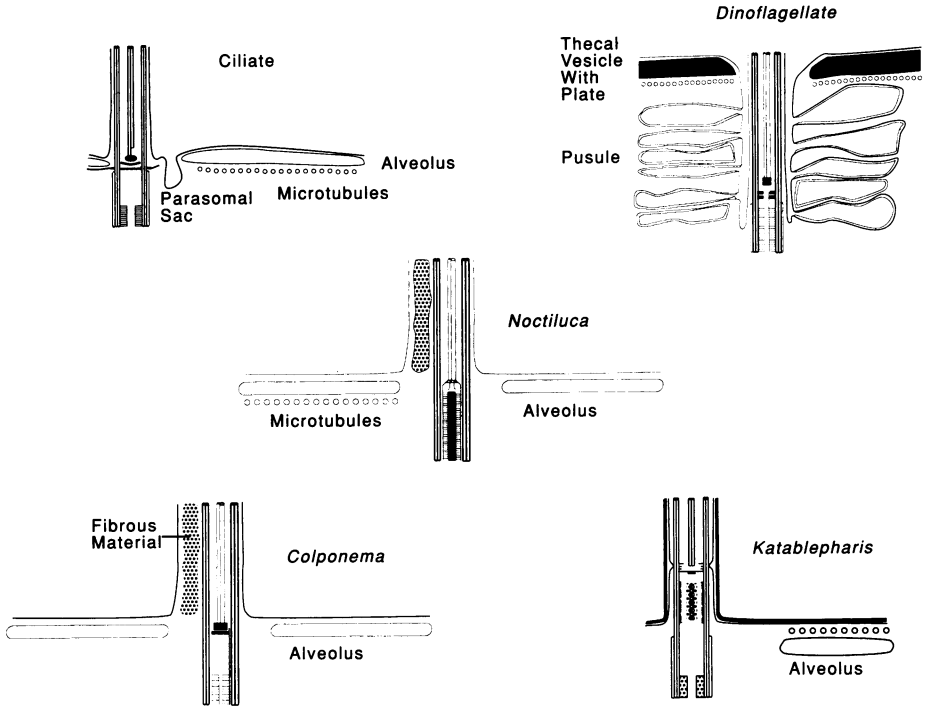

FIG. 4. Cortical and kinetosome structures of a ciliate, a typical dinoflagellate, and the genera *Noctiluca*, *Colponema*, and *Katablepharis*.

mitochondrial cristae (Fig. 1) (121); however, it should be mentioned that Gunderson et al. (43) argue that organelle characteristics such as mitochondrial cristae and chlorophyll type are not reliable phylogenetic indicators in early-diverging plants. The evolutionary lines based on the shape of the mitochondrial cristae generally agree with evolutionary lines based on other characteristics. Both the ciliates and dinoflagellates have tubular mitochondrial cristae. This indicates that the two groups are probably in the same evolutionary line, although it does not necessarily indicate that they are close, since this line includes half of the protozoa.

(iii) Cilia, flagella, and associated structures. Cilia and flagella have the same basic structure; they are about 0.25 μm in diameter and are composed of an axoneme surrounded by cytoplasm and the plasma membrane. The microtubules of the axoneme are arranged as nine peripheral doublets with two separate central microtubules. Each peripheral doublet consists of a complete A microtubule that shares part of its wall with an incomplete B microtubule. Each A microtubule has lateral dynein arms. Although this is the basic structure of cilia and flagella, there are variations involving the grouping and number of flagella or cilia, structures on the surface and beneath the surface, the structure of the basal body, the ciliary necklace and the type of ciliary or flagellar roots.

(a) Grouping and number of cilia and flagella. In the ciliates, related basal bodies (kinetosomes) with their associated ciliary roots are called a kinetid (77–79, 115). Kinetids may have one, two, or more basal bodies in each kinetid (monokinetid, dikinetid, or polykinetid). Different ciliate groups are characterized by the type of kinetid in the group. It has been postulated that the dikinetid is the ancestral condition in the ciliates. All dinoflagellates are dikinetid and therefore, as far as this character is concerned, qualify as ancestors of the ciliates.

In a ciliate the kinetids are linked together to form kineties or rows of cilia. It is the large number of ciliary rows that distinguish a ciliate from a flagellate. Some dinoflagellates, however, are able to change from a cell with a single dikinetid to one with many dikinetids. In the parasitic subclass Amoebophryidae, the dinospore initially contains only two flagella (Fig. 5) (16, 18). After the dinospore has infected the host, the cell elongates considerably, with the girdle making many helical coils around the cell. As the girdle elongates, many new flagellar pairs are produced along the girdle. The resulting multiflagellated and multinucleated cell illustrates that the production of a dikinetid multiflagellated cell leading to cilia is not a large evolutionary step for the dinoflagellates.

(b) Surface and subsurface of cilia and flagella. Cilia have no hairs, tubules, or theca on their surface, and the interior contains a normal axoneme. Dinoflagellates have a trans-

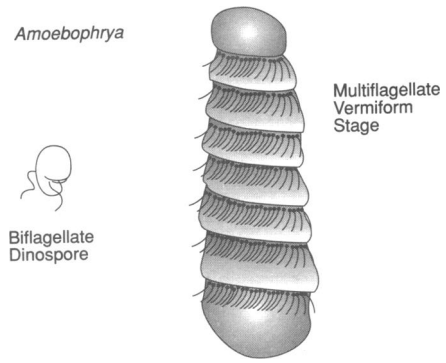

FIG. 5. The dinoflagellate *Amoebophrya* showing a biflagellate dinospore and the multiflagellate, multinucleate vermiform stage.

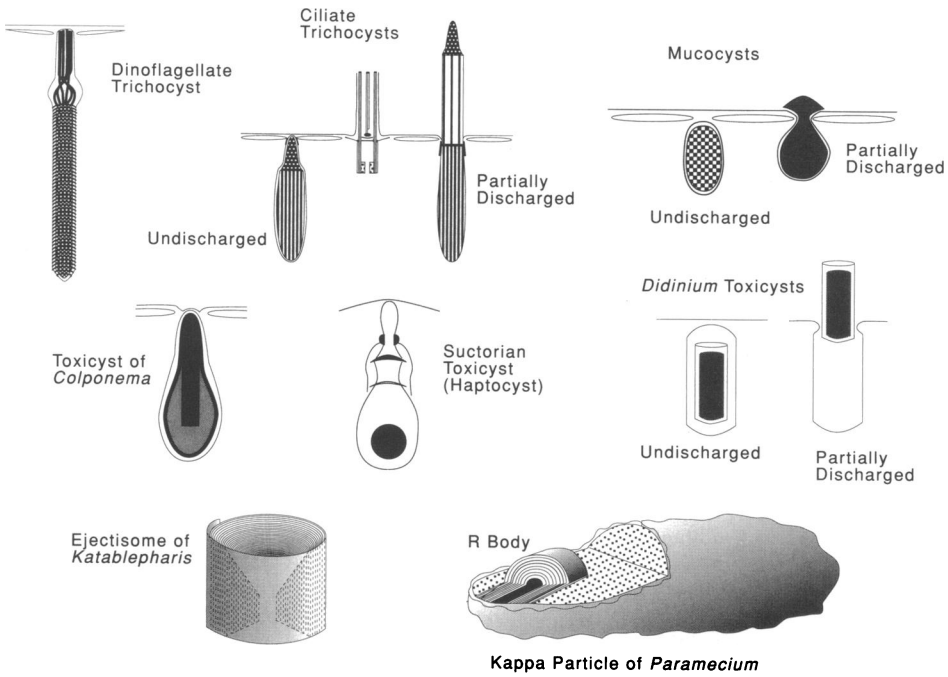

FIG. 6. The types of extrusive organelles found in organisms discussed in the text.

verse flagellum in the girdle (cingulum) that runs around the cell and a longitudinal or posterior flagellum that protrudes from the cell (Fig. 3). The transverse flagellum has a striated strand next to the axoneme (10, 11). The striated strand is shorter than the axoneme and distorts the transverse flagellum into an undulating structure. Fibrillar hairs are present on the surface of the transverse and longitudinal flagellum (32).

(c) Basal body structure. The structure of the basal body is characteristic of a group of flagellates or ciliates (39). The structure of the basal body of the dinoflagellates is different from that of ciliates (Fig. 4). At present, no one has presented an evolutionary progression of basal body structures, so it is not clear how far removed in evolution the basal bodies of the dinoflagellates are from those of the ciliates.

(d) Type of ciliary necklace. Ciliary necklaces, patterns of intramembrane particles in the plasma membrane at the base of cilia, have been used to characterize groups of ciliates (7, 39). These structures have not yet been examined in the dinoflagellates.

(e) Type of ciliary and flagellar roots. Cytoplasmic root structures associated with basal bodies (kinetosomes) can be composed of either microtubules or striated fibers (39). In ciliates these structures are represented by the microtubules that make up the transverse and postciliary microtubular ribbons and the striated fibers that make up the kinetodesmal fibril (77, 114, 115). The dinoflagellates also have microtubular and fibrillar roots (34, 35, 107), although not in the same configuration as those in the ciliates.

(f) Summary of similarities in cilia and flagella of the ciliates and dinoflagellates. There is little in common between the cilia of ciliates and the flagella of dinoflagellates to suggest that they are related. About the only positive aspect is the dikinetid nature of the dinoflagellates and the proposed ancestor of the ciliates and the tendency of some dinoflagellates to produce multiflagellated cells.

(iv) Parasomal sac and pusule. The dinoflagellates have water-regulating structures, pusules, associated with the flagella (Fig. 4) (30). The pusule is similar to a contractile vacuole. There are usually two pusules, one associated with each flagellar canal. The pusule has about 40 globe-shaped indentations that open into the flagellar canal. This relationship is somewhat similar to the association between a parasomal sac and cilium in the ciliates (Fig. 4).

(v) Extrusive organelles. The most common types of extrusive organelles in the ciliates, trichocysts and mucocysts, have similar counterparts in the dinoflagellates (Fig. 6) (32, 51). The undischarged trichocyst of the ciliate *Paramecium* is contained in a spindle-shaped vesicle beneath the plasma membrane and is situated between basal bodies or paired basal bodies in the cortex. The undischarged trichocyst is composed of a shaft of crystalline material with 7-nm striations (2, 52). At the top of the shaft, under the plasma membrane, is the trichocyst tip, which is also crystalline with striations that are 7 nm apart. Three different types of sheaths surround the shaft and tip, and the whole structure is contained in a vesicle. On discharge, the shaft expands from about 3 μm in length to 25–35 μm, driving at its apex the unexpanded tip. The discharged trichocyst has about 500 striations (as does the undischarged trichocyst), but the striations are now 55 nm apart.

A dinoflagellate trichocyst is similar in structure to that of a ciliate trichocyst. An undischarged dinoflagellate trichocyst (Fig. 6) (14) has a rod-shaped crystalline core, as does the ciliate trichocyst. At the top of the crystalline core are 20 to 22 fibers that extend from the core to the enclosing membrane. Just within the enclosing membrane are five hoops. The anterior part of the trichocyst membrane is attached to the plasma membrane between thecal vesicles. On discharge, the trichocyst elongates to a tapering rod up to 200 μm long containing 50- to 80-nm striations.

Thus, the undischarged trichocysts of both ciliates and dinoflagellates are composed of a crystalline shaft with an apical structure all enclosed in a vesicle. On discharge, both

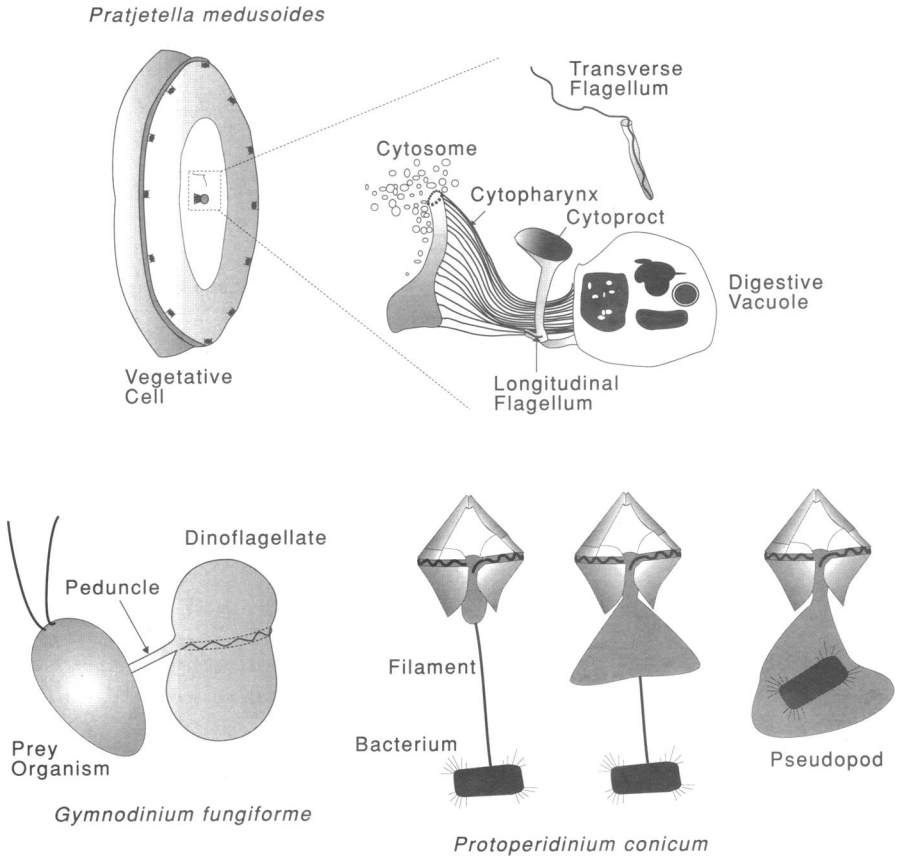

FIG. 7. Representative types of feeding apparatuses found in dinoflagellates.

types of trichocysts expand into long, rod-shaped projectiles with about 50- to 80-nm striations.

Both the ciliates and the dinoflagellates also have mucocysts, extrusive organelles that discharge mucus. The mucocysts of the ciliates are composed of a spindle-shaped core made up of regular subunits surrounded by a membrane (Fig. 6) (51). On discharge, the mucocyst membrane fuses with the plasma membrane and an elongated amorphous or striated rod is produced. Dinoflagellate mucocysts (32) are flask-shaped vesicles under the plasma membrane that contain a finely granular material that is secreted onto the outer surface of the cell. In both the ciliates and the dinoflagellates, therefore, the mucocysts have similar structures and functions.

(vi) Feeding apparatus. Most ciliates are phagotrophic, taking food particles or organisms in through a mouth (cytosome, cytopharynx) that is commonly surrounded by rows of cilia (oral kinetids) (Fig. 2). The mouth and the oral cilia make up the oral apparatus, which can be on the cell surface or in a depression of the cell surface. Once taken up, the food passes into food vacuoles, where it is digested. The undigested components of the food pass out through the anus of the cell, which can be a well-defined structure (cytoproct) or a less well-defined structure (cytopyge) (115).

Although this is the complex feeding and digestive system of most ciliates, some ciliates have a simpler system. The ciliate class Karyorelictea is generally considered to be ancestral among ciliates because of its nuclear characteristics (26, 77, 79, 102, 115). The simplest feeding apparatus in the ciliates is found in the karyorelictean *Kentrophoros* and *Trachelonema* spp. (115). In these organisms, the cytosome is an apical dome-shaped or elongate region. The cytosome is not a permanent organelle but is formed only as prey is captured. If a cytopharynx is present, it is supported by transverse microtubules that originate at the ciliary basal bodies.

Relatively simple feeding apparatuses also occur in some dinoflagellates in the order Noctilucales (123). *Noctiluca* spp. have a relatively undifferentiated cytosome that consists of a fold in the cell surface that is supported by groups of fibrils (117). More complex digestive systems, similar to the complex systems in the ciliates, are found in other members of the order. A dinoflagellate such as *Pratjetella medusoides* (Fig. 7) (17) has a digestive system composed of a cytosome, cytopharynx, food vacuoles, and a cytoproct. Thus, the dinoflagellates in the order Noctilucales have a range of feeding apparatuses that are similar to those in the ciliates.

A second type of feeding apparatus in the dinoflagellates involves the extension of a peduncle (70, 118) or pseudopod (38, 67) that attaches to, or engulfs, the prey organism (Fig. 7). A peduncle is a projection of cytoplasm that contains an array of microtubules (70). The peduncle is extendable and can attach to and make a hole in the prey organism (118, 119). The cytoplasm of the prey is taken up into the peduncle and streams back into the dinoflagellate, where it is digested in food vacuoles. This method of feeding is not unlike that in suctorian ciliates. The suctoria have tentacles that attach to prey by chance contact. The tentacle shortens and broadens while the prey is held at the tip of the tentacle. A stream of

tiny granules moves up the tentacle, and the prey cytoplasm flows through the tentacle into food vacuoles in the body of the suctorian (5, 109, 110). A cross-section of the suctorian tentacle shows two concentric arrays of microtubules with associated vesicles and toxicysts. The dinoflagellate peduncle also contains microtubules, although they are not in the same configuration as in the suctorian tentacle.

In summary, the dinoflagellates show the same general types of feeding apparatuses that occur in the ciliates.

(vii) Nucleus. The dinoflagellate nucleus is unique among living organisms. The chromosomes remain condensed during the cell cycle and consist almost entirely of approximately 2.5-nm-diameter DNA fibrils with no histone protein and no nucleosomes (29, 32, 76, 104–106). The chromosomes contain large amounts of a fifth DNA base, hydroxymethyluracil (101), and lack nucleosomes (13, 60). There is a large amount of DNA in the dinoflagellate nucleus, with values ranging from 3 pg per cell in *Amphidinium* spp. to 200 pg per cell in *Gonyaulax* spp. (compared with 0.1 to 0.2 pg per cell in most flagellates) (105). During nuclear division, the nuclear envelope remains intact and spindle microtubules occur in the cytoplasm outside the nucleus (32), although there is one reported exception, the dinoflagellate *Oxyrrhis* sp., which has spindle microtubules inside the nucleus (124, 128). At metaphase, the spindle microtubules occur in cytoplasmic tunnels that pass through the nucleus, outside the intact nuclear envelope. The chromosomes lack the differential heterochromatic cross-banding that occurs in metaphase chromosomes of other organisms (44). The nucleolus does not disperse during nuclear division; instead, it pinches in two during anaphase.

All of the characteristics of the dinoflagellate nucleus are different from those of the generative nucleus (micronucleus) in ciliates (25, 103). The ciliate nucleus has about 0.2 pg of DNA per cell, and the chromosomes are dispersed during interphase; it contains no hydroxymethyluracil; and it has nucleosomes and spindle microtubules in the nucleus during nuclear division. The only similarity is the larger amount of DNA in the macronucleus of ciliates, although the ciliate macronucleus still has less DNA than that in a dinoflagellate nucleus. Heath (53) assembled data on mitosis from a large number of protists and analyzed the data by using two types of algorithms. Mitosis in the dinoflagellates clustered into two separate groups. The ciliates clustered into one group, which was no surprise considering the uniformity of mitosis in the organisms. The dinoflagellates clustered closer to the green algae *Valonia* and *Bulbochaete* than to the ciliates.

Summary of the similarities between dinoflagellates and ciliates. The similarities between the two groups include (i) the similarity in the cortical alveoli of the ciliates and the thecal vesicles of the dinoflagellates; (ii) the similarity in the tubular cristae of mitochondria; (iii) the similarity of the parasomal sac of ciliates to the pusule of dinoflagellates; (iv) the similarity in the structure of trichocysts and mucocysts in the two groups; and (v) some similarity in the feeding apparatuses of the two groups. Dissimilarities include (i) the structure of flagella and (ii) the structure and composition of the nucleus.

Comparison of *Colponema loxodes* and Ciliates

Colponema loxodes is a colorless, phagocytic flagellate (Fig. 3) that is characterized by Small (113) as having some similarities with the ciliates. *C. loxodes* has many of the characteristics of the dinoflagellates (22, 82), and so a comparison of *C. loxodes* with the ciliates is similar to the comparison of the dinoflagellates with the ciliates. As such, a relatively brief comparison of *C. loxodes* with the ciliates is presented. (i) *C. loxodes* has cortical alveoli similar to those in ciliates and like the thecal vesicles in the dinoflagellates (Fig. 4). Like the ciliates, the alveoli are empty in *C. loxodes*. (ii) Similar to the dinoflagellates and ciliates, *C. loxodes* has tubular cristae in the mitochondria. (iii) One flagellum in *C. loxodes* has a wing (Fig. 3), similar to the transverse flagellum of the dinoflagellates. The other flagellum has fibrillar hairs attached to the surface. The basal body is somewhat similar in construction to that of the ciliates and dinoflagellates (Fig. 4). The flagella have both fibrillar and microtubular roots. The structure of the ciliary necklace is not known. (iv) *C. loxodes* has a contractile vacuole near the flagellar basal bodies (Fig. 3) in much the same position as the parasomal sac in the ciliates and the pusule in the dinoflagellates. (v) *C. loxodes* has toxicysts that are discharged when the flagellate is feeding (Fig. 3 and 6). The undischarged toxicyst is a spindlelike structure in an oval vesicle beneath the plasma membrane and is composed of a capsule surrounding a tube. Toxicysts of somewhat similar structure occur in ciliates (Fig. 6) (51). (vi) *C. loxodes* does not have a specialized feeding apparatus. Instead, prey organisms are engulfed by the posterior portion of the cell. The method is somewhat similar to that used by some of the karyorelictean ciliates. (vii) The nucleus of *C. loxodes* appears to be similar to that of most flagellates. The details of nuclear division have not been reported.

Similarity between Suctorian Ciliates and the Flagellate *Katablepharis*

The structural similarities between the suctorian ciliates and the flagellate *Katablepharis* are the strongest between any group of ciliates and flagellates. The feeding apparatuses of *Katablepharis* spp. and the suctorian ciliates are virtually the same, they both have alveoli in their cortical cytoplasm, the flagella are subapical, there are no appendages on the flagellar surface, projectiles are present, and there are certain similarities in nuclear division.

Characteristics of *Katablepharis* spp. *Katablepharis* is a genus of unicellular colorless flagellates found in freshwater and marine environments. The two flagella are inserted subapically into a raised area of the cell (Fig. 8) (72). The flagella have scales on their surface and are covered by the cell covering, which also covers the rest of the cell. The cell covering is attached to the plasma membrane by a couple of attachment strips which resemble hemidesmosomes (71). The cell has one or more posterior food vacuoles, a central nucleus, and a Golgi body. There are two rows of large ejectisomes posterior to the area of flagellar attachment, and smaller ejectisomes are found under the plasma membrane in the posterior and medial areas of the cell.

The feeding apparatus occupies the anterior portion of the protoplasm of *Katablepharis* spp. (Fig. 8 and 9) (71, 73). The mouth of the feeding apparatus is an oval depression at the anterior end of the cell. The mouth is covered by only the inner component of the two-layered cell covering. Two arrays of microtubules, one inside the other, begin in the anterior cytoplasm behind the mouth. Each array contains groups of two to eight microtubules.

Characteristics of suctorian ciliates. The suctoria are unique among the ciliates in that they do not have cilia during their adult life. Adults are sedentary and are attached to a substrate by a disc. They are characterized by the presence of tentacles which are used to capture their prey

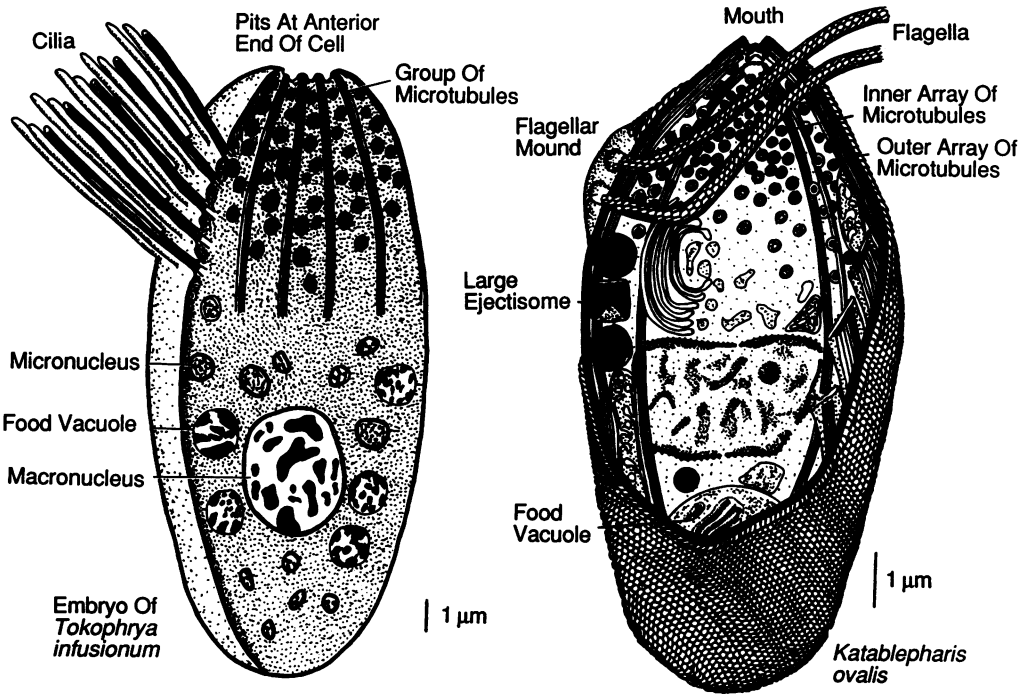

FIG. 8. Structures of an embryo of the ciliate *Tokophrya infusionum* and a cell of the flagellate *Katablepharis ovalis*.

(Fig. 9) (6, 110). The tentacle has a broad anterior knob on top of the narrower shaft, which is connected to the body. In cross section, the shaft is composed of microtubules arranged in two concentric circles. The broad anterior knob contains haptocysts (Fig. 6) that are discharged at the prey, with the result that the prey becomes attached to the tentacle.

The suctoria reproduce by an unequal division in a process known as budding, which results in the formation of a motile ciliated cell called a migrant or embryo (4, 28, 36, 42). In *Tokophrya* spp. a brood pouch is formed by invagination of the pellicle and plasma membrane (47). The ciliated embryo is formed in this brood pouch and, when mature, released through an opening to the medium, where it swims by means of its cilia. The embryo of *Tokophrya infusionum* is oval and about 20 μm long (Fig. 8). The embryo has several hundred cilia arranged in five rows circling the anterior end of the cell. A tuft of several cilia is also present at the posterior end. The anterior end of the embryo is indented into pitlike invaginations. Bundles of microtubules pass from under these pits toward the middle of the cell. Two types of vesicles are found between the bundles of microtubules.

Comparison of suctorian ciliates and *Katablepharis* spp. There are more similarities between the suctoria and *Katablepharis* spp. than between any groups of ciliates and flagellates. The most striking similarity is in the structure of the feeding apparatus. The feeding apparatus of the suctorian ciliates is contained within the tentacles of the nonmotile adult. In most suctoria, such as *Tokophrya* spp. (Fig. 8 and 9), prey is caught by chance contact of the tip of a tentacle with another ciliate. The prey is held to the tip of the tentacle, the tentacle shortens and broadens, a stream of tiny granules starts to move up the tentacle, the prey becomes paralyzed, and the cytoplasm of the still-living prey begins to flow through the center of the tentacle into the body of the suctorian (5, 109, 110, 125).

The suctorian tentacle is composed of a terminal knob on a shaft (Fig. 9). The knob is the only part of the tentacle which attaches to the prey. Under the anterior membrane of the knob are the haptocysts (49) or missilelike bodies (109, 110) (Fig. 6). On contact with prey, the haptocysts discharge and puncture the pellicle of the prey, thereby giving rise to a firm connection between the suctorian tentacle and the prey. The complex structure of the tentacle suggests the presence of several enzymes that may be responsible for puncturing the pellicle, stopping ciliary motion, and producing partial solubilization of the cytoplasm of the prey (6, 51, 110). Also within the knob are three types of vesicles. One type contains a spherical membrane within the vesicle membrane. The second type has an electron-dense core within the vesicle membrane. The third type contains electron-translucent contents, except for a thin electron-dense cap on one side. The knob is surrounded by only a single membrane, whereas the shaft of the tentacle is surrounded by two sheaths. The shaft contains two microtubular arrays, one inside the other (Fig. 9) (5, 6, 9, 49, 50, 61–62, 86, 104). The microtubules of each circular array are arranged in groups of about five to seven, depending on the species. During ingestion of the protoplasm of the prey, the microtubules of the inner array move out and become dispersed among the microtubular groups of the outer array, and the plasma membrane at the center of the knob invaginates, carrying the protoplasm of the prey with it into food vacuoles in the body of the suctorian.

The similarities between the tentacle structure of the suctoria and the feeding apparatus of *Katablepharis* spp. include the structure of the two concentric microtubular arrays and the structure of the vesicles associated with the microtubular arrays (Fig. 8 and 9). *Katablepharis* spp. have an anterior feeding apparatus composed of two concentric arrays of microtubules. The microtubular arrays are arranged the same way that they are arranged in the tentacles

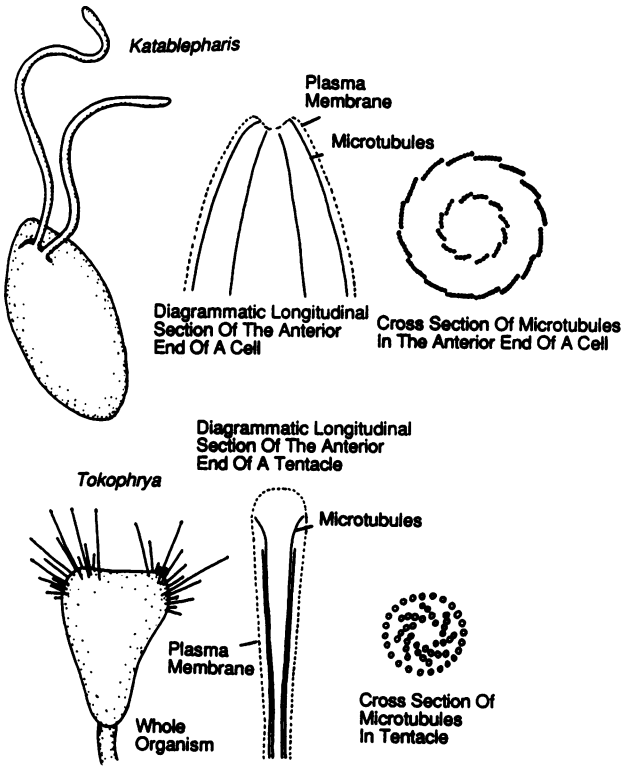

FIG. 9. Comparison of the feeding apparatus of the flagellate *Katablepharis* with that of the suctorian ciliate *Tokophrya*.

of the suctoria. Each array consists of groups of microtubules that are aligned slightly off center. The microtubular arrays are separated by cytoplasm that contains vesicles. Two of the types of vesicles in the suctorian tentacles are also present in *Katablepharis* spp. These are the vesicles containing a single spherical membrane and those containing electron-dense cores.

The suctoria produce a ciliated motile reproductive cell, called an embryo or migrant. The anterior end of the single-celled embryo is very similar to the anterior end of the *Katablepharis* cell (Fig. 8). The anterior end of the suctorian *Tokophrya* cell (47, 83) is composed of groups of microtubules that terminate under the anterior end of the cell. The anterior end of the embryo is invaginated into pits, with a microtubular group terminating between adjacent pits. The base of the pits is covered by a single membrane, while the rest of the pit, and the cell, has a thicker covering. There are two types of vesicles in the cytoplasm between groups of microtubules. Both *Tokophrya* and *Katablepharis* spp. contain groups of longitudinally arranged microtubules that terminate under the invaginations of the anterior end of the cell. In *Katablepharis* spp. there is only a single invagination (the mouth) (72, 73). In *Katablepharis* spp., the mouth is covered only by the plasma membrane and a layer of fibrils, while the rest of the cell has the thicker covering. This situation is analogous to that of the anterior pits in the *Tokophrya* embryo.

The system of cortical alveoli in the ciliates (Fig. 4) (26, 111, 115) is similar to that in *Katablepharis* spp. (71–73). In *Katablepharis* spp., the alveoli are continuous with the outer membrane of the nuclear envelope.

Adult suctorians are not ciliated. However, the embryo has rows of subapical cilia. In the suctorian *Tokophrya* spp. (47), there are several hundred cilia that encircle the anterior end of the cell in five rows (Fig. 8). In *Katablepharis* spp., there are only two subapical flagella (72). However, the flagella in *Katablepharis* spp. are located in the same part of the cell as are the ciliary rows in *Tokophrya* spp. The flagella and cilia do not have any hairs on their surface in either *Katablepharis* spp. or the suctorian ciliates, although the *Katablepharis* flagella are covered with a theca. The flagellar and ciliary roots in *Katablepharis* spp. and the suctorian ciliates are similar in that they both have microtubular and fibrillar roots (9).

The ciliates have mitochondria with tubular cristae (120, 121). *Katablepharis* spp. also have mitochondria with tubular cristae (72, 73).

The tentacles of the suctorian ciliates have toxicysts (haptocysts), vesicles containing a tube, that discharge to hold a prey organism on the tentacle (110). *Katablepharis* cells contain extrusive organelles called ejectisomes that are discharged into the medium (72). An ejectisome is a vesicle containing a tightly wound tape in the peripheral cytoplasm that unwinds to a spiraled tube on discharge. The ejectisomes are similar to the R bodies in the kappa particles of the ciliate *Paramecium aurelia* (66, 96). R bodies consist of a tightly wound tape contained within the kappa particle.

Nuclear division in *Katablepharis* spp. and the micronucleus of ciliates (85, 103) has more similarities than differences. The similarities include (i) no participation of basal bodies or centrioles in nuclear division, (ii) spindle microtubules not focused to a single pole during metaphase and anaphase, and (iii) daughter chromatin masses that are moved apart during anaphase by elongation of the spindle microtubules. The major difference between nuclear division in *Katablepharis* spp. and suctorian ciliates is in the behavior of the nuclear envelope. In the suctorian ciliates the nuclear envelope in intact throughout nuclear division whereas in *Katablepharis* spp. the nuclear envelope breaks up in prophase and reforms during telophase. Another difference is that the of ciliate micronuclei have no nucleoli, whereas *Katablepharis* micronuclei do, as do the nuclei of all flagellates.

Kinetid structure in *Katablepharis* spp. is different from that in the suctorian ciliates. In the suctorian ciliates, the kinetosomes (basal bodies) occur singly, not associated with other kinetosomes (4, 36, 47, 49, 77–79, 84, 115). *Katablepharis* spp. have the dikinetid structure (basal bodies associated in pairs) that is characteristic of most flagellates.

Despite the difference in kinetosome grouping into kinetids, it would appear that the strongest cytological and structural relationship between the ciliates and the flagellates is that between the suctorian ciliates and the flagellate *Katablepharis*. Evolutionary schemes of the ciliates often have the suctoria in a derived and isolated position (26). Corliss (26) refers to the suctoria as "a most unique protozoan group" and recognizes that there is a considerable gap between the suctoria and the rest of the ciliates on the basis of unusual "key" characteristics of the suctoria. These key characteristics are the presence of tentacles with haptocysts and stalks, the lack of cilia in the adult stage, and the use of the budding types of reproduction.

COMPARISONS BASED ON MOLECULAR STRUCTURE

The most significant data based on molecular structure that have been used to produce phylogenetic trees have come from the sequencing of nucleotides from rRNA. A

second source of information has been from stop codons used by mRNA to produce polypeptides.

rRNA Nucleotide Sequencing

Cells contain three kinds of RNA: (i) rRNA, which makes up most of the ribosome; (ii) tRNA, which carries amino acids in an activated form to the ribosome for peptide bond formation; and (iii) mRNA, which is the template for protein synthesis. In eukaryotic cells, the rRNA makes up about 85% of the RNA, tRNA makes up about 11%, and mRNA makes up about 4%.

The 80S ribosomes of eukaryotic cells contain 60S and 40S subunits. The 60S subunit contains 45 to 50 different polypeptides and three types of rRNA (5S, 5.8S, and 28S). The 40S subunit contains 30 to 35 different polypeptides and 18S rRNA.

The rRNAs provide molecular markers that are informative in phylogeny because their structure and function have been largely conserved during evolution. Comparisons of base sequences in these rRNA molecules provide information on how far organisms have diverged during evolution. Many systematists have come to believe that determining the nucleotide sequences of rRNA of different organisms will clearly delineate all of their evolutionary relationships. Rothschild et al. (108) stated that "Systematists have long yearned for the magic characteristics that would reveal the 'natural' system of classification." They cautioned against using rRNA nucleotide sequencing to determine evolutionary relationships alone without regard to other structural and biochemical information. Initially, investigations involving extensive rRNA base sequencing looked at the 5S rRNA molecule, which contains 120 sites. The change in 5S RNA nucleotides has been very conservative over time; in mammals there has been about 1% change in 25 million years. This change is too small to be of value in determining evolution in mammals. On the other hand, attempts to use 5S rRNA to determine relationships among organisms of very ancient common origin have encountered the opposite problem; i.e., too much change has occurred in these molecules over the appropriate time spans. Many molecular evolutionists now believe that studies on the 5S rRNA molecules are of limited, if any, value in the study of ancient evolutionary events (87, 88). Hendricks et al. (58), referring to a study of arthropod affinities, noted that "5S rRNA sequences by a clustering algorithm, yielded a tree topology which was inconsistent with common evolutionary views." Hori and Osawa (64) attempted to derive eukaryotic phylogenies of 350 species by determining relationships in 5S rRNA sequences and found that they were unable to consistently generate appropriate groupings whose affinities had been established by other means.

Some investigators have used the larger 5.8S rRNA molecule (154 bases) to prepare sequences used in phylogeny (89). However, more reliable data are being obtained from 18S rRNA of the small-subunit rRNA (1, 12, 33, 40, 41, 56, 57, 80, 91, 116, 127) and the 28S rRNA of the large-subunit rRNA (8, 74, 75, 98, 99). These rRNAs are larger, although the degree of evolutionary diversity is more critical than the molecular size. The evolutionary diversity varies from molecule to molecule and within molecules (126). Base sequences that change relatively rapidly provide information about recent evolutionary events, but they obscure ancient events through their multiple changes and reversions.

The 18S rRNA of the small-subunit rRNA has 1752 bp and therefore provides more base pairs with which to assess evolutionary drift than does 5S or 5.8S rRNA (15). Also, regions in the 18S rRNA with differing degrees of sequence conservation can be used to span a broad range of phylogenetic distances (12). The hypervariable regions aid in comparison of closely related taxa, whereas the more highly conserved regions aid in comparison of more distantly related taxa with a statistically larger number of sites with which to derive a homology value (116).

The 28S rRNA from the large subunit has a largely conserved structural core which, in eukaryotes, is interdispersed with 12 divergent, more rapidly evolving domains (D1 through D12) (48, 75, 81). The conservative core (over 2,000 nucleotides) has been constrained by heavy selective pressure and is suitable for phylogenetic evaluation among distant taxa. Partial sequences limited to conservative domains near the 5′ end of 28S rRNA have been used to infer phylogenetic relationships among protists (8) and algae (94). The divergent domains of 28S rRNA display a high rate of sequence variation and therefore do not provide useful information for the comparison of distant organisms. However, some of these domains (mainly D1, D3, D8, and, to some extent, D2) have the potential to be useful for phylogenetic and taxonomic analyses of closely related species (8, 74, 100).

The data from the nucleotide sequences of the 18S rRNA of the small subunit (1, 12, 40, 41, 56) and the 28S rRNA of the large subunit (75, 89, 98, 99) both show dinoflagellates ancestral to the ciliates (Fig. 10). Within the dinoflagellates, nucleotide sequencing of divergent domains D1 and D8 of 28S rRNA has shown that *Oxyrrhis marina* emerged early, followed by the order Peridinales. The unarmored Gymnodiniales and the Prorocentrales appeared more recently (75).

Within the ciliates, the phylogeny based on rRNA sequence comparisons (40, 41, 78) is remarkably congruent with that inferred from ultrastructural data. On the basis of nucleotide sequences, the heterotrich *Blepharisma* appears to be the oldest ciliate investigated so far (karyorelicteans have not yet been investigated). The hypotrichs, stichotrichs, nassophoreans, and hymenostomes diverge after *Blepharisma*.

Use of mRNA Codons

The nucleotides in DNA control the genetic information in a cell. RNA polymerase synthesizes RNA by transcription of the DNA template. The single-stranded mRNA molecules contain the encoded information for the synthesis of polypeptides. The code in mRNA consists of groups of three nucleotides containing the bases uracil (U), cytosine (C), adenine (A) or guanine (G). The four bases in three positions result in 64 possible combinations or triplet codons. Of these, 61 codons are used for specific amino acids and 3 are used to terminate the production of polypeptides during translation. These three termination or stop codons are UAA, UAG, and UGA and are often called the "universal" stop codons because they were thought to occur in all prokaryotes and eukaryotes. Recently, however, it has been found that some of the ciliates do not use two of these stop codons, UAA and UAG, for terminating polypeptide synthesis (93). Instead, UAA and UAG are used as codons for glutamic acid or glutamine by *Paramecium* (19, 37, 95, 97), *Stylonchia* (54, 55), *Tetrahymena* (3, 37, 45, 65, 69, 90), and *Oxytricha* (59) spp. On the basis of these investigations, it seemed that UAA and UAG in these organisms are not stop codons and that UGA is the only functioning stop codon. Although UAA is not a stop codon in these organisms, a

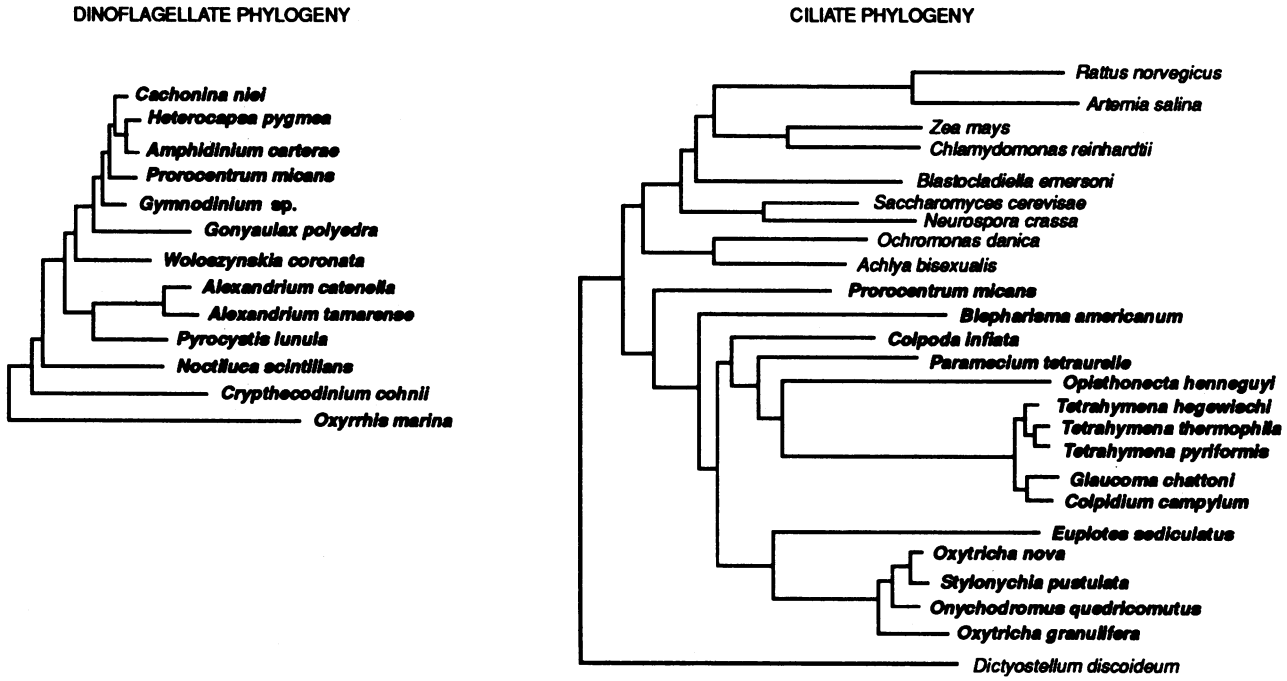

FIG. 10. Phylogeny of the dinoflagellates as deduced from nucleotide sequencing of 24S rRNA (75), and phylogeny of the ciliates as deduced from nucleotide sequencing of the small-subunit rRNA (41).

more recent investigation has found that UAA is a stop codon in the ciliate *Euplotes crassus*, although how UAG and UGA (the other "universal" stop codons) are used was not determined (46). Harper and Jahn (46) suggest that the use of the codon UAA in *E. crassus* and information from rRNA sequencing indicate that the euplotids show divergence from *Tetrahymena*, *Paramecium*, *Oxytricha*, and *Stylonchia* spp.

Although codon use appears to be of use in determining phylogenetic relationships within the ciliates, it appears to be of only limited use so far in determining the relationship with the flagellates, since the flagellates appear to use the universal codons to stop polypeptide synthesis. Interestingly, two *Acetabularia* species also use UAA and UAG to code for glutamine (112). *Acetabularia* is a relatively large siphonaceous green alga that produces flagellated swarmers. It is, however, far from the ciliates phylogenetically, and it is probable that the change in codon use arose independently from that in the ciliates.

CONCLUSION

After more than a century of speculation on the ancestors of the ciliates, the dinoflagellates still remain the most likely candidate. The nucleotide sequencing data from rRNA place the dinoflagellates before the ciliates. Structurally, the ciliates and dinoflagellates have a number of similarities, which include cortical alveoli and thecal vesicles, tubular cristae in mitochondria, parasomal sacs and pusules, trichocysts and mucocysts, and some similarities in the feeding apparatus.

Structurally, the similarity between the flagellate *Katablepharis* spp. and the ciliated swarmer (embryo) of the suctorian ciliates is quite striking. Reduction in the number of cilia to two and elimination of macronuclei in the suctorian embryo would produce a cell very similar to the *Katablepharis* cell. These structural similarities could, however, be a result of parallel evolution. Presumably, molecular evidence, particularly sequencing of rRNA, will provide more insight into the relationship between *Katablepharis* spp. and the suctorian ciliates.

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