Somatic kineties or paroral membrane: which came first in ciliate evolution?

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The ciliate species which lack a distinctive oral ciliature are considered to represent an ancestral state in ciliate evolution. Consequently, the somatic kineties composed of kinetids (kinetosomes plus cilia and associated fibrillar systems) are thought to be the ancestral ciliature. Results on stomatogenesis in ‘gymnostomial ciliates’ have shown that these ciliates probably have evolved from ancestors already equipped with an oral ciliature. Thus instead of the somatic, the oral ciliature may be regarded as ancestral. Based on these ideas a hypothesis on the evolution of the ciliate kinetome (assembly of all kinetids covering the body of a given ciliate) is presented. The first step in the evolution of the kinetome was the formation of a paroral membrane, a compound ciliary organelle lying along the right side of the oral area which historically but falsely is termed membrane. It was composed of kinetosomal dyads (dikinetids), derived from the kinetid of a dinoflagellate-like ancestor. From the beginning the paroral membrane was responsible for locomotion, ingestion and for the formation of a cytopharyngeal tube which the first ciliate probably had inherited from its flagellate ancestor. In the second step a first somatic kinety was formed from the right row of kinetosomes of the paroral membrane as a result of a longitudinal splitting of the paroral membrane and a subsequent migration of the forming kinety to the right into the somatic cortex. To increase the number of somatic kineties this process was repeated until the kinety produced first reached the left border of the oral area. By this step the locomotive and the nutritional functions were differentiated between somatic and oral structures. In a third step the adoral organelles were formed from somatic kinetids left of the oral area. The primitive type of stomatogenesis was a buccokinetal one derived from the mode the flagellate ancestor used to distribute its replicated kinetosomes to the offspring cells (buccokinetal means that at least parts of the oral anlage for the posterior offspring cell has its origin in the parental oral apparatus). This hypothesis, based on comparative studies on ciliate morphogenesis, is corroborated by molecular data from other laboratories.

Keywords: Phylogeny; Ciliate evolution; Ciliate kinetome; Ultrastructure; Morphogenesis.

1. Introduction

It is largely accepted by ciliatologists, that ciliates without an elaborated oral ciliature show a more primitive stage in evolution of the ciliate kinetome than species with a complex oral ciliature. Following this line, most systems of ciliate classification such as the Kahlian and the Fauré-Fremiet schemes (Kahl, 1930–1935; Fauré-Fremiet, 1950), the classifications of de Puytorac et al. (1974) and Corliss (1979) and recent proposals made by Small and Lynn (1985) and by de Puytorac et al. (1987), regard the ‘gymnostomial ciliates’ as a basic branch of the tree. This holds also for the hypotheses on ciliate evolution proposed by Orias (1976) and Small (1984).

Small (1984) tried to explain the origin of prostomial and ventrostomial ciliates from an astomial Kentrophoros-like ancestor, which itself should have originated from a flagellate ancestor as follows: passing through a Polykrikos-like intermediate stage, a dinoflagellate-like organism with two flagella should have given rise to an organism with a single somatic kinety. Multiplication of this first kinety should have led to a flan-like ciliate ancestor with a ciliated dorsal surface and a nonciliated ventral
surface capable of ingesting food by simple membrane infolding. The prostomial and the ventrostomial ingestatory areas of recent ciliates should have evolved from the nonciliated ventral surface of the *Kentrophoros*-like ancestor. Derived from a truly gymnostomatous condition, the ciliated oral structures and the cytopharyngeal apparatus are assumed to have evolved from the anterior tips of somatic kineties in ciliates with a prostomial ingestatory area and from somatic kineties on both sides of the oral opening in ciliates with a ventrostomial ingestatory area.

In recent years investigations on morphology and morphogenesis of the prostomial ciliate *Coleps* (Huttenlauch, 1987; Huttenlauch and Bardele, 1987) and other gymnostomial ciliates (Gärtnert-Schür, 1990; Hiller, 1990, 1992; Muñoz et al., 1989) indicate that these ciliates lacking an elaborate oral ciliature probably have evolved from ancestors with a true adoral and paroral ciliature (Bardele, 1991a). The oral structures of these ciliates therefore should be interpreted as an apomorphous instead of a plesiomorphous character state. The assumption that the possession of a prostomial and gymnostomial oral apparatus might be a derived character within the ciliates makes it possible to go one step further and to ask whether in extant ciliates, parts of the oral ciliature instead of the somatic kineties could be interpreted as the remnant of the very first ciliature of the ciliate ancestor.

The results of various studies on ciliate morphology and morphogenesis reveal striking similarities concerning ultrastructure and morphogenetic functions of the paroral membrane within different ciliate groups. Based on these observations, a basic pattern of the paroral membrane, illustrated below in detail, is recognizable. This paroral pattern is widespread among ciliates and is connected with very important functions during ciliate morphogenesis. Therefore the paroral membrane of extant ciliates and not a somatic kinety, as assumed by several authors (Foisner et al., 1988; Orias, 1976; Small, 1984), is proposed to be the extant derivative of the ancestral ciliate kinetome.

This led to a hypothesis on the evolution of the ciliate kinetome comprising the following steps. In a first step of evolution a single row of paired kineties, the ancestral paroral membrane, which at first was responsible for locomotion, nutrition and for the construction of a cytopharyngeal tube has evolved from the flagella of a dinoflagellate-like organism. In a second step the paroral membrane gave rise to the somatic kineties. Finally, in a third step of evolution, the adoral organelles have evolved from somatic kineties left of the cytostome.

2. The paroral membrane and its part during stomatogenesis

The cytostome of ventrostomial ciliates usually is surrounded by a distinct paroral and adoral ciliature, composed of a paroral membrane (also called undulating membrane, endoral membrane or haplokinety) on the right side of the oral aperture and several adoral membranelles on its left side (Fig. 1). The postciliary microtubules emerging from these organelles on both sides of the oral opening run towards the cytostome-cytopharyngeal complex. In many ventrostome ciliates these microtubules are not in contact with the cytopharyngeal apparatus in the adult stage. During stomatogenesis however, the postciliary microtubules of the paroral membrane and often the adoral membranelles, as well, provide the microtubular ribbons of the cytopharynx which are involved in ingestion and in the formation of food vacuoles. It is important to mention that the dyads of the paroral membrane show an orientation which differs by about 90° compared with somatic kineties. It is this orientation which enables the postciliary microtubules of the posterior kineties of the dyads to run towards the oral aperture. In somatic kineties the postciliary microtubules run in a posterior right direction and therefore in kineties located to the right of the oral opening they run away from the oral region. In adoral membranelles, however, the kineties may be orientated in a quasi somatic orientation to provide their postciliary microtubules for the construction of a cytopharyngeal tube.
Fig. 1. The oral ciliature of a ventrostomial ciliate. (a) General view of a ventrostomial ciliate with somatic kineties and oral organelles. Cytostome (cs), contractile vacuole pore (cvp) and cytoproct (cp) are labeled. (b) Schematic drawing of the oral area at higher magnification. The postciliary microtubules (pcmt) of both the paroral membrane (pm) and the adoral membranelles (m₁, m₂, m₃) also point towards the cytostome (cs). The paroral membrane is composed of kinetosomal dyads, the posterior kinetosome of each dyad is rotated 90° compared with kinetosomes of somatic kineties (k₁, kₙ, kₙ₋₁, kₙ₋₂).

Many variations of this basic pattern of the ventrostomial oral ciliature can be found in extant ciliates. In the last decade however, studies on ciliate stomatogenesis, especially those investigations using electron microscopical techniques, made it possible to recognize a basic pattern of the paroral membrane present in a huge number of ciliate taxa.

This basic pattern of a paroral membrane (Fig. 2), comparable with a stichodyade in the sense of de Puytorac and Grain (1976) and Grain (1984), can be defined as follows:

- The kinetosomes forming the paroral membrane are arranged in pairs that are orientated nearly perpendicular to the longitudinal axis of the entire organelle.
- The anterior-posterior axis of the posterior kinetosome of each pair is orientated perpendicular to the longitudinal axis of the paroral membrane. Therefore these pairs may be called dyads in the sense of Noirot-Timothee (in Grain, 1969). (The anterior-posterior axis of a kinetosome, see arrows in Fig. 2, enters the kinetosomal cylinder between triplet 1 and triplet 9 and leaves the kinetosome passing triplet 5. Triplet 9 is
Fig. 2. Basic pattern of the paroral membrane. The kinetosomal dyads are orientated almost perpendicular to the anterior-posterior axis (a, p) of the organelle. The anterior-posterior axis (arrow) of the posterior kinetosome of each dyad, detectable by the presence of postciliary microtubules (pcmt), is orientated perpendicular to the anterior-posterior axis (a, p) of the paroral membrane. The kinetosomes of each dyad are connected by desmoses (d). (a) Dyad in the sense of Noirot-Timothée, the orientation of the anterior kinetosome (?) is not detectable due to the lack of fibrillar systems. (b) Plagiodyad, the anterior kinetosome has fibrillar systems attached and is orientated in a somatic way (arrow) with its anterior-posterior axis parallel to the longitudinal axis of the paroral membrane but perpendicular to the axis of the posterior kinetosome. (c) Isodyad, the anterior kinetosome has fibrillar systems attached, e.g. transversal microtubules and postciliary microtubules (tmt, pcmt) and its orientation (arrow) is the same as in the posterior kinetosome. (d) Antidyad, the anterior kinetosome has fibrillar systems attached and is orientated in an opposite direction (arrow) compared with the posterior kinetosome.

- Concerning the orientation of the anterior kinetosomes of the dyads, four different types of dyads may be distinguished within the basic paroral pattern, thus extending the definition of the dyad given by Noirot Timothée (in Grain, 1969):

1. The axis of the anterior kinetosome is unknown due to lacking fibrillar systems (Fig. 2a). This is the situation in the adult stages of hymenostome and peritrich ciliates and fits exactly to the definition of Noirot-Timothée. Such kinetosomal arrangements may be called dyads.

2. The anterior kinetosome has fibrillar systems attached and is orientated in a somatic way with its anterior-posterior axis parallel to the longitudinal axis of the paroral membrane (Fig. 2b). This situation is reported from early stages of division in the scuticociliate Dexiotricha (Peck, 1974) and in the peritrich ciliate Opisthонecta (Bradbury, 1965) as well as from the adult stage of the nassulid ciliate Furgasonia (Eisler 1986, 1988). Dyads fitting to this description may be called plagiodyads (Greek: plagios, transverse, oblique).

3. The anterior kinetosome has fibrillar systems attached and its orientation is the same as in its posterior partner (Fig. 2c). For example this pattern is found in the...
adult stage of *Loxodes* (de Puytorac and Njiné, 1970) and in *Furgasonia, Paraurostyla* and *Colesp* during stomatogenesis (Eisler, 1986, 1989; Jerka-Dziadosz, 1981; Huttenlauch and Bardele, 1987) at a time when the postciliary microtubules of both kinetosomes of the dyads provide the microtubular lamellae of the cytopharyngeal apparatus. Such dyads may be called isodyads (Greek: isos, equal).

(4) The anterior kinetosome has fibrillar systems attached and is orientated in the opposite direction compared with the posterior one. This pattern is described in the colpodid ciliate *Colpoda variabilis* (Hofmann-Münz, 1991) and may be called antidyad (Greek: anti, opposite of).

As was shown in *Furgasonia* (Eisler, 1989), the anterior kinetosomes of the paroral dyads may successively show several of these orientations (Fig. 2) during stomatogenesis in one and the same species.

Even if the paroral ciliature of the adult stage is highly modified, at least in the stage of stomatogenesis when the new cytopharyngeal apparatus is formed, the basic pattern of the paroral membrane is recognizable. A paroral membrane composed of one of the dyads mentioned above is to be found in the following ciliate taxa: in the karyorelictide (Nouzaréde, 1976; de Puytorac and Njiné, 1970) and heterotrich ciliates (Fischer-Defoy and Hausmann, 1981; Peck et al., 1975; Pelvat, 1985), the genus *Protoctuzia* (Groïlère et al., 1980), the hypotrich (Jerka-Dziadosz, 1981) and nassulid ciliates (Eisler, 1986, 1988, 1989; Grain et al., 1978), the hymenostome (e.g. Didier, 1971; Lynn and Didier, 1978; Peck, 1978; de Puytorac et al., 1974) and prostome ciliates (Huttenlauch and Bardele, 1987) and in the colpodid (e.g. Didier et al., 1980; Hofmann-Münz, 1991; Wirsberger et al., 1985) and peritrich ciliates (Bradbury, 1965).

The paroral membrane not only shows a broad distribution among ciliates but it also plays a central part during stomatogenesis. Even in ciliates with a somatic stomatogenesis the paroral membrane gives rise to one of the most important organelles of a heterotrophic organism – the cytopharyngeal apparatus with its vital functions during food ingestion. In ciliates with a buccokinetal type of stomatogenesis (according to the definitions given by Corliss, 1973), it is the parental paroral membrane and never the parental adoral organelles which provide the anlagen of the new oral organelles for the opisthe.

In the course of stomatogenesis in some ciliates the paroral membrane is also responsible for the formation of new somatic structures. In *Paraurostyla*, after the formation of the cytopharynx, the anlage of the paroral membrane splits longitudinally forming the inner paroral membrane (endooral membrane) and the outer paroral membrane. At the end of stomatogenesis, the outer paroral membrane, the kinetosomes of which are orientated in a somatic way, forms a somatic cirrus, the frontal cirrus no. 1 (Jerka-Dziadosz, 1981).

In *Furgasonia*, at the beginning of stomatogenesis, the old paroral membrane splits longitudinally and the right row of kinetosomes, the former anterior kinetosomes of the dyads, which are already orientated in a somatic way, form a new somatic kinety 1' in the proter (Eisler, 1986, 1989; Eisler and Bardele 1986).

Due to the wide distribution of the paroral membrane among ciliates and its important functions in stomatogenesis, it seems reasonable to assume that the central ontogenetic part of this unique organelle is correlated with a central part in phylogeny. This assumption led to the hypothesis on the evolution of the ciliate kinetome presented below.

3. The postulated steps in the evolution of the ciliate kinetome

3.1. The dawn of the ciliates and the formation of the paroral membrane

The first hypothetical step in the evolution of the ciliate kinetome (Fig. 3) is the formation of a paroral membrane composed of dyads emerging from the kinetid of a flagellate with two flagella at its apical pole.
It is often assumed that the ancestor of ciliates might have been a dinoflagellate-like organism or, in other words, the ciliates and dinoflagellates might be sister groups. Many common characters such as the possession of pellicular alveoli and spindle-shaped trichocysts and the sequence similarities of the ribosomal RNA, support a sister group relationship between dinoflagellates and ciliates (e.g. Corliss, 1988; Greenwood et al., 1991; Gunderson et al., 1987; Schlegel, 1991; Schlegel et al., 1991). Dinoflagellates, with the exception of the Prorocentrales, divide perpendicular to their longitudinal axis (this axis is defined by the main direction of cell movement). Since this mode of division is also widespread in ciliates, it seems to be likely that the common ancestor of ciliates and dinoflagellates already had a division plane perpendicular to its main direction of locomotion.

The common ancestor of ciliates and dinoflagellates (Fig. 3b) is presumed to have evolved from an organism with apical flagella and a direction of locomotion aligned in the division plane (Fig. 3a). To achieve the transverse or homothetogenic mode of division typical for most dinoflagellates and ciliates, the direction of locomotion must have changed on the branch leading to ciliates and dinoflagellates while the division plane must have been retained still ensuring an equal allocation of the replicated kinetosomes.

In the evolution of the ciliate ancestor from a dinoflagellate-like organism (Fig. 3b) replication of the kinetid in the absence of cytokinesis could have led to an organism comparable with the recent multiflagellated dinoflagellate *Polykrikos*. This was already assumed by Small (1984) in his hypothesis on the evolution of the ciliate kinetome. Small interpreted the alignment of paired kinetosomes (dikinetids) in the cortex of this

**Fig. 3.** The first step in the evolution of the kinetome, the formation of the paroral membrane. (a) Flagellate ancestor with apical flagella, the direction of movement which determines the longitudinal axis of the cell is in the fission plane. (b) Common ancestor of dinoflagellates and ciliates with ventral flagella accompanied by a cytopharyngeal tube. The direction of movement is perpendicular to the fission plane. (c) Ciliate ancestor with an early paroral membrane composed of plagiodyads and a cytopharyngeal tube on its ventral surface.
Polykrikos-like ciliate ancestor as an early stage of a somatic kinety. In my opinion this row of paired kinetosomes resembles a precursor of the paroral membrane described above.

It is assumed that in this early paroral membrane, the kinetosomal pairs (dikinetids) have already been orientated perpendicular to the longitudinal axis of the cell. The kinetosomes of each pair are proposed to have been arranged in a way comparable with Fig. 2b. The anterior-posterior axis of the left kinetosome, that is the posterior kinetosome of the pair, is orientated perpendicular to the longitudinal axis of the cell. The right kinetosome, the anterior one of the pair, is orientated in a somatic way with an anterior-posterior axis parallel to the longitudinal axis of the cell. This pattern found in the paroral membrane of adult or ontogenetic stages in extant ciliates as mentioned above (Bradbury, 1965; Eisler, 1986, 1988; Peck, 1974) is comparable to a certain degree with the arrangement of kinetosomes of the longitudinal and transverse flagella in extant dinoflagellates (e.g. Farmer and Roberts, 1989; Roberts, 1985, 1989; Roberts and Timpano, 1989). The transverse flagellum would resemble the posterior kinetosome of a paroral dyad while the longitudinal flagellum would resemble the anterior kinetosome.

Left of its early paroral membrane the ciliate ancestor (Fig. 3c) is assumed to be furnished with a tube-like cytopharyngeal apparatus thus determining the ventral side of the cell. Since comparable structures are reported from some phagotrophic dinoflagellates (Dodge, 1971, 1983), ciliates were not forced to re-invent 'cytopharyngeal baskets'; they may have inherited them from their dinoflagellate-like ancestors (Fig. 3b).

Concerning the mode of locomotion, the ciliate ancestor (Fig. 3c) may have performed the same swimming behaviour as many extant dinoflagellates with their longitudinal flagellum mainly responsible for the forward movement and their transverse flagellum which, apart from its contribution to the forward swimming, gives rise to a stabilizing spin of the cell. If one presumes that only these cilia of the right kinetosomes of the paroral membrane with an effective stroke towards the posterior pole of the cell are involved in the generation of forward force, the resulting direction of locomotion is parallel to the new longitudinal axis of the cell. In addition to their functions in food uptake the cilia of the left kinetosomes of the paroral membrane beating towards the cytostome may generate a spin like the transverse flagellum of dinoflagellates.

Thus, it is assumed that the kinetome of the ciliate ancestor (Fig. 3c) consisted exclusively of a paroral membrane responsible for both the locomotion of the cell and the ingestion of food as well. The basic structures to perform these vital tasks may have been inherited from a dinoflagellate-like organism.

3.2. The second step, the acquisition of somatic kineties

In a postulated second step in the evolution of the ciliate kinetome the ciliate ancestor described above obtained its somatic kineties, now enabling the cell to allocate the different tasks in locomotion and ingestion of food to different cortical organelles.

The somatic kineties are assumed to have evolved as derivatives of the paroral membrane. As shown in Fig. 4, the somatic kineties were formed from the anterior kinetosomes of the paroral dyads by a longitudinal splitting of the paroral membrane and a subsequent migration of these kinetosomes to the right into the somatic cortex. The paroral dyads were reconstructed by the formation of new kinetosomes in front of the old left kinetosomes of the paroral membrane, the former posterior kinetosomes of the dyads. The number of somatic kineties is assumed to have increased by a multiple repetition of this process until kinety n reached the left border of the oral opening.

These hypothetical mechanisms are strongly supported by ontogenetic events to be found in some extant ciliates. A longitudinal splitting of the paroral membrane with a subsequent migration of the former anterior kinetosomes of the paroral dyads to the right is to be found during
stomatogenesis in many ciliates (e.g. Bakowska et al., 1982; Eisler, 1989; Eisler and Bardele, 1986; Grolière, 1974; Grolière and Detcheva, 1974; Jerka-Dziadosz, 1981; Nelsen, 1981). There are further results on morphogenesis from several ciliates verifying the use of these kinetosomes in the formation of new somatic structures.

As already mentioned above in the hypotrich ciliate *Paraurostyla weissei* (Jerka-Dziadosz, 1981), the former anterior kinetosomes of the paroral dyads take part in the formation of the outer paroral membrane which at the end of stomatogenesis is responsible for the formation of the somatic frontal cirrus no. 1.

In the nassulid ciliate *Furgasonia blochmanni* (Eisler, 1989; Eisler and Bardele, 1986), the former anterior kinetosomes of the parental paroral dyads released from the paroral membrane by a longitudinal splitting at the beginning of stomatogenesis give rise to a new somatic kinety 1' in the proter. To compensate for the increased number of kineties, the kinety n disappears in the proter. This phenomenon leads to a shifting of kineties with a source of new kineties to the right of the paroral membrane and a sink
on the left side of the oral opening. In *Paramecium*, the rotation of the kinetome already shown by Beisson and Sonneborn (1965) is explained by Iftode (1991) and Iftode and Adoutte (1991) with a similar mechanism that leads to a renewal of the kinetome within 20–30 division cycles.

In *Paramecium* like other hymenostome ciliates, the former anterior kinetosomes of the paroral dyads are involved in the formation of the oral anlagen for the opisthe (Beran, 1990; Grolière, 1974; Grolière and Detcheva, 1974; Jones, 1976; Peck, 1974; de Puytorac et al., 1974; Roque, 1961).

In *Tetrahymena*, however, the oral anlagen for the opisthe are provided by the postoral kinety 1, without any participation of the parental paroral membrane (Nelsen, 1981; Bakowska et al., 1982). Nevertheless, in the course of stomatogenesis during the reorganisation of the paroral membrane of the proter, the old paroral membrane splits longitudinally as in other hymenostomes and the anterior kinetosomes of the dyads move to the right into the somatic cortex. Finally, in *Tetrahymena* these kinetosomes are resorbed. However, under certain conditions, e.g. during the oral replacement (Frankel, 1969) and during the transformation of the microstome into the macrostome form (Stone, 1963), the paroral membrane of *Tetrahymena* is also able to participate in the formation of new oral anlagen. During the regulation of corticotype through kinety insertion, the additional postoral kinety is also provided by the paroral membrane (Nelsen and Frankel, 1979).

Having reached this stage in the ciliate evolution (Fig. 4), the kinetome consisted of an oral ciliature composed of a paroral membrane and of somatic kinetics running from the anterior to the posterior pole of the cell. Adoral organelles were not yet present.

At this stage the functions of the kinetome for locomotion and nutrition may have been allocated to different cortical structures or domains. Therefore, in further evolutionary events, the paroral membrane could have been modified in many ways adapting to the different modes of ingestion or even reduced in the adult cell if the paroral membrane has lost its importance in ingestion. In some extant ciliates, e.g. *Pseudomicrothorax dubius*, the paroral membrane is therefore only recognizable as such during stomatogenesis (Peck, 1974; Thompson and Corliss, 1958).

### 3.3. The third step, the formation of adoral membranelles

The next stage proposed in this hypothesis on the evolution of the ciliate kinetome is characterized by the acquisition of adoral membranelles derived from somatic kinetics bordering the oral area at its left edge (Fig. 5).

In the formation of these ciliary organelles, proliferation of somatic kinetids, without subsequent segregation of the single kinetosomes, supplied kinetosomal pairs or triads, which later on aligned laterally. No significant rotation of kinetosomes was necessary to achieve an adequate orientation of kinetosomes, which enabled the postciliary microtubules of the posterior kinetosomes of the adoral organelles to run towards the cytostome.

In many ciliates extant today, the adoral organelles are ontogenetic derivatives of somatic kinetics. This not only holds for species characterized by a somatic stomatogenesis, such as tetrahymenids (Nelsen, 1981; Bakowska et al., 1982) and heterotrichs (Bohatier et al., 1976; Dubochet et al., 1979; Pelvat and de Haller, 1979), but also for nassulid ciliates with a bucckinetal stomatogenesis regarding the paroral membrane (Eisler, 1989; Eisler and Bardele, 1986).

However, in ciliates with an exclusively bucckinetal type of stomatogenesis, such as the scuticociliates, the anlagen for the adoral membranelles of the opisthe are provided by the parental paroral membrane. But as mentioned above, in contrast to the new paroral membrane of the opisthe, the new adoral membranelles are like ontogenetic dead ends, they never participate in the formation of oral anlagen in the subsequent division cycle. This holds also for the process of oral replacement in *Tetrahymena* where the old paroral membrane but never the
Fig. 5. The third step in the evolution of the kinetome, the formation of adoral membranelles. The adoral membranelles are formed by proliferation and alignment of kinetosomes within somatic kineties left of the oral area. No rotation of kinetosomes is necessary during this process since the postciliary microtubules of somatic kinetosomes are already orientated properly to extend towards the cytostome.

4. The implications for ciliate stomatogenesis

It is assumed that the paroral membrane of ciliates is homologous to the flagellar apparatus of the ancestor and therefore it may also be assumed that a type of stomatogenesis should be considered as primitive which allocates the kinetosomes of the paroral membrane equally to both offspring cells, as is done with the kinetosomes in flagellates. This basic type of stomatogenesis (Fig. 6) is therefore a buc- cokinetal or autonomous one, characterized by the formation of the oral anlagen of both off-
Fig. 6. The early type of ciliate stomatogenesis. This process may be subdivided into different steps (a)–(g). (a) Longitudinal splitting of the paroral membrane (arrowhead). (b) Transverse division of the old paroral membrane on a level with the presumptive cleavage furrow, first round of paroral kinetosomal proliferation. (c) Incorporation of new kinetosomes within the paroral membrane. (d) Somatic kinetosomal proliferation. (e) Second round of paroral kinetosomal proliferation. (f) Formation of the cytopharyngeal tubes. (g) Rotation of the anterior kinetosomes of the dyads by 90° to achieve a somatic orientation.
presumptive cleavage furrow. Both segments move apart, and new kinetosomes are formed in a first round of kinetosomal proliferation at the right side of these segments, respectively in the front of the old posterior kinetosomes of the dyads. In both offspring cells these new kinetosomes join the anlagen of the new paroral membrane, thus the halving of its length is compensated for (Fig. 6, step d). Now, resulting from a second round of kinetosomal proliferation, the kinetosomes of both paroral anlagen get new anterior kinetosomes, thus becoming dyads again (Fig. 6, step e). In the next step the new cytopharyngeal tubes are formed (Fig. 6, step f). Now both kinetosomes of the dyads are turned by 90° compared with the longitudinal axis of the cell. Therefore both kinetosomes can send postciliary microtubules towards the forming cytopharyngeal tubes of the two offspring cells. At the end of stomatogenesis (Fig. 6, step g) the anterior kinetosomes of the paroral dyads again take up a somatic orientation.

The replication of the paroral ciliature, the reconstruction of the cytopharynx in both offspring cells and the formation of new somatic structures is already explicable by this basic type of stomatogenesis derived from the mode of kinetosomal allocation found in flagellates and supplemented by a second round of kinetosomal proliferation.

In ciliates extant today this mechanism is modified in many ways. Mostly the oral apparatus is not located in the area of the presumptive cleavage furrow. Instead it is shifted towards the apex of the cell – a phenomenon surely correlated with the advantage of a more or less apical cytostome in the search for food. As a consequence of this location of the oral opening, in buccokinetal stomatogenesis, the anlage for the posterior offspring cell, the opisthe, has to migrate backwards from the area of the old oral opening beyond the presumptive cleavage furrow. A rather primitive type of stomatogenesis with a buccokinetinal origin of the paroral membrane of the proter and a somatic origin of the adoral organelles is seen in the nassulid ciliate Furcagonsia (Eisler, 1989; Eisler and Bardele 1986).

During ciliate evolution, the part that the old paroral membrane plays in stomatogenesis is assumed to have changed in different ways. On one hand the ontogenetic importance of the paroral membrane may have increased as in scuticociliates (Grolière, 1974; Grolière and Detcheva, 1974; Peck, 1974; de Puytorac et al., 1974) and certain hymenostomes (Beran, 1990; Jones, 1976; Peck, 1974; Roque, 1961) where the old paroral membrane provides all anlagen for the oral apparatus of the opisthe. On the other hand the direct ontogenetic dependency of the oral anlagen on the parental paroral membrane may have decreased in several branches. In some ciliates, e.g. in the nassulid genus Nassula (Eisler and Bardele, 1986), the paroral membrane produces arrays of kinetosomes that produce oral anlagen not until the subsequent division cycle. In other groups, e.g. heterotrich, hypotrich, colpodid and tetrahymenid ciliates, the paroral membrane has lost its dominance in stomatogenesis. This transition from a buccokinetal mode of stomatogenesis to a somatic one certainly occurred several times during ciliate evolution enabling the parental cell to provide the opisthe with an oral ciliature without a migration of kinetosomes from the old oral apparatus to the new one.

Another line of ciliate evolution may have led to a total loss of the original oral organelles, the paroral membrane and the adoral membranelles. Concerning the mode of stomatogenesis, these ciliates show a holotelokinetal mode (Bardele, 1989), e.g. in haptorid ciliates. Instead of postciliary microtubules, their cytopharynx is supported by transversal microtubules emerging from somatic kinetosomes at the anterior tips of somatic kineties surrounding the cytostome. Therefore, their cytopharyngeal apparatus may be described by the term rhadob in the sense of Small (1976) to distinguish it from the cyrtos, which is lined by postciliary microtubules.

5. Conclusions

Based on results on ciliate ultrastructure and morphogenesis, a hypothesis on the evolution of the kinetome is presented comprising the follow-
ing assumptions. In a first step the paroral ciliature of the ciliate ancestor was developed from the flagellar apparatus of a dinoflagellate-like organism. In a second and third step the somatic kineties and finally the adoral organelles have evolved as derivatives of the paroral ciliature. The transverse mode of cell division, characteristic for most ciliates and dinoflagellates, resulted from a changed direction of locomotion while the division plane was retained unchanged. Therefore the old apical pole of the cell was transformed into the new ventral pole. Concerning stomatogenesis, a primitive buccokinetal mode is assumed to be ancestral. The parakinetal, telokinetal and apokinetal modes (according to the definitions given by Corliss, 1973) in extant ciliates are assumed to be derived characters.

Compared with the previous hypotheses presented by Orias (1976) and Small (1984), the present hypothesis has some striking advantages. The ciliate prototype already had a cytopharyngeal apparatus, which is inherited from its flagellate ancestor. The ciliature of the prototype consisted of a paroral membrane with morphological and ontogenetic properties to perform vital tasks in locomotion, food-uptake and in the construction of new cytopharyngeal tubes as well as in the reorganisation of the kinetome during cell division. Phylogenetic events such as the formation of somatic kineties and adoral membranelles, can be explained by ontogenetic mechanisms found in extant ciliates. The ciliate prototype already had a primitive mode of a buccokinetal stomatogenesis. While in previous hypotheses the complex stomatogenetic events are assumed to have developed de novo during ciliate evolution, in the present hypothesis stomatogenesis has its roots in the mechanisms flagellates use to allocate their kinetosomes to the offspring cells.

It may be argued that in extant ciliates, buccokinetal stomatogenesis has a rather restricted distribution and therefore it should be assumed as a derived rather than an ancestral character state. But, at least in the hymenostome ciliate *Tetrahymena* with its parakinetal stomatogenesis, it is rather probable that this ciliate has evolved from an ancestor with a buccokinetal type of stomatogenesis. It is also striking that buccokinetal stomatogenesis is mainly found in small ciliates (many scuticociliates) and in ciliates with an oral opening located near the presumptive cleavage furrow (e.g. *Paramecium*) while somatic stomatogenesis is often found in large or elongate ciliates (many heterotrichs) or in ciliates with an oral opening in a more or less apical position (prostomes, haptorids, *Tetrahymena*). It seems plausible that in these ciliates showing a long distance between old oral opening and the new cytophage of the opisthe, the migration of kinetosomes for the oral anlage of the opisthe was omitted. It may be advantageous to transfer only the information of the oral pattern to the opisthe without being forced to move kinetosomes over long distances. Consequently in these ciliates, the presumptive oral kinetosomes are provided by the somatic cortex.

Applying the present hypothesis to ciliate systematics, some characters like the possession of a paroral membrane, the ventrostomial oral opening, the cytopharyngeal tube equipped with postciliary microtubules (cyrtos) and a buccokinetal type of stomatogenesis previously regarded as apomorphic within the ciliates should now be regarded as plesiomorphic characters. The lack of a true oral ciliature, the prostomial oral opening, the cytopharynx lined by transversal microtubules (rhabdos) and the para-, telo- and apokinetal modes of stomatogenesis should be regarded as apomorphic characters.

As already proposed by Bardele (1990, 1991b), this changed valuation of morphological and ontogenetical characters leads to a ciliate tree turned upside down compared with traditional suggestions. Ciliate groups, such as the prostomes and haptorids, previously thought to represent an early branch of the tree, should now be regarded as highly derived ciliates. On the other hand, heterotrich, nassulid and hymenostome ciliates show character states proposed for the hypothetical ciliate ancestor.

Recently these assumptions based on morphological and morphogenetic data were confirmed by distance matrix trees based on ribosomal RNA sequence comparisons. Phylogenetic analyses derived from small subunit
ribosomal RNA sequence comparisons (Greenwood et al., 1991; Schlegel, 1991, Schlegel et al., 1991) and sequence comparisons of parts of the large subunit ribosomal RNA (Delgado et al., 1991) confirm a sister group relationship between dinoflagellates and ciliates and reveal that ciliates with a distinct oral ciliature, such as the heterotrichs, are to be found in the earliest branches of the tree. The prostome and haptorid ciliates are highly evolved and branch off later.

Although the suggestions presented here are corroborated by molecular data there are many problems still to be solved. I am convinced that a further expansion of our ultrastructural and molecular database and especially a combination of the morphological and the biochemical approach will substantially help to lift the veil of ciliate evolution and to reconstruct the phylogeny of these unicellular organisms.

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References


