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Behaviour of the Chrysoflagellate Alga, *Dinobryon divergens*, During Lorica Formation

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Summary

A timed series of light micrographs illustrates the process of lorica formation in *Dinobryon divergens*. Analysis of this series reveals that the *Dinobryon* cell forms the lorica in two distinct phases: First, the stalk region, without rotation of the cell, and second, the vase-shaped upper part of the lorica, with five slow rotations of the cell yielding the helicoidal band structure of the lorica. Complex changes of shape by the cell body provide the model for lorica diameter.

Keywords: Cell rotation; Cell shape change; *Dinobryon;* Lorica formation.

1. Introduction

Dinobryon is a very sensitive organism in light microscopical observation. Nevertheless, KLEBS (1893), PASCHER (1913), KRIEGER (1930), FRITSCH (1948), BOURRELLY (1957) and PRINGSHEIM (1963) have previously described the process of lorica formation in *Dinobryon* and its relatives, claiming a rotation of the cell and secretion of the lorica at the flanks of the cell. Since recent analysis has shown that the lorica of *Dinobryon* contains cellulose (HERTH and ZUGENMAIER 1979), details of lorica formation are of great importance for discussion of the controversial question of the cytological site of cellulose formation (O'BRIEN 1972, PRESTON 1974, SCHNEPF 1974, BROWN *et al.* 1973, ROBINSON 1977, SCHNEPF and HERTH 1978). I have recently succeeded in obtaining a timed series of light micrographs during lorica formation of *Dinobryon divergens*, and have analyzed it to test the old claims.

2. Materials and Methods

Dinobryon divergens was collected and cultivated as described by HERTH and ZUGENMAIER (1979). Prolonged observation in the Zeiss plankton chamber was possible with low illumination and heat filters, the Zeiss IM 35 microscope with Plan 100 objective and differential interference contrast, using the Zeiss microflash for the micrographs.

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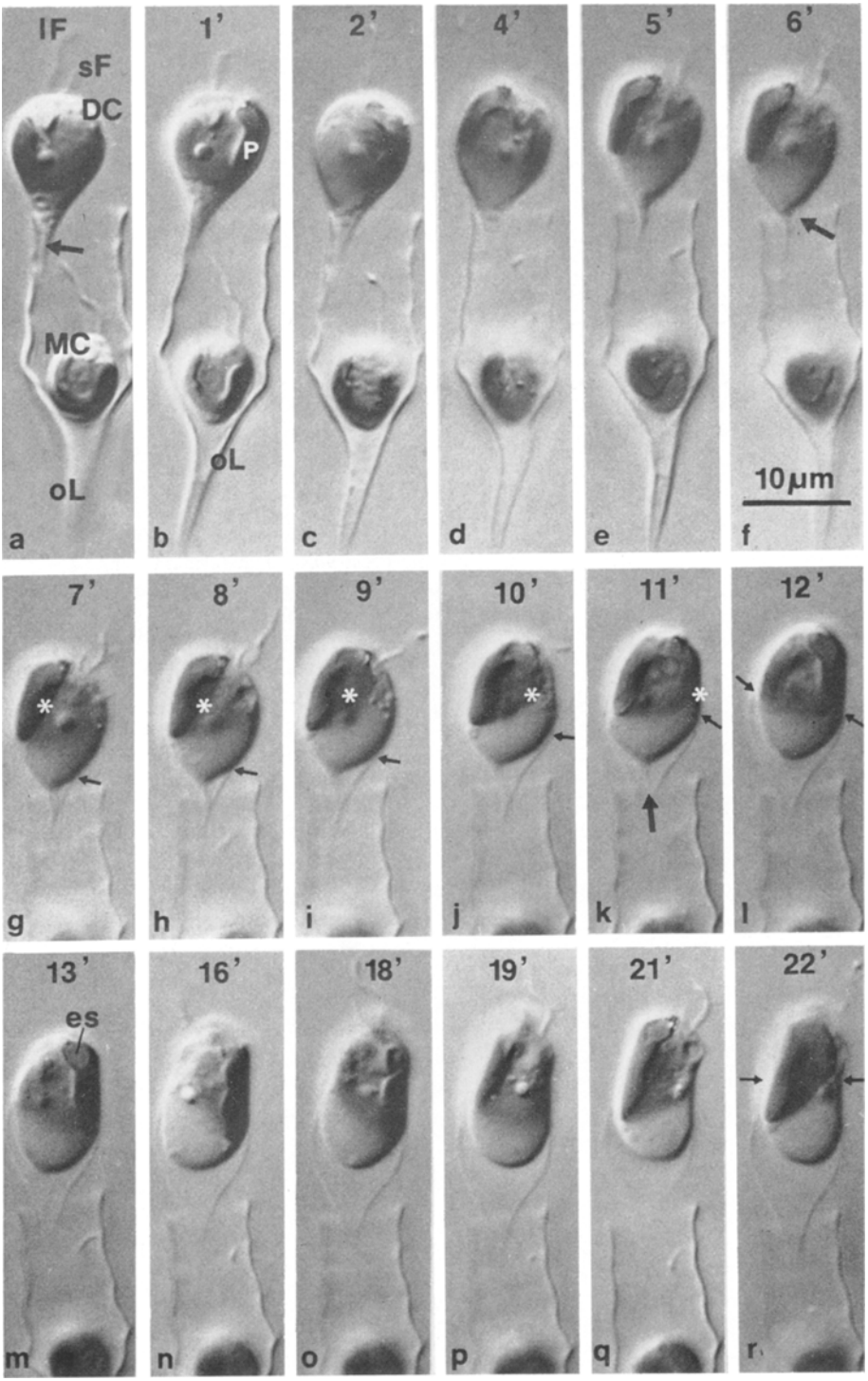

Fig. 1. *a–r*. Timed series of light micrographs of *Dinobryon divergens* during lorica formation. Time in minutes from beginning of lorica formation in (*a*) is given in upper part of each figure. Abbr.: *lF* = long flagellum; *sF* = short flagellum; *P* = chloroplast; *oL* = old mother cell lorica; *yL* = young lorica of the daughter cell; *LV* = leucosin vacuole; *MC* = mother cell; *DC* = daughter cell; *ps* = pseudopodial extension. Magn. ×1,500

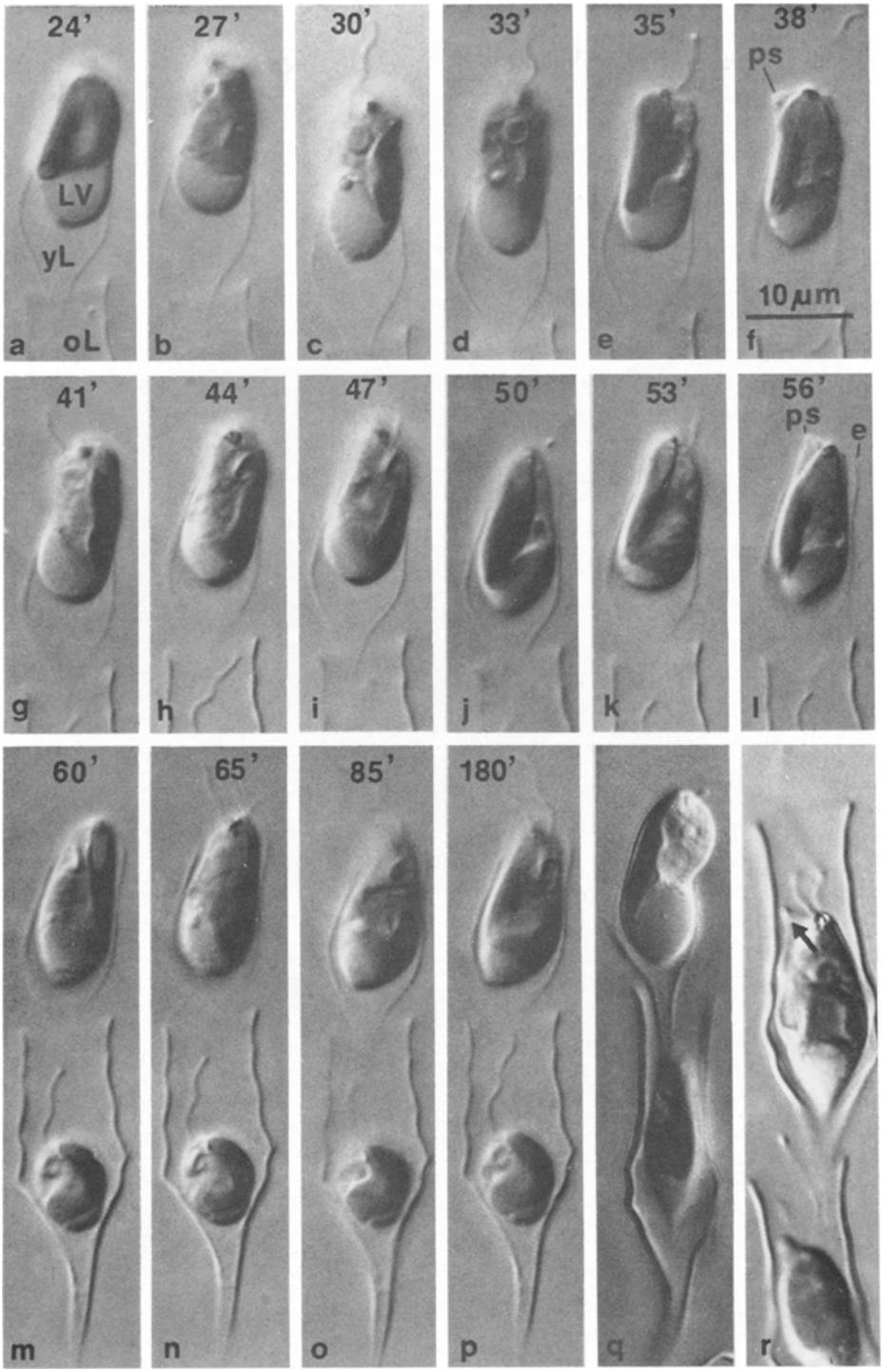

Fig. 2. *a–p*. Continuation of lorica formation. *q* Representative intermediate stage from another timed series taken during lorica formation. *r* Complete mother and daughter loricae; the fully grown daughter cell shows a small pseudopodial extension of the anterior end (arrow). Abbr. as in Fig. 1. Magn. ×1,500

3. Results

After cell division, the daughter cell moves to the edge of the mother cell lorica and there attaches itself, whereas the mother cell rounds up in the basal part of the old lorica. The timed series of micrographs starts at this moment (Fig. 1 *a*). During the next 5 minutes, the cell extends its straight cytoplasmic tail and forms the basal stalk region of the lorica around this tail (Figs. 1 *a–e*). The cell does not rotate during this stage, but this is hard to see in these micrographs owing to focus differences from movements of the cells. With respect

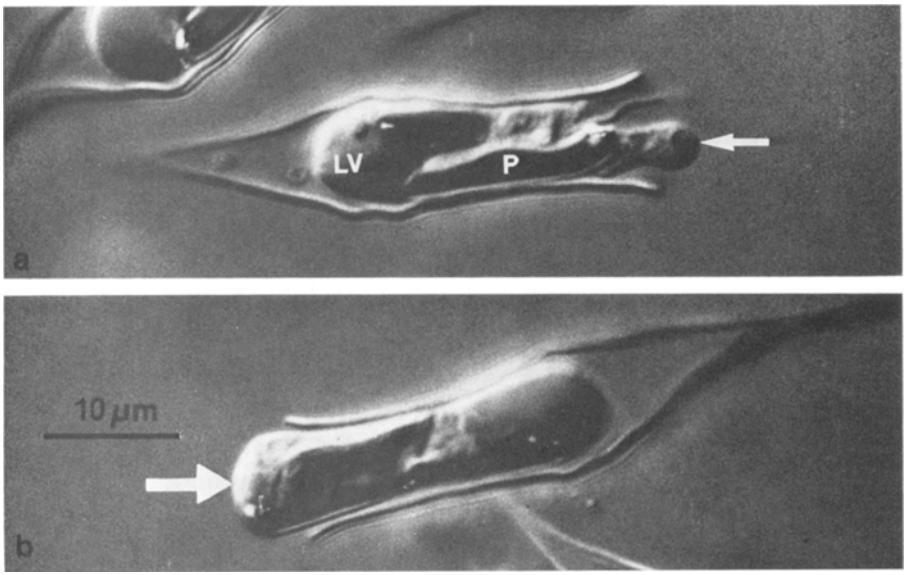

Fig. 3. Amoeboid changes of shape of *Dinobryon*. *a* Tongue-like pseudopodial extension of the anterior end region (arrow). *b* Changed cell shape of a *Dinobryon* cell, probably a division stage. Magn. ×1,800

to the point of attachment, the cell moves slightly forwards during this process. Then the cell starts to retract its tail, gets broader posteriorly and the vase-like part of the lorica is begun (Fig. 1 *f*, arrow). The cell begins a slow rotation, best seen by the relative movement of the edge of the chloroplast (asterisk) and of the flagella (Figs. 1 *g–k*). One rotation takes ca. 10 minutes. The region of lorica formation gradually moves from the posterior end to the flanks of the cell (arrows in Figs. 1 *g–l*). At the same time, the cell moves forward and retracts its cytoplasmic tail (Fig. 1 *k*, arrow). During the next slow rotation, the cell shape changes (Figs. 1 *m–r*), reducing the cell diameter to the value necessary for the upper part of the lorica (Fig. 1 *r*, arrow). The cell further rotates (Figs. 2 *a–f*) and becomes still more elongate. The region of lorica formation moves to the anterior part of the cell, where it is obviously associated with a small pseudopod-like extension (Fig. 2 *f*). Two

further rotations are seen in Figs. 2 *g–p*. Unfortunately, the cell stopped at this stage of lorica formation.

The leucosin vacuole, first appearing as a voluminous sphere in the posterior part of the cell (*e.g.*, Figs. 1 *g–r* and 2 *a*) is more and more reduced during lorica formation (compare with Fig. 2 *p*), indicating utilization during this process.

The behaviour of *Dinobryon* during lorica formation is very reproducible (compare Fig. 2 *q*, from another timed series, with Figs. 1 *r* and 2 *a–f*).

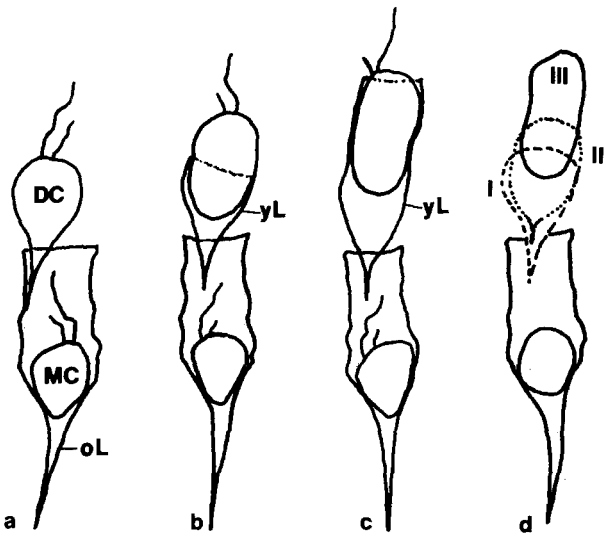

Fig. 4. Schematic drawing of the behaviour of *Dinobryon* during lorica formation. *a* Early stage. *b* Intermediate stage. *c* Late stage. *d* Changes of shape of the *Dinobryon* cell during lorica formation; *I*, *II*, *III* corresponding to early, intermediate and late stage. Abbr. as in Fig. 1

A comparison with a fully formed daughter lorica (Fig. 2 *r*) reveals that the young lorica formed in my timed series is less substantial, as judged from the wall thickness, and does not show the undulated appearance, probably owing to an inhibitory effect of the conditions required for observation.

The *Dinobryon* cells were frequently observed to form an anterior pseudopod-like extension (Figs. 2 *r* and 3 *a*, arrow) during times other than lorica formation. Rotations, movements up and down in the lorica, extension or retraction of the cytoplasmic tail and changes of shape, to the extreme of Fig. 3 *b* (arrow), probably a division stage, were also frequently observed. The *Dinobryon* cell thus is almost amoeboid.

4. Discussion

If the movements of the *Dinobryon* daughter cell during lorica formation are traced, it becomes obvious that there are distinct phases: In the first phase, the cell moves slightly forward with some elongation of the cytoplasmic tail (Fig. 4 *a*), without observable rotation. Then the tail is retracted, the cell

rotates and at the same time moves forward (Fig. 4 *b*). Later, the cell becomes elongate during formation of the upper part of the lorica (Fig. 4 *c*), with further rotations. The changes of shape are summarized in Fig. 4 *d*. Lorica formation thus is the product of complex movements and changes of shape of the *Dinobryon* cell. In part, these micrographs confirm the old claims (see Introduction) that the lorica is formed according to the "potter's wheel" principle, but the process is more complex than as given in the old descriptions. The number of rotations observed corresponds to the helicoidal band structure of the lorica (HILLIARD 1971, HERTH and ZUGENMAIER 1979). These slow rotations now documented (0.1 rpm) are probably different from those of the old descriptions, since disturbed *Dinobryon* and *Poterioochromonas* cells tend to detach from the substratum with a fast rotation (HERTH, unpublished). As in the chrysoflagellate *Poterioochromonas*, which forms a chitinous lorica (HERTH *et al.* 1977), first the cytoplasmic tail region, then the posterior flanks of the cell are involved in lorica formation (SCHNEPF *et al.* 1975). The *Poterioochromonas* cell, according to KRAMER (1970, 1972), seems to rotate during lorica stalk formation, but this needs to be reexamined. Lorica formation of *Dinobryon* began fast, but then was slowed down during observation. If this slow speed of lorica formation was a consequence of inhibitory conditions, both *Dinobryon* and *Poterioochromonas* form their lorica in almost the same time (SCHNEPF *et al.* 1975).

The probable involvement of pseudopod-like anterior extensions in lorica formation has not been observed in *Poterioochromonas*. This organism is also very flexible and capable of shape changes, as is *Ochromonas* (OLTMANNS 1922, FOTT 1969). In *Poterioochromonas*, it is assumed (SCHNEPF *et al.* 1975) that the fibrils are formed at the plasma membrane without exocytotic phenomena. Lorica formation in *Dinobryon* is more likely to be a secretion process with involvement of vesicles, as vesicles were frequently seen close to the region of lorica formation. However this question cannot be decided by light microscopy (for further discussion see FRANKE and HERTH 1973, and HERTH and ZUGENMAIER 1979).

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