Isolation and Phenotypic Characterization of Chlamydomonas Mutants Defective in Cytokinesis

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ABSTRACT. Two genetically independent Chlamydomonas mutants, oca1 and oca2, that display abnormal cell division were isolated by DNA insertional mutagenesis. The culture of these mutants contained large abnormally-shaped cells with multiple pairs of flagella. DAPI staining showed that those aberrant cells carried the same number of nuclei as that of flagella pairs. Time-lapse video microscopy revealed the following characteristics of the cell division process in the mutants: i) although the mutants, like wild-type cells, had a potential to divide into eight daughter cells by successive three rounds of division cycle, they frequently failed to cleave and formed a fused cell in the course of division; ii) a cell often grew into an extremely large cell with many pairs of flagella; iii) the large cell was suddenly divided into a number of daughter cells by simultaneously forming multiple cleavage furrows; iv) alternatively, an extremely large cell stopped dividing although many cleavage furrows were formed on its surface. These observations suggest that these mutants are partially deficient in the progression of furrowing, and that Chlamydomonas is capable of undergoing cytokinesis between many pairs of nuclei simultaneously, as in the cellularization process of insect eggs.

Cytokinesis in animal cells takes place through the constriction of a contractile ring situated at the cleavage furrow. It is usually tightly coupled with mitosis in that the progression of mitosis is prerequisite to the contractile ring formation. However, how the position and timing of the cleavage furrow formation are controlled is largely unknown, although many protein factors involved in cytokinesis have been identified, including actin and myosin as the main components of the contractile ring (1, 2, 5, 7, 11, 13, 14, 15).

Studies using mutants would be important for the elucidation of this problem, which must involve interactions between a number of different proteins. We have thus started isolating cytokinesis mutants from the bi-flagellate alga Chlamydomonas reinhardtii, of which the cell division machinery has been considered to be similar to that of animal cells. Its nucleus divides by the process of mitosis in which the basal bodies function as centrosomes (4), and its cell body divides by means of a cleavage furrow where actin is localized (3, 8).

Here we report the isolation and initial characterization of two Chlamydomonas mutants displaying abnormal cell division, produced by insertion of plasmid DNA into the nuclear genome. These mutants appear to be partially deficient in progression of cytokinesis and, unexpectedly, have an ability to undergo a novel mode of cell division in which a single multi-nuclear cell is simultaneously divided into multiple daughter cells.

MATERIALS AND METHODS

Strains. The Chlamydomonas reinhardtii 137c (wild type), nit1/cw15, and the newly isolated mutants, oca1 and oca2, were used. These mutants were isolated from cells transformed by the method of Kindle (10) using glass beads. Cells were treated with autolysin and then transformed with 1 μg of pMN24 plasmid DNA digested with EcoRI. The colonies of transformants on SGII-NO3 plates were placed into 96-well titer plates and screened for abnormal cell shapes. Mutants obtained were back-crossed with wild type cells, and third-generation daughter cells were used as mutants. Sexual crosses were performed by a standard method (9). These mutants usually did not yield complete numbers of tetrads in crosses with wild type cells, thus we did not attempt to map their genetic loci.

DAPI-staining. Nuclei were stained with DAPI according to the method described by Nishii and Ogihara (Nishii, I. and S. Ogihara, "Actin filament-dependent inversion of the posterior hemisphere of Volvox embryos", manuscript in preparation). Briefly, cells cultivated for 2 days in TAP medium (6) were fixed with 3.6% formaldehyde and 0.1% Triton-X100 in

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a basic solution (1 mM DTT, 2 mM MgCl2, 5 mM EGTA, 150 mM KCl, 10 mM sodium glycerophosphate, 10 mM HEPES, pH 7.1), and permeabilized with 1% NP-40 and 1% BSA in the basic solution. After washing with PBS containing 0.1% BSA 3 times, the cells were stained with 4 μg/ml of DAPI for 10 min. Fluorescence in cells and differential interference contrast images were observed under a Zeiss Axiosplan microscope.

Time-lapse video micrographs. To follow the growth and division process for up to 3 days, Chlamydomonas cells were immobilized within the field of microscope by sandwiching between a thin agar sheet and a coverslip. The agar sheet was prepared from TAP medium containing 1% agar. Margins of the coverslip were sealed with Vaseline to protect the cells from drying. In this condition, cells were able to live and grow for at least 3 days. Their phase-contrast images were continuously recorded for 1 or 2 days with a time-lapse video recorder (Panasonic, AG-6730).

RESULTS

Of about 6500 cells transformed with plasmid DNA, two mutants were found to have abnormal shapes. The two mutants were indistinguishable by their phenotypes. Sexual crosses between them yielded daughter cells with a wild-type phenotype as well as cells with abnormal shapes, indicating that the two mutants differ genetically. We named them oca1 and oca2 for occasion-al cytokinesis arrest. In the following descriptions of phenotypes we will not distinguish the two mutants.

The cultures of both mutants contained normally-shaped cells with two flagella and abnormally-shaped cells with multiple pairs of flagella. The number percentage of the abnormal cells in the total was typically 30–50%. The number of flagella pairs in an abnormal cell varied from two to as many as 13. The cell size increased with the number of flagella. Typical shapes of the abnormal cells with two, three and four pairs of flagella are shown in Fig. 1. However, various shapes were frequently observed other than those shown. Cells with more than five flagella pairs displayed quite irregular shapes and no typical ones.

In wild-type Chlamydomonas, the pair of flagellar basal bodies is connected to the nucleus in interphase (17). During mitosis, the basal body pair is separated into two halves and function as the poles of the mitotic spindle, which distributes the duplicated chromosomes into two daughter nuclei (4). Thus, there is an exact correspondence between the number of nuclei and that of basal body pairs in the whole population of cells. If the basal bodies in the mutant function in the same manner as those in the wild type, then the number of nuclei in a mutant cell also should agree with that of the flagella pairs. We thus counted the number of flagella pairs and nuclei visualized by DAPI staining. We found that the numbers of those agreed with each other. Furthermore, the nuclei were always located near the flagellar roots (Fig. 1). This observation suggests that the mutants underwent nuclear division normally with functionally active basal bodies, but were somehow abnormal in completion of cytokinesis.

To understand how those abnormal cells emerged and why the cultures of the mutants contained cells with both normal and abnormal shapes, we followed the cell
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![Fig. 2. An oca mutant undergoing normal division. A series of phase-contrast images. A mutant cell divided evenly into 8 daughter cells through 3 successive rounds of division. Numbers denote the time after the onset of cell division (hour: minute). Bar, 10 μm.](image)

...fates for 1 or 2 days with a video camera and a time-lapse video recorder. The mutants were sandwiched between a thin agar sheet and a coverslip and immobilized within the area of observation. Under this condition, cells divided and grew for at least 4 days.

*Chlamydomonas* cells generally divide consecutively 2 or 3 times within the original cell wall, forming four or eight cells which are released at once after the division (3). As shown in Fig. 2, oca mutants were also found to divide into eight daughter cells through three consecutive division cycles. In another case shown in Fig. 3, however, an abnormally large and aberrantly-shaped cell appeared among the daughter cells originated from the same parent cell. Because the number of the daughter cells in this case was seven, this large cell was thought to be resulted from partial failure in cell division in the third round of division cycle.

Fig. 4 shows another example in which a cell with an abnormal shape continued to grow without cell division and became about four times larger in diameter than a normal cell. This cell appeared to have lost the ability to divide when it grew to this size. Unexpectedly, however, it then divided into more than 15 daughter cells within a short period of time. The manner of division significantly differed from that of a normal cell division; namely, multiple furrows were simultaneously formed on the surface of this large cell, and daughter cells were produced by an apparently single division event. In a still another case, a cell was observed to stop the progression of simultaneous furrowing, at which point the furrows then disappeared and the cell grew further in size (Fig. 5).

**DISCUSSION**

Using DNA insertional mutagenesis, we isolated two mutants whose cultures contain abnormally-shaped cells. A similar mutant has previously been isolated from the cells mutagenized with N-nitrosoguanidine (16). This mutant (*cyt-1*) and our mutants (*ocal* and *oca2*) share phenotypes in common; the culture contains both the normal and abnormally-shaped cells, and the abnormal cells have the same numbers of multiple nuclei and pairs of flagella. However, how normal and abnormal cells emerge in the culture of *cyt-1* has not been made clear. At present, we have no information about the genetic relationship between *cyt-1* mutant and *ocal* or *oca2*.

The presence of the same number of flagellar pairs and nuclei in these mutant cells suggests that nuclear division is accomplished normally with proper functioning of the basal bodies as centrosomes. The observation that each nucleus is located near a flagellar base indicates that the nucleus is associated with the basal body as in wild-type cells (17). The mutants *ocal* and *oca2* have multiple nuclei probably because of some deficien-
cies in a process following the nuclear division.

Time-lapse observations of the mutants have revealed a striking feature, which explains why cells with normal and abnormal shapes are present simultaneously in a culture. The mutant cells have a potential to accomplish cytokinesis (Fig. 2), but some of the daughter cells occasionally fail to cleave and assume aberrant shapes (Fig. 3). Once a cell of this type has emerged, it skips over the furrowing phase of the cell cycle, eventually becoming an extremely large cell (Fig. 4). Stochastic emergence of such cells that fails to cleave can explain the simultaneous presence of cells with a variety of size, shape, and number of nuclei.

Unexpectedly, those cells with abnormally large numbers of nuclei were observed to divide simultaneously into many daughter cells with normal and abnormal shapes (Fig. 4). This process is reminiscent of the cellularization process of insect eggs, where a single multi-nuclear cell is divided into a number of daughter cells simultaneously. In the cellularization process, the cleavage furrow is produced by a network of actin bundles, as evidenced by staining with fluorescence-labeled phalloidin (12). With Chlamydomonas, however, we have been unable to detect any phalloidin binding along the

Fig. 4. A sequence showing growth and division of an oca mutant cell with an aberrant shape. A cell carrying 3 pairs of flagella grew into a round cell with a diameter about 4 times as large as that of the normal cell, and then divided into > 15 cells in a single division event. Bar, 10 μm.

Fig. 5. Failure in the progression of furrowing in an abnormally-shaped cell. Although many furrows (indicated by arrowheads) once formed, those disappeared as the cell grew. Bar, 10 μm.
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cleavage furrow either in the mutants or in the wild type cells, although the same fluorescent phalloidin did reveal an F-actin bundle in the fertilization tubule (data not shown). Hence the mechanism for the cleavage furrow formation and function remains to be studied.

From these observations, we concluded that the mutants reported here are normal in mitosis and initiation of cytokinesis but partially deficient in the progression of furrowing. Because the genomes of these mutants carry the inserted plasmid sequence, we may be able to clone the mutant genes using the plasmid sequence as a tag. Information from the mutant genes may possibly help us to unlock the molecular mechanism for cytokinesis.

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