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Nuclear migration during wound-healing process in three ceramiacean species: *Antithamnion nipponicum, Aglaothamnion oosumiense* and *Platythamnion yezoense* (Rhodophyta)

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The wound-healing process of three filamentous red algae, *Antithamnion nipponicum, Aglaothamnion oosumiense* and *Platythamnion yezoense* was examined using the fluorescent nuclear stain DAPI. The nuclear movement during the process was followed to observe how the plants manage the heterogenous nuclear condition caused by the process. A strong relationship between nuclear movement and wound-healing response during the process was observed. In all three species the process included the early migration of adjacent cell nuclei toward the wounded region and their later return to the central portion. When the nuclei of adjacent cells remained at the central portion, the repair process did not proceed. The ratio of nuclear DNA to cytoplasmic volume seemed to break down during wound-healing response. The ratio increased in *Antithamnion nipponicum* and *Aglaothamnion oosumiense*, but it decreased in *Platythamnion yezoense*.

Key Index Words: Aglaothamnion oosumiense—Antithamnion nipponicum—*nuclear DNA to cytoplasmic volume ratio—nuclear movement*—Platythamnion yezoense—*wound-healing response*

Red algae are exceedingly diverse with respect to their nuclear features. The number, size and position of nuclei in cells as well as their DNA content (ploidy) vary considerably in different taxa (Goff and Coleman 1990). One constant nuclear trait expressed in the class Florideophyceae, whether in uninucleate or multinucleate plants, is that the total nuclear DNA within a cell is very closely correlated with the total cytoplasmic volume; there is a reasonably constant ratio between nuclear DNA and cytoplasmic volume (Goff and Coleman 1986, 1987, 1990).

This constant ratio appears to break down during cellular events involving nuclear migration resulting in secondarily derived multinucleate cells (L'Hardy-Halos 1969, Cabioch 1972, Goff and Coleman 1984a, 1985, 1986). Perhaps the best known example is nuclear transfer during the formation of secondary pit-connections (Rosenvinge 1888 Goff and Coleman 1984b, 1986). The nuclei transferred from one cell to another by somatic cell fusion, sometimes fuse with resident nuclei, thereby resulting in an increase in cell ploidy levels (Goff and Coleman 1985).

An increase in cell ploidy levels may also occur during the wound-healing response. In many filamentous red algae the damaged intercalary cells are replaced through a process of cell repair. In a detailed study of red algal wound-healing, Kim *et al.* (1988) observed the process in 11 genera, 16 species and grouped them into three typical patterns; fusion type, non-fusion type and elongation type. During the fusion-type wound-healing response two kinds of specialized somatic cells are produced, one to several upper repair rhizoid cells and a lower repair shoot cell.

These cells grow to each other and fuse to replace the dead intercalary cell (Waaland and Clreland 1974, Kim *et al.* 1988, Kim and Fritz 1993). Because the region occupied by the intercalary cell maintains a relatively constant volume, the fused repair cell prossesses at least two times more DNA than the prior intercalary cell, thus resulting in an imbalance in the DNA to cytoplasmic volume ratio. Imbalance of the ratio can be induced in non-fusion type and elongation type as well.

In this study, using the fluorescent nuclear stain DAPI, we examined the nuclear movement during the wound-healing process in three filamentous red algae representing each of the typical patterns of wound-healing response (Kim *et al.* 1988); *Antithamnion nipponicum* (fusion type), *Aglaothamnion oosumiense* (non-fusion type), and *Platythamnion yezoense* (elongation type), with an aim toward understanding how the plants manage the heterogenous nuclear condition caused by the process.

Materials and Methods

The marine red algae *Antithamnion nipponicum* Yamada, *Aglaothamnion oosumiense* Itono and *Platythamnion yezoense* Yamada et Inagaki (Rhodophyta, Ceramiales) were cultured as described previously (Kim *et al.* 1988) in modified Provasoli's enriched medium (PES; Provasoli 1968) at 20°C on a 16h light: 8h dark cycle under 12 $\mu Em^{-2}s^{-1}$ cool-white fluorescent lamp.

For wound-healing experiment, intercalary cells in the upper part of an actively growing thallus were wounded with a razor blade and the cytoplasm was carefully removed so as not to sever the wall. Wounded plants were placed in fresh culture medium and observed every hour. For observations of cell nuclei, wounded plants were transferred into a solution of DAPI nuclear stain (0.5 μg/ml) and fixed by microwave at high level for 10-15 seconds (Goff and Coleman 1987).

All specimens were examined with a Reichert-Jung Polyvar and Olympus BH-2 microscope equipped with epifluorescence illumination and differential interference optics.

Results

***Antithamnion nipponicum* (Fusion type):** This species is composed of uninucleated cells (Fig. 1-8). The size of nuclei in the intercalary cells is in direct proportion to their cytoplasmic volume (Fig. 1). Each axial cell is in contact with four cells, two adjacent axial cells and two small basal cells, all of which are involved in the wound-healing response.

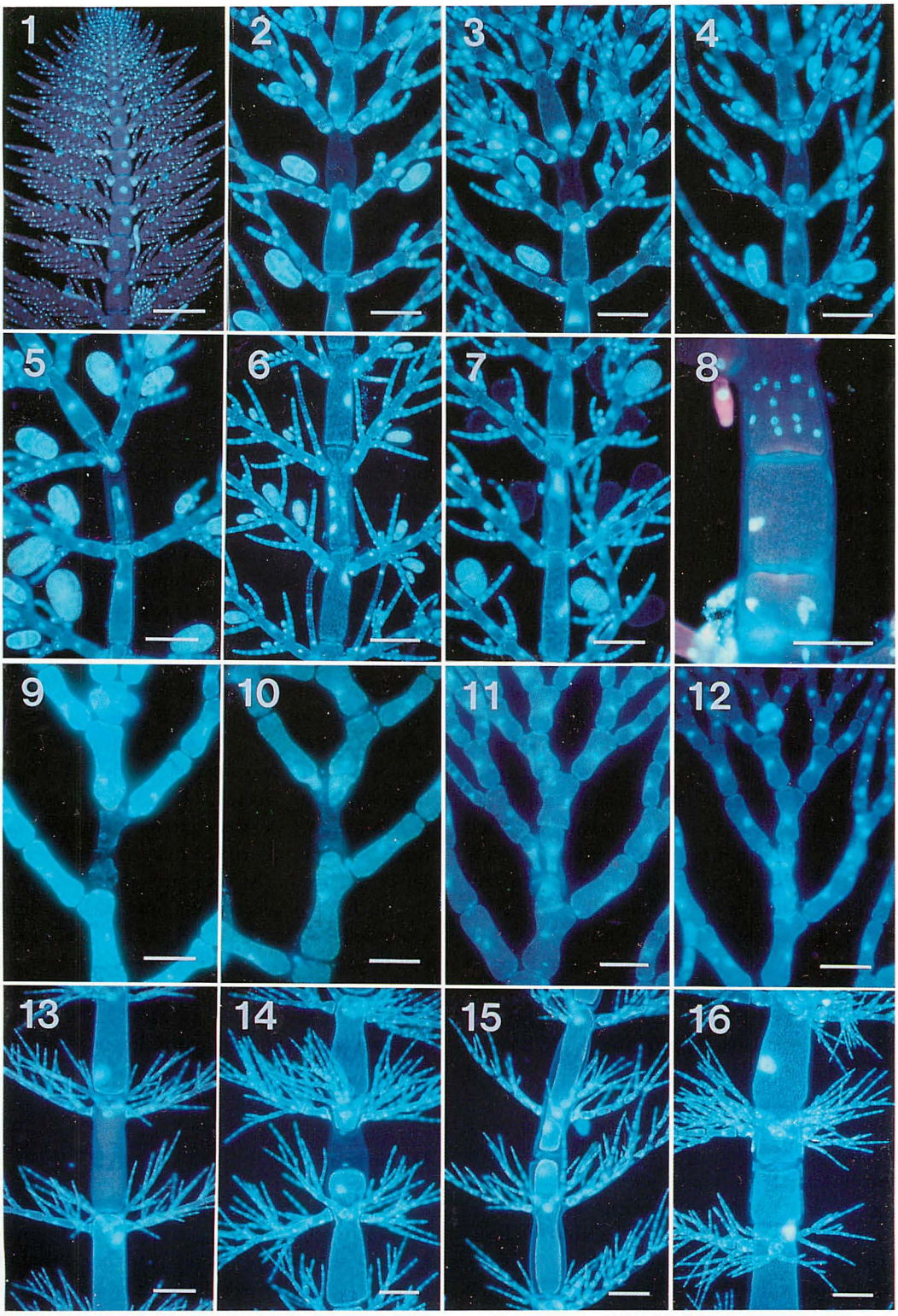
When the nuclear stain DAPI was added immediately after wounding, nuclei of adjacent cells were observed to be located close to the center of each cell (Fig. 2). Within two to three hours after wounding the nuclei of adja-

Figs. 1-16. Nuclear movement during wound-healing response in three ceramiacean algae.

Figs. 1-8. *Antithamnion nipponicum* (Fusion type). Fig. 1. Before wounding. Nuclear size is in proportion to cell size. Scale bar=200 μm. Fig. 2. 1 h after wounding. Nuclei of adjacent cells are situated in the center of cells. Scale bar=100 μm. Fig. 3. 3 h after wounding. Nuclei of adjacent cells moved toward wounded portion. Scale bar=100 μm. Fig. 4. 6 h after wounding. Each adjacent cell divided repair cell. Scale bar=100 μm. Fig. 5. 12 h after wounding. The repair cells grew toward each other. Scale bar=100 μm. Fig. 6. 18 h after wounding. The repair cells were fused. Four nuclei were present in the fusion cell. Scale bar=100 μm. Fig. 7. 24 h after wounding. Nuclei from each repair cell fused into one big nucleus. Scale bar=100 μm. Fig. 8. 48 h after wounding. A fusion cell divided into three small cells containing various number and size of nuclei. Scale bar=50 μm.

Figs. 9-12. *Aglaothamnion oosumiense* (Non-fusion type). Scale bars=50 μm. Fig. 9. 2 h after wounding. Nuclei of adjacent cells moved toward wounded portion. Fig. 10. 6 h after wounding. Repair cells were formed from each adjacent cell. Fig. 11. 18 h after wounding. Repair cells grew in the wounded portion. Fig. 12. 24 h after wounding. Repair cells were attached to each other without cell fusion.

Figs. 13-16. *Platythamnion yezoense* (Elongation type). Scale bar=100 μm. Fig. 13. 6 h after wounding. Nuclei of adjacent cells moved toward wounded portion. Fig. 14. 10 h after wounding. Elongation of adjacent cells began. Fig. 15. 18 h after wounding. Fig. 16. 24 h after wounding. Two adjacent cells attach to each other and nuclei of both cells moved back to the center.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cent cells have moved closer to the wounded region (Fig. 3), and have divided to form daughter nuclei. In the rare cases where the wound-healing process was not initiated even in 24 hours after wounding, the nuclei of adjacent cells remained at the central position of the cell and never underwent division.

In the normal situation, four to six hours after wounding each of the adjacent axial cells divided to produce small repair cells with nuclei approximately the same size as those of the large adjacent cells (Fig. 4). Upon completion of repair cell formation the nuclei of adjacent cells moved back to their earlier central position. Each basal cell of lateral branches also produced a respective repair cell with a single small nucleus. The fusion of the repair cells occurred first among the three upper repair cells, and by 12 hours post-wounding three nuclei (a large one and two small ones) were present in the upper repair cell (Fig. 5). The upper and lower repair cells grew and fused at about 18 hours after wounding (Fig. 6). At this time four nuclei were distributed irregularly within the single fused repair cell. Twenty four hours after wounding the nuclei migrated toward the center of the cell and fused (Fig. 7).

Although nuclear fusion was a rather common process after somatic cell fusion, all fused repair cells did not result in fused nuclei. By 72 hours after wounding, 56% (14/25) of fusion cells had fused nuclei and 24% (6/25) had partially fused ones, but 20% (4/25) of them still had four nuclei. At five days after wounding it became very hard to distinguish the fused repair cell from other adjacent cells. In rare cases (1/25), however, the fusion cell divided again into three small cells which had various number (1-13) and size of nuclei (Fig. 8).

When the filament was severed just after the fusion of three upper repair cells, three nuclei in the cell divided once or twice, resulting in six to twelve heterogenous nuclei. Later, the rhizoidal initial cell divided to form a new cell with two of four nuclei. A subsequent divisions of this daughter cell resulted in a cell with only one nucleus.

***Aglaothamnion oosumiense* (Non-fusion type)**: This species has non-polyploid uninucleate cells. The size of nucleus is relatively constant throughout the filament (Figs. 9-12), and intercalary cells are in contact with three cells. When an intercalary cell was wounded, the nuclei of adjacent cells moved from their central position toward the wounded region (Fig. 9). Four to six hours after wounding each of the adjacent cells divided to produce a respective repair cell (Fig. 10). The nuclei in the repair cells were approximately in same size as those of the mother cells. When the formation of repair cells was completed, the nuclei of adjacent cells moved back to their central position (Fig. 11). Within 24 hours after wounding the repair cells made contact with each other but without cell fusion (Fig. 12).

***Platythamnion yezoense* (Elongation type)**: This species also has uninucleate cells (Figs 13-16). The size of the nuclei in intercalary cells is in proportion to their cytoplasmic volume similar to *A. nipponicum*. Each axial cell is in contact with six cells, two adjacent cells and four small basal cells of lateral branches (Fig. 13). During wound-healing, however, only two adjacent axial cells were involved in the process. Four basal cells elongated a little. At the time of wounding, the nuclei of adjacent cells were located at the center of each cell. At six hours after wounding the nuclei of adjacent cells moved toward the wounded region (Fig. 13), and the adjacent cell began to elongate (Fig. 14). Both of the nuclei remained at the tip of the adjacent cell throughout the process (Figs 13-15). When the two adjacent cells came in contact with each other, both nuclei moved back to their central position (Fig. 16).

Discussion

The data presented show that there is a very close relationship between nuclear movement and wound-healing response. In all three species the wound-healing process begins with the migration of adjacent cell nuclei

toward the wounded cell, and upon completion of the process the nuclei return to a central position. As ceramiacean species are supported to lack cyclosis and their organelles are fixed in the peripheral cytoplasm that surrounds the large central vacuole (Goff and Coleman 1987, Koslowsky and Waaland 1984), the nuclear migration suggests a major rearrangement of cell organelles.

To ensure concurrent migration of adjacent cell nuclei and development of compatible repair cells, transmission of chemical messages between the cells is necessary during the wound-healing process. To date, only one endogenous development regulating substance has been isolated from a red alga, rhodomorphin of *Griffithsia pacifica* (Watson and Waaland 1983, 1986). Rhodomorphin is an α-D-mannosyl-linked glycoprotein, which is purported to induce cell division, to control cell elongation and morphogenesis in the cells involved in wound-healing response (Waaland 1990). Recently, Kim and Fritz (1993) reported a signal glycoprotein with α-D-mannosyl residues is involved in the wound-healing response of *Antithamnion sparsum*. By the use of FITC-conjugated lectins combined with the fluorescent nuclear stain DAPI, they distinguish the wound-healing process into three principle steps and suggest that the first step of the process which comprises with concurrent migration of adjacent nuclei may be dependent on another cellular signal because there is no apparent labelling of the signal glycoprotein at this step (Kim and Fritz 1993). The concurrent migration of adjacent cell nuclei observed in non-fusion type (*Aglaothamnion oosumiense*) and elongation type (*Platythamnion yezoense*) wound-healing response which seems to lack cell fusion hormone may support the idea.

In both prokaryotic and eukaryotic cells strong correlations have been reported between genome size and cell volume and there is a reasonably constant ratio between nuclear DNA and cytoplasmic volume (Cavalier-Smith 1978, 1985, Watanabe and Tanaka 1982, Shutter *et al.* 1983, Brodsky and Uryvaena 1985, Lewis 1985, Goff and Coleman 1986, 1987, 1990).

During the wound-healing process of filamentous red algae, however, this constant ratio appears to break down. In *Antithamnion nipponicum*, two large nuclei and two small nuclei participate in the fused repair cell, thereby increase the ratio more than two times. In *Aglaothamnion oosumiense*, the ratio also increases three times because the region of the dead cell is replaced by three repair cells which have their own nuclei. In contrast, in *Platythamnion yezoense* both of the adjacent cells increase in cytoplasmic volume about 1.5 fold without apparent increase in DNA amount, which may result in decrease of the DNA to cytoplasmic volume ratio.

Heterokaryons can be produced experimentally by fusing male and female gametophytes during the fusion-type wound-healing process (Waaland 1978, Hwang *et al.* 1991). The regenerated plants were shown to be morphologically and genetically different from either gametophyte, giving rise to tetrasporangia characteristic of the tetrasporophyte (diploid) generation. Waaland (1978) suggested that tetrasporangia formed on regenerated plants might be a result of a co-action between male and female nuclei in the cell. Hwang *et al.* (1991), however, obtained viable tetrasporangia from the regenerated plants, and suggested that at least some of the nuclei might fuse with each other to form diploid nuclei. Our data from *Antithamnion nipponicum* of which wound-healing response resulted in fusion of the nuclei involved in the process appears to support this hypothesis.

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イギス科3種，*Antithamnion nipponicum, Aglaothamnion oosumiense* および *Platythaminion yezoense* でみられた創傷治癒における核移動

3種の糸状紅藻，*Antithamnion nipponicum, Aglaothamnion oosumiense* および *Platythamnion yezoense* の創傷治癒過程をDAPI蛍光核染色で調べた。藻体が創傷治癒で引き起こされた異質の核条件にどの様に対応するか，核の移動を追跡した。核の移動と創傷治癒反応には強い関係がみられた。3種すべてにおいて傷を受けた細胞に隣接する細胞の核は傷に向かってすみやかに移動した。隣接した細胞の核が中心部に残っている場合には傷の修復が行われなかった。細胞質量に対する核DNAの割合は創傷治癒に際し破られるようである。*A. nipponicum* と *A. oosumiense* ではこの割合は増加し，*P. yezoense* では低下した。(*Development of Biology, Seoul National University, Seoul, 151–74 Korea; **Department of Biology, Kongju National University, Kongjushi, Chungnam, 314–702 Korea; ***Institute for Marine Bioscience, National Research Council of Canada, Halifax, Nova Scotia, B3H3ZI, Canada)

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