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Cytomorphology of the 'wound healing' process in the green filamentous algae, *Ulothrix zonata* (F. Weber & Mohr) Kützing 1833

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ABSTRACT. For the first time, we present a cytomorphological description of the self-healing (repair) process of damaged thalli sites, 'wound healing', in the green filamentous algae, *Ulothrix zonata* (F. Weber & Mohr) Kützing 1833. In the filaments of this species from Lake Baikal and the Angara (Baikal outflow), Zhilishche (Baikal inflow) and Ida (Angara inflow) rivers, light microscopy methods revealed dome-like and conical protrusions and elongations of transverse cell walls directed into adjacent dead (without protoplast) and defective (with deformed chloroplasts) cells. At the same time, there were mostly patterns where two cells formed protrusions directed into the same damaged filament site between them, i.e. towards each other. The growth of previously unconnected cells towards each other led to their convergence and adjacency. This had two important physiological consequences that ensured the restoration of the filament integrity. The first consequence was the formation of intercellular junctions. The second one was the fusion of the protoplasts and nuclei of the adjacent cells (cell fusion) with the formation of vegetative polyploid cells with increased size. During subsequent divisions of these cells, extended areas emerged with a two- to three-fold increase in the diameter of the algal filaments. It was also found that the process of 'wound healing' promoted the development of giant hypnospores. We showed that the H-shaped septa between cells of filamentous algae were not thickenings of the outer walls but the sheaths of the dead cells preserved after this reparation process. Analysis of the 'wound healing' patterns revealed that the *Ulothrix* cell nuclei did not migrate to the polarized regions of the cells but retained their central position, which testifies to their fixation in the protoplast. We observed sporadic cases of the development of lateral filaments in *U. zonata* were due to the self-repair of defective cells and their subsequent division during 'wound healing'. A comparison of various cell deformations allowed us to determine the characteristic stages of the 'wound healing' process in *U. zonata*, which have some similarities and differences with those in marine red filamentous algae. Our study indicates that 'wound healing' is an evolutionarily developed and genetically programmed adaptation that may be widespread in the population of filamentous algae.

Keywords: filamentous algae, *Ulothrix zonata*, wound healing, repair, cell fusion

1. Introduction

The integrity of algal thalli is constantly exposed to various environmental factors: abiotic and biotic. They include water currents and wave action, abrasion from sand and other fine-grained soils, grazing of fish and benthic animals (molluscs, crustaceans, etc.) as well as attacks by pathogenic fungi, bacteria and protozoa (Gachon et al., 2010). Moreover, programmed cell death may also be a mechanism of thalli damage, especially for filamentous algae (Dingman and Lawrence, 2012; Garbary et al., 2012). If damage is not quickly repaired, the body can fall apart at the sites of damaged or dead cells. Therefore, repair is an important life process for representatives of aquatic flora. Information about the ability of macroalgae to repair the regions of thalli with dead or damaged cells began to appear in the scientific literature as early as in the 19th century (Janczewski, 1876 cited by Lewis, 1909). Interestingly, the term 'regeneration' was originally used to describe such cases. However, already in subsequent publications concerning the study of repair in algae, there were expressions most likely appropriate for representatives of animal world: 'methods of healing' and 'wound healing' (Nichols, 1922; Höfler, 1934). According to the literature, to date, these expressions have also become generally accepted for the description of repair processes in macroalgae (Waaland and Cleland, 1974;

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La Claire, 1982; Kim et al., 1988; Menzel, 1988; Kim and Fritz, 1993; Mine et al., 2008; Hoermayer and Friml, 2019).

The process of 'wound healing' in the modern sense and in the application to the animal body is a complex of reactions and interactions of cells and 'mediators' that ensure the replacement of damaged cells or tissues with new healthy ones (Wang et al., 2018; Wilkinson and Hardman, 2020). Unlike animal cells, plant cells are enclosed in rigid walls and cannot migrate to the damaged site. Therefore, their protective mechanisms, namely regeneration and repair, are based on three cytological strategies: oriented cell division, acquisition of new cell fates and directional cell elongation (polarized growth or tropism) (Hoermayer and Friml, 2019; Mironova and Xu, 2019). Oriented cell division is the involvement in mitosis of any cell adjacent to the damaged site, even of differentiated cells, i.e. those that have previously left the cell cycle (Hoermayer and Friml, 2019). Acquisition of new cell fate means either dedifferentiation or transdifferentiation of cells surrounding the damaged site. Dedifferentiation refers to any cellular change towards a less specialized, more juvenile and more pluricompetent state (Sugiyama, 2015; Mironova and Xu, 2019), while transdifferentiation is defined as a switch from one specialized cell type to another (Nguyen and McCurdy, 2016; Mironova and Xu, 2019). Directional (polarized) cell growth (or tropism) occurs thanks to irreversible elongation, a very rapid growth type that is only characteristic of plant cells (Polevoy, 1998; Baluska et al., 2003; Cole and Fowler, 2006; Girloy, 2008; Obroucheva, 2008; Cells, 2007; Jaffar and Davidson, 2013). This process is based on three coordinated stages: i) swelling of the central vacuole resulted from the influx of water; ii) loosening of the cell wall allowing its expansion and iii) formation of the cell swelling force vector in a certain direction, i.e. cell polarization. It is also generally accepted that growth by elongation is one of the ways, in which plants move. It first appeared in filamentous algae, enabling the attached plants to move to food sources and other resources (Esmon et al., 2005).

In the vast majority of studies on the 'wound healing' process in algae, representatives of the marine flora with a siphonal and siphonocladal arrangement of thalli, as well as with giant coenocyte (multinucleate) cells reaching millimeter and even centimeter sizes, served as study objects (for reviews, see Menzel, 1988; Mine et al., 2008). Mechanisms of 'wound healing' in macroalgae from freshwater bodies were studied only in a few species that also had giant coenocytic cells (Nichols, 1922; Foissner, 1988; Foissner and Wasteneys, 1997). To date, it has been shown that, unlike higher plants, individual damaged cells can be restored in algae. For instance, the process of 'wound healing' in cells of siphon algae includes the rapid repair of cytoplasmic damage, the preliminary sealing of wounds in cell wall with plugs consisting of polysaccharides or peptides, or their mixture and then the final regeneration of a new cell wall over the restored surface (Burr and West, 1972; Colombo and De Carli, 1980; Menzel, 1988; Mine et al., 2008). The restored cells continue to function normally. Most siphonocladal algae respond to mechanical trauma by contraction of the protoplast or by the formation of numerous cytoplasmic aggregates, from which regeneration of new thalli can subsequently occur (Tatewaki and Nagata, 1970; La Claire, 1982; Kim et al., 2001). In marine red algae with branching filamentous thalli, damaged intercalary cells are not repaired but replaced by new ones (Lewis, 1909; Waaland and Cleland, 1974; Kim et al., 1988). At the same time, two types of specialized somatic cells are formed: from one to several upper rhizoid cells and lower shoot cells. They grow from the transverse walls of adjacent cells towards each other into the damaged cells and either fusion to form a single cell or a pit connection. Thus, these new cells ultimately replace the dead cell, completing the wound healing response and restoring the continuity of the filament. Also, it was shown that adjacent cells themselves can continue to grow into the lumen of damaged cells and form pit connections upon meeting, thereby repairing the filament (Kim et al., 1988). Notably, all published results were obtained empirically, i.e. this process was studied in the thalli of macroalgae with intentionally damaged or killed cells. Such an experimental and rather intensive study of 'wound healing' process in algae not only allowed the description of its pattern but also elucidated the role of cell organelles as well as provided identification and isolation of the substances involved in this process (Burr and West, 1972; Waaland and Watson, 1980; Watson and Waaland, 1986; Menzel, 1988).

Despite many years of intensive studies of 'wound healing' process in macroalgae, the information about this phenomenon in freshwater inhabitants is very limited. There is also a lack of scientific information about how 'wound healing' occurs in algae under natural conditions. In this study, we demonstrate that this process can be observed and studied not only in artificially damaged cells but also in naturally damaged ones in samples of macroalgae taken from their habitat.

2. Materials and methods

U. zonata (girdle-shaped Ulothrix) is a member of rather large genus from the order of Ulothrix algae (Ulotrichales). It is widespread in the coastal zone of clean and well-aerated fresh and brackish water bodies, forming dark and yellow-green fouling on various objects submerged in water: stones, wood residues, hydraulic structures, ship bottoms, etc. (Fig. 1A, 1B). This typical representative of macrophytobenthos is considered one of the most studied species of the genus with a clearly established development cycle (Shyam and Saxena, 1980). The history of the study of *U. zonata* dates back to 1804, the year of its first description when this species was named *Conferva zonata*, and in 1833 it was first classified as *Ulothrix zonata* (Weber and Mohr, 1804; Kützing, 1833; Lokhorst and Vroman, 1972). Foreign and Russian algologists indicated a high degree of polymorphism of this species both in nature and in cultivation, which resulted in the description of many

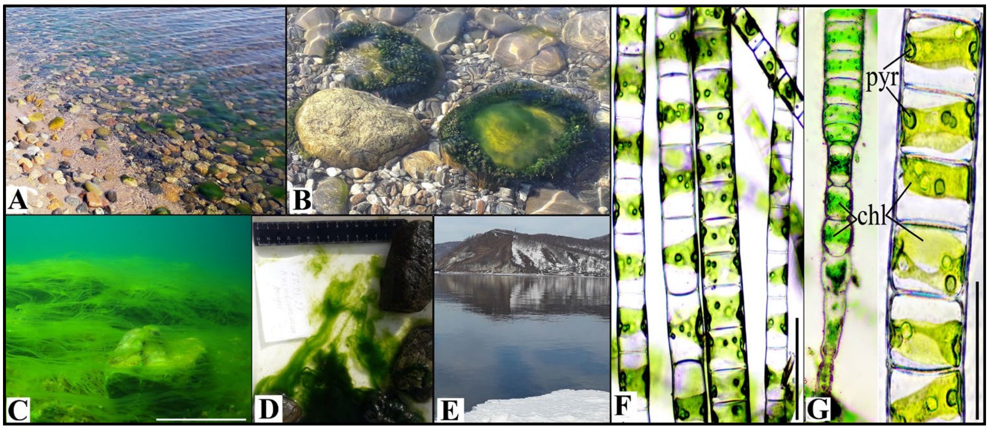

Fig.1. General information about the green filamentous algae, *Ulothrix zonata* (F. Web. & Mohr.) Kützing 1833 (girdle-shaped Ulothrix). One of the coastal areas of Lake Baikal with stones overgrown with Ulothrix in summer (A, B); *U. zonata* bloom with filament strands reaching a meter depth at a depth of 5 m (C, D) in the Angara River source in late March 2021 (E); live filaments of *U. zonata* under a light microscope (F, G); basal (left) and intercalary (right) fragments of a live filament with typical vegetative cells (G). Designations: chl – chloroplasts and pyr – pyrenoids. Scale bars: 0,5 m (C); 50 µm (F, G).

of its varieties (Lokhorst and Vroman, 1972; Shyam and Saxena, 1980; Moshkova and Gollerbakh, 1986; Izhboldina, 2007). In Lake Baikal, *U. zonata* begins to develop in April in the coastal zone on the lower surfaces of ice hummocks (Bondarenko et al., 2009). After the release of the water column from the ice, its large-scale vegetation begins along the entire coast at depths from 0 to 1.5 m, with subsequent formation of an entire vegetation belt (Meyer, 1930). At the same time, in the past decade, abundant Ulothrix blooms have begun in March at depths of 0.5 to 3–5 m near the source of the Angara River free of the ice (Fig. 1C–1E). Despite the still snowy and icy shore, *U. zonata* successfully vegetates here, and strands of its filaments reach a meter length at depths of 3 to 5 m. This is associated with an intense anthropogenic impact on the aquatic environment in the Listvyanka settlement (Kravtsova et al., 2014). Taxonomic analysis revealed only two varieties of this algae in Lake Baikal: *U. zonata* and *U. zonata* var. *zonata* (Izhboldina, 2007).

U. zonata thalli samples were collected from stones during the expedition in June 2020 at Lake Baikal in the coastal splash zone at depths of 0 to 1 m near the following sampling stations: Polovinny Cape (51°47’52.8”N 104°21’04.8”E), Kultuk (51°43’39.9”N 103°43’21.3”E) and Marituy (51°47’13.5”N 104°13’16.2”E) settlements, and the Katorzhanka River (51°47’59.7”N 104°37’40.6”E). In August 2020, samples were taken from stones near the shore from the Angara River basin: (52°14’23.4”N 104°19’27.6”E), (52°15’00.5”N 104°17’05.7”E). Thereafter, in March 2021, Ulothrix thalli were sampled in the Angara River source in the vicinity of the Listvyanka settlement from stones near the shore and from a depth of 5 m (51°50’51.5”N 104°52’17.4”E). Additionally, we studied summer samples of *U. zonata* from the Zhilishche River (July 2021) (51°54’00.7 “N 105°03’51.4 “E) flowing into Lake Baikal and the samples from the Ida River, the Angara’s tributary (53°09’54.6”N 104°12’43.1”E), which were collected in July 2017. For light microscopy, temporary cytological preparations were made from live and vitally (in vivo) unfixed filaments stained with acetoorcein (La Cour, 1941; Pausheva, 1988). For vital staining, live samples were immediately placed and stored in Eppendorf tubes with dye. Temporary cytological preparations were also prepared from Ulothrix thalli fixed in a mixture of ethyl alcohol and acetic acid (3:1) and stained with hematoxylin (Wittmann, 1965; Pausheva, 1988). Live filaments were distributed using tweezers or needles under a stereomicroscope on a glass slide in a drop of water, covered with a cover slip and examined under an Olympus CX23 LED microscope with a ToupCam 9.0 MP series digital camera. Thalli samples stained with acetoorcein and hematoxylin were rinsed in 45% acetic acid, dried on filter paper and distributed in a drop of lactic acid (80%) on a glass slide under a stereomicroscope (La Cour, 1941; Wittmann, 1965; Pausheva, 1988). Then they were covered with a cover slip and were also examined under a microscope coupled with photodocumentation. The species identification of the specimens was carried out according to N.A. Moshkova and M.M. Gollerbakh (1986) and L.A. Izhboldina (2007).

3. Results and discussion

Based on morphological characteristics, all algae samples collected were determined as the species *U. zonata* (girdle-shaped Ulothrix). According to the taxonomic description (Lokhorst and Vroman, 1972; Moshkova and Gollerbakh, 1986; Izhboldina, 2007), these algae have the shape of long (from 5 cm to 5 dm), strait or variously curved green or yellow-green (unbranched) filaments consisting of one series of cells tightly attached to each other (Fig. 1F, 1G). Typical vegetative cells of this species are characterised by cylindrical or barrel shape and chloroplasts in the form of median ring (girdle) with several large pyrenoids (Fig. 1G). During the transition of cells to reproductive functions (sporogenesis, gametogenesis or formation of hypnospores), their shape does not change, i.e. sporangia, gametangia and cells with hypnospores have the same cylindric shape (Lokhorst and Vroman, 1972). Cell width determines the width of filamentous Ulothrix thalli, varying from 11 to 83 µm. The length of cells varies depending on the width of the cells (Fig. 1F, 1G): it can be six times longer, equal to or three times shorter than the width, while usually in the juvenile filaments of the species of the genus *Ulothrix*, the length of cells significantly exceeds the width, i.e. they look narrow and long (Moshkova and Gollerbakh, 1986; Izhboldina, 2007). Throughout their length, filaments often consist of cells with the same width (Fig. 1F, 1G), but there are also thalli with sites containing a series of cells having a shorter or longer width than the entire filament (Fig. 1G, basal fragment).

In all studied samples of *U. zonata* filaments, we revealed various deviations from the typical cilindrical shape in vegetative and reproductive cells with zoo- and hypnospores, which indicated the ‘wound healing’ process previously described in marine red filamentous algae (Lewis, 1909; Waaland and Cleland, 1974; Kim et al., 1988; Kim and Fritz, 1993).

3.1. Directional (polarized) cell growth in filaments, a characteristic sign of the onset of the ‘wound healing’ process in the *U. zonata* green algae

Directional (polarized) growth of typical vegetative cells is a characteristic morphological sign of the onset of the ‘wound healing’ process in the filaments of *U. zonata*, as evidenced by dome-shaped (Fig. 2A–2G) and conical (Fig. 2F, 2H) protrusions and elongations of their transverse walls. At the same time, frequent episodes of protrusions of both transverse walls of one cell indicate that polarized growth can occur in two directions and change her shape to spherical (Fig. 2D). It should be noted that such protrusions of cell wall always closely adjacent to dead cells (without protoplast) and defective cells (Fig. 2A–2H, marked with asterisks) and, moreover, are always directed inside their space. Anatomically altered cells with chloroplasts that were deformed or changed colour due to physiological or mechanical damage are called here as defective. The number of dead or defective cells can be one-two (Fig. 2A, 2B, 2D, 2E, 2G, 2H) or more (Fig. 2C, 2F,), i.e. they can occupy rather long sites in the filaments and are always clearly visible between dome-shaped and conical protrusions and elongations of cell walls. Also, sporangia (the reproductive cells) carry out directional growth into adjacent cells released from zoospores (Fig. 2E). Notably, protrusions of the walls only in one cell adjacent to defective filament sites are rather rare. As a rule, such deformations of transverse walls occur in both cells, between which one or several defective or dead adjacent cells appear. For example, Figure 2F shows a fragment of a filament, in which one of the vegetative cells (on the left) formed a clearly visible dome-shaped protrusion of the transverse wall directional to a series of several defective cells. On the opposite side of this series (on the right), another vegetative cell transformed into a rather extended cone that was also directional to the same series. Thus, these cells grow directionally towards each other.

Noteworthy is that polarized growth in *U. zonata* does not prevent mitosis and does not change the behaviour of the nuclei in the growing cells. Nuclei retain their location in the centre of the cell and their role in cell division. For instance, Figure 2G shows that a cleavage furrow (cf) and two juvenile nuclei (N) occurred in the centre of one of the cells (on the left). In another cell (on the right), cleavage furrow (cf) began to form, and already two sets of genetic material were visible in the nucleus (N). In other words, these cells performed division and directional growth simultaneously. At the same time, patterns of deformation and rupture of filaments at the most vulnerable sites with defective or dead cells testify to the possible interruption of the ‘wound healing’ process and another likely outcome such as filament rupture (Fig. 2I, 2J). The size and fate of the fragments can be different because they both would become independent plants. Attached thallus fragment would continue its activity. The free fragment of the filament would be carried away from the original filament, and, only after attaching to the substrate, it would be able to grow further. In this case, it would serve to spread the species

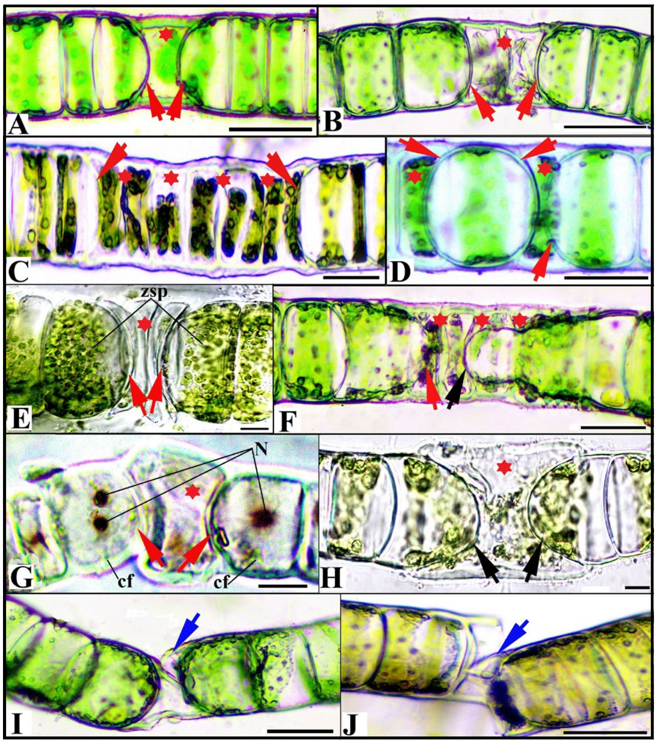

Fig.2. Typical signs of the onset of the ‘wound healing’ process and its interruption in filaments of the *U. zonata* green algae. Protrusions and elongations of the cell walls are marked with arrows: red – dome-shaped (A–G) and black – conical (F, H). The blue arrows indicate cases of interruption of the ‘wound healing’ process: deformation (I) and rupture (J) of filaments at vulnerable sites with dead cells. Asterisks mark defective (anatomically altered cells with chloroplasts that were deformed or changed colour due to physiological or mechanical damages) and dead (without protoplast) cells. (E) – filament fragment with sporangia containing zoospores. Designations: zsp – zoospores; cf – cleavage furrow and N – cell nuclei. Photomicrographs of live filament specimens (A–F and H–J) and photomicrograph of a filament specimen fixed in ethyl alcohol and acetic acid (3:1) and stained with hematoxylin (G). Scale bars: 50 μm (A–D, F, I, J) and 10 μm (E, G, H).

because thalli fragmentation is one of the methods of vegetative propagation in all algal divisions, especially in filamentous algae (South and Whittick, 1990).

3.2. Convergence and adjacency of previously unconnected cells during 'wound healing' in filaments of *U. zonata*

Analysis of images of directional cell growth, namely dome-shaped and conical protrusions of their transverse walls, revealed that such modifications are dynamic. Progressive lengthening of the cell wall bulges led to convergence (Fig. 3A–3C) and adjacency of previously unconnected vegetative cells (Fig. 3D–3J). By the moment of adjacency, transverse cells could either retain the correct form or deform. In the first case, both cells developed equally convex transverse walls that were evenly adjacent to each other in the centre of a dead or defective cell (Fig. 3D, 3E). In the second case, cells formed different curvatures in walls free of interconnection so that their attachment to each other looked uneven (Fig. 3F–3H) and even as avoidance of contact (Fig. 3I, 3J). Obviously, with uneven attachment during directional growth, such cells experienced resistance from defective cells, in which vital processes were still preserved. This could cause curvature of cell walls and change in the direction of cell growth. The presented images also indicate that during the convergence and attachment of cells to each other, their nuclei retained their central position and continued to perform their functions in the cell cycle (Fig. 3C, 3J). For example, Fig. 3C shows that the nucleus occupies the central area of the cell with a pronounced conical elongation (on the right), and doubled genetic material is also visible in it, indicating nuclear division. There is also an emerging cell cleavage furrow (cf).

Notably, intercellular convergence and adjacency during 'wound healing' occurred within defective and dead cells that were still visible (Fig. 3A–3J, red asterisks). In other words, directional growth, convergence and adjacency of previously unconnected cells took place in a space protected by cell walls. This suggests that intercellular recognition and adhesion occured under these conditions, which was an important result at this stage (Jarvis et al., 2003).

3.3. Completion of the 'wound healing' process in filaments of *U. zonata*

Comparison of shapes of vegetative cells and configurations of their intercellular walls in filaments of the studied *U. zonata* specimens revealed that completion of the 'wound healing' process (i.e. the restoration of the filament integrity) in this green algae occurred according to the following different cytological scenarios: i) formation of junctions between cells directionally growing into the same defective section of the filament, which were not previously interconnected; ii) fusion of cells directionally growing into the same defective area of the filament, which were not previously interconnected; iii) restoration of viability and preservation of individuality of the defective cells during the directional growth of adjacent cells into their space. The first two scenarios led to the same result: the complete replacement of defective and dead cells by adjacent cells. The third scenario is an occasional event that leads to the development of a lateral filament from repaired defective cells.

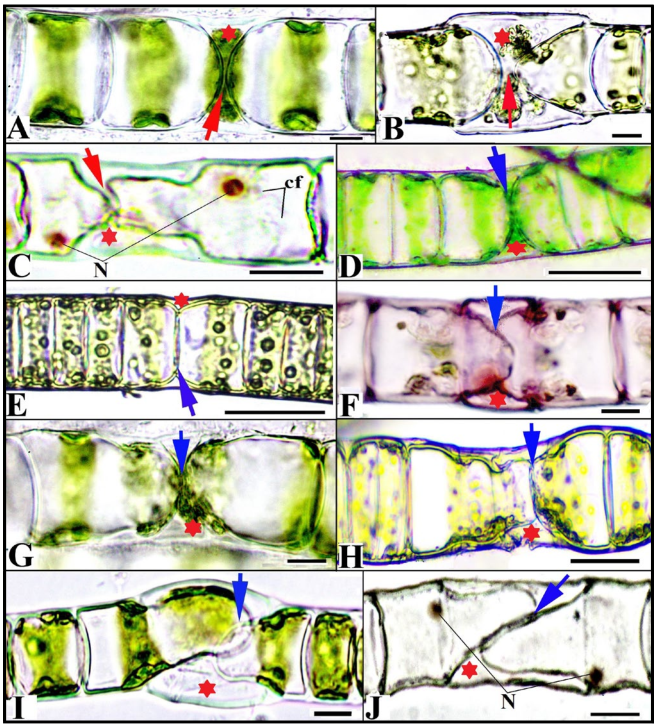

Fig.3. Cell convergence and adjacency during the 'wound healing' process in *U. zonata* filaments. Convergence of transverse walls of vegetative cells inside defective and dead adjacent cells through dome-shaped and conical protrusions and elongations (A–C). Attachment of previously unconnected vegetative cells to each other (D–J). The red arrows indicate narrow spaces between cells growing towards each other; blue arrows indicate areas of mutual cell contacts. Asterisks mark defective (anatomically altered cells with chloroplasts that were deformed or changed colour due to physiological or mechanical damages) and dead (without protoplast) cells. Designations: N – cell nuclei and cf – cleavage furrow. Photomicrographs of live specimens (A, B, D, E, and G–I); photomicrograph of a specimen with in vivo staining with acetoorcein (F); photomicrographs of specimens fixed in ethyl alcohol and acetic acid (3:1) and stained with hematoxylin (C and J). Scale bars: 10 μm (A, B, F, G, I, and J) and 50 μm (C–E and H).

3.3.1 Formation of junctions in previously unconnected cells

Based on the curved intercellular septa, trapezoid and some other non-cylindrcal shapes of vegetative cells in the *U. zonata* filaments among the studied samples, the convergence and adjacency of previously unconnected cells during directed growth to each other might result in the formation of an intercellular junction (Fig. 4). At the same time, in the case of even adjacencies of the adjoining cell walls, typical cellular septa were formed (Fig. 4A, 4B). They looked parallel to other transverse septa in filaments, and the connected cells themselves might not be deformed and be almost indistinguishable from the adjacent ones. With uneven wall adjacencies, the conncted cells did not have a typical cylindrical shape and parallel intercellular septa (Fig. 4C–4G). In some cases, parallel septa of reduced diameter were formed between cells in the form of closed truncated cones (Fig. 4C). Moreover, sometimes there were connected cells of a very bizarre shape with significantly curved intercellular septa (Fig. 4E–4G). Such patterns arose in filaments apparently due to incompletely suppressed vital processes in defective cells, in which adjacent cells carried out directional growth. In these cases, there also might be the competition of cells for vital resources, which takes place in the recovery processes (Kravez, 2008). Light microscopy analysis of the samples revealed that the sheaths of dead cells could sometimes be preserved (Fig. 4H) and subjected to bilateral compression of adjacent cells growing within them. They could remain between cells in compressed form and contribute to thickening of the intercellular septa. Light microscopy did not allow us to identify and determine the type of emerging intercellular junctions. Nevertheless, it is acknowledged that a common type of intercellular junction in Ulothrix algae is plasmodesmata similar to those in higher plants (Floyd et al., 1971; Ehlers and Kollmann, 2001; Cells, 2007). They are thin tubular cytoplasmic channels that pass through the cell wall and connect two adjacent cells. The number and structure of plasmadesmata can vary depending on the cell and change in individual cells. This type of intercellular junction is a functional analogue of gap junctions in animals (Cells, 2007). Primary plasmadesmata are formed during cell division at the cytokinesis stage. In cells that are not products of a single division, there is also a mechanism for the formation of secondary plasmadesmata by partial destruction of the cell wall (Ehlers and Kollmann, 2001).

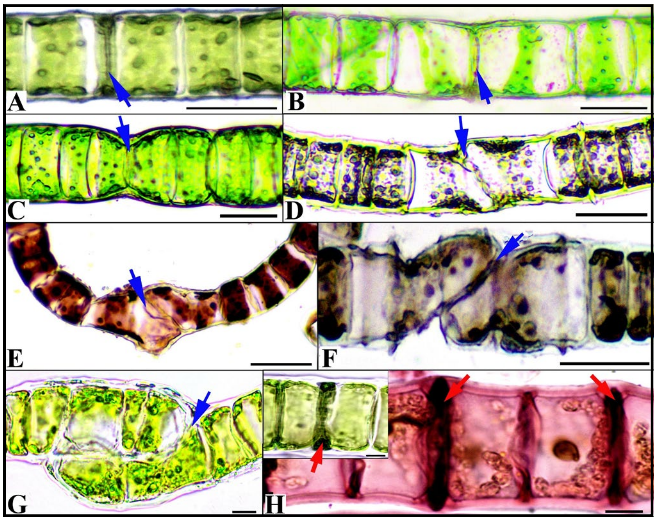

Fig.4. Completion of the ‘wound healing’ process in *U. zonata* filaments by formation of intercellular junctions. The blue arrows indicate intercellular septa formed with regular and even adjacencies (A, B) and irregular and uneven adjacencies of the adjoining walls (C–G). In the first case, intercellular walls are not deformed and are parallel to others; the shape of the cells almost does not differ from the typical one. In the second case, the formed intercellular septa have reduced diameter (C) or are deformed and not parallel to other septa (D–G), while the shape of the cells is significantly altered. The red arrows (H) mark the thickening of the intercellular septa due to compressed sheaths of dead cells (the insert shows live cells between which there is a compressed sheath of a dead cell). Photomicrographs of live specimens (A–D, G, and H (insert)) and photomicrographs of specimens stained in vivo with acetoorcein (E, F and H). Scale bars: 50 μm (A–F) and 10 μm (G, H).

3.3.2. Cell fusion and formation of giant vegetative cells

In addition to the cells of atypical noncylindrical shape in the studied samples, there were vegetative cells that were two to four times larger than the size of typical cells. These giants are evidence of cell fusion during ‘wound healing’ in *U. zonata*. Perhaps, in some cases of adjacent cells in the state of growth by elongation and with loosened cell walls, conditions arise, which promote the restructuring and fusion of their sheaths followed by the fusion of their protoplasts and nuclei. The fusion mechanism of plant vegetative cells with solid cell walls has not yet been fully elucidated. The possible expansion of intercellular channels (plasmodesmata) in the zones of contact cells and the effect of viruses or animal larvae on the dissolution of the solid cell wall were hypothesized (Maruyama et al., 2016). Thus, during directional cellular growth towards each other, interacting cells not only form intercellular junction, but also fuse into one giant vegetative cell, thereby ‘healing the wounds’ by restoring morphological integrity of the filament (Fig. 5A, 5B). During subsequent divisions, this cell gives rise to the same generation, leading to the appearance of extended sites with a sharp two- to four-fold increase in the filament diameter (Fig. 5C, 5D). This is reflected in one of the species traits of *U. zonata*: “…filamets of the same thickness throughout the length or randomly thickening or thinning…” (Moshkova and Gollerbakh, 1986). The cell fusion process is probably especially relevant for the onset of algal filament growth, which begins with a rather narrow basal cell with a lower level of ploidy than in other cells (Fig. 2C, basal filament fragment on the left). Furthermore, the localization and size of cell nuclei in such giant cells in cytological preparations from the *U. zonata* filaments fixed in ethyl alcohol and acetic acid fixative and stained with hematoxylin indicated karyogamy (cell fusion). In these giant cells, patterns of convergence (Fig. 5E, 5F) and fusion (Fig. 5G) of two nuclei from former typical cells, as well as patterns of significant differences of their nuclei from the nuclei of adjacent cells, were clearly visible. For example, Fig. 5H shows that the nucleus (1) of the giant cell is two to four times greater than the size of adjacent dividing (2) and juvenile nuclei (3), respectively. Obviously, the nuclei of giant cells contain an increased amount of genetic material, i.e. they are polyploid. Notably, the proximity of two nuclei is typical with the completion of division of typical vegetative cells, but in these cases, cell cleavage furrows can always be visible between the two nuclei (Fig. 2G; 5H – cf), which was not detected

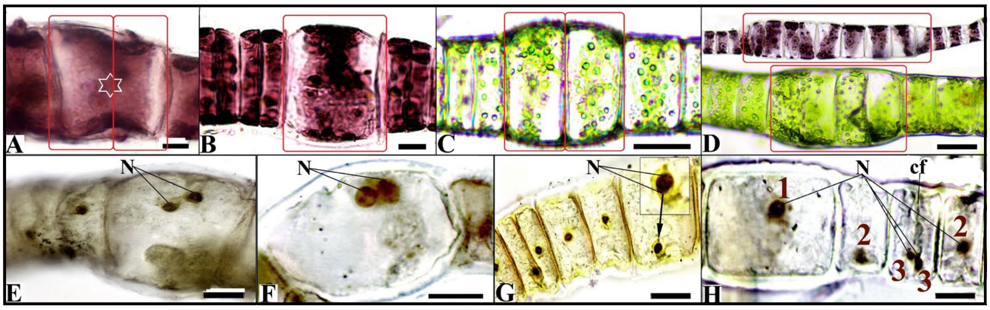

Fig.5. Completion of the 'wound healing' process in the *U. zonata* filaments by cell fusion and the formation of giant (polyploid) vegetative cells. The red rectangular contours indicate two vegetative cells in the fusion phase (A) (an asterisk marks the area of dissolved intercellular walls); the giant vegetative cell resulted from the fusion of two typical cells (B); two giant vegetative cells formed after division of one giant cell (C); thickened sections of the filaments formed by the division of giant vegetative cells (D); convergence of nuclei in giant cells (E, F); karyogamy (fusion of nuclei) in the giant cell (G) (the insert shows enlarged area indicated by the arrow); nuclei marked with the following numbers (H): (1) in the giant cell; (2) in dividing cells and (3) in juvenile typical vegetative cells. It is obvious that the nucleus of the giant cell (1) is two times larger than the nuclei of dividing cells (2) and four times larger than the nuclei of juvenile cells (3). Designations: N – cell nuclei and cf – cleavage furrow. Photomicrographs of specimens with in vivo staining with acetoorcein (A, B and D (upper filament fragment)), photomicrographs of live specimens (C, D (lower filament fragment)) and photomicrographs of specimens fixed in ethyl alcohol and acetic acid fixative (3:1) and stained with hematoxylin (E–H). Scale bars: 10 μ (A, B and E–H) and 50 μ (C, D).

during the convergence of nuclei in giant cells. Owing to their sizes (up to 100 μm in length and width) that are two to four times larger than the width or/and the length of typical filament cells, such vegetative cells were clearly distinguished in filaments even at the smallest magnification of a light microscope (x40).

3.3.3. Restoration of viability in defective cells during 'wound healing'

Microscopy of *U. zonata* filament samples indicated that sometimes defective cells themselves resotored their viability during "wound healing" (Fig. 6). Under the influence of directional growth of adjacent cells, they underwent significant deformation (Fig. 6A–6C). The presented figures indicate self-restoration of defective cells even in a significantly deformed cellular shape (Fig. 6B, 6C). The increased turgor pressure exerted on them by polarized growth of adjacent cells may also change the orientation of the preprophase bands of microtubules and actine filaments of the cortex, i.e. cytoplasmic structures that determine the direction of the cell division plane in vegetative cells (Brown and Lemmon, 2001; Cells, 2007). This is evidenced by sporadically occurring patterns of lateral filament development from deformed cells, the division of which was directed perpendicular to the main filament (Fig. 6B, 6D). Nevertheless, the images presented show that the directional growth of adjacent cells ends according to one of the above scenarios: the formation of intercellular junctions and typical transverse septa (Fig. 6C, 6D). Perhaps, there also may be cell fusion.

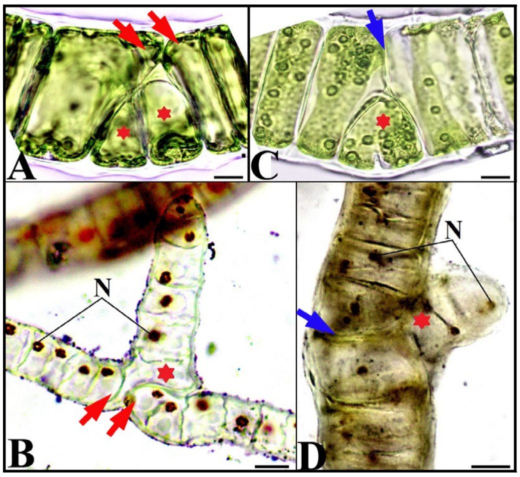

Fig.6. Restoration of viability in defective cells during the 'wound healing' process in *U. zonata*. Defective cells are marked with red asterisks. The red arrows indicate cells with directional growth (A, B) into adjacent defective cells. The blue arrows indicate transverse intercellular septa (C, D) formed during the directional growth of cells into defective cells. Sites of the thalli with the onset of the growth of new filaments from the defective cells, the mitotic division of which occurs in a plane perpendicular to the direction of the filament (B, D). Designations: N – cell nuclei. Photomicrographs of live specimens (A, C) and specimens fixed in ethyl alcohol and acetic acid (3:1) and stained with hematoxylin (B, D). Scale bars: 10 μm.

3.4. 'Wound healing' promotes the development of giant hypnospores in *U. zonata*

In addition to giant vegetative cells, there were huge cells with gigantic hypnospores (up to 100 μm in diameter) in the *U. zonata* specimens. Hypnospores are structures for asexual reproduction (Starmach, 1972; Bullock, 1978; Vasser et al., 1989). According to available descriptions, from 1 to 4 large and up to 32 small hypnospores can develop in the normally vegetating cell of *U. zonata*, which completely fill its volume. In other words, a single hypospore can grow to the size of a typical filament cell. Their characteristic features are a thick dense sheath and the lack of flagella, and during the development, the cytoplasm of the parent cell is not used entirely, and its sheath does not participate in the formation of their integuments. Hypnospores can remain inside the parent cell for a long time and germinate into numerous filaments there. Our analysis revealed that sometimes typical vegetative cells carried out directional growth into cells with developing hypnospores (Fig. 7A). Perhaps, the transition of cells to this form of reproductive function significantly changes their typical vegetative state, which is a stimulus for adjacent cells to directed growth. Their growth into the same cell with developing hypnospore may also result in the fusion of all the three into one vast cell section (Fig. 7B, 7C). Thus, the conditions for the further development of the hypnospore are created, leading to the formation of its enormous size, 4–6 times the size of typical cells (Fig. 7D, 7E). The patterns that we identified also indicated that developing hypnospore can be displaced by two vegetative cells beyond the algal filament but remain connected to it and continue its growth in the already free space, where it can also reach gigantic sizes (Fig. 7F–7I). Hypnospore-displacing vegetative cells can fusion to form an enlarged polypoid vegetative cell (Fig. 7H) or build an intercellular junction (Fig. 7I). The presented photos clearly show the honeycomb structure of hypnospores, testifying to the development of numerous microspores in them, each of which can give rise to an individual algal filament (Bullock, 1978). Therefore, the 'wound healing' process in the *U. zonata* filamentous algae not only leads to the restoration of the filament integrity but also contributes to the reproductive function of the species.

3.5. Scheme of the 'wound healing' process in *U. zonata* and comparison of the results with data from the literature

The above patterns of the 'wound healing' process in green filamentous algae, *U. zonata*, are shown schematically in Figure 8. Based on the morphological changes in the cells, the entire process can be divided into the following three stages: (I) the emergence of directional (polarized) cell growth, the sign of which are dome-like and conical protrusions of transverse walls in cells adjacent to the defective and dead cells (marked in red); (II) convergence and adjacency of previously unconnected cells that carry out directional growth; (III) completion of the 'wound healing' process, i.e. restoration of the filament integrity through the formation of intercellular junction or cell fusion. The scheme demonstrates that, at the initial stage, a rupture may occur at the site of the algal filament with defective or dead cells, dividing this filament into two fragments, one of which would remain attached, and the other would be free (dashed arrow). Moreover, the scheme reflects that, in rare cases at the first stage, defective cells can restore their vital functions themselves and, hence, carry out the 'wound healing' process with subsequent mitotic division and formation of a lateral filament (dashed arrow). At the last third stage of the 'wound healing' process in *U. zonata*, two scenarios are employed through directional growth, and in both cases, the lesions are eliminated by filling the spaces of defective or dead cells with live cells. Furthermore,

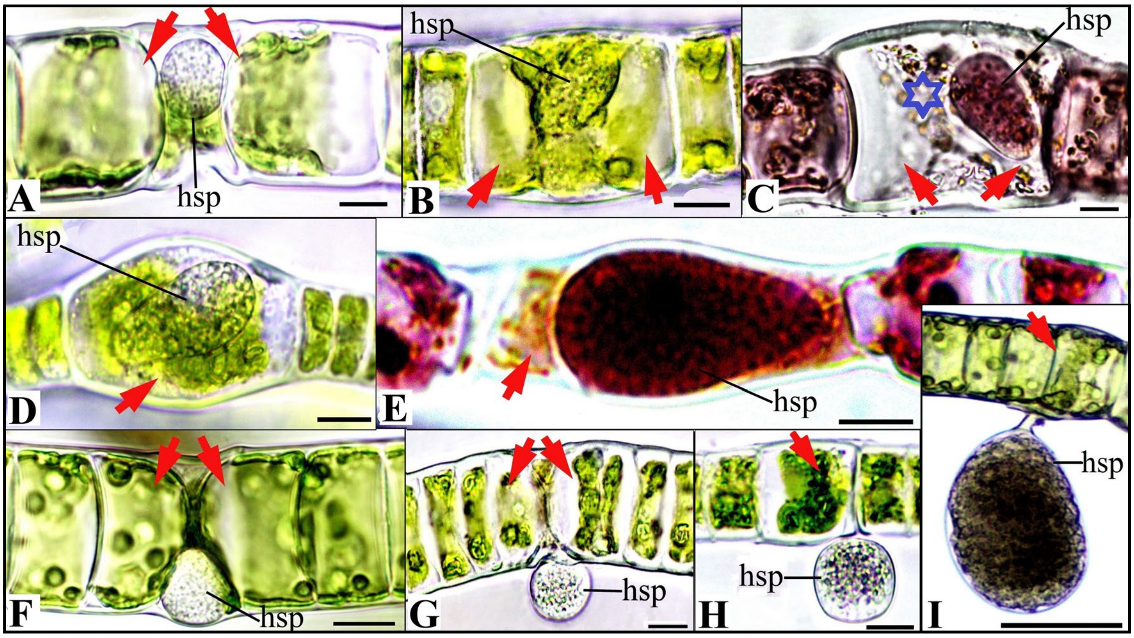

Fig.7. Development of giant hypnospores caused by the 'wound healing' process in *U. zonata*. The red arrows indicate (A) two vegetative cells that carry out directional growth to each other into the adjacent cell with a developing hypnospore (B, C) two vegetative cells in different phases of fusion during the directional to each other into the space of the adjacent cell with a developing hypnospore (the asterisk marks the area of dissolution of cell walls (C)), (D) giant cell with growing hypnospore (after completion of the fusion of two vegetative cells), (E) giant cell, inside which a giant hypnospore has developed, (F, G) vegetative cells displacing the hypnospore during their directional growth, (H) large vegetative cell that has formed after the fusion of typical vegetative cells during their directional growth and displacement of the growing hypnospore beyond the filament, and (I) intercellular septa formed by typical vegetative cells during their directional growth and displacement of the growing hypnospore beyond the filament. Designations: hsp – hypnospore. Photomicrographs of live specimens (A, B, D, and F–I) and photomicrographs of specimens with in vivo staining with acetoorcein (C, E). Scale bars: 10 μ (A–D and F–H) and 50 μ (E, I).

cell fusion leads to the emergence of a giant polyploid vegetative cell, the mitotic division of which contributes to an increase in the diameter of the filament. The scheme shows the fate of the cell and the hypnospore developing in it, which were under the pressure of the directional growth of adjacent cells. Transformations in such a cell are similar to those that occur in typical defective or dead cells during the 'wound healing' process. There are two pathways of development of the hypnospore: either inside the fused cells or beyond the filament after being displaced by cells that grow towards each other. Both cases lead to its development to a gigantic size (up to 100 μm in diameter).

The intensive development of *U. zonata* in flowing waters and surf zones of water bodies suggests that the 'wound healing' process preventing rupture of its filaments occurs rather quickly. Perhaps, its duration is the same as in the red algae, *Griffithsia pacifica* Kylin 1925, the experiments on which revealed that the repair of the filament at the site of the damaged cell occured within 24 to 30 hours (Waaland and Cleland, 1974).

The presented cytomorphological description of the 'wound healing' process in the *U. zonata* green filamentous algae had some common features with those in red algae (Lewis, 1909; Waaland and Cleland, 1974; Kim et al., 1988; Kim and Fritz, 1993). In the latter, three fundamentally different stages of this process were distinguished (Kim and Fritz, 1993). At the same time, the study of 16 species of red algae revealed three types of 'wound healing' scenarios: the fusion type detected in 13 species, the non-fusion type identified only in one species and the elongation type found in two species (Kim et al., 1988). In the first two scenarios, new (repairing) cells in the algal filaments grew towards each other from the transverse walls of the cells adjacent to the damage into the lumen of the dead cell. After adjoining each other, they mostly fusioned into a single typical shoot cell, and, in rarer cases, they formed intercellular junctions, retaining their individuality. Thus, in the vast majority of the studied species, dead cell in the filament was replaced by one cell, and only in one species – by several cells. Similar phenomena but without the formation of 'repairing' cells and involving adjacent cells themselves to the damage site also occurred in the *U. zonata* filaments, which the description, figures (Figs. 4, 5) and the scheme (Fig. 8, stage III) above reflect. In the elongation type scenario, the cells adjacent to the wound elongated, growing into the lumen of the dead cell. When they adjoined, they formed a 'pit connection' (Hawkins, 1972; Kim et al., 1988). Based on our description of the 'wound healing' process in *U. zonata*, this scenario takes place in its filaments (Figs. 3, 4, 8 (stage III)). Another cytological similarity of the 'wound healing' process between *U. zonata* and marine filamentous red algae is that during the fusion of mononuclear cells in the red algae, *Antithamnion nipponicum* Yamada et Inagaki, in the process of 'wound healing', nuclei also fusioned with an increase in their ploidity level, corresponding to an increase in cell volume (Kim et al., 1995). In other words, our hypothesis about the polyploid status of giant cells in *U. zonata* filaments formed during the fusion of two cells in the process of 'wound healing' can be considered correct because it is consistent with previous data on other filamentous algae. The fusion of somatic cells as one of the polyploidy mechanisms was established already in the second half of the past century in both plants and animals (Brodsky and Uryvaeva, 1981; Anatskaya and Vinogradov, 2022). Polyploid plants have larger sizes of the body itself and individual organs (e.g. fruits and seeds) as well as greater resistance to adverse effects. Therefore, the formation of giant polyploidy cells in the *U. zonata* filaments not only ensures the repair of thalli but also contributes to an increase in the thickness of the filaments, thereby enhancing the viability of this species.

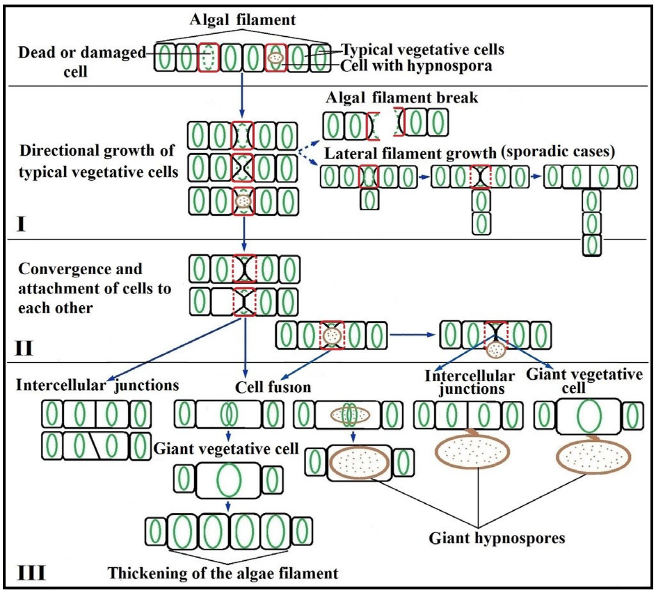

Fig.8. Three stages of the 'wound healing' process in the green filamentous algae, *U. zonata*. At stage I, signs of directional (polarized) growth appear, namely, dome-like and conical protrusions of the transverse walls in cells associated with dead and defective cells as well as with cells containing hypnospores (marked in red). During this stage, a defective cell may be restored with the development of a lateral filament from it, and also the filament may fragment at sites with dead and defective cells (dashed arrows). At stage II, the cells that carry out oppositely directional growth into the space of dead, defective and hypnospore-containing cells converge and attach to each other. Stage III is the completion of the 'wound healing' process through the formation of intercellular junctions, or through intercellular fusions. The latter leads to the emergence of giant (polyploid) vegetative cells, whose further mitotic divisions cause thickening of the original filament. The development of hypnospores during this process occurs both inside the giant cells and outside the filament. In both cases, they can grow to gigantic sizes (up to 100 μm in diameter). Green ovals are chloroplasts.

The nuclear dynamic movement to proper positioning at the correct functional location is essential for cellular functions in animal, fungal, and plant cells (Gundersen and Worman, 2013; Klimenkov et al., 2018; Wada, 2018). In particular, nuclear migration is required in apical, directionally growing cells to maintain a constant distance between the cell tip and the nucleus, probably to maintain an efficient supply of mRNAs necessary for tip growth and the formation of new chloroplasts and other organelles (Baluska et al., 2003). At the first stage of ‘wound healing’ in filamentous red algae, the nuclei migrated to the transverse cell walls adjacent to the dead cell (Kim et al., 1995). Their mitotic division occurred there, after which, from the outer side of these walls, new ‘repairing’ cells replacing it began to grow into the inside of the dead cell. In *U. zonata*, we did not identify the migration of nuclei into the polarized sites of cells either during their directional growth (Fig. 2G) or during their convergence and close interaction (Fig. 3C, 3J). The convergence and fusion of nuclei in *U. zonata* occurred only after the fusion of protoplasts of two cells that converged and came into interaction (Fig. 5E–5G). Thus, the fixed position of nuclei in directionally growing cells, which we identified, indicates that they are probably somehow fixed in the protoplast. Perhaps, like in green filamentous algae of the genus *Spirogira* Link., 1820 (Fowke and Pickett-Heaps, 1969), thin cytoplasmic strands hold *U. zonata* cell nuclei in the cells centre. The German researchers H. Sawitzky and F. Grolig (1995), called these strands visible under a light microscope the nucleus positioning scaffold or NPS. In the central region of *U. zonata* cells where the nucleus is located, the chloroplast also is located in the form of a girdle. This may be the reason why such strands are difficult to detect.

Other researchers also revealed during cultivation the cases of branching in the species of the genus *Ulothrix*, including *U. zonata*, which are not typical of the common filamentous algae (Lokhorst and Vroman, 1972; 1974a; 1974b; Floyd et al., 1972; Lokhorst, 1985). The list of taxonomic features of *U. zonata* may also include the following property: «In culture, this alga show branching» (Lokhorst, 1985). Notably, all these researchers only stated this fact without providing detailed descriptions. Our study demonstrates, firstly, that branching also occasionally occurs in natural specimens and, secondly, one of the likely cytological mechanisms of the lateral filament development during ‘wound healing’.

In the publication of English algologists (Jane and Woodheat, 1941), we also saw the patterns of directional growth of cells in *U. zonata* and another species of filamentous algae, *Hormidium* ref. *flaccidum* (Küntz) (this name is currently regarded a synonym of Klebsormidium flaccidum (Kützing) P.C.Silva, K.R.Mattox & W.H.Blackwell), which testified to the ‘wound healing’ process. However, this publication states the formation of H-shaped structures or thickenings in the walls of vegetative cells that, as noted by the authors, were previously described in the species of the genus *Microspora* as well as in other Ulothrix species due to the development of aplano- and macrozoospores (Fig. 9A, 9B marked in yellow). According to the opinion of F.W. Jane and N. Woodheat (1941), such thickenings appeared in algal filaments from exposure to cold because the samples were collected in the winter. Histochemical tests conducted in their study revealed that the H-shaped thickenings were the products of the outer cell walls. We believe that these H-shaped structures in algal filaments are rather sporangia without spores or former defective and dead cells that were found in winter samples of the *U. zonata* and *H. flaccidum* filaments than thickened outer cells. Figures from that publication clearly show the characteristic protrusions of the transverse walls of adjacent cells into these H-shaped structures that, in fact, are former deformed cells (Fig. 9A, 9B, red arrows). In other words, reproductive transformation or the death of cells in the filaments caused directional growth of their adjacent cells with the corresponding morphological changes. In Figures 2 and 3, the *Ulothrix* filaments are located horizontally. However, if they are turned vertically, it would be seen that the cells, into which the directional growth of adjacent cells is carried out, are deformed and H-shaped. H-shape results from the compression of defective and dead cells on the two sides by the growing adjacent cells. The authors of the indicated publication also stated that at the sites of H-shaped thickenings of the species *H. flaccidum* the filaments often ruptured, while in *U. zonata*, these H-shaped structures did not cause the fragmentation of the filaments. Therefore, in *U. zonata*, the sites of filaments where the ‘wound healing’ process has begun can be rather resistant to rupture. Our observation that retained sheaths of dead cells contribute to the thickening of the intercellular walls of vegetative cells (Fig. 4H) corresponds to the results in F.W. Jane and N. Woodhead (1941). Perhaps, thanks to this publication, H-shaped structures or septa (or the remains of dead cells, as we define them) were taken into account during taxonomic descriptions of the Ulothrix algae (Lokhorst and Vroman, 1972; 1974a; 1974b; Floyd et al., 1972; Lokhorst, 1985). For example, the morphological characterization of cultivated specimens of *U. zonata* indicated that «H-pieces may be present in all filamentous stages, but are more common in older cultures» (Lokhorst and Vroman, 1974b). Similar situations were observed in the cultivation of other species of the genus *Ulothrix* (Lokhorst and Vroman, 1974a). This confirms our conclusion about the formation of H-shaped septa from

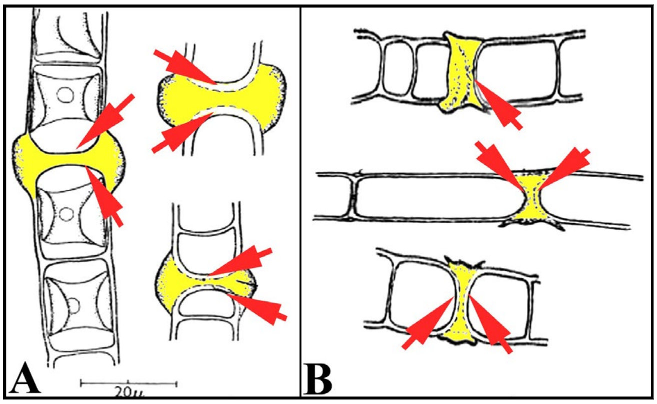

Fig.9. H-shaped structures in the walls of *U. zonata* (A) and *Hormidium* ref. *flaccidum* (Küntz) (B) (according to F.W. Jane and N. Woodheat, 1941). H-shaped structures (former defective and dead cells, according to our definition) are marked in yellow, and arrows indicate the protrusions of transverse cell walls that carry out growth towards each other.

the sheaths of dead cells: in old (depleted) cultures, cell death rate was naturally higher than in other cultures.

Image of giant vegetative cells that underwent divisions in the species of green filamentous algae, *Rhizoclonium subtile* Z. Zhao & G. Liu, sp. nov., was presented in the article on the phylogenetic analysis of the genus *Rhizoclonium* Kützing, 1843 (Cladophoraceae, Cladophorales) from China (Zhao et al., 2018). The authors called them 'swollen cells' without explaining their origin. As seen in our description, in *U. zonata*, these 'swollen cells', i.e. giant cells, resulted from cell fusion during directional growth. Moreover, in this publication, photomicrographs of this algae clearly showed dome-like protrusions of transverse cells in some cells directed into the empty spaces of filaments towards each other where dead cells were likely located. Also, cells of an atypical cylindric shape are also visible in the filaments, indicating contact and subsequent cell fusion during growth towards each other (Zhao et al., 2018). According to modern taxonomy (Algae Base, https://www.algaebase.org/), the genera *Rhizoclonium* and *Ulothrix*, belong to the same class of green algae, Ulvophyceae. This probably determines the existence of similar cytological mechanisms in *U. zonata* and *R. subtile*, in particular, the 'wound healing' process.

According to modern taxonomy (Algae Base, https://www.algaebase.org/), the above-mentioned taxa, which, in our opinion, have characteristic features of "wound healing", represent three phyla (types) and four families of filamentous algae. Two species, *U. zonata* (***Family*** Ulotrichaceae) and *Rhizoclonium subtile* (***Family*** Cladophoraceae), represent the Phylum Clorophyta, the green filamentous algae. The species *H. flaccidum* or *Klebsormidium flaccidum* (***Family*** Klebsormidiaceae) belongs to the ***Phylum*** Charophyta, charophytes. The marine red algae, *G. pacifica* **(*Family*** Wrangeliaceae), are members of the ***Phylum*** Rhodophyta, a type of red algae. Based on this information about the occurrence of repair in different taxonomic groups, as well as from our study, we conclude that the 'wound healing' process due to cell growth towards each other with their subsequent fusion or connection is a natural biological property of the species *U. zonata* and possibly is also present in natural populations of other filamentous algae. Cytological patterns that we described, as well as H-shaped intercellular septa in filaments detected previously (Jane and Woodheat, 1941), are markers of both this process and its preceding abnormalities and death of cells. Thus, the frequency of the 'wound healing' processes in the prepared filaments can also indicate the degree of damage to the algal thalli. For instance, microscopy of the studied *U. zonata* specimens revealed that they can be divided into two groups that dramatically differ in the number of cases of filament repair in one cytological preparation. The first group with a low occurrence of the 'wound healing' episodes included the samples collected in Lake Baikal opposite the Kultuk and Marituy settlements as well as near the Katorzhanka River (brook 1.5 m wide and 15 cm deep) (Fig. 10A). From 20 to 50 episodes of filament repair could be observed in one cytological preparation ($S = 7.5$ cm^2) of the *U. zonata* thalli from these sites. The second group included the *U. zonata* specimens with a high occurrence of 'wound healing', which were collected near Polovinny Cape (Lake Baikal, estuary of the Bolshaya Polovinnaya River), in the Zhilishche River, the Angara River: the Angara source, downstream of the Irkutsk Hydroelectric Power Station and one of its coves, as well as in the Ida River (the Angara tributary) (Fig. 10B–10D). In these samples, 1000 or more above-described patterns of different stages of 'wound healing' could be counted in one cytological preparation (with the same area: $S = 7.5$ cm^2). In other words, in each field of vision of the microscope at 100x magnification, there were ten or more cases of filament reparation. Furthermore, in such samples, individual filaments might also have many sites with 'wound healing' (Fig. 10C, 10D). Therefore, algal thalli had a higher degree of damage in rivers and their estuaries than in the lake.

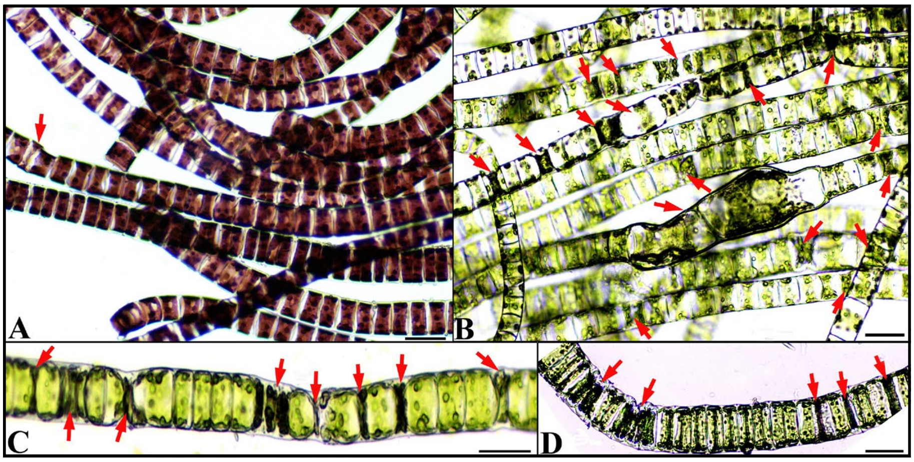

Fig.10. Low (A) and high (B) frequency of the 'wound healing' episodes (indicated by arrows) in the thalli of the filamentous algae, *U. zonata*. Samples collected in Lake Baikal opposite the Kultuk settlement (A) and in the source of the Angara River from a 5 m depth (B). Individual filaments with numerous 'wound healing' episodes (indicated by arrows) from the samples collected in the Angara (downstream of the Irkutsk Hydroelectric Power Station) (C) and Zhilishche (D) rivers. Photomicrographs of filaments stained with vivo in acetoorceine (A) and photomicrographs of live filaments (B–D). Scale bar: 50 μ.

Based on modern concepts, directional growth (polarized growth or tropism) in plants is associated with a redistribution of auxin phytohormones in cells (Blum et al., 2012; Kuluev and Safiullina, 2015). The root meristem of the known model plant, *Arabidopsis thaliana* (L) Heynh, revealed that activation of auxin production in its surrounding cells was one of the most substantial responses to damage (Hoermayer and Friml, 2019). This phytohormone plays a key role not only in the processes of growth and development of plants but also in the restoration of tissues and organs. To date, it has been also shown that phytohormones play an important role in the vital activity of algae, and green algae (Chlorophyta) are one of the taxonomic groups where their greatest number has been characterised (Tarakhovskaya et al., 2007; Kiseleva et al., 2012). In particular, the studied spectrum of auxin action in macroalgae vegetation indicated its role in growth regulation (thallus branching and rhizoid formation) and formation of reproductive structures, which corresponds to the functions of phytohormones in higher plants. In the red filamentous algae, *G. pacifica*, during the rupture of the filament in the basal cell of one of the fragments, the rhodomorphin phytohormone is released, under the influence of which the apical cell of the second fragment forms a specific 'repairing' cell that ensures cell fusion and restoration of filaments (Waaland and Watson, 1980). Other biologically active substances can also play an important role in the regulation of the 'wound healing' process in algae. The species *U. zonata* descdibed in our study is an easily accessible model object to search for these components and determine their role in biochemistry of the repair process.

4. Conclusions

For more than two hundred years of studies of the *U. zonata* green filamentous algae, we have revealed and described for the first time a cytomorphological pattern of self-healing (repair) of damaged filament sites (with defective and dead cells), which is called 'wound healing' in scientific literature, including algological literature. Moreover, for the first time in the field of algology, we have studied this process in the samples collected from natural populations. In this regard, we think that the following fact is of interest. To date, due to the lack of information on repair of macroalgae filaments in natural conditions, some researchers have concluded that such an adaptation cannot appear in them in the course of evolution. In other words, these researcher believe that only rupture and fragmentation of filaments (water immediately separates fragments from each other) occur due to cell damage in algae under natural aquatic conditions; therefore, in thei opinion, algae could not develop a 'wound healing' mechanism. For example, the 'wound healing' process that occurred in the experiment with red filamentous algae, according to one of these researchers, can be considered a model of cell intelligence (Ford, 2010; 2017). They stated that cells in an unpredictable situation were able to 'guess' how to act correctly, 'turning on' a genetically unprogrammed mechanism. Brian J. Ford (2017, caption to Fig. 11) wrote about this: "Within thirty hours, the damaged cell has been restored in its entirety and is functioning as before. Since this is an unprecedented eventuality for which the cells invoke remedial action, it has clear connotations of intelligent behavior." Our and other studies have revealed that restoration of thalli in algae through directional cell growth is a rather widespread phenomenon in their natural population, which is very important: to ensure the preservation of vital activity of individuals (i.e. filaments), which works not only in artificial but also in natural conditions. In this regard, 'wound healing' is an evolutionary developed cytological mechanism encoded in cell genomes. Furthermore, as our study indicates, directional cell growth is carried out not only into defective but also into live cells with developing hypnospores, reproductive structures, in which thousands of spores mature, giving rise to new filaments. Sometimes, in the course of such growth, hypnospores are even displaced beyond the filaments where their development can be complicated or stopped. In these cases, deirectionally growing cells suppresses the reproduction process, that is unreasonable in terms of species development. However, in the course of evolution of the species *U. zonata*, the 'wound healing' process with its pressure of directional cell growth, on the contrary, has developed to contribute to the formation of giant hypnospores. This adaptation increases the reproductive abilities of *U. zonata* and contributes to its successful dispersal throughout the globe.

The natural phenomenon of somatic cell fusion in the representative of the lower plants, the *U. zonata* filamentous algae, revealed in our study can serve as a convenient model for studying its mechanism because the information about it is limited.

The various revealed deformations of typical vegetative cells in the studied samples indicate that 'wound healing' in these filamentous algae is the process of a successive alteration of the characteristic morphological transformations of the cell walls, based on which we can determine the stages of filament restoration. A comparison of our data with the results of other studies has revealed that the cytological patterns of filament repair in *U. zonata* have some similarities and differences with those in marine red filamentous macroalgae. Moreover, substances accompanying the 'wound healing' process in marine algae appeared to be attractive for medical purposes (Kuznetsova et al., 2020). Therefore, we believe that further study of this significant self-healing ability in this typical and widespread representative of aquatic vegetation may also be promising.

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Conflict of interests

The authors declare no conflict of interests.

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