

Prolonged Effects of He-Ne Laser Irradiation on Ultrastructure of Mitochondria in Successive Generations of Yeast Cells

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The data on the aftereffect of He-Ne laser light ($\lambda = 632.8$ nm) on mitochondria of yeast reached to late log phase of growth are reviewed. The quantitative analysis of the ultrathin cell sections demonstrated a variable thickness of the giant branched mitochondrion in cells, precursors of which were sham-irradiated or irradiated at 460 J/m^2 . Exposure to a dose of 460 J/m^2 (accelerating cell proliferation and activating cytochrome c oxidase and NADH dehydrogenase), results in changing macrostructure of the giant mitochondrion in the cells of $\sim 7^{\text{th}}$ generation: much of the narrow regions of the mitochondrial tube with profiles $\leq 0.06 \mu\text{m}^2$ were expanded (while no signs of organelle damage were observed). At the same time, mitochondria are characterized by increasing relative surface area of the cristae, which can be due to the activation of respiratory chain enzymes and ATP synthesis. In contrast, the irradiation at higher dose (1150 J/m^2) resulted in damages of mitochondria in the progeny yeast cells, among them fragmentation of the giant organelles and appearance of aberrant mitochondria, which can be due to the inhibition of main mitochondrial functions. It is supposed that dose-dependent modifications of mitochondrial ultrastructure in the progeny of the irradiated cells may be consequence of DNA mutations induced by some secondary messengers.

Keywords: He-Ne laser; mitochondrial ultrastructure; progeny cells, respiratory chain; *Torulopsis sphaerica*

1. Introduction

Radiation of red-to-near IR spectral region (600-1000 nm) is used in laser phototherapy to treat in a non-destructive and non-thermal fashion various soft-tissue and neurological conditions. One of the practically not investigated problems in laser phototherapy is whether irradiation with monochromatic light of visible-to-near IR spectral region can cause long-term effects, which appear in following cell generations. Experimental data considering possible hazardous (e. g., mutagenic) effects of such light are not numerous so far. It is known that the irradiation with a He-Ne laser ($\lambda=632.8$ nm) increased the frequency of chromosome aberrations in diploid cells of human fibroblasts [1]. Irradiation with a semiconductor laser at 660 nm increased the output of single-strand breaks of DNA in a dose-dependent manner [2]. The number of sister chromatid exchange frequencies in sheep peripheral blood mononuclear cells was found to be significantly increased after irradiation with a He-Ne laser at doses from 2 to 24 J/cm^2 , whereas by increasing the dose, the effect decreased [3]. Chromosomal aberrations in pig kidney cells were induced by far red light [4]. However, the irradiation in red region at 632.8 or 660 nm used in these experiments can not cause mutations through direct action upon DNA. The energy of these photons is too low (~ 1.7 eV) to cause ruptures of covalent bonds in the macromolecule. DNA (and RNA also) do not have absorption bands in the visible spectral region. So, the results of works [1-4] can not be explained by a direct action of visible light on DNA, and one has to suppose indirect effects. On the other side, the proliferation rate of mammalian cell cultures [5] as

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well as yeasts cultures (Fig. 1) was increased in next generations after a short-time irradiation. These data evidence that some genetic effects can be involved.

Microorganisms are convenient models to investigate changes in the growth and division of the next generations of initially irradiated cells. The reason is that the generation times (the periods between two successive divisions) are much shorter for yeast (some hours) as compared to the cell cycle duration for mammalian cells. It has been shown that irradiation with a He-Ne laser causes a substantial shortening of generation time (from 5 h to 3.5 h) occurred in the log-phase of growth of yeast cultures (Fig. 1). This is evident from biomass' accumulation (by the amount of total cell protein). The increased accumulation of the biomass is directly proportional to increase in the number of cells and buds in the log-phase of growth [6]. It means that the size of the cells and the amount of the total protein in the single cell do not differ for the exposed and unexposed cultures. Thus, irradiation with a He-Ne laser leads to the intensification of protein synthesis and speeds of cells division. It is significant that maximal level of protein synthesis is achieved to 14–18 h after irradiation at dose of $4.2 \times 10^2 \text{ J/m}^2$ (Fig. 1).

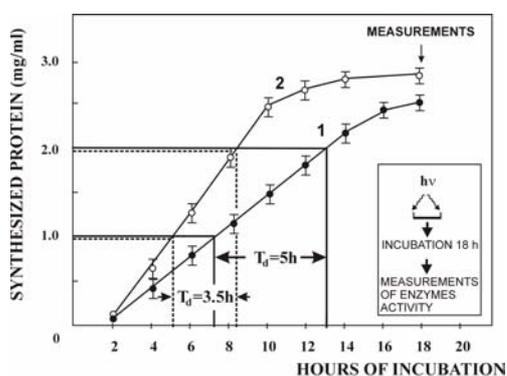


Figure 1. Growth curves of *T. sphaerica* yeast measured as a change in the amount of total protein in (1) intact and (2) irradiated (with a He-Ne laser at dose of $4.2 \times 10^2 \text{ J/m}^2$) cultures. Generation time in the exponential phase of growth is marked for control and irradiated cells. The inset illustrates the experimental scheme for measurements of enzymes activity [6,8,10].

Progenies of the irradiated yeast cells are characterized by changes in activity of various enzymes located in the relevant cell organelles (Table 1). An important point is that enzymes of oxidative metabolism (localized in mitochondria) are significantly activated. Thus, laser-enhanced activity of respiratory chain in mitochondria corresponds to higher level of protein synthesis in whole cell. It is not surprising, considering that protein synthesis in cell is substantially due to intensity of mitochondrial metabolism. It is necessary to stress, that the above listed signs of cell rearrangement are retained in the cells of $\sim 7^{\text{th}}$ generation (after 18h cultivation of irradiated cells). This circumstance allows us supposing that genetic mechanisms might be involved in these long-term effects.

Table 1. Changes in the activity of enzymes, localized in different cytoplasm organelles in *T. sphaerica* yeast cells after initial irradiation with a He-Ne laser ($4.6 \times 10^2 \text{ J/m}^2$) and following cultivation for 18 h [7, 9].

Enzymes	Localization	Activity (% of control value)
NADH-dehydrogenase	Mitochondrion	241.2±10.8 (p< 0.001)
Cytochrome c oxidase	Mitochondrion	121.0±9.6 (p< 0.05)
Acid phosphatase	Lysosome	48.1±6.3 (p< 0.005)
Catalase	Peroxisome	75.0±2.1 (p< 0.05)
Superoxide dismutase	Cytoplasm	103.0±3.0 not significant

This review summarizes our experiments performed to study the long-term effects of He-Ne laser radiation on *T. sphaerica* yeast cells in culture. The main goal of these studies was to investigate whether the functional activation of mitochondria in successive generations of initially irradiated cells is accompanied by ultrastructure changes of these organelles. The reasons for studying the mitochondria were first, the location of the photo-acceptors in the mitochondria [11]. Secondly, the mitochondria have their DNA and genes that encode some subunits for NADH-dehydrogenase, cytochrome c oxidase, ATP synthase and other proteins. It is also known that mitochondrial gene expression is partially regulated by energy demand [12]. It is notable that genetic screens (performed first in yeast) have identified a set of proteins required for mitochondrial morphology maintenance and mitochondrial inheritance during mitotic division [13].

2. Ultrastructure of mitochondria in the progeny *T. sphaerica* yeast from cultures irradiated with He-Ne ($\lambda = 632.8$ nm) laser light

2.1 Main features of mitochondrial organization after microscopic studies

In living cells the mitochondria often form a tubular network that permanently moving, dividing, and fusing [14, 15]. In budding *Saccaromyces cerevisiae* cells under active respiration 1-2 giant branched mitochondria constitutes ~ 50% of the total mitochondrial reticulum [14]. In dividing yeast there is a branched mitochondrial network (reticulum) during the whole cell cycle except mitosis, when it is considerably fragmented (Fig. 2) [13, 16]. The presence of branched mitochondrial reticulum in the yeast as well in some specialized mammalian cells [17-19] is significant for the maintenance of the mitochondrial DNA level [20], provision of daughter cells with the complete set of mitochondrial genes [21] as well as energy transfer in the form of the membrane potential [22]. High-dynamic behavior of actively respiring mitochondria is in equilibrium [23-25], which is regulated by the genes of mitochondrial fusion and division [13, 26].

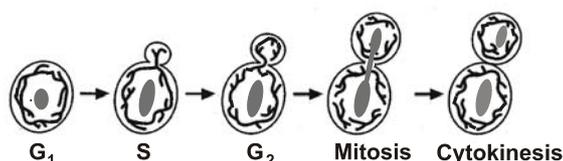


Figure 2. The integration of mitochondria into the common network at all stages of the cell cycle of budding yeast cells (G₁, S, G₂) except mitosis. Mitochondria and nucleus are shown in black and gray, respectively. Adapted from [13, 16].

The structure of the inner mitochondrial membrane, the surface of which is increased by the cristae, is highly variable and depends on the type and metabolic activity of the cell [27, 28]. The number of cristae and the surface area of their membranes relative to the mitochondrial section area correlate with the energy activity of the organelles [28, 29]. According to current views, the cristae are not just folds of the inner confining membrane but contact it via narrow tubules, which can serve as a barrier for the diffusion of ions, membrane proteins, and other macromolecules [30]. A recent model of mitochondrial structure is displayed in Fig. 3, adapted from review which is concerned with microscopic organization of mitochondria [31].

In addition to the main function, generation of energy, mitochondria play an important role in the transport of Ca²⁺ that is a polyfunctional secondary messenger indirectly affecting the metabolic processes, gene transcription as well as cell proliferation [32-35]. Ca²⁺ transport from the endoplasmic reticulum (ER) to the mitochondria takes place in the regions where these organelles approach each other.

The general principles of structural organization of the mitochondria of yeast cells (primitive eukaryotes) [13, 17] are the same as those of the higher eukaryotic cells. As a consequence, the ultrastructural analysis of mitochondria in yeast is subjected to He-Ne laser irradiation should provide general insight into the mechanism of this agent action in all eukaryotes.

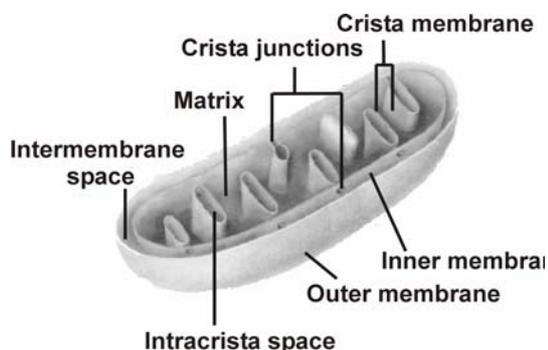


Figure 3. A current model of mitochondrial organization. Adapted from [31].

2.2. Growth stimulation of the irradiated *T. sphaerica* cultures is dose-dependent

Low-intensity monochromatic light with $\lambda = 632.8$ nm exerts an activating effect on the vegetative growth of yeast organisms cultivated under aerobic conditions in a nutrient medium containing 1% of glucose [6-10]. Yeast cells studied (*Candida boidinii*, *C. maltosae*, *S. cerevisiae*, *Saccharomyces ludwigii*, *T. sphaerica*) appeared to differ in photosensitivity, depending on their metabolic pattern. It is higher in cells capable of rapid rearrangement in response to changes in cultivation conditions and to the action of various chemical and physical agents. A pronounced sensitivity to monochromatic light with $\lambda=632.8$ nm is characteristic of the facultative anaerobes *T. sphaerica* [7]. The optimal dose of irradiation by this light was established on the basis of the dose dependence found from the amount of the whole cellular protein in the yeast cultivated 18 h after the irradiation (Fig. 4). It can be seen in Fig. 4 that the change of this parameter is characterized by a bell shaped curve, its maximum being reached at a dose of 460 J/m^2 . The maximum protein synthesis level was correlated with an elevated activity of NADH-dehydrogenase and cytochrome *c* oxidase (Fig. 4, point A). The lowering the protein synthesis level following irradiation at a dose of 1150 J/m^2 (Fig. 4, point B) was accompanied by a decrease in the activity of the both respiratory chain enzymes in mitochondria in comparison with that in the cells irradiated at a dose of 460 J/m^2 .

The above-listed data [7, 8] formed the basis for an electron microscopy study of *T. sphaerica* yeasts in ultrathin cell sections. The impact of He-Ne laser on the mitochondrial ultrastructure was studied in the successive generations of *T. sphaerica* cells [36-44]. For this purpose non dividing cells were irradiated in phosphate buffer and then both sham-irradiated (control) and irradiated cells (experiment) were transferred to Rider nutrient medium with 1% glucose and incubated in the presence of O_2 . Finally, successive generations of cells were studied.

2.3. Ultrastructure of the giant mitochondria in the progeny yeast cells after radiation at 460 J/m^2 corresponds to the signs of activation of the respiratory chain enzymes

Invariable qualitative features of giant mitochondrion. Structure of mitochondrial profiles was indistinguishable in the irradiated and control cultures. The mitochondria in progeny of irradiated yeast cells contained developed cristae with a common orientation and nearly constant widths of the intracrista and intermembrane spaces (~ 30 nm). No signs of damage were observed in the mitochondria such as swelling or contraction of matrix as well as membrane destruction, which points to normal function of the organelles. The above microstructure of the mitochondria is typical for various budding yeast cells with aerobic metabolism [14]. The spatial reconstruction of the chondriome of the budding cells, the

precursors of which were sham-irradiated (Fig. 5 A) or irradiated at 460 J/m² (Fig. 5 B) evidenced the presence of mitochondrial reticulum, distributed between the mother and the daughter cells. Whole mitochondrial reticulum involved 1 giant branched and 2-3 small mitochondria. It is known that giant mitochondrion can provide normal oxidative phosphorylation [45].

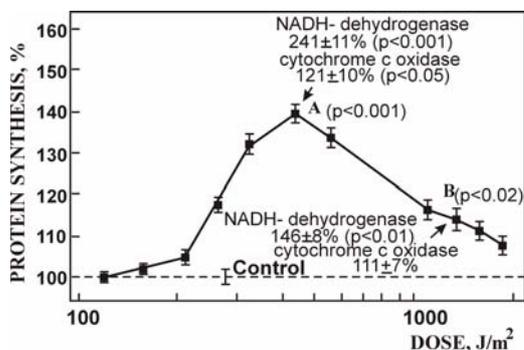


Figure 4. The amount of total cell protein (% of control) synthesized in cultured *T. sphaerica* yeast 18 h after exposure to different doses of He-Ne laser light. NADH-dehydrogenase and cytochrome c oxidase activities relative to control after irradiation at 460 and 1150 J/m² are shown in points A and B, respectively. Data from [7, 8].

Changes in quantitative features of the giant mitochondrion. According to our data, relatively straight regions of the giant mitochondrion amounted to around 90% of the chondriome in total both in control and after irradiation at 460 J/m². Accordingly, the mitochondrial profiles revealed on the ultrathin cell sections largely belonged to the giant organelle. Table 2 shows the results of the quantitative analysis of mitochondria. For this purpose point-counting method on the random ultrathin cell sections was used [46].

No differences in the number and total area of mitochondrial profiles related to the cytoplasm area have been revealed between the experiment (irradiation at 460 J/m²) and control. The invariable total mitochondrial area per cytoplasm area (which corresponds to unchanged volume of mitochondria in the cell [46]) could be due to asynchronous division of the yeast cells in both populations irrespective of a high (in the experiment) and low (in the control) cell cycle rate. The total chondriome volume is known to increase with the cell volume at successive stages of the yeast division [26].

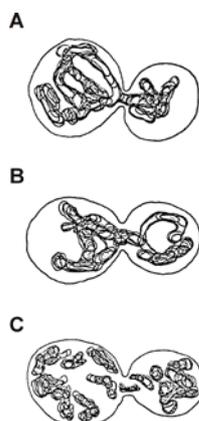


Figure 5. The spatial reconstruction of the mitochondria in the progeny of *T. sphaerica* cells after (A) sham-irradiation, (B) irradiation with a He-Ne laser at 460 J/m² and (C) 1150 J/m². Data from [38–40].

At the same time, other properties of mitochondria differed. The mean area of the mitochondrial profile increased after irradiation at 460 J/m² by 53% (Table 2). This increase of the average mitochondrial profile was due to the expansion of the matrix, since the widths of the intermembrane and intracrista spaces remained nearly constant (~ 30 nm). It is pertinent to repeat that matrix of such mitochondria did not swell as observed in certain pathologies. The increase of matrix volume within the physiological limits can be accompanied by the accumulation of inorganic pyrophosphate and activation of the mitochondrial respiratory chain [47]. Besides, the mean distance between the nearest mitochondrial

profiles is increased in progeny of irradiated cells (Table 2). The latter corresponds to a less dense mutual arrangement of the organelle regions, which can facilitate their oxygen supply. Increased oxygen consumption was observed after *in vitro* exposure of mitochondria to He–Ne laser light [48, 49].

Changes in the configuration of the giant mitochondrion. Quantitative analysis demonstrated significant variation in the mitochondrial section area (from $0.04 \mu\text{m}^2$ to $0.69 \mu\text{m}^2$) in cells, precursors of which were sham-irradiated or irradiated at 460 J/m^2 .

The size distribution of mitochondrial profiles is shown in Fig. 6. It is seen that in the case under consideration (Fig. 6 B) the distribution is shifted to the right at the expense of decreasing a fraction of small mitochondrial profiles (section area $\leq 0.06 \mu\text{m}^2$). The test of proportion differences demonstrated about twice lower relative number of small mitochondrial profiles ($S \leq 0.06 \mu\text{m}^2$) in experiment: 41% and 22% in control and experiment, respectively ($p < 0.05$, Table 2). As a consequence mean area of mitochondrial profiles is 1.5 times greater vs. the control (Table 2).

Hence, the giant mitochondrion in budding *T. sphaerica* cells is a dynamic organelle composed of regions with different thickness. Activation of respiratory chain enzymes (after initial exposure of yeast culture to 460 J/m^2) was accompanied by an expansion of a considerable number of narrow regions of the giant mitochondrion. Such rearrangement of the mitochondria may promote accelerated energy transport along lengthy membranes of the giant mitochondrion.

Increase in the relative surface area of mitochondrial cristae. Considering that cristae play the leading role in respiration and oxidative phosphorylation, their quantitative estimation is widely used in microscopy [e. g., 50, 51]. In such cases the total area of cristae relative to the mitochondrial profile area is calculated, using point-counting method on the random cell sections [46].

Morphometric analysis of mitochondria in the subsequent generations of yeast cells after initial irradiation of the cultures at 460 J/m^2 demonstrated an increased relative surface area (or surface density) of cristae by 25% as compare to control (Table 2).

An increased surface density of cristae is presently attributed either to new cristae formation on the inner mitochondrial membrane [29] or to growth of pre-existing cristae, which can presumably branch, fuse, and divide [29, 52]. Correlation between the relative surface area of cristae and activity of the respiratory chain enzymes as well as between the surface area of cristae and intensity of ATP synthesis has been demonstrated for different cells [28, 29, 50, 53, 54]. Besides, an increased number of cristae proved to correlate with the increased activity of the electron transport chain and ATP synthase [55, 56]. It is of interest that the increased surface density of cristae as a result of the aftereffect He-Ne laser light is correlated with the activation of NADH-dehydrogenase and cytochrome c oxidase (Fig. 4, point A).

2.4. Increased number of mitochondrion–endoplasmic reticulum associations is a result of irradiation of the yeast at 460 J/m^2

The presence of the regions of association between mitochondria (MT) and endoplasmic reticulum (ER) is beyond question now [34, 35, 57]. Apposition of the mitochondria and ER cisterns at a distance of 50–100 nm establishes a firm junction between their outer membranes, which is required for Ca^{2+} transfer into mitochondria [32, 58] and/or for phospholipids exchange between these organelles [35]. We considered the mitochondria–endoplasmic reticulum (MT–ER) associations when the distance between their outer membranes was $\leq 50 \text{ nm}$.

After exposure of the yeast cells to 460 J/m^2 and the following cultivation for 6 h, the number of MT–ER association regions relative to cytoplasm area increased by 69% versus the control ($p < 0.05$). In addition, the total perimeter and the number of ER cisterns per cytoplasm area increased by 43 and 48%, respectively. This means that the rate of ER formation increased in budding cells in the experiment. As this takes place, the total area and the number of mitochondrial profiles per cytoplasm area remained virtually unaltered (the total perimeter of mitochondrial profiles per cytoplasm area also did not change). However, the mitochondria from irradiated cell cultures were characterized by an increased variability [43]. The dispersion of the mean indices of mitochondrial profiles (area, number of profiles and number of dumbbell-shaped profiles relative to cytoplasm area etc) was higher in the experimental group than in

the control according to the *F*-test ($p < 0.001$). The significant variability of mitochondria in *T. sphaerica* cells exposed at 460 J/m^2 —was attributed to the division of most cells after 6 h cultivation in the experiment, while majority of cells in the control did not start division [42]. It has been known that mitochondria in dividing *S. cerevisiae* have actually unstable structure [14], which is due to the high mobility of these organelles during yeast budding [59].

Table 2. Changes of mitochondria in *T. sphaerica* cells 18h after irradiation ($\bar{X} \pm S_{\bar{X}}$) [44].

Indices of mitochondrial (MT) profiles on cell sections	Sham-irradiation of cells-precursors	Irradiation of cells-precursors at 460 J/m^2	Irradiation of cells-precursors at 1150 J/m^2
Number of MT profiles per μm^2 of cytoplasm	0.22 ± 0.02	0.17 ± 0.02 NS	0.28 ± 0.02 $p < 0.05$ $p' < 0.001$
Total area of MT profiles per cell section, μm^2	0.64 ± 0.07	0.89 ± 0.11 NS	0.67 ± 0.07 NS
Mean area of MT profile, μm^2	0.17 ± 0.02	0.26 ± 0.02 $p < 0.001$	0.14 ± 0.01 NS $p' < 0.001$
Distance between neighboring MT profiles μm	0.39 ± 0.02	0.50 ± 0.03 $p < 0.01$	0.25 ± 0.02 $p < 0.001$ $p' < 0.001$
Cristae/MT profile area ratio	0.20 ± 0.01	0.25 ± 0.01 $p < 0.001$	0.19 ± 0.01 NS $p' < 0.001$
Content of small MT profiles ($S \leq 0.06 \mu\text{m}^2$), %	41	22 $p < 0.05$	55 $p < 0.05$ $p' < 0.001$
Content of large MT profiles ($S > 0.33 \mu\text{m}^2$), %	12	28 $p < 0.01$	7 NS $p' < 0.001$

In Table 2: p and p' , significance of differences from control and first experiment, respectively; NS, differences from control were not significant

Spearman's test allowed us to reveal a positive correlation between the number of MT–ER associations and the total perimeter of the perimitochondrial ER cisterns ($r = 0.52$ and 0.71 for experiment and control, respectively; $p < 0.001$) [43]. Such correlation can be due to the presence of numerous clusters of Ca^{2+} -releasing channels on the ER surface [32]. At the same time, no correlation has been revealed between the number of MT–ER associations and the total mitochondrial perimeter.

The absence of the correlation in the latter case can be due both to the involvement of as low as 5–20% of the mitochondrial surface in junction formation [31] and to the integration of Ca^{2+} released from numerous channels on the ER membrane to not numerous uptake sites on the mitochondrial membrane [32]. In mechanistic terms, the increased number of MT–ER associations in yeast cells after irradiation can result from both the increased length of the ER membranes and the structural variability of mitochondria.

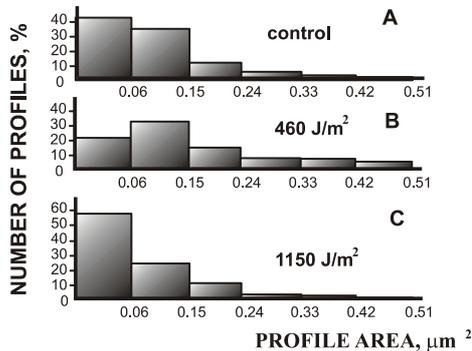


Figure 6. The distribution of mitochondrial profile areas in progeny cells after (A) sham irradiation, (B) irradiation with He-Ne laser at 460 J/m² and (C) 1150 J/m². Adapted from [40]. Foot-note: in Figs. A and B few higher-area mitochondria profiles ($S > 0.51 \mu\text{m}^2$) were omitted.

It was shown previously that exposure of isolated mitochondria and whole cells to He-Ne laser light increases the capacity of the organelles to accumulate Ca²⁺ [49, 60, 61], as revealed within the first tens of minutes after irradiation. In addition, similar exposure increases the electric membrane potential of mitochondria [62, 63]. These phenomena can be mutually related considering that only highly energized mitochondria can uptake Ca²⁺ [64]. Until recently, an increased concentration of mitochondrial Ca²⁺ in response to He-Ne laser irradiation was documented within the first minutes [60, 61, 65, 66]. Our data may indicate a long-term (during 6 h) effect of laser light on the mitochondrial capacity to Ca²⁺ uptake. The latter can be based on the functioning of mitochondria as a Ca²⁺ buffer, which slowly releases Ca²⁺ as its concentration in cytoplasm decreases [67].

2.5. Damaging effect of laser irradiation at higher dose on mitochondria in the progeny yeast cells

Structural damage of mitochondria. Cells of the cultures initially exposed to 1150 J/m² were characterized by the presence of mitochondrial profiles of an irregular shape with curved contours. Cases of mitochondrial aggregation were observed, when two or three organelles contacted or even interfused. Mitochondrial profiles with the matrix separated by solid crista could be observed [39]. The matrix of some aggregated mitochondria contained areas of low electron density without cristae or areas with irregular cristae orientation. Such injured mitochondria were absent from the control cells and from cells, precursors of which were irradiated at 460 J/m².

Fragmentation of mitochondria. Yeast cells, precursors of which were irradiated at 1150 J/m², are characterized by numerous discrete mitochondria (Fig. 5 C) resulting from the fragmentation of the giant mitochondrion. This agrees with morphometric data: the average number of mitochondria profiles per cytoplasm area significant increased in successive generations of cells irradiated at 1150 J/m² (Table 2).

Decrease of mitochondrial areas of profiles. Fragmentation of giant mitochondrion (after irradiation at 1150 J/m²) was accompanied by a decrease in their mean section area by comparison with that in other experimental variant ($p < 0.001$; Table 2). The distribution of mitochondria by profile area was shifted to the left (Fig. 6 C). This is due to an increase in the proportion of small mitochondrial profiles with section areas $\leq 0.06 \mu\text{m}^2$ ($p < 0.05$; Table 2). In the case under consideration distance between neighboring mitochondrial profiles decreased significantly (Table 2).

The modifications of mitochondria in successive generations of cells after initial irradiation of yeast cultures at 1150 J/m² were comparable to the changes of mitochondria after metabolism inhibition by a number of specific agents. For instance, inhibition of the respiratory activity of *S. cerevisiae* introduced mitochondrial profiles with curved contours [14]. Matrix partitioning was observed after functional disturbances of mitochondria including uncoupling of respiration and oxidative phosphorylation [68] as well mutations of genes (DPR1 and MDM33) [69, 70]. Fragmentation of the giant mitochondrion accompanied by the changes in the internal structure of the organelle was observed in different pathologies [68] and after inhibition of respiration and oxidative phosphorylation [45, 71-73]. Mitochondrial

fragmentation in yeast cells is attributed to the disturbed balance between the fusion and division of the organelle towards the latter process [74]. It is remarkable that the mutations of *S. cerevisiae* genes encoding mitochondrial fusion factors (MGM1, FZO1, UGO1, and MDM30) induced fragmentation and damage of mitochondria [26, 75-77]. The above-listed changes in the structure of mitochondria in various cells are similar to those observed in successive generations of *T. sphaerica* cells, the precursors of which were irradiated at 1150 J/m².

Thus, structural damages of mitochondria demonstrated by us can evidence a disturbance of respiration and oxidative phosphorylation. At the same time, the dysfunction of these organelles is not critical since the *T. sphaerica* cells continued to divide after laser irradiation at 1150 J/m² [6]. A similar phenomenon was observed in *S. cerevisiae* (the yeast cells were divided while their mitochondria were damaged) [78].

3. Concluding remarks

The bulk of the chondriome in budding *T. sphaerica* cells was represented by the giant branched mitochondrion (in the cells, the precursors of which were either sham-irradiated or irradiated at 460 J/m²). Quantitative analysis of the ultrathin cell sections demonstrated the uneven thickness of different branch regions of the giant mitochondrion in such cells. The revealed heterogeneity of the mitochondrial tube thickness in budding yeast agrees with the data for other unicellular organisms [16, 69, 79]. It was suggested that presence in cell of the giant mitochondrion favor the optimal transfer of the transmembrane electric potential [22]. This agrees with the data on the appearance of the giant mitochondrion in human peripheral lymphocytes irradiated with He–Ne laser light (synthesis of ribosomal RNA precursors in such lymphocytes was activated) [80, 81, 82].

The irradiation at 460 J/m² accelerated cell proliferation, activated enzymes of the mitochondrial respiratory chain, and at the same time induced the following ultrastructure changes in the giant mitochondria in yeast cells cultured for 18 h after the exposure. *First*, the relative content of small organelle profiles (with the profile area $\leq 0.06 \mu\text{m}^2$) on the random cell sections decreased and the mean mitochondrial profiles area increased which points to the dilatation of narrow regions of giant mitochondrion branches. This modification of mitochondria is due to the matrix expansion. At the same time, the organelle microstructure remains unchanged. The dilatation of narrow regions of the giant mitochondrial tube as a result of prolonged effect of irradiation can provide conditions that are more favorable for energy transfer as compared to the conditions for the giant mitochondrion in the intact cells. *Second*, the mean distance between the nearest mitochondrial profiles increased, which indicates a less dense packing of the mitochondrial branches. *Third*, the relative surface area of the cristae, whose membranes involve the respiratory chain enzymes and ATP synthase, increased. Similar changes of mitochondrial cristae observed in other biological models are attributed to the activation of respiratory chain enzymes and ATP synthesis. The analogous functional shift was revealed as a result of aftereffect of He–Ne laser light not only in *T. sphaerica* [8] but in *HeLa* cells as well [83].

Irradiation at a higher dose (1150 J/m²) induced fragmentation of giant mitochondria as well as structural damages of discrete mitochondria in the progeny of exposed cells. Such changes in mitochondria are commonly attributed to the inhibition of their respiratory and phosphorylation activities. Essentially, the changes in most mitochondrial indices in cells, precursors of which were irradiated at 1150 J/m² had the opposite direction relative to that in the cells, precursors of which were irradiated at 460 J/m² (Table 2).

Thus, the successive generations of cells from cultures initially exposed to He–Ne laser light demonstrated a number of dose-dependent quantitative and qualitative changes in mitochondria. Conservation of the modified mitochondria in the progeny cells can be due to the inheritance of changes in the mitochondrial (and possibly nuclear) DNA. It is known that rearrangements in the mitochondrial genome can be inherited in up to 20 generations of yeast cells [84]. As mentioned above, no direct impact of 632.8 nm radiation on DNA is possible, since this macromolecule absorbs no visible light. DNA mutations responsible for the mitochondrial changes under irradiation can be due to the impact of secondary messengers such as reactive oxygen species, calcium ions etc.

Summing up, the presented experimental material allows to conclude that the irradiation at $\lambda=632.8$ nm exerts prolonged influence on the mitochondrial ultrastructure in the yeast cells of successive generations.

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