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Influence of a short-time He-Ne laser irradiation on the next generations of yeast cells: Prolonged effects on mitochondrial ultrastructure

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Abstract

The influence of irradiation with He-Ne laser (λ=632.8 nm) on ultrastructure of mitochondria of next generations of Torulopsis sphaerica yeast cells was studied. Exposure of yeasts to He-Ne laser in a dose of 460 J/m^2 resulted in activation of cytochrome c oxidase and NADH-dehydrogenase as well as in

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changes of the configuration of the giant mitochondrion of cells of 7^{th} successive generation. Narrow regions of mitochondrial tube were expanded (relative quantity of small organelle profiles with area ≤0.06 μm^2 was reduced by half vs. the control, $p<0.05$). This shift was a result of matrix expansion. Such mitochondria were characterized by increasing relative surface area of the cristae by 25% ($p<0.001$). Similar changes of cristae are usually connected with the activation of the respiratory chain and oxidative phosphorylation. Irradiation of yeasts at 460 J/m^2 resulted in increasing number of mitochondrion- endoplasmic reticulum associations per cytoplasm area by 70% ($p<0.05$), which can reflect a raised capacity of mitochondria to Ca^{2+} uptake. It is generally assumed, that the capture of Ca^{2+} is realized only by highly energized mitochondria and the increase of concentration of Ca^{2+} in mitochondria leads to both enhancing mitochondrial ATP production and releasing Ca^{2+} to cytoplasm. The irradiation at 1150 J/m^2 resulted in various damages of mitochondria in yeast cells of successive generations, among them fragmentation of the giant organelle, appearance of aberrant organelles as well as decreasing mean area of mitochondrial profiles, which points to the inhibition of main mitochondrial functions. It is supposed, that dose-dependent modifications of mitochondria in the progeny of the irradiated cells may be consequence of DNA mutations induced by some secondary messengers.

1. Introduction

Radiation of red-to-near IR spectral region is used both in photodynamic therapy (PDT) and laser phototherapy. PDT is the method based on combined use of a photosensitizer and light. As the combined action of two different agents is utilized, an investigation of action of these agents in separate is important for a full understanding of processes occurring during PDT.

One of the practically not investigated problems in both laser phototherapy and PDT 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 light in visible-to-near IR region are not numerous so far. The irradiation with a He-Ne laser was shown to increase 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 DNA breaks in a dose-dependent manner [2]. The frequency of sister chromatin exchanges 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 could not cause mutations through direct action on DNA. The energy of these photons is too low (~1.7 eV) to cause ruptures of covalent bonds in a molecule. 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 well as the division rate of yeasts (Fig. 1) was increased in next generations after a short-time irradiation. These data evidence that some genetic (mutagenic?) effects can be involved.

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. 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 time (the period between two successive divisions) is much shorter (some hours for yeasts) as compared to that of mammalian cells.

Irradiation with a He-Ne laser did not change the duration of the lag-period of the growth curves of yeasts neither estimated by biomass' accumulation (protein synthesis) nor by number of buds [6]. A substantial shortening of generation time occurred in the log-phase of growth as illustrated in Fig. 1. For example, in *T. sphaerica* yeast, the generation time shortened from 5 to 3.5 h. This finding shows that the cell proliferation rate increases in subsequent generations of initially irradiated cells.

The increased accumulation of the biomass (i.e., the amount of synthesized protein) of the exposed culture was followed by a strictly proportional increase in the number of cells and buds in the logarithmic phase of growth [6]. It means that the size of the cells and the amount of the protein in the single cell apparently 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 up the preparation of cells for division. The latter is confirmed shortening the generation time in log-phase from 5h to 3.5h (Fig.1). The maximal level of protein synthesis was achieved 14 – 18 h after irradiation at dose of 4.2×10^2 J/m^2.

Progenies of the irradiated cells are characterized by changes in activity of various enzymes located in the relevant cell organelles (Table 1, Fig. 1). An important point is that enzymes of oxidative metabolism (localized in mitochondria) were significantly activated. Thus, laser-enhanced activity of mitochondrial respiratory chain corresponded to higher level of protein synthesis. It is not surprising, because the protein synthesis in cells is

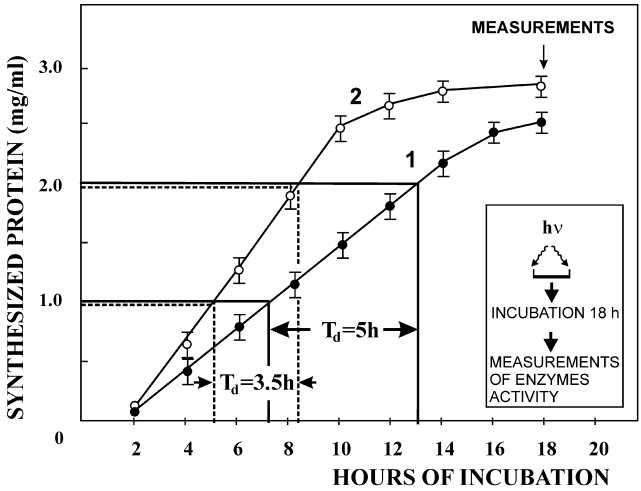

Figure 1. Growth curves of *T. sphaerica* yeast measured as a change in the amount of synthesized protein in (1) intact and (2) irradiated with a He-Ne laser at dose of 4.2×10^2 J/m^2 cultures. Generation times in the exponential phase of growth are marked for control and irradiated cells. The inset on the left side illustrates the experimental scheme and that on the right side shows the location of enzymes under study. Data from the papers of [6-10] are used in this Figure.

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

Enzymes	Location in the cytoplasm	Activity (% of control value)
NADH dehydrogenase	Mitochondrion	241.2±10.8 ($p< 0.001$)
Cyt. 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 ($p>0.05$)

substantially related to the intensity of mitochondrial metabolism. It is necessary to stress, that the above listed signs of cell rearrangement were retained in the cells of 6-7 generations (after 18 h cultivation of irradiated cells). This circumstance suggests that genetic mechanisms might be involved in these long-term effects.

These experiments showed that irradiation had a prolonged influence on the growth of eukaryotic microorganisms. It means that effects of a short-term exposure are manifested in the progeny of the irradiated cells. In Sect. 2 below

we will look at the changes in the mitochondrial ultrastructure in the cells-descendants. The reasons for studying the mitochondria were first, the location of the photoacceptors in the mitochondria [11]. Secondly, the mitochondria have their own DNA and genes that encode some subunits for NADH-dehydrogenase, cytochrome c oxidase, and ATP synthase. It is also known that mitochondrial gene expression is partially regulated by energy demand [12]. Last, but not least, He-Ne laser irradiation of mitochondria *in vitro* induced a new subpopulation of these organelles [13, 14].

Mitochondrial ultrastructure in the successive generations of *T. sphaerica* cells irradiated by He-Ne (λ=632.8 nm) laser light

Main features of mitochondrial organization in budding yeast cells

The studies on living cells have demonstrated the plasticity of mitochondria. These organelles are permanently moving, dividing, and fusing [15]. As demonstrated for *Saccharomyces cerevisiae* cell, the equilibrium is maintained between these processes, which shape the chondriome as a continuous network [16-18]. Such equilibrium is regulated by the genes of mitochondrial fusion and division [19, 20].

Various eukaryotic cells and yeast cells in particular, contain a branched giant mitochondrion [21-23]. The presence of the mitochondrial reticulum in the cell is significant for the maintenance of the mitochondrial DNA level [24], provision of daughter cells with the complete set of mitochondrial genes [25] as well as the energy transfer in the form of the membrane potential [26]. The organization of mitochondria as a continuous network is maintained during the whole cell cycle of budding yeasts except mitosis, when the giant mitochondrion is fragmented (Fig. 2). After mitosis, the discrete mitochondria fuse again to form the giant organelle [19, 27].

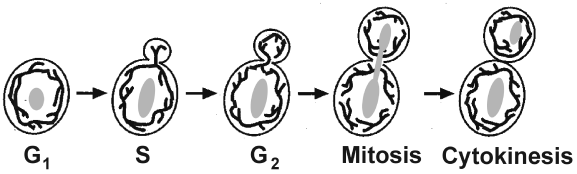

Figure 2. The integration of mitochondria in budding yeast cells into the common network at all stages of the cell cycle (G_1, S, and G_2) except mitosis (M). Modified from [19, 27].

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 [28, 29]. The number of cristae and their surface correlate with the energetic activity of these organelles [29, 30]. According to current views, the cristae are not just folds of the inner confining membrane but contact it via narrow tubules (Fig. 3). These crista junctions can serve as a barrier for the diffusion of ions, membrane proteins, and other macromolecules [31].

In addition to the main function, generation of energy, mitochondria play an important role in the transport of Ca^{2+} that is a polyfunctional secondary messenger indirectly affecting the metabolic processes, gene transcription and cell proliferation [32]. Ca^{2+} transport from the endoplasmic reticulum (ER) to the mitochondria takes place in the regions where these organelles approach each other. Application of an intracellular Ca^{2+} -sensitive marker allowed tracing Ca^{2+} migration in living cells from the ER to the narrow cytoplasm layer between ER and mitochondrion and further into the mitochondrion [33, 34].

The general principles of organization of the mitochondrial apparatus of budding yeast cells (primitive eukaryotes) [17, 19] are the same as those of the higher eukaryotic cells, characterized by an active energy metabolism [26, 29].

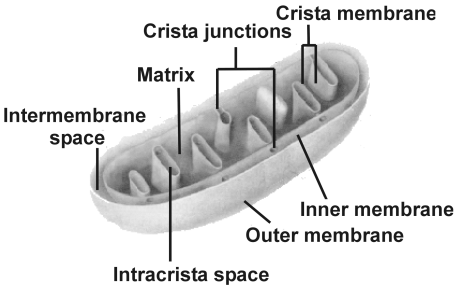

Figure 3. A current model of mitochondrial structure as adapted from [35].

Stimulation of growth of the irradiated *T. sphaerica* yeast cultures is dose-dependent

Low-intensity monochromatic light with λ = 632.8 nm exerted an activating effect on the vegetative growth of yeast organisms that were cultivated under aerobic conditions in a nutrient medium containing 1% of glucose [6-10]. The studied yeast cell lines (*Candida boidinii*, *C. maltosae*, *Saccharomyces cerevisiae*, *Saccharomycodes ludwigii*, *T. sphaerica*) differed in photosensitivity that depended on the metabolism character. It was higher in

cells capable of rapid rearrangement in the response to changes in cultivation conditions and to the action of various chemical and physical agents. A pronounced sensitivity to monochromatic light with λ = 632.8 nm was 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 yeast cultivated 18 h after the irradiation (Fig. 4). The change of this parameter was characterized with a bell shaped curve, its maximum being reached at a dose of 460 J/m^2. The maximal protein synthesis level was correlated with an elevated activity level of NADH-dehydrogenase and cytochrome *c* oxidase (Fig. 4. point A). The lowering of 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 enzymes.

The above-listed biochemical data [7, 8] formed the basis for an electron microscopy study of *T. sphaerica* yeasts. The impact of He–Ne laser on the mitochondrial ultrastructure was studied in the successive generations of *T. sphaerica* cells [36-44]. Non dividing cells were irradiated in phosphate buffer and then both sham-irradiated (control) and irradiated cells were transferred to Rider medium with 1% glucose and incubated in the presence of O_2. Finally, successive generations of the sham-irradiated or irradiated cells were studied. In the first case, the proliferation was induced by the medium (in the control) and in the second – by the medium plus laser light (in the experiment).

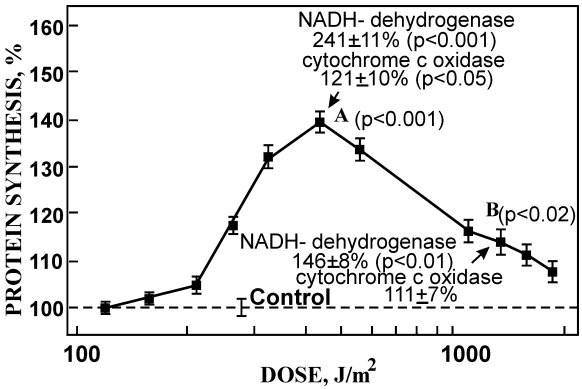

Figure 4. The amount of protein (% of control) synthesized in cultured *T. sphaerica* cells 18 h after exposure to different doses of He-Ne laser light. NADH-dehydrogenase and cytochrome c oxidase activities (relative to control) after irradiation at 460 and 1150 J/m^2 are shown in points A and B, respectively (the data from [7, 8]).

The spatial reconstruction of the chondriome of the budding cells, the progenitors of which were sham-irradiated (Fig. 5 A) or irradiated at 460 J/m^2 (Fig. 5 B) evidenced the presence of one giant mitochondrion. The giant mitochondrion was distributed between the mother cell and the bud. The chondriome also involved 2-3 ordinary small mitochondria. Cells, the progenitors of which were irradiated at a dose of 1150 J/m^2 (Fig. 5 C) were characterized by changing of spatial organization of the chondriome as compared with both previous groups (Fig. 5 A, B). A great number of small organelles were revealed in these budding cells. The giant mitochondria were not found (Fig. 5 C).

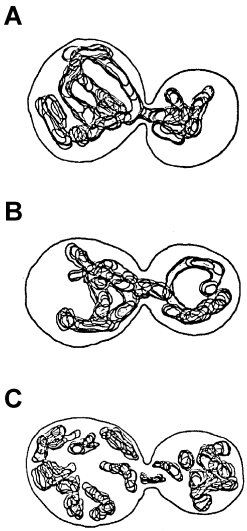

Figure 5. The spatial reconstruction of the chondriome in the progeny *T. sphaerica* cells initially sham-irradiated (A), irradiated with a He-Ne laser at doses of 460 J/m^2 (B), or 1150 J/m^2 (C). Adapted from [38, 39, 41].

Ultrastructure of the giant mitochondria in the progeny of cells irradiated at 460 J/m^2 corresponds to the signs of activation of the respiratory chain

Invariable qualitative characteristics of giant mitochondrion

The mitochondrial structure was indistinguishable in the irradiated and control cultures. The mitochondria in progeny of irradiated yeast cells contained developed cristae with a normal orientation and nearly constant widths of the intracristal and intermembrane spaces (~30 nm). No signs of

damage such as matrix swelling, its contraction or membrane destruction were observed in the mitochondria.

The same microstructure of the giant mitochondrion was observed in *T. sphaerica* cells, precursors of which were irradiated. It was typical for budding yeast cells with active aerobic metabolism [45, 19]. It is well known that the giant mitochondrion in eukaryotic cells with active energy consumption [26] is fragmentized in response to injured oxidative phosphorylation (e. g. in conditions of inhibition of respiratory chain activity or mitochondrial de-energization) [46].

Changes in quantitative characteristics of the giant mitochondrion

According to our data, relatively straight regions of the giant mitochondrion amounted to around 90% of the chondriome in total. 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 on the random ultrathin cell sections. No differences in the number of mitochondrial profiles and their total area related to the cytoplasm area have been revealed between the experiment (irradiation at 460 J/m^2) and control. The invariable mean mitochondrial area of the cell section (proportional to the volume of whole cell [47]) could be due to asynchronous division of the yeasts in both populations irrespective of a high (in the experiment) and low (in the control) cell cycle rate. The total mitochondrial volume is known to increase with the cell volume at all stages of the cell cycle [15, 27].

At the same time, other properties of mitochondria differed. The mean area of the mitochondrial profile increased by 53% in the successive generations of irradiated cells (460 J/m^2, Table 2). This increased mitochondrial surface area (or their relative volume according to [47]) was due to the expansion of the matrix, since the widths of the intermembrane and intracrista spaces remained nearly constant. It is pertinent to repeat that matrix of such mitochondria did not swell – its electron density did not decrease 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 [48].

The mean distance between the nearest mitochondrial profiles is increased in progeny of irradiated cells (460 J/m^2, Table 2). This points out 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 [49, 50].

Table 2. Changes of mitochondria in *T. sphaerica* cells 18 h after irradiation ($\overline{X} \pm S_{\overline{X}}$) [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 1 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
Area of an 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
Ratio of cristae/MT profile areas	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≤ 0.06 μm^2), %	41	22 p<0.05	55 p<0.05 p'<0.001
Content of large MT profiles (S>0.33 μm^2), %	12	28 p<0.01	7 NS p'<0.001

The significance of differences from control and the first experiment: p and p', respectively;
NS: the difference was not significant.

Changes in the configuration of the giant mitochondrion

Quantitative analysis demonstrated significant variation in the mitochondrial section area in cells, progenitors of which were sham-irradiated or irradiated at 460 J/m^2 (Fig. 6A, B). The test of proportion differences demonstrated twice lower relative number of small mitochondrial profiles (S≤0.06 μm^2) and a higher relative number of large profiles (S>0.33 μm^2) in experiment versus control ($p < 0.05$ and $p < 0.01$, respectively, Table 2).

Interpretation of this result was complicated by the fact that a fraction of the mitochondrial profiles represented oblique sections of the mitochondrial tube at various angles to the longitudinal axis, which could cause variations in the section areas of mitochondria even with constant diameter. Analysis of the areas of round or slightly elliptic sections (with the maximum/ minimum diameter ratio ≤1.65) demonstrated that the profiles with the area >0.33 μm^2 indeed represented oblique sections of mitochondria. These experiments support the fact of the thickness variation for different regions of the mitochondrial tube.

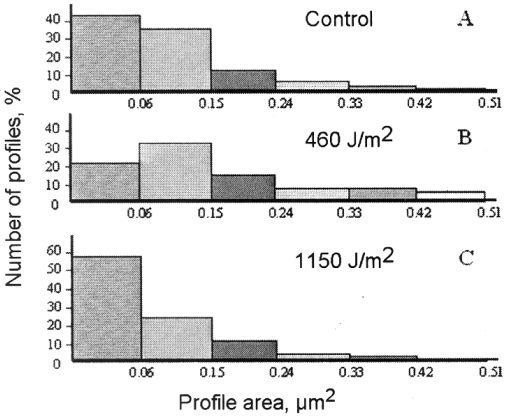

Figure 6. The distribution of mitochondrial profile areas in progeny cells after (A) sham irradiation or (B) irradiation with He-Ne laser at 460 J/m^2 and (C) 1150 J/m^2 (adapted from [39]).
Foot-note: in Figs. A and B few higher-area mitochondria profiles (S>0.51 μm^2) were omitted.

Hence, the giant mitochondrion in budding yeast cells is a dynamic structure composed of regions with different thickness. Activation of respiratory chain enzymes (after initial cell exposure to 460 J/m^2) was accompanied by an expansion of a considerable number of narrow regions of the giant mitochondrion (as relative quantity of small mitochondrial profiles is substantially decreased). Such rearrangement of the mitochondrial macrostructure may promote accelerated energy transfer along lengthy mitochondrion membranes.

Increase in the relative surface area of mitochondrial cristae

Considering that cristae play the leading role in respiration and oxidative phosphorylation, their quantization is widely used in microscopy [51, 52]. In the present case, the number of cristae or their area were calculated relative to the mitochondrial profile area on ultrathin sections.

Morphometric analysis of mitochondria in the subsequent generations of yeast cells initially irradiated at 460 J/m^2 demonstrated an increased relative surface area of cristae by 25% relative to control (Table 2).

An increased relative surface of cristae is presently attributed either to new cristae formation [30] or to growth of pre-existing cristae, which can branch, fuse, and divide [30, 53]. Correlation between the relative surface area of cristae and enzyme activity of the respiratory chain as well as between the

cristae surface area and activity of ATP synthesis has been demonstrated for different cells [29, 30, 51, 54, 55]. An increased number of cristae proved to correlate with the increased activity of the membrane-bound enzymes of the electron transport chain and ATP synthase [56, 57]. It is of interest that the increased surface density of cristae in yeast cells activated by red monochromatic light correlated with the activation of NADH-dehydrogenase and cytochrome c oxidase (Fig. 4, point A).

Increased number of mitochondria–endoplasmic reticulum associations induced by an "activating dose"

The presence of the regions of association between mitochondria and endoplasmic reticulum (ER) is beyond a question now [35, 58, 59]. Apposition of the mitochondrion and ER at a distance of 50–100 nm establishes a firm junction between their outer membranes, which is required for Ca^{2+} transfer into mitochondria [33, 60]. We considered the mitochondria–endoplasmic reticulum (MT–ER) associations when the distance between the heterogeneous membranes was ≤50 nm.

After cell exposure to 460 J/m^2 and the following 6 h cultivation, the relative number of MT–ER association regions increased (Table 3). In addition, we established that the total perimeter and the number of cisterns of the cortical ER per cytoplasm area considerably increased relative to the control (Table 3). This means that the rate of ER formation increased in budding cells in the experiment. However, 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 (data not shown). At the same time, the mitochondria from irradiated cells were characterized by an increased variability [40]. The dispersions of the mean mitochondrial indices (profile area, elongation index, number of profiles relative to 1 μm^2 of cytoplasm, and relative number of dumbbell-shaped mitochondria) were higher in the experimental group than in the control ($p < 0.001$; *F*-test). This significant variability of mitochondria in cells exposed to He–Ne laser light was attributed to the division of most cells after 6 h cultivation in the experimental group, while the majority of intact cells did not start division [43]. It has been shown that mitochondria in dividing yeast have an unstable structure [45] due to the high mobility of these organelles during cell budding [61].

Spearman's test showed 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 the experiment and control, respectively; $p < 0.001$ in both cases) [43]. Such correlation could be due to the presence of numerous clusters of Ca^{2+}-releasing channels on the ER surface [32]. At the

Table 3. Changes in endoplasmic reticulum and mitochondria ($\overline{X} \pm S_{\overline{X}}$) in *T. sphaerica* cells 6 h after irradiation with a He-Ne laser [40].

Indices of organelle profiles on cell sections	Control	Irradiation (460 J/m^2)	Difference from control, %	Significance: $p<$
Number of MT-ER associations				
Per 1 μm^2 of the cytoplasm	0.13±0.03	0.22±0.03	69	0.05
Per 1 μm of the mitochondrial perimeter	0.10±0.02	0.18±0.02	80	0.01
Per 1 μm of the perimitochondrial perimeter of ER cisterns *	0.62±0.17	1.16±0.20	87	0.01
ER cistern perimeter per cytoplasm area (μm/μm^2)				
Total ER	0.98±0.06	1.40±0.07	43	0.001
Cortical ER	0.78±0.05	1.06±0.06	36	0.001
Number of ER cisterns per cytoplasm area				
Total ER	1.81±0.12	2.67±0.12	48	0.001
Cortical ER	1.43±0.09	2.02±0.10	41	0.001
Mitochondrial profiles				
Total mitochondria/cytoplasm area ratio	0.103±0.007	0.106±0.007	3	NS**
Number of mitochondria per cytoplasm area	0.79±0.04	0.89±0.06	13	NS

*The cisterns located closer than 160 nm from mitochondria were considered as perimitochondrial;
**NS- not significant difference from control; ER – endoplasmic reticulum; MT- mitochondria

same time, no correlation has been revealed between the number of MT–ER associations and the total mitochondrial perimeter. The absence of correlation in the latter case could be due to both the involvement of as low as 5–20% of the mitochondrial surface in junction formation [33] 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 associations between the mitochondrial and ER membranes in yeast cells after irradiation can result from both the increased length of the ER membranes and the structural variability of mitochondria. 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 Ca^{2+} [50, 62, 63]. At the same time, such irradiation increases the electric membrane potential of mitochondria [64, 65]. These phenomena can be mutually related considering that only highly energized mitochondria can uptake Ca^{2+} [66]. Until recently, an increased concentration of mitochondrial Ca^{2+} in response to He–Ne laser irradiation was documented only within the

first tens of minutes [67, 68, 63]. Our data may indicate a long-term effect of laser light on the mitochondrial capacity to Ca^{2+} uptake.

Damaging effect of laser irradiation at higher doses on mitochondria of the yeast cells of successive generations

Structural damage of mitochondria

Cells exposed to 1150 J/m^2 featured the presence of mitochondrial profiles of an irregular shape with curved contours. Cases of mitochondrial aggregation when two or three organelles contacted or even interfused were observed. Mitochondrial profiles with the matrix separated by solid cristae membrane could be observed [41]. 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 in the control cells and in the cells, precursors of which were irradiated at 460 J/m^2.

Fragmentation of mitochondria

As noted above, yeast cells, progenitors of which were irradiated at 1150 J/m^2, are characterized by numerous discrete mitochondria (Fig. 5 C) resulting from the fragmentation of the giant mitochondrion. This agrees with the morphometric data: the number of mitochondrial profiles per cytoplasm area increased in successive generations of cells initially irradiated at 1150 J/m^2 (Table 2).

Decrease of mitochondrial profiles

Mitochondrial fragmentation was accompanied by a decrease in their mean section area. The distribution of mitochondria by profile area was shifted to the left (1150 J/m^2, Fig. 6 C) relative to control (Fig. 6A) and another experimental variant (460 J/m^2, Fig. 6 B). This is accompanied with a significant increase in the relative content of small mitochondrial profiles with the section areas ≤ 0.06 μm^2 as compared to cells, progenitors of which were sham-irradiated or irradiated at 460 J/m^2 ($p < 0.05$ and 0.001, respectively, Table 2). Moreover, the distance between neighboring mitochondrial profiles decreased after irradiation of progenitors at 1150 J/m^2.

The modifications of mitochondria in successive generations of cells after initial irradiation at 1150 J/m^2 were comparable to the changes of mitochondria after inhibition of metabolism by a number of specific agents. For instance, inhibition of the respiratory activity of *S. cerevisiae* induced mitochondrial profiles with curved contours [45]. Matrix partitioning was observed after functional disturbances of mitochondria including uncoupling of respiration

and oxidative phosphorylation [70] and mutations of the DPR1 and MDM33 genes [71, 72]. Fragmentation of the giant mitochondrion accompanied by the changes in the internal structure of the organelle was observed in different pathologies [70] and after inhibition of respiration and oxidative phosphorylation [46, 73-75]. Mitochondrial fragmentation in yeast cells is attributed to the disturbed balance between the fusion and division of the organelle towards the latter process [17, 76]. 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 [77-79]. The above-listed changes in the mitochondrial structure are similar to those observed in successive generation of *T. sphaerica* cells, the precursors of which were irradiated at 1150 J/m^2.

Thus, damages of mitochondria revealed a disturbance of mitochondrial respiration and oxidative phosphorylation. At the same time, the dysfunction of these organelles is not critical since the yeast cells can divide [6]. A similar phenomenon was observed in *S. cerevisiae* [80].

Concluding remarks

The bulk of the chondriome in budding *T. sphaerica* cells was represented by the giant branched mitochondrion (in the cells, the progenitors of which were either sham-irradiated, or irradiated at 460 J/m^2). Quantitative analysis of the ultrathin cell sections demonstrated the uneven thickness of different branch regions of the organelle. The revealed heterogeneity of the mitochondrial tube thickness in budding yeast cells agrees with the data for other unicellular organisms [16, 71, 81]. It was suggested that the structure of the giant mitochondrion favor the optimal transfer of the transmembrane electric potential [26, 46]. This agrees with the data on the appearance of the giant mitochondrion in human peripheral lymphocytes exposed to He–Ne laser light [82]. The synthesis of ribosomal RNA precursors was activated in the lymphocytes simultaneously [83, 84].

The irradiation at 460 J/m^2 accelerated cell proliferation, activated enzymes of the mitochondrial respiratory chain, and induced three types of changes in the giant mitochondria of yeast cells cultured for 18 h after the light exposure. *First*, the relative content of small organelle profiles (with the section area ≤0.06 μm^2) decreased and the mean profile area increased by 53% that pointed out to the dilatation of narrow regions of mitochondrial branches. This modification of mitochondria was due to the matrix expansion, while the organelle microstructure remained normal. The dilatation of narrow regions of the giant mitochondrial tube as a result of prolonged effect of irradiation could provide more favorable conditions for energy transfer as compared to the conditions for the giant mitochondrion in the corresponding control cells.

Second, the mean distance between the nearest mitochondrial profiles increased that indicates a less dense packing of the mitochondrial branches. *Third*, the relative surface area of the cristae, whose membranes contain enzymes of the respiratory chain 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 [e. g., 30]. The similar functional changes were revealed in cultured yeast and HeLa cells after exposure to He–Ne laser light [8, 85].

A higher irradiation dose (1150 J/m^2) induced fragmentation and structural damage of mitochondria in the progeny cells. Such changes in mitochondria are commonly attributed to the inhibition of their respiratory and phosphorylation functions. Essentially, the changes in most mitochondrial indices in cells, the progeny of which were irradiated at this dose, had the opposite direction relative to those in the cell cultures initially irradiated at 460 J/m^2 (Table 2). Hence, prolonged effect of laser irradiation on the structure and function of mitochondria is dose dependent: irradiations at 460 J/m^2 or at 1150 J/m^2 produce either activating or inhibiting effects, respectively.

Thus, the successive generations of cells initially exposed to He–Ne laser light demonstrated a number of dose-dependent quantitative and qualitative changes in their mitochondria. The conservation of the modified mitochondria in the progeny cells could be due to the inheritance of the 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 [86]. Recall that 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 could be due to the impact of secondary messengers such as reactive oxygen species and calcium ions as well [32].

In addition, our experiments showed increasing number of MT–ER associations in cultured yeast cells 6 h after exposure to 460 J/m^2 [44]. This shift was accompanied by lengthening of ER profiles and increasing in variability of mitochondrial structure (Table 3). It is well documented that appositions of mitochondria and ER at 50–100 nm enhance contacts between heterogeneous outer membranes of these organelles [33, 58, 60]. The contacts between membranes can have bifunctional significance. First and foremost, these contacts of membranes take part in transfer of phospholipids ([58] and Refs therein). It is well established that phospholipids are necessary for growth of cellular membranes during yeast budding. An increase in the number of MT–ER associations in our case could be necessary for enhancement of cell proliferation rate after laser irradiation at 460 J/m^2 [40]. On the other hand, it was observed that labeled Ca^{2+} (in living HeLa cells) transfers from the ER to mitochondria just in the regions of MT–ER associations [33]. The

accumulation of Ca^{2+} in mitochondrial matrix results in activation of some metabolic processes not only in the mitochondrion, but in the cytoplasm as well [34, 60]. Accordingly, the increased number of MT–ER associations in yeast cells induced by irradiation can reflect (at least in part) the increased Ca^{2+} uptake by mitochondria followed by intensification of Ca^{2+}-dependent metabolic processes, including mitochondrial ATP synthesis. It is known that mitochondria can function as a Ca^{2+} buffer, which slowly releases Ca^{2+} as its cytosolic concentration decreases [69]. Also, a short Ca^{2+}-flash into the mitochondrion and cell can result in metabolic shifts continuing several hours [69].

Summing up, the presented experimental material allows to conclude that the irradiation at λ=632.8 nm induces some changes (possibly of genetic origin) in the mitochondrial ultrastructure simultaneously with an enhancement of activity of respiratory chain enzymes that are expressed in ~7^{th} generation of initially exposed cells.

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