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REARRANGEMENTS OF MITOCHONDRIAL ULTRASTRUCTURE IN DESCENDANTS OF YEAST CELLS IRRADIATED WITH He-Ne LASER

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Abbreviations: ER – endoplasmic reticulum
mit – mitochondrial
PDT – photodynamic therapy
ER-M– links between endoplasmic reticulum and mitochondria
ER-PM – links between endoplasmic reticulum and plasma membrane

Abstract

He–Ne laser irradiation of *Torulopsis sphaerica* yeast at 460 J/m^2 accelerates cell proliferation and activates mitochondrial (mit) respiratory chain enzymes NADH-dehydrogenase and cytochrome c oxidase. Mit rearrangements in the descendants of the cells irradiated and then cultivated for 18 h were investigated by transmission electron microscopy. Morphometric analysis revealed the mitochondria changes on the thin sections passing through the branched mit network of the cells from yeast cultures initially irradiated at 460 J/m^2: increasing of the relative surface area of the cristae responsible for oxidative phosphorylation as well as loosening of the packing organelle branches. Also, the mit network was expanded: relative quantity of the smallest mit profiles decreased. On the contrary, the yeasts initially irradiated at 1150 J/m^2 were characterized by fragmentation of mit network and damaging organelle microstructure. The mit structural modifications under the laser irradiation aftereffect at 460 J/m^2 may be due to primary of photon absorption by cytochrome c oxidase followed by elevating bioenergetics and forming secondary messengers. We received indirect evidences of enhanced Ca^{2+} concentration in mitochondria and endoplasmic reticulum (ER) in the yeast, cultivated for 6 h after irradiation at 460 J/m^2: increasing the number of association sites of (a) ER with mitochondria and (b) ER with plasma membrane. Raising quantity of these organelle links may provide long-term Ca^{2+} signaling, influence on gene expression and cell proliferation.

Keywords: He-Ne laser irradiation, Budding yeast, Electron microscopy, Mitochondria, ER–mitochondria associations, ER–plasma membrane associations, Ca^{2+} signaling.

1. Introduction

Radiation of red-to-near IR spectral region is used both in photodynamic therapy (PDT) and laser phototherapy. One of practically not investigated problems in both laser phototherapy and PDT is whether low-intensity irradiation with monochromatic light of visible-to-near IR spectral regions can cause long-term effects, appearing in following cell generations. It is well documented that monochromatic light in this spectral region can be used on cell and organism level for therapeutically purposes (review [1]). Experimental data considering possible hazardous (e. g. mutagenic) effects of the light are not numerous so far. 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 chromatid exchanges in sheep peripheral blood mononuclear cells was found to be significantly increased after irradiation with a He-Ne laser 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 at 632.8 or 660 nm used in works [1–4] could 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 DNA (and RNA also), as these molecules do not have absorption bands in the visible spectral region. So, these results can not be explained by action of visible light on DNA, and one has to suppose indirect effects. On the other side, the proliferation rate of mammalian cells *in vitro* [5] as well as yeasts cultures [6, 7] was increased after short-time irradiation. These data evidence that some mutagenic effects can be involved. It is possible that the effects were developed through secondary messengers.

This review summarizes our experiments performed to study the long-term effects of low-energy He-Ne laser (λ=632.8 nm) irradiation on yeast cells cultured in aerobic conditions [6-10]. The main goal of these studies was to investigate whether the activation of mitochondrial metabolism in successive generations of initially irradiated cells is correlated with ultrastructure changes of the mitochondria. Budding yeasts are convenient models to investigate prolonged effects of the radiation on the growth and division of initially exposed cells. The reason is that the generation time (the period between two successive divisions) for yeast is much shorter (some hours) as compared to that for mammalian cells.

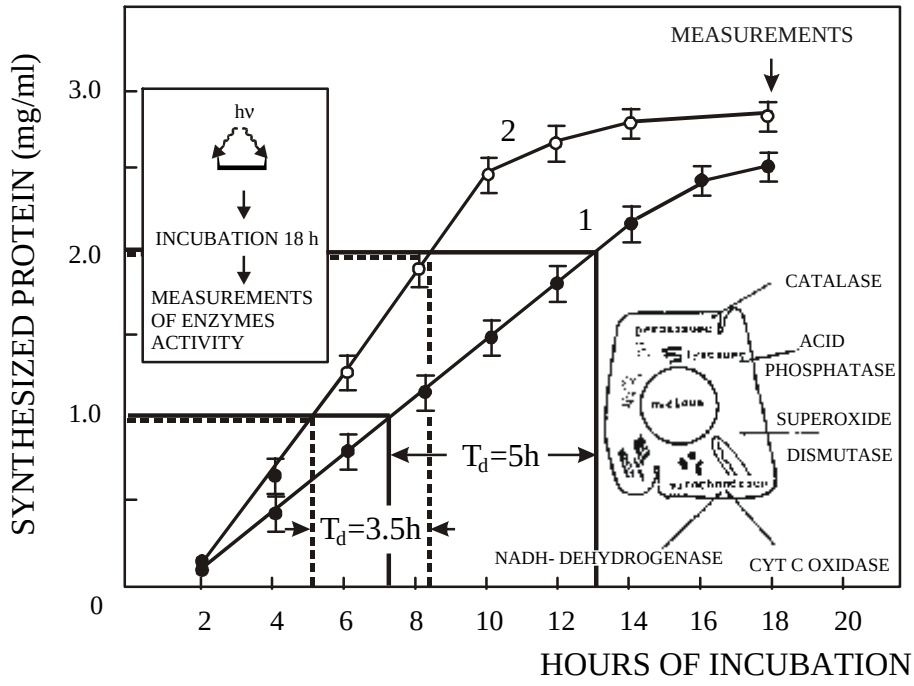

Fig. 1 Growth curves of *Torulopsis sphaerica* yeast measured as a change in the amount of synthesized protein in (1) intact and (2) irradiated with a He-Ne laser at dose of 4.2×10^3 J/m^2 culture. Generation times in the exponential phase of growth are marked for control and irradiated cells. The inset 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 [6-10] is used in this Figure.

Exposure of *Torulopsis sphaerica* cells with He-Ne laser light at 4.2 x 10^2 J/m^2 did not change the duration of the lag-period of the growth of the cultures estimated by both biomass accumulation (i.e. the amount of total protein) as well number of cells and buds [6, 7]. Substantial shortening of generation time (or increasing proliferation rate) occurred in the log-phase of growth of the yeast culture. Figure 1 illustrates decreasing the generation time from 5 to 3.5 h of the *T. sphaerica* cells, which were initially irradiated with He-Ne laser. The maximal level of protein synthesis was achieved 14 – 18 h after irradiation. The increased accumulation of the yeast biomass was directly proportional to the increase in the number of cells and buds within log-phase of growth. What this means is that the size of the cells and the amount of total protein per single cell apparently do not differ for the exposed and unexposed cultures. Thus, irradiation with He-Ne laser speeds the cells division at dose of 4.2 x 10^2 J/m^2.

Table1. Changes in the activity of enzymes, localized in different organelles in descendants of *T. sphaerica* cells, initially irradiated with a He-Ne laser (4.6x 10^2 J/m^2), then cultivated for 18 h (adapted from [9])

Enzymes	Localization	Activity (% of control level)	
NADH-dehydrogenase	Mitochondrion	241.2±10.1	$p < 0.001$
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

Descendants of the exposed yeasts are characterized by change in activity of various enzymes located in the relevant cell organelles [8-10] (Table 1). An important point is that enzymes of oxidative metabolism, localized in mitochondria, were significantly activated by the irradiation. It is necessary to stress, that the rearrangements of cell metabolism were retained in the cells of approximately 7th generation (after 18 h cultivation of initially exposed cells).

Thus, the low-intensity laser irradiation activated the growth of yeast cultures. Moreover, the effects of a short-term exposure were manifested in the distant progeny of irradiated cells. Below we will examine changes in mitochondrial ultrastructure of the descendants of the irradiated yeast cells. The reasons for studying the mitochondria were, first, the location of photoacceptor in the mitochondria [11]. Second, the mitochondria have their own DNA and genes that encode some subunits for NADH-dehydrogenase, cytochrome *c* oxidase, ATP synthase and other proteins. It is also known that expression of mitochondrial genes is partially regulated by energy demand [12]. It is notable that yeast cells are characterized by non stable mitochondrial genome and the mutations of mitochondrial DNA result in appearance of mitochondria with changed phenotype [13].

2. Mitochondrial ultrastructure in eukaryotic cells

The studies on living cells demonstrated the plasticity of mitochondria: they are permanently moving, dividing and fusing [14]. As demonstrated for budding yeast *Saccharomyces cerevisiae*, these processes are responsible for the shape of the mitochondria network [15-18]. The equilibrium between processes of dividing and fusing is regulated on the genetic level [19, 20]. The majority of eukaryotic cells, including yeasts, has branched mitochondrial network [15, 21-23]. Such network is significant for the maintenance of the mitochondrial DNA level [24], provision of daughter cells with the complete set of mitochondrial genes [25] and energy transfer [26]. Mitochondrial network (or reticulum) is maintained during the whole cell cycle of budding yeasts except mitosis, when the mitochondrial network is fragmented (Figure 2). After mitosis, the small discrete mitochondria fuse forming branched elongated network [19].

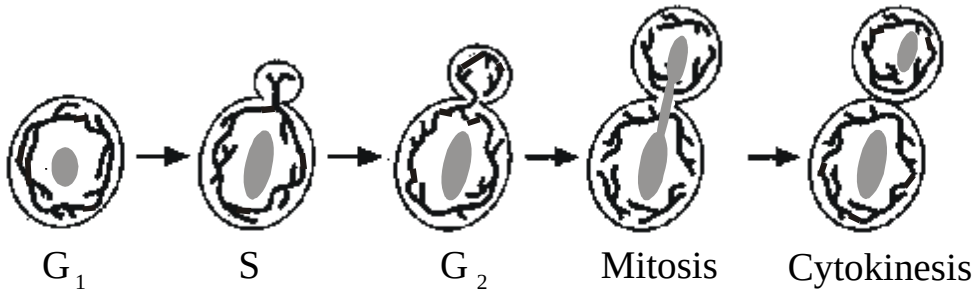

Fig. 2. Integration of mitochondria in budding yeast cells into the common network at all stages of the cell cycle (G_1, S, and G_2) except mitosis. Mitochondria and nucleus are shown in black and gray, respectively. Modified from [19].

The inner boundary of mitochondrial membrane adjacent to the outer membrane increases its surface by the cristae. The length of the cristae membranes is in average 1.5-fold longer compared to that of the inner boundary membrane [27]. Oxidative phosphorylation complexes are preferentially located in the cristae membranes [28]. The cristae are highly variable, depending on the type and metabolic activity of the cells [29]. It is known that the number of cristae and their surface area correlate with the energetic activity of the respiratory chain [30, 31]. According to current views, the cristae are not just folds of the inner boundary membrane but associate with it via narrow tubules (Figure 3), which are dynamic structures, influenced on the cristal organization [32].

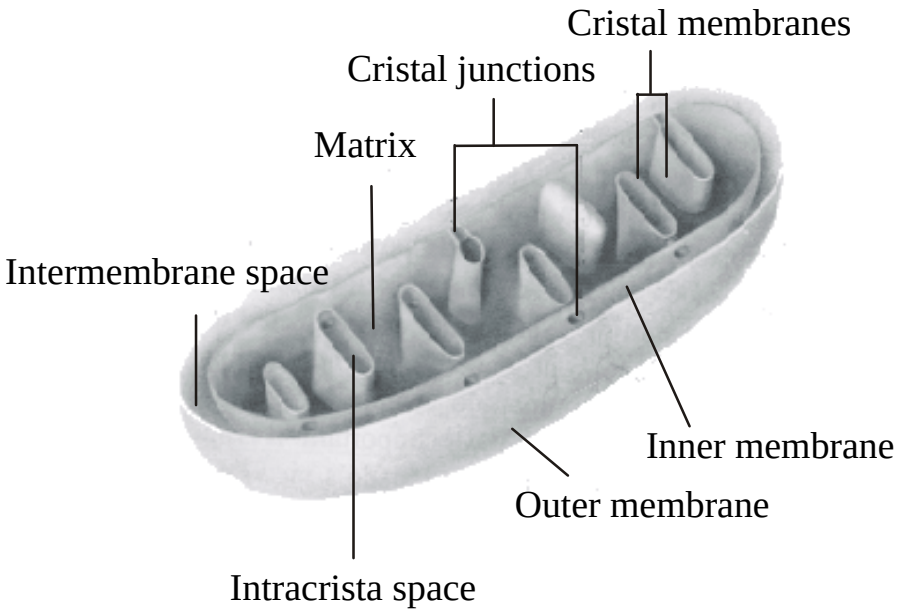

Fig. 3. A current model of mitochondrial structure ([92], with modifications).

In addition to the energy generation, mitochondria are the hub of cellular Ca^{2+} signaling [33]. It is known that long-term Ca^{2+} signals may control transcriptional programmes and cell proliferation [34, 35]. Ca^{2+} transport from endoplasmic reticulum (ER) to mitochondria takes place in sites of association of both organelles. Application of Ca^{2+}-sensitive marker allowed tracing Ca^{2+} migration in living HeLa cells from ER to the narrow cytoplasm layer between ER and mitochondrion and further into the mitochondrial matrix [36]. Space interactions of ER and mitochondria (ER-M) as well ER and plasma membrane ER-PM) are important for regulation of Ca^{2+} signaling in cells [37] as are for exchange of phospholipids between membranes of the linked organelles [38-40]. $Ca^{2+}{}_m$ signals (via ER-M links) influence both mitochondrial bioenergetics and cascades of Ca^{2+} signaling pathways in cells under various stimuli [41-43]. The ER-PM links serves to refill exhausted ER Ca^{2+}store by calcium from extra cellular space [34].

High changeability is characteristic of the mitochondria, which was observed both in physiological conditions (in aerobic budding yeast) and at pathologies (in various mammalian cells). For example, fragmentation of mitochondrial network accompanying of damaging the organelles has been studied extensively at apoptosis, cellular aging, and mitochondrial diseases as well as under inhibition of the oxidative phosphorylation complexes [29]. However, very few studies consider mitochondrial structure in metabolically activated cells. The activation of mitochondrial respiratory chain and ATP synthesis were typical for yeast cells irradiated by low-energy laser [8, 44]. Below is shown that similar functional shifts are correlated with mitochondrial rearrangement as well as increase in number of ER-M and ER-PM association sites. Changing spatial organization of the mitochondrial network, its inner structure as well interrelation of mitochondria with other organelles influenced on proper mitochondrial bioenergetics as well as essentially cellular functions [29]. It has been supposed that changes in mitochondrial metabolism under low-energy laser light (in red-to near IR spectral regions) resulted in transmission of signals from mitochondria to the nucleus via cytoplasm [11, 50]. However, details of the retrograde mitochondrial signaling mechanism remain to refine using the electron microscopy.

3. Stimulation of growth of *T. sphaerica* culture under the laser irradiation is dose-dependent

Low-intensity monochromatic light with λ = 632.8 nm at respective doses exerted an activating effect on the vegetative growth of yeasts that were cultivated under aerobic conditions in a nutrient medium containing 1% glucose [6, 7]. Yeast cells studied (*Candida maltosae, S. cerevisiae, Saccharomycodes ludwigii, T. sphaerica*) differed in photosensitivity, which depended on cell metabolism pattern [7, 10]. It was higher in cells capable for rapid changes in response to change in cultivation conditions and to the action of various chemical and physical agents.

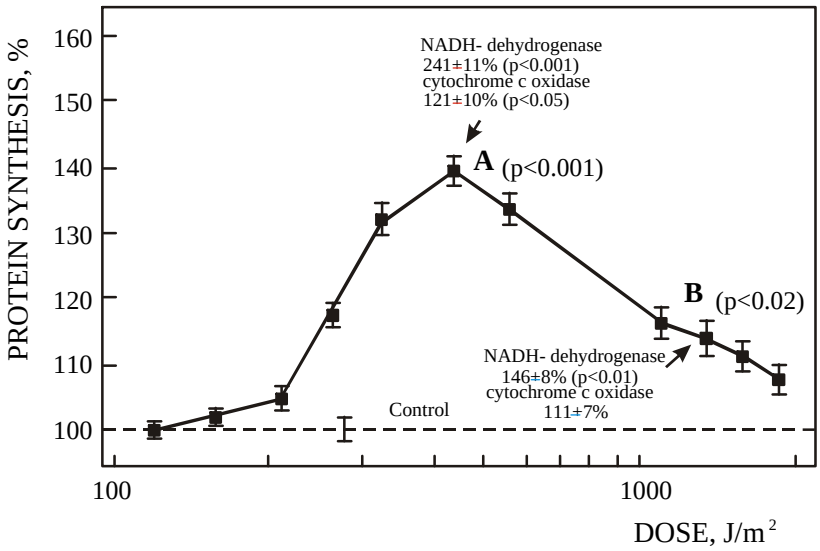

Fig. 4 The amount of protein (% of control) synthesized in cultured *T. sphaerica* cells 18 h after exposure to different doses of He-Ne laser light. NADH-dehydrogenase and cytochrome c oxidase activities relative to control after irradiation at 460 and 1150 J/m^2 are shown in points A and B, respectively.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

A pronounced sensitivity to monochromatic light with λ=632.8 nm is characteristic for the yeast of T. *sphaerica* relating to facultative anaerobe [7, 8]. The optimal dose of irradiation by this light was established on the basis of the dose dependence found from the amount of the total cellular proteins in yeast cultivated 18 h after the irradiation (Figure 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 elevated activity of NADH-dehydrogenase and cytochrome *c* oxidase (Figure 4, point A). The lowering of the protein synthesis level following irradiation at a dose of 1150 J/m^2 (Figure 4, point B) was accompanied by a decrease in the activity of both the respiratory enzymes.

Above-mentioned results formed the basis for electron microscopic study of budding *T. sphaerica* yeasts. The impact of He-Ne laser irradiation on the mitochondrial ultrastructure was studied in approximately 7th generation of exposed yeast cells [45-47]. No dividing cells were irradiated in phosphate buffer and then both sham-irradiated and irradiated cells were transferred to synthetic Reader' medium [48] with 1% glucose and incubated in darkness at intensive aeration. Finally, the sham-irradiated and irradiated cells were studied in late log-phase of growth (after 18 h of cultivating). So, in the control, the proliferation was induced by the nutrient medium and in the experiment – by the nutrient medium plus laser light.

4. Modification of mitochondria structure in the yeast-descendants after initial irradiation at 460 J/m^2 correlates with activation of respiratory chain enzymes

4.1. Conservation of some qualitative features of the mitochondria

The spatial reconstruction of mitochondria by the serial thin sections of the *T. sphaerica* yeast cell from cultures reaching late log phase of growth, show the presence of elongated branched mitochondrial network and 3-4 of small organelles per cell [46, 47]. These organelles were localized at periphery of the cell that was completing nuclear dividing.

3D mitochondrial organization was similar in the cells from irradiated and no irradiated cultures (Figure 5 A, B). Mitochondrial profiles on the random thin sections in the descendants of irradiated cells were characterized by developed cristae having the usual orientation and constant width of the intracristal and intermembrane spaces (~30 nm). The cells did not show any of the signs of the mitochondrial damage (matrix swelling or contraction, as well as membrane destruction). This finding pointed to the normal functioning of the organelles.

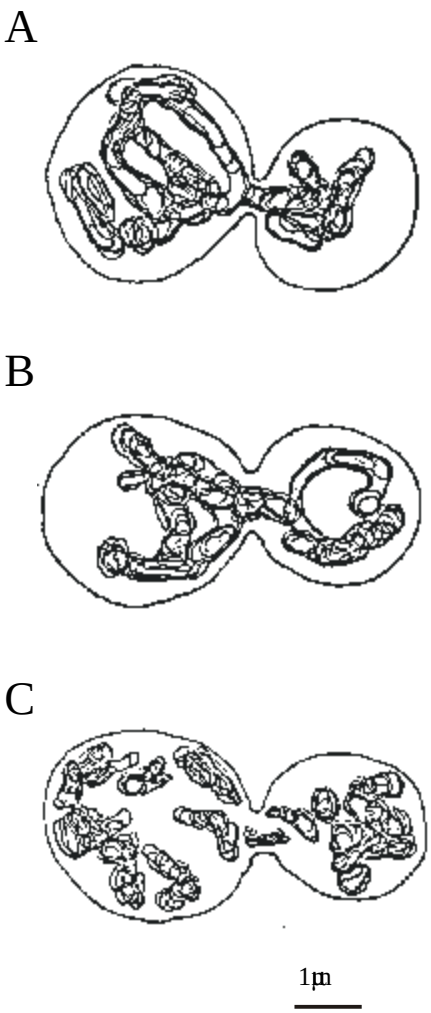

Fig.5. The spatial reconstruction of the mitochondria in the yeast cells-descendants after (A) sham-irradiation or irradiation with a He-Ne laser at a dose of (B) 460 J/m^2 or (C) 1150 J/m^2. Mitochondria in budding yeasts were reconstructed in the cells when dividing of nucleus was completed. Adapted from [46].

4.2. Changes in the global configuration of mitochondrial network

Quantitative analysis performed on random cell sections demonstrated significant variation in the area of mitochondria profiles (Figure 6 A, B). The test of proportion differences demonstrated nearly twice lower relative number of smallest mitochondrial profiles ($\leq 0.06\ \mu m^2$) and higher relative number of large profiles ($> 0.33\ \mu m^2$) in experiment vs. the control (Table 2).

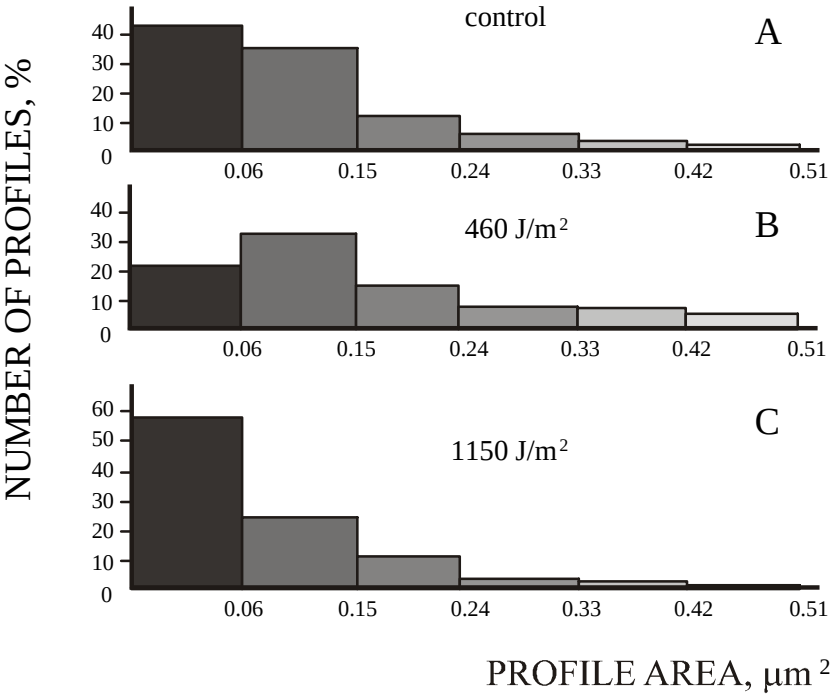

Fig.6 The distribution of mitochondrial profile areas in cells-descendants after (A) sham-irradiation or irradiation with a He-Ne laser at (B) 460 J/m^2 and (C) 1150 J/m^2 and following cultivation for 18 h (adapted from [45, 47]).

Table 2. Changes in mitochondria on ultrathin sections of *T. sphaerica* yeasts in response to the irradiation of the cells-progenitors following by 18 h of cultivation ($\overline{X} \pm S_{\overline{X}}$). Adapted from [45, 47].

Indices of mitochondrial (mit) profiles on cell sections	Control	Irradiation at 460 J/m^2	Irradiation at 1150 J/m^2
Number of mit 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 mit profiles per cell section, μm^2	0.64±0.07	0.89±0.11 NS	0.67±0.07 NS
Area of mit profile, μm^2	0.17±0.02	0.26±0.02 $p < 0.001$	0.14±0.01 NS $p' < 0.001$
Distance between nearest mit profiles, μm	0.39±0.02	0.50±0.03 $p < 0.01$	0.25±0.02 $p < 0.001$ $p' < 0.001$
Cristae area /mit profile area, μm^2 / μm^2	0.20±0.01	0.25±0.01 $p < 0.001$	0.19±0.01 NS $p' < 0.001$
Content of smallest mit profiles (≤ 0.06 μm^2), %	41	22 $p < 0.05$	55 $p < 0.05$ $p' < 0.001$
Content of large mit profiles (>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– not significant difference from control.

Figure 6, B shows that the distribution of mitochondrial profiles by areas in the experiment was shifted to the right as a result of decreased percentage of the smallest organelle profiles.

Thus the mit network in the budding yeast cells consists of regions of uneven thickness, whose distribution depends on a dose of the laser irradiation [45-47]. Confocal fluorescent microscopic studies has been conformed to these results [29, 49]. The authors showed that the interior of elongated mitochondrion was separated into fragments. It has been supposed that the matrix compartment can create barriers which retard the diffusion of energy substrates and metabolites as well Ca^{2+} diffusion along mitochondrial tube [29]. Our results from decreasing significant quantity of the smallest mitochondrial were due to the expansion of very thin regions of the elongated mitochondrial tube. The expansion of mitochondrial network in the result of laser irradiation aftereffect can be also connected with increasing energy production as well as more effective transfer of signal elements. Elevating ATP synthesis and appearance a number of signal elements inside mitochondria have been observed in different cells under low energy laser irradiation [11, 44, 50-52].

4.3. Quantitative changes in mitochondrial structure in the progeny of irradiated yeast

According to the results of 3D analysis, relatively straight regions amounted to around 90% of the mitochondria network both in control and after irradiation at 460 J/m^2. Consequently, the mitochondrial profiles revealed on the thin cell sections largely belonged to the mitochondrial network. No differences in the number of mitochondria and their total area related to the cytoplasm area have been revealed between the experiment (irradiated at 460 J/m^2) and the control (Table 2). It is necessary to underline that the area of different cell structures, measured on random cell sections was proportional to their volume in the whole cell [53]. The invariable volume of mitochondria per cell volume could be due to asynchronous division of yeast cells in both cell populations, irrespective of high (in the experiment) and low (in the control) cell cycle rate. Total mitochondrial volume is proportional to the cell volume at all stages of the cell cycle [29].

At the same time, other properties of mitochondria differed. The mean area of mitochondrial profiles was increased by 53% in the descendants of irradiated cells in comparison with the control (Table 2). This increased mitochondrial surface area was due to the expansion of the matrix, since the width of the intermembrane and intracristal spaces remained nearly constant. The data above mentioned were conformed to the results of previous part 4.2. It is pertinent to repeat that matrix of such mitochondria did not swell. It has been shown that the increased matrix volume in the physiological limits can be accompanied by the accumulation of inorganic pyrophosphate and activation of the mitochondrial respiratory chain [54].

In addition, in the cells from the exposed cultures the average distances between nearest mitochondrial profiles were increased vs. the control ($p<0.01$) (Table 2).The more loose packing of the branches of mitochondrial network may facilitate its oxygen supply.

4.4 Increase in the surface area of the cristae

As far as the cristae play a leading role in respiration and oxidative phosphorylation [28], their quantitative estimation is widely used in microscopy. Morphometric analysis of the mitochondria in the progeny of irradiated cells demonstrated an increased surface area of cristae by 25% relative to control (Table 2). This fact points to increased surface area of the cristae membranes, since the width of intracristal space was not changed [55].

An increased relative surface area of the cristae is presently attributed either to new cristae formation on the inner mitochondrial membrane [32] or to growth of pre-existed cristae [30, 56]. Correlations between the surface area of cristae and activity of the respiratory chain enzymes as well as between the cristal surface areas and energy activity of mitochondria have been demonstrated for different experimental models [30, 57-59]. An increased number of cristae proved also to correlate with the activity of the membrane-bound enzymes of electron transport chain and ATP synthase [28, 60]. In our case increasing the relative area of the cristae in yeast cells, whose progenitors were irradiated at 460 J/m^2, is consistent with the activation of NADH-dehydrogenase and cytochrome *c* oxidase, i. e. with the mitochondrial respiratory chain activation (Figure 4, point A). Considering that electron transport via enzyme complexes of respiratory chain is completed by ATP production [29], above-mentioned cristae changes can connect with rising mitochondrial bioenergetics. Evidence of enhancing ATP level was obtained in the dividing HeLa cells initially irradiated by He-Ne laser [44].

5. Increase in number of association sites of the organelles (ER-M and ER-PM), responsible for Ca^{2+} signaling after 6 h cultivation of the yeast irradiated at 460 J/m^2

Action of different stimulating agents results in appearance Ca^{2+} signaling. Ca^{2+} signals influence both short-term (for some minutes) and long-term (for some hours and more) functional changes in cells. Prolonged increasing of Ca^{2+} signals in the cell activates its growth and proliferation [34, 35]. Physical links between ER and mitochondria (ER-M) [36, 38, 43, 61] as well between ER and plasma membrane (ER-PM) are related to Ca^{2+} signaling [35-37, 39, 61-63].

The mitochondria play an important role in forming of Ca^{2+}signaling [33, 43]. It is known that respiring and energized mitochondria are capable of (i) uptake of Ca^{2+} released from ER; (ii) buffering Ca^{2+} inside the matrix, and (iii) efflux of Ca^{2+} into cytoplasm [33].

Formation of connections between ER and mitochondria in yeast and mammalian cells is favored by localization of the most parts of ER and mitochondrial network in cortical cytoplasm layer, where both mobile networks interweave with each other [36]. Close appositions of the cortical ER to PM are favorable to form physical and functional connections of the organelles [63]

Table 3. Changes in the number of association sites of ER with mitochondria (ER-M) and ER with plasma membrane (ER-PM) on ultrathin sections of T. *sphaerica* yeasts in response to 460 J/m^2 irradiation of the cells-progenitors following by 6 h of cultivation ($\overline{X} \pm S_{\overline{X}}$) ([64,65]).

Number of the association sites of the organelles on cell sections	Control	Irradiation at 460 J/m^2	Difference from control, %	$p <$
Number of ER-M association sites / cytoplasm area	0.13 ± 0.03	0.22 ± 0.03	69	0.05
Number of ER-PM association sites / cytoplasm area	0.70 ± 0.14	1.14 ± 0.08	63	0.01

It is known that the apposition of ER to mitochondria and ER to plasma membrane at a distance of about 50 nm establishes a firm junction between membranes of the organelles that is required for Ca^{2+} transfer from extra cellular medium inside ER and from ER into mitochondria [36, 38, 40]. We examined the ER-M and ER-PM association sites when the distance between suitable adjacent membranes was ≤ 50 nm. After the exposure of the yeast to He-Ne laser irradiation at 460 J/m^2 and following 6 h of cultivation, the number of membrane links of both ER-M and ER-PM per cytoplasm area increased by 63% and 69%, respectively (Table 3) [64].

It is notable that the measured features of the ER and mitochondria in the exposed cells differ (Table 4). The relative length of total and cortical ER membranes after irradiation increased vs. control. It may mean that the rate of ER cisterns formation prevails over their separation between two dividing cells. At the same time, the area of mitochondrial profiles per cytoplasm area (proportional to their relative perimeter) remained virtually unaltered. However, the mitochondria were characterized by increased variability, which was attributed to the division of the most yeast cells initially irradiated at 460 J/m^2 with following 6 h of cultivation [65]. It is known that mitochondria in dividing yeasts have an unstable structure [15], which is connected with the high mobility of the mitochondria in the budding yeast [66]. In mechanistic terms, the increased number of ER-M association sites in the progeny of irradiated yeast is a result from increasing length of the ER membranes as well as from structural variability of mitochondria.

Table 4. Changes in quantitative parameters of *T. sphaerica* cells, after irradiation of initial yeast cultures at 460 J/m^2 following by cultivation for 6 h ($\overline{X} \pm S_{\overline{X}}$) (after [64, 65]).

Features of cellular compartments on ultrathin sections	Control	Irradiation at 460 J/m^2	Difference from control, %	$p <$
Relative length of ER membranes/ cytoplasm area total ER cortical ER	 0.98 ± 0.06 0.78 ± 0.05	 1.40 ± 0.07 1.06 ± 0.06	 43 36	 0.001 0.001
Relative area of mitochondria/ cytoplasm area	0.103 ± 0.007	0.106 ± 0.007	2.9	0.75 not significant
Area of cell profile, μm^2	6.48 ± 0.22	5.09 ± 0.18	−21.5	0.001
Area of cytoplasm profile, μm^2	5.70 ± 0.22	4.43 ± 0.16	−22.3	0.001
Area of mitochondria in the cell section, μm^2	0.61 ± 0.05	0.46 ± 0.03	−24.6	0.017

It is of interest the changes in quantitative parameters of the yeast cells cultivated 6 h after the irradiation at 460 J/m^2. The analysis revealed that mean areas of the cell, the cytoplasm and all mitochondria per the cell section decreased in the irradiated cells vs. control (Table 4). This means that after 6 h of cultivation the majority of initially irradiated cells enters in early log phase of the culture growth (possibly, in the first division cycle), while their intensive growth is still not beginning. To this time the majority of the intact cells do not start division.

On the basis of present knowledge to functional significance of the ER-M and ER-PM connections [33, 67], it can accept following interpretation of the data given in Table 3.The effects of the irradiation on the yeast cells-descendants include enhancing mitochondrial uptake of calcium released from ER (quantity of ER-M links increased). Simultaneously ER Ca^{2+} store is refilled by calcium from extracellular space (quantity of ER-PM links increased). Strengthening of the organelle association process during rather long period after initial radiation of the yeast is a new finding.

6. Damaging effect of the laser irradiation at 1150 J/m^2 on mitochondria in the yeast cells-descendants

6.1. Fragmentation of mitochondrial network

Aftereffect of the irradiation at 1150 J/m^2 caused fragmentation of the mitochondrial network, revealed by 3D reconstruction of the organelles. With reference to Fig. 5-C, it can be seen that the cells-descendants were characterized by numerous discrete mitochondria. This fact was agreed with quantitative data received on random thin cell sections: the number of mitochondrial profiles per cytoplasm area increased in descendants initially irradiated cells (Table 2) [45, 47]. Fragmentation of mitochondrial network was accompanied by a decrease in mean area of mitochondrial profiles (Table 2). The distribution of mitochondria by profile area shifted to the left (Figure 6-C) relative to control (Figure 6-A) and to other experimental variant (Figure 6-B). The reason is that content of the smallest mitochondrial profiles (with area ≤ 0.06 μm^2) increase by 34% vs. the control and by 150% - vs. experiment using 460 J/m^2 dose (at $p <$ 0.05 and < 0.001, respectively (Table 2).

Besides, of the distances between nearest mitochondrial profiles decreased in the cells under

consideration (Table 2). Thus the discrete mitochondria were closely packed as compared to packing branches of the mitochondrial network in the control and in the experiment using 460 J/m^2 dose (Table 2).

The cell population under consideration is characterized by presence of dumbbell-shaped mitochondria with deep constrictions [68]. 3D analysis showed that such constriction no divides the organelle completely.

6.2. Damages in mitochondrial microstructure

Cells of the cultures exposed to 1150 J/m^2 and then cultivated 18h featured the presence of mitochondrial profiles of irregular shape. The cases of mitochondrial aggregation were also observed, when two or three organelles contacted or even interfused. Mitochondrial profiles with the matrix separated by septa have been also found. The matrix of some aggregated mitochondria contained areas of low electron density without cristae or with changed cristae orientation. Such injured mitochondria were absent in the control and in the population of cells from the yeast cultures initially irradiated at 460 J/m^2.

Thus, the changed mitochondria in cells-descendants of yeast culture irradiated at 1150 J/m^2 evidence the fragmentation and damaging of the discrete organelles, which may be connected with their dysfunction. However, these cells retain capacity to diving [8]. A similar phenomenon was observed in *S. cerevisiae* [69].

7. Concluding remarks

The bulk of the mitochondrial profiles on random cell sections through budding *T. sphaerica* yeast are related in 3D branched mitochondrial network. Such network is inherent in descendants of both control and irradiated at 460 J/m^2 cells. Quantitative analysis of mitochondria on random ultrathin cell sections demonstrated the uneven thickness of different regions of mitochondrial network. Similar mitochondrial heterogeneity was inherent in some other unicellular organisms [70, 71]. Previously we observed appearance of lengthened branched mitochondria in human blood lymphocytes under low-energy He–Ne laser irradiation [72] with simultaneous rearrangements of nucleolus and nuclear chromatin, which reflected the activation of synthesis of rRNA and mRNA precursors [73, 74].

The irradiation of *T. sphaerica* cells at 460 J/m^2 accelerated cell proliferation, activated enzymes of the mitochondrial respiratory chain as well as induced the following ultrastructural changes in mitochondria in the cell-descendants.

First, the relative content of small organelle profiles with the area ≤ 0.06 μm^2 decreased by 46 % and the mean mitochondrial profiles area is increased by 53%. In other words, significant part of mitochondrial network is expanded. This modification of mitochondrial network is due to expansion of the matrix. An important point is that the microstructure of these organelles remained unchanged. The dilatation of a great deal of narrow regions of mitochondrial network under low-energy He-Ne irradiation can provide conditions that are more favorable for ATP synthesis [54], transfer of energy ($\Delta\psi$) [26] as well as transmission of Ca^{2+} signals.

Second, the mean distance between the nearest mitochondrial profiles increased that indicates a more loose packing of the mitochondrial branches. This may promote accessibility of the organelles for energy metabolites and O_2.

Third, the surface area of cristae, whose membranes contain enzymes of the electron transport chain and ATP synthase, increased. Similar changes of cristae observed in other biological models are attributed to the activation of respiratory chain enzymes and ATP synthesis. The analogous functional shifts were revealed as a result of aftereffect of He–Ne laser irradiation on cultured cells of *T. sphaerica* yeast (increasing activity of NADH-dehydrogenase and cytochrom c oxidase) and *HeLa* cells (increasing ATP production) [8, 44]

On the contrary, irradiation at 1150 J/m^2 induced fragmentation and structural damages of mitochondria microstructure in the progeny of exposed cells (after 18 h cultivating). The modification of mitochondria in cells-descendants after irradiation of initial yeast cultures at 1150 J/m^2 was similar to the changes of mitochondria after cell metabolism inhibition by a number of specific agents. For instance, inhibition of respiratory activity of *S. cerevisiae* induced appearance of mitochondrial profiles with curved contours [15]. Matrix partitioning by solid cristae was observed after functional disturbances of mitochondria under uncoupling agents [75] as well as of the DPR1 and MDM33 gene mutations [70, 76]. Fragmentation of mitochondrial network accompanied by the damages in internal structure of the organelles was observed in different pathologies [75] and after inhibition of mitochondrial respiration and oxidative phosphorylation [77-79]. As a whole, mitochondrial fragmentation in yeast cells is

attributed to the disturbed balance between the fusion and fission processes towards the latter one [80]. It is remarkable that the mutations of *S. cerevisiae* genes encoding mitochondrial fusion factors (MGM1, FZO1, UGO1, and MDM30) induced fragmentation and various structural damages of mitochondria [81-84].

Essentially, the changes in the most mitochondrial indices in cells of the cultures initially irradiated at 460 J/m^2, had the opposite direction relative to those in the cultures irradiated at 1150 J/m^2 (Table 2). In other words, prolonged effects of laser irradiation on microstructure and global organization of mitochondria are dose-dependent: exposure at 460 J/m^2 and 1150 J/m^2 produce activating or inhibiting effects, respectively.

Thus, descendants of the yeast cells initially exposed to He–Ne laser light at 460 J/m^2 were characterized by various modifications of the mitochondrial structure. Conservation of the modified mitochondria in the cell-descendants 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 [85].

It is known that no direct impact of 632.8 nm irradiation on DNA is possible, since this macromolecule absorbs no visible light. Gene mutations responsible for the mitochondrial changes under low-energy laser irradiation can be due to the impact of secondary messengers produced in mitochondria. Mitochondria are just targets for red and near IR light, as they contain cytochrome c oxidase which is considered as the photoacceptor [11]. Intensification of mitochondrial bioenergetics exerts influence not only on mitochondrial but on nuclear DNA as well.

The analysis of signal transmission from the mitochondrion to the nucleus via the cytoplasm under red and near-IR light was presented in review [50]. Changes in different elements of mitochondrial retrograde signaling: $\Delta\psi_m$, ROS, Ca^{2+}, NO*, ATP were also discussed [11].

Some new insight into the long-term Ca^{2+} signaling in the yeast under He-Ne laser irradiation has been gained as summarized in the present paper. The results discussed below have essential significance as Ca^{2+} is an important polyfunctional secondary messenger. It was shown that in the yeast cells, cultivated after irradiation (at 460 J/m^2) for 6 h, the number of ER–M association sites in regions of contacting the organelles increased. It points on the effective mitochondrial Ca^{2+} uptake [36]. Also, it was established that quantity of the ER–PM contacts (serving for entering Ca^{2+} inside ER from extracellular space [86]) increased. It has been accepted that for sustained Ca^{2+} capture by mitochondria (during several hours), continuous refilling exhausting ER Ca^{2+}-store is required [62].

However, there is information that monochromatic red light has also short-term effect on mitochondrial capacity to accumulate Ca^{2+} (within the first minutes after exposition) [87-89]. It is possible that the light exerts influence both short- and long-term Ca^{2+} signaling. For instance, it was established that due to fast uptake and slow efflux of Ca^{2+}, mitochondria are capable to influence on transients of cytoplasm Ca^{2+}, creating long-term Ca^{2+} signals [41].

Capacity of mitochondria to accumulate Ca^{2+} inside matrix results in intensification of ATP synthesis through activation of Ca^{2+}-dependent dehydrogenases in the TCA cycle [41]. High level of ATP influences the cascade of reactions forming long-term Ca^{2+} signals in the cell. It was known that the increased Ca^{2+} level for several hours activated transcriptional factors, which transferred to the nucleus, bound with DNA and finally activates cycle-related genes [90, 91].

It has been known that the maintenance of Ca^{2+} signaling under low power laser irradiation influences on the upregulation of the genes, which results in acceleration of cell proliferation [50, 52]. It can be proposed that the laser irradiation influences both early raising ATP synthesis (ensuring short-term Ca^{2+} signaling) and late $Ca2^{+}$-dependent ATP synthesis (ensuring long-term Ca^{2+} signaling).

Recently, it has been revealed additional function of ATP, as signaling molecule, playing significant role in mitochondrial mechanisms of Ca^{2+} signaling under low intensive laser irradiation [93].

Also, an important peculiarity of mitochondria has been discussed, namely, a connection of oxidative phosphorylation and Ca^{2+} signals propagation with mitochondrial morphogenesis [29]. It was supposed that heterogeneous configuration of mitochondrial network, capable of changing under different conditions, modulates calcium waves and Ca^{2+} signaling [63]. Observed by us expansion of the narrow regions of mitochondrial network in the progeny of irradiated yeast may favor the propagation of Ca^{2+} signals. Another essential phenomenon, revealed in successive generations of the irradiated yeast, is the increase in surface area of the cristae (main carriers of the oxidative phosphorylation complexes) which reflects activation of ATP synthesis. Above mentioned expansion of mit network may also facilitate the distribution of the enery, forming in a result of ATP hydrolisis.

However, the exact correlations between structure and function of the mitochondria in the descendants of the irradiated cells are to be established in the future.

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