ELSEVIER

J. Photochem. Photobiol. B: Biol. 49 (1999) 1–17

Journal of Photochemistry and Photobiology B:Biology

Invited Review

Primary and secondary mechanisms of action of visible to near-IR radiation on cells

Tiina Karu *

Laser Technology Research Center of Russian Academy of Sciences, 142092 Troitsk, Moscow Region, Russia

Received 17 March 1998; accepted 9 November 1998

Abstract

Cytochrome *c* oxidase is discussed as a possible photoacceptor when cells are irradiated with monochromatic red to near-IR radiation. Four primary action mechanisms are reviewed: changes in the redox properties of the respiratory chain components following photoexcitation of their electronic states, generation of singlet oxygen, localized transient heating of absorbing chromophores, and increased superoxide anion production with subsequent increase in concentration of the product of its dismutation, H_2O_2. A cascade of reactions connected with alteration in cellular homeostasis parameters (pH_i, $[Ca_i]$, cAMP, E_h, [ATP] and some others) is considered as a photosignal transduction and amplification chain in a cell (secondary mechanisms).

Keywords: Cytochrome *c* oxidase; Electronic excitation; Local transient heating; Low-power laser therapy; Redox state; Singlet oxygen; Superoxide anion

1. Introduction

The most frequently used mechanism of photon energy conversion in laser medicine is heating. Average heating of irradiated samples occurs with all methods of tissue destruction (cutting, vaporization, coagulation, ablation). Many of these surgical laser techniques are reviewed elsewhere.

At low light intensities the photochemical conversion of the energy absorbed by a photoacceptor prevails. This type of reaction is well known for specialized photoacceptors such as rhodopsin or chlorophyll. In medicine light absorption by non-specialized photoacceptor molecules (i.e., molecules that can absorb light at certain wavelengths, but that are not integral to specialized light-reception organs) is used rather extensively (Fig. 1). The absorbing molecule can transfer the energy to another molecule, and this activated molecule can then cause chemical reactions in the surrounding tissue. This type of reaction is successfully used in photodynamic therapy (PDT) of tumors. Alternatively, the absorbing molecule in a light-activated form can take part in chemical reactions, as occurs in treatment of skin diseases with psoralens and UV-A radiation (PUVA). Importantly, in both PDT and PUVA therapy the photoabsorbing molecules are artificially introduced into a tissue before irradiation.

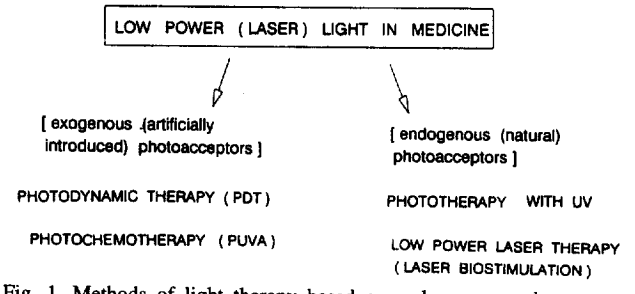

Fig. 1. Methods of light therapy based on endogenous and exogenous photoacceptors in cells.

Irradiation of cells at certain wavelengths can also activate some of the native components. In this way specific biochemical reactions as well as whole cellular metabolism can be altered. This type of reaction is believed to form the basis for low-power laser effects [1–3]. One should note that light-therapy methods based on photochemical conversion of photoabsorbing molecules (Fig. 1) are not laser-specific methods. Conventional light sources generating the appropriate wavelength can also be used (as is done in PUVA and UV therapy). Laser sources are just handy tools providing many practical advantages (e.g., efficient fiber-optic coupling to irradiate interior body parts, high monochromaticity and easy wavelength tunability, simplicity of use and electrical safety in the case of semiconductor lasers).

* Fax: +7-095-335-08-86; E-mail: kara@isan.troitsk.ru

1011-1344/99/$ - see front matter
PII S1011-1344(98)00219-X

Low-power laser effects and, in particular, their primary and secondary mechanisms, are the topic of the present paper. During the past few years numerous reviews on this topic have been written describing various aspects of the problem: history [1,4], controversies [3–9], quantitative laws of visible-light action on cells [1,2,10], photobiological fundamentals [4,9], and molecular mechanisms [11,12]. Specific data about the effects of irradiation on various cells have also been reviewed, including: cell cultures in vitro [13,14], *Escherichia coli* [15], microorganisms [16], and human lymphocytes [17,18]. In addition, clinical applications of low-power laser therapy have been surveyed [19–22]. As a rule, the material from these previous reviews is not repeated here.

2. Photoacceptors

A photobiological reaction involves the absorption of a specific wavelength of light by the functioning photoreceptor (photoacceptor) molecule. To distinguish specialized photoreceptor molecules such as rhodopsin, phytochrome, bacteriorhodopsin, and chlorophylls from nonspecialized chromophores, let us call the last ones photoacceptors. The photoacceptors take part in a metabolic reaction in a cell which is not connected with a light response. After absorbing the light of the wavelength used for irradiation, this molecule assumes an electronically excited state from which primary molecular processes can lead to a measurable biological effect in certain circumstances. To work as a photoacceptor taking part in photobioregulation, this molecule must be part of a key structure that can regulate a metabolic pathway. Redox chains are an example of this type of key structure which fits these requirements.

Several pieces of evidence show that mitochondria are sensitive to irradiation with monochromatic visible and near-infrared (IR) light. The illumination of isolated rat liver mitochondria increased adenosine triphosphate (ATP) synthesis and the consumption of O_2 [23–25]. Irradiation with light at wavelengths of 415 [23], 602 [26], 632.8 [24], 650, and 725 nm [25] enhanced ATP synthesis. Light at wavelengths of 477 and 554 nm [23] did not influence the rate of this process. Oxygen consumption was activated by illuminating with light at 365 and 436 nm, but not at 313, 546, and 577 nm [26]. Irradiation with light at 633 nm increased the mitochondrial membrane potential ($\Delta\psi$) and proton gradient (ΔpH), caused changes in mitochondrial optical properties, modified some NADH-linked dehydrogenase reactions (NADH is a reduced form of nicotinamide adenine dinucleotide) [27], and increased the rate of ADP/ATP exchange (ADP is adenosine diphosphate) [28], as well as RNA and protein synthesis in the mitochondria [29]. In the case of state 4 respiration, 351 and 458 nm laser irradiations accelerated the oxygen consumption of rat liver mitochondria; such an acceleration was not observed with 514.5 nm irradiation. On the contrary, in the case of state 3 respiration, 514.5 nm laser irradiation activated the oxygen consumption of mitochondria. Activation did not occur with 458 nm irradiation and 351 nm irradiation reduced the oxygen consumption in state 3 [30]. 660 nm irradiation increased state 3 oxygen consumption at both coupling II and III sites, as well as increasing the respiratory control ratio [31].

It is also believed that mitochondria are the primary targets when the whole cells are irradiated with light at 630 [29], 632.8 [32–34], or 820 nm [35]. Irradiation with light at 812 [36] or 632.8 nm [37] altered the rhodamine 123 uptake by fibroblasts. These results were interpreted by the authors as inducing the perturbation of mitochondrial energy production [36] and membrane potential [37].

The question is, which molecule in a mitochondrion is responsible for the effects mentioned above? When considering the cellular effects, this question can be answered with the aid of action spectra. We know that within certain limits, an action spectrum follows the absorption spectrum of the photoacceptor molecule [38]. On the other hand, the action spectrum is insufficient to distinguish between potential photoacceptor pigments with very similar absorption spectra. Moreover, the absorption spectrum for the photoacceptor pigment may depend strongly on its environment, but this environment remains unknown as long as the photoacceptor pigment itself is unknown. Because of these inadequacies of the photoacceptor pigment, some other criteria for identification are also used.

Action spectra for the DNA and RNA synthesis rate in HeLa cells in the exponential and plateau phase of growth as well as those for the adhesive properties of HeLa cellular membranes were published by Karu et al. [39–41]. Fig. 2 shows a generalized action spectrum for HeLa cells (the sum of the four spectra for nucleic acid synthesis stimulation, which were very similar to each other) taken from Refs.

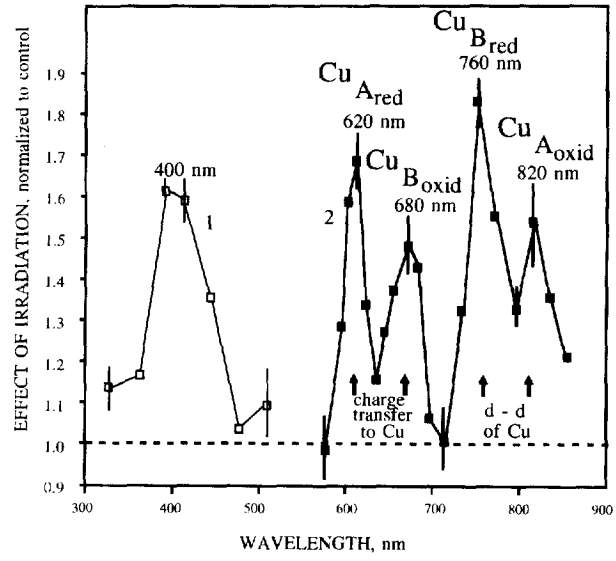

Fig. 2. The generalized action spectrum of proliferation increase of HeLa cells for $\lambda = 330$–860 nm [39,40]: curve 1, dose 10 J m^{-2}; curve 2, dose 100 J m^{-2}. A possible assignment of peaks to absorbing chromophores is marked as suggested in Ref. [61].

[39,40]. Recall that in the wavelength range 310–500 nm, a maximum stimulating effect was obtained with a radiation dose one order of magnitude less than in the longer-wave spectral range [39,40].

Fig. 2 shows that the action spectrum in the range 580–860 nm consists of two series of doublet bands in the ranges 620–680 and 760–830 nm with well-pronounced maxima at 620, 680, 760, and 820 nm. In the violet–blue region there is one maximum at 400 nm with the edge of the envelope near 450 nm.

It is known that the action spectrum is roughly the same shape as the absorption spectrum of the photoacceptor [38]. Therefore, the bands in the action spectra were identified by analogy with the metal-ligand system absorption spectra characteristic of this spectral range [42–44]. The regions 400–450 and 620–680 nm are characterized by the bands pertaining to complexes with charge transfer in a metal-ligand system, and within 760–830 nm, these are d–d transitions in metals. The region 400–420 nm is typical of π–π^* transitions in a porphyrin ring [45].

Comparative analysis of spectral data for transition metals and their complexes on one hand, and biomolecules participating in the regulation of cellular metabolism on the other, allows us to suggest that multinuclear enzymes containing Cu(II) may be participating [12,44,45]. Analysis of the electron excitation transitions of participating molecules containing Cu(II) [46–49] shows that metal-ligand transitions in the range 400–450 nm correspond to the $N_{imidazole} \rightarrow Cu$ transition, at 620 nm, to the $S_{cysteine} \rightarrow Cu$ transition, and at 680 nm to the $S_{methionine} \rightarrow Cu$ transition.

Comparing the lines of possible d–d transitions and charge-transfer complexes of Cu [44,47–50] with our action spectrum (Fig. 2) allows us to assume that the photoacceptor molecule has different types of centers containing Cu(II) in the ranges 420–450 and 760–830 nm. In the range 420–450 nm, this may be a combination of centers of types I and II (for the characteristics of centers of types I, II and III, see Ref. [46]) though a center of type I may be present. At 330 nm, a center of type III may be present, and in the range 760–820 nm centers of types I and III coexist. Within 620–680 nm, there is a center of type I and a combination of centers of different types is unlikely.

The above analysis allows us to conclude that all bands in the action spectrum in Fig. 2 may be related to the cytochrome *c* oxidase. The fact that the photoacceptors are components of the respiratory chain was considered earlier [4]. Cytochrome *c* oxidase (or cyt a/a_3) is the terminal enzyme of the respiratory chain in eukaryotic cells (Fig. 3(a)), which mediates the transfer of electrons from cyt *c* to molecular oxygen. Ferrocytochrome *c* is oxidized, dioxygen is reduced, and protons are pumped vectorially from the mitochondrial matrix to the cytosol. Free energy resulting from this redox chemistry is converted into an electrochemical potential across the inner membrane of the mitochondrion, which ultimately drives the production of ATP. Accordingly, cytochrome *c* oxidase plays a central role in the bioenergetics of the cell.

Cytochrome *c* oxidase of mammalian cells is a large multicomponent membrane protein of considerable structural complexity (molecular size 200 kDa). Two heme moieties (heme *a* and heme a_3), two redox-active copper sites (Cu_A and Cu_B), one zinc, and one magnesium are the possible absorbing chromophores for visible light. Recently, the high-resolution three-dimensional X-ray structures of cytochrome *c* oxidase of bovine heart [51,52] and *Paracoccus denitrificans* [53] were reported. These studies indicated that Cu_A is a dinuclear copper center with an unexpected structure similar

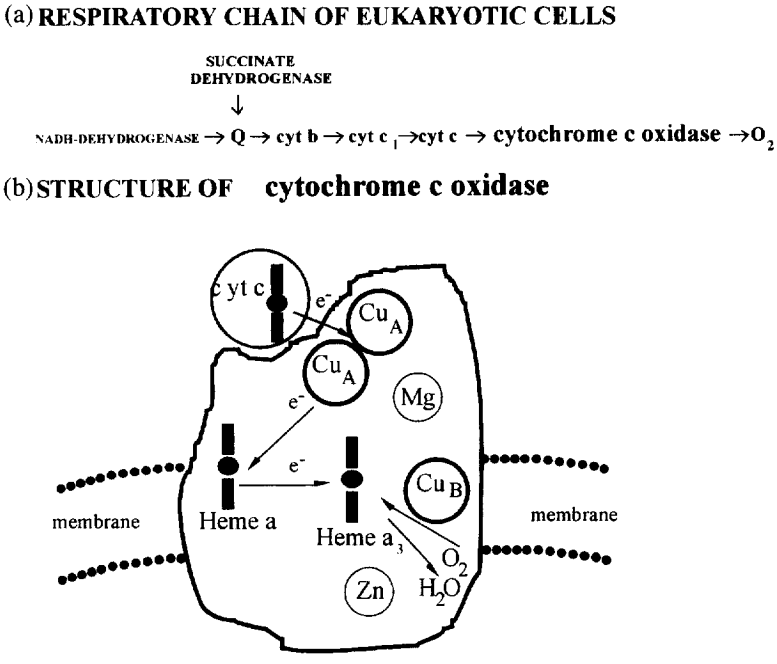

Fig. 3. (a) Mitochondrial respiratory chain of eukaryotic cells. (b) A scheme of the structure of cytochrome *c* oxidase (after [51,52,54]).

to a [2Fe–2S]-type iron–sulfur center in which the Fe ions and inorganic sulfur atoms are replaced with Cu ions and cysteine sulfur atoms, respectively. The O_2 binding site contains heme a_3, iron, and Cu_B; there is no detectable bridging ligand between the iron and copper atoms. Heme *a* is coordinated with two imidazoles of histidine residues. The fifth ligand of heme a_3 is an imidazole, whereas Cu_B is coordinated by three imidazoles of histidine. The Cu_A (Cu–Cu) center is coordinated by residues of two cysteins, two histidines, one methionine, and one peptide carbonyl of a glutamate [51].

In the catalytic cycle of cytochrome *c* oxidase electrons are transferred sequentially from water-soluble cytochrome *c* to Cu_A, then to heme *a* and to the binuclear center a_3–Cu_B, where oxygen is reduced to water (Fig. 3(b)). Oxygen binds to heme a_3 and is reduced to water through a series of short-lived elusive intermediates. Singular value decomposition analysis indicated the presence of at least seven intermediates [55]. The best-characterized species up to now are ferrous-oxycomplex and peroxy species [55–57].

Generally speaking, the cytochrome *c* oxidase can be fully oxidized (four redox-active metal centers: Cu_A, Cu_B, and iron in hemes *a* and a_3, are in their common higher oxidation state; 3^+ for iron and 2^+ for copper), or fully reduced (four metal centers are in their common lower oxidation state; 2^+ for iron and 1^+ for copper). A partially reduced enzyme, usually called a mixed-valence enzyme, has some metal centers in their higher oxidation state and the remainder in their lower oxidation state. There are also a number of forms of oxidized enzyme: fast enzyme (reacts relatively rapidly with cyanide), slow enzyme (reacts at about of 1% of the rate of the fast enzyme, also called resting enzyme), pulsed enzyme (obtained by reducing slow enzyme and oxidizing it with oxygen under conditions in which the production of H_2O_2 is avoided), and oxygenated enzyme (subjected to a cycle of reduction and reoxidation under conditions in which H_2O_2 is produced) [58–60]. These details are given to illustrate how complicated and controversial the overall picture of the functioning of cytochrome *c* oxidase still is.

Coming back to the comparative analysis of the action spectrum in Fig. 2 and available spectroscopic data on cytochrome *c* oxidase cited above, it was suggested [61] that the 825 nm band belongs to the oxidized Cu_A, the 760 nm band to the reduced Cu_B, the 680 nm band to the oxidized Cu_B, and the 620 band to the reduced Cu_A (Fig. 2). The 400–450 nm band is more likely to be the envelope of a few absorption bands in the range 350–500 nm (i.e., a superposition of several bands). This means that the main contribution to the mentioned absorption bands is made by these particular chromophores. It does not mean that other chromophores are not participating to a lesser extent. The band with a maximum near 404–420 nm can be assigned to the oxidized heme, whereas the longer-wave edge of the envelope at 450 nm (due to its asymmetry) should evidently be assigned to the reduced Cu_B. The participation of the heme in the action spectra is confirmed by the optimal dose ratio (10 and 100 J m^{-2}, respectively, for 404 nm and other visible-region maxima [39,40]). It should be noted that the Soret band of heme compounds (i.e., the band in the range 400–420 nm) is more intense by an order of magnitude than the absorption bands of these compounds in the visible region [45]. The weak band at 330 nm may belong to the oxidized Cu_B. Thus, the bands at 330, 404–420, 680, and 825 nm can be attributed to the oxidized form of cytochrome *c* oxidase; the edge of the blue–violet band at 450 nm and the distinct bands at 620 and 760 nm belong to the reduced form of the enzyme.

Analysis of the band shapes in the action spectra (Fig. 2) and the line intensity ratios enables us to conclude that cytochrome *c* oxidase cannot be considered as a primary photoacceptor when it is fully oxidized or fully reduced, but only when it is in one of the intermediate forms (partially reduced, or mixed valence enzyme).

The conclusion that the action spectrum (Fig. 2) reflects the absorption spectrum of one of the intermediate forms of the cytochrome *c* oxidase complex is supported by the results of experiments using simultaneous dichromatic irradiation (Fig. 4(b)). As well as irradiating the cells with light of different wavelengths as normal (shown on the abscissa of Fig. 4(b)), they were simultaneously irradiated with light at

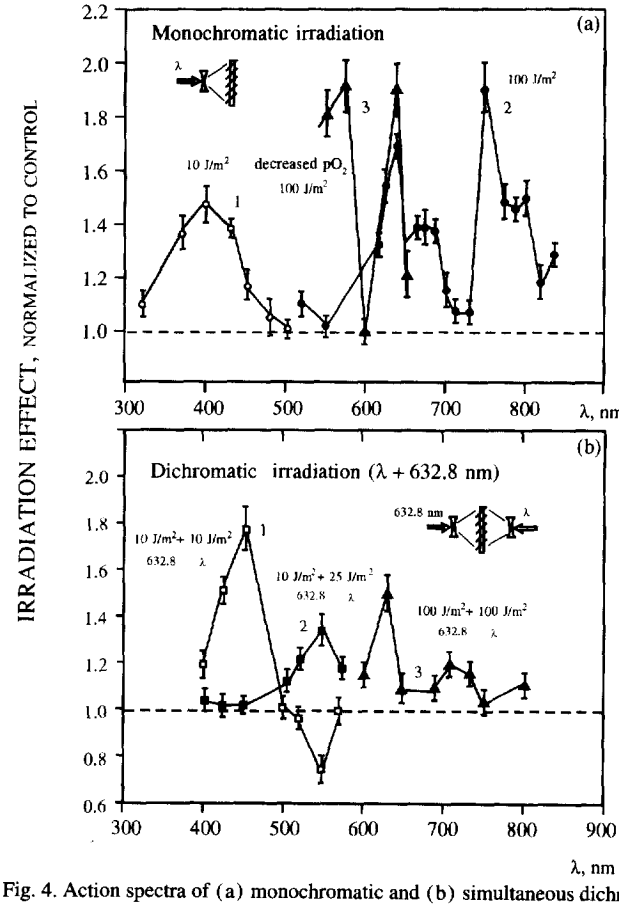

Fig. 4. Action spectra of (a) monochromatic and (b) simultaneous dichromatic irradiation with $\lambda = 632.8$ nm and λ (wavelengths shown on the abscissa) on the DNA synthesis rate in exponentially growing HeLa cells (adapted from [39,62]). Fluences used (10 J m^{-2} for violet–blue region and 100 J m^{-2} for yellow to near-IR region) were earlier [39] found to be optimal for these spectral regions.

633 nm. This wavelength was close to the position of one maximum in the action spectrum in Fig. 2 (620 nm). The technique of this experiment is described in Ref. [62]. The light doses in both irradiation processes were either optimal (100 J m^{-2} in the region 600–860 nm, Fig. 4(b), curve 3, and 10 J m^{-2}, Fig. 4(b), curve 1) or increased (25 J m^{-2} in the blue–green region, Fig. 4(b), curve 2). So, in the case of simultaneous dichromatic irradiation a new action spectrum is formed. The comparison of the action spectra in Fig. 4(a) (curves 1 and 2) and (b) reveals essential differences between them: the shift of the blue–violet maximum from 404 to 450 nm or its absence in the spectrum; new bands in the green region (550–560 nm); the absence of bands at 680, 760, and 825 nm. It is known [59] that the shift of the band from 400 to 450 nm can be observed in the course of cytochrome *c* oxidase reduction. It should be noted that in the range 550–560 nm, one can observe an absorption band of one of the intermediate forms [50]. These results enable us to conclude that simultaneous dichromatic irradiation changes the ratio of the reduced and oxidized forms of the enzyme as compared with ordinary irradiation.

The suggestion that an intermediate form of cytochrome *c* oxidase is the primary photoacceptor is also supported by the results of Pastore et al. [63]. The fully oxidized form of the enzyme appeared to be insensitive to He–Ne laser radiation as revealed by absorption spectra. The irradiation increased the absorption of the partially reduced enzyme as well as its proton pump activity.

It is worth noting that when the cellular monolayer was irradiated simultaneously with $\lambda = 633$ nm and various wavelengths of visible light, the red and far-red peaks at 680 and 760 nm disappeared (Fig. 4(b)). When the cells were irradiated consecutively with wavelengths 633 and 760 nm and the time interval between the two irradiation events was varied, the DNA synthesis rate depended on the order in which the wavelengths were used (Fig. 5(a)). Irradiation first with the light at 760 nm and then with the red light ($\lambda = 633$ nm) stimulated the DNA synthesis, whereas irradiation in the reverse order (633 nm followed by 760 nm) inhibited it. These effects reached their maxima when the time interval between the successive irradiation events was between 1 and 3 min, and became progressively less pronounced with a further increase in the interval. It should be noted that the effects were not equal in magnitude: stimulation amounted to 60%, while inhibition was 20% (Fig. 5(a)). This result supports the conclusion made above that the 620 and 760 nm bands do not belong to the one photoacceptor but to its different absorbing centers (probably Cu_A and Cu_B). Electronic excitation of these centers in a different sequence may influence the electron transfer in cytochrome *c* oxidase in a different way which has an effect on the final photobiological response, namely, DNA synthesis.

When the consecutive irradiation was performed with red (633 nm) and blue (404 nm) light, the sequence 633 followed by 404 nm had no effect on DNA synthesis, but the sequence 404 followed by 633 nm stimulated it (Fig. 5(b)). Again, as in the previous case (Fig. 5(a)), the effects did not occur until the interval between the two irradiation events reached 10 s, then increased as the interval was increased. The difference between the results of the two experiments presented in Fig. 5(a) and (b) is that in the case of far-red–red light irradiations (Fig. 5(a)) the effect disappeared (was reduced to control levels) when the time interval was increased. In the second case, when irradiation was performed in the sequence 404 nm + 633 nm (Fig. 5(b)), the effect was still maximal at the same time interval of 10^4 s. It is quite possible that chromophores absorbing at 404 and 633 nm do

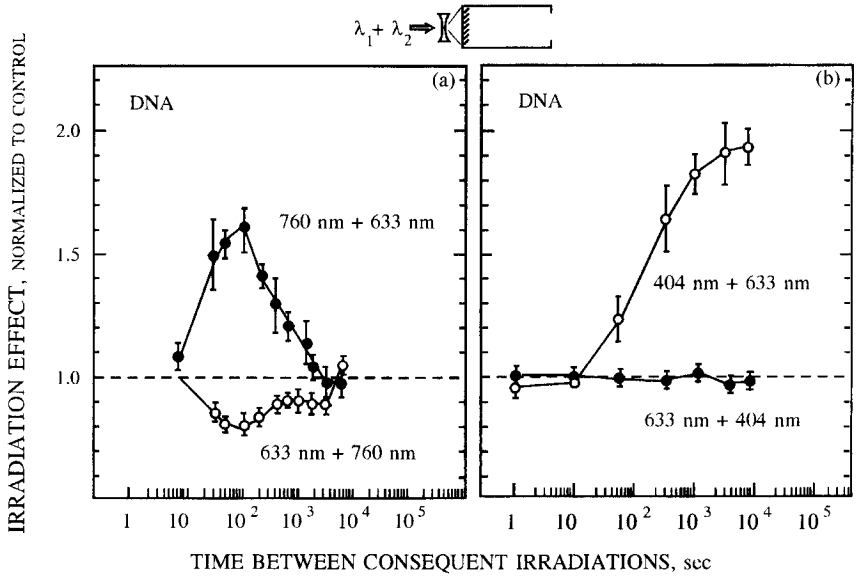

Fig. 5. Stimulation of DNA synthesis in HeLa cells measured after consecutive irradiation with (a) far-red and red, or (b) blue and red light as a function of irradiation wavelength sequence and the time interval τ between the two irradiation events (shown on abscissa) (adapted from [62]). The doses were 100 J m^{-2} for radiation at 760 and 633 nm and 10 J m^{-2} for radiation at 404 nm (optimal for these particular wavelengths [39]).

not belong to one molecule but to different molecules of the same redox chain.

Let us recall here three results indicating the antagonistic action of red and far-red light. First, when irradiating isolated rat liver mitochondria, Gordon and Surrey [25] found that red light at 650 nm increased oxidative phosphorylation, but far-red light at 725 nm inhibited it. Irradiating hamster fibroblasts with red (632.8 nm) and far-red (760 nm) light caused a change in the intracellular concentration of cAMP [64], the cAMP concentration behavior in the former case being opposite to that in the latter case (increase and decrease, respectively). Irradiation at 670 and 830 nm stimulated the proliferation of the Schwann cells, but irradiation at 780 nm inhibited it [65]. These results could be explained by taking into account the fact that the wavelengths mentioned above are absorbed by different chromophores in a different redox state: λ_{max} at 620 nm by Cu_A (reduced), λ_{max} at 680 nm by Cu_B (oxidized), λ_{max} at 760 nm by Cu_B (reduced), and λ_{max} 820 nm by Cu_A (oxidized). One can also suggest that different absorbing chromophores may play a different role in driving the metabolism.

One important step in identifying the photoacceptor molecule is to compare the absorption and action spectra [38]. Recording an absorption spectrum of a cellular monolayer or individual cell is not an easy task. The absorption spectra of individual cells up to 700 nm were recorded years ago with the aim of identifying respiratory chain carriers [66]. The absorption spectrum for the visible-to-near-IR region of eight parallel monolayers of human fibroblasts was recorded using a commercial double-beam spectrophotometer [67]. This spectrum is presented in Fig. 6(a). For recording the absorption of one layer with the aim of studying the irradiation-induced changes in absorption of cell chromophores, a sensible multichannel registration method was developed [68]. The first results of these experiments are presented in Fig. 6(b) and Fig. 7(a) and (b).

First, we recorded the absorption spectra of a dry monolayer (the monolayer was held in air at room temperature for 30 min after being rinsed with Hanks' solution). An example of such a spectrum is presented in Fig. 6(b). The spectrum exhibits distinct absorption bands with maxima at 620, 680 (with a shoulder manifest at 665), 810, and 870 nm, weak bands being observed at 715, 730, and 765 nm. Irradiating

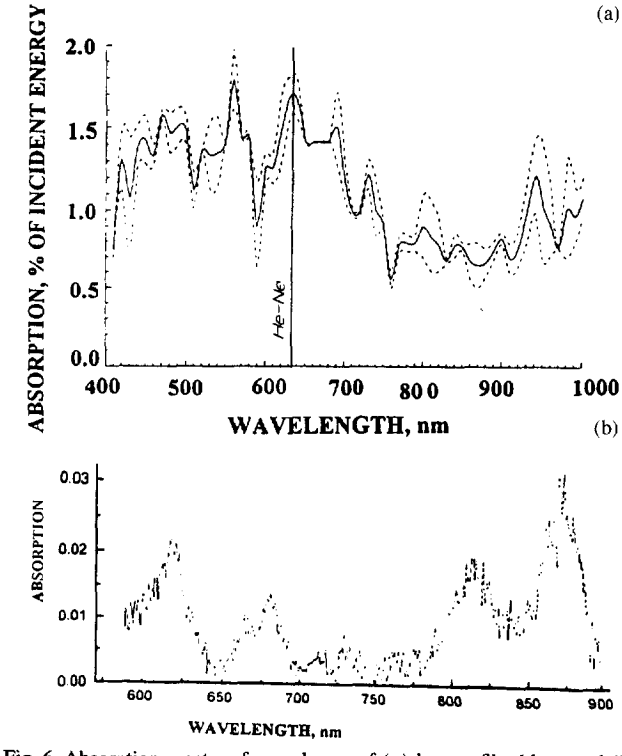

Fig. 6. Absorption spectra of monolayers of (a) human fibroblasts and (b) dry HeLa cells. The experimental details are described respectively in Ref. [67] and Ref. [68].

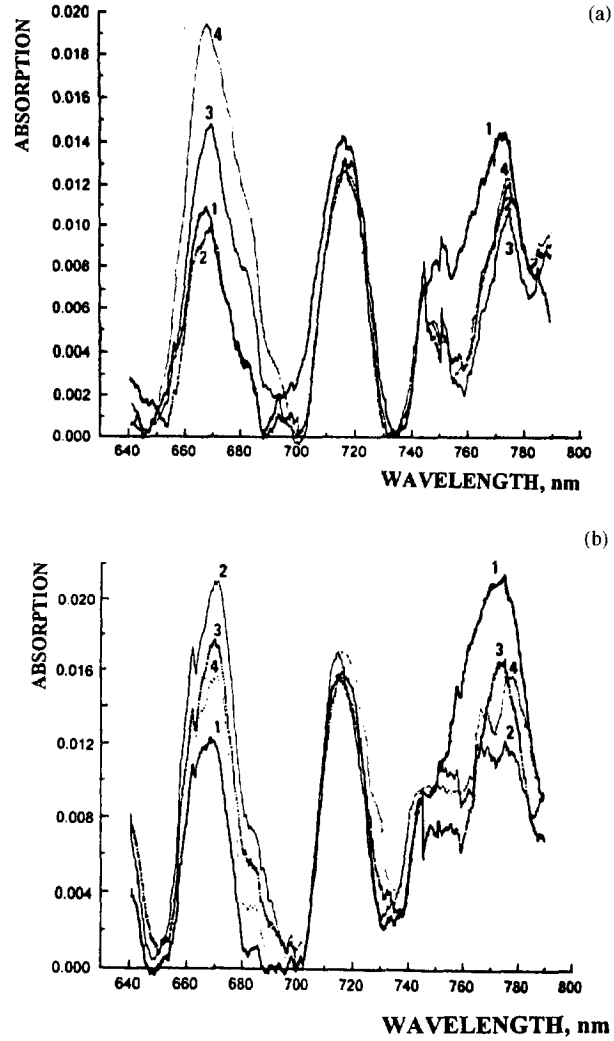

Fig. 7. (a) Absorption spectrum of the HeLa monolayer immediately after the removal of the nutrient medium (curve 1) and following exposure to radiation with $\lambda = 670$ nm for the first time (curve 2), second time (curve 3), and third time (curve 4) (each exposure, 10 s for a dose of 6.3×10^3 J m^{-2}) [68]. (b) Absorption spectrum of the HeLa monolayer immediately after the removal of the nutrient medium (curve 1) and following exposure to radiation with $\lambda = 820$ nm for the first time (curve 2), second time (curve 3), and third time (curve 4) (each exposure, 10 s for a dose of 6.3×10^3 J m^{-2}) [68].

this type of monolayer with laser radiation had no effect at all. This circumstance is explained by the fact that virtually no cells survived the drying, which was verified through staining with trypan blue.

Further experiments were aimed at recording the absorption spectra of a wet monolayer immediately after rinsing with Hanks' solution. To improve sensitivity, the spectra in this series were recorded in the wavelength range 640–790 nm (585–900 nm in the preceding series). In the given wavelength range (640–790 nm), there are clearly manifest absorption bands at 670, 718, and 775 nm, and also a less distinct band or a band shoulder in the vicinity of 750 nm (curves 1 in Fig. 7(a) and (b)).

Exposing the sample for 10 s to laser radiation with a wavelength of 670 nm and a dose of 6.3×10^3 J m^{-2} once (curve 2 of Fig. 7(a)), twice (curve 3 of Fig. 7(b)), or three times (curve 4 of Fig. 7(a)) caused changes in its absorption bands around 670, 750, and 775 nm, the absorption band at 718 nm remaining unchanged (see Fig. 7(a)). In the action spectra, the band in the neighborhood of 670–680 nm supposedly belongs to the chromophore Cu_B in the oxidized state, while that in the vicinity of 760–770 nm belongs to the chromophore Cu_B in the reduced state [61]. If there is a correspondence between the action spectra bands (Fig. 2) and the absorption spectra bands presented in Fig. 7(a), the results presented in Fig. 7(a) are quite natural: as laser irradiation increases absorption in the band at 670 nm, and hence the concentration of the chromophore in the oxidized state, absorption near 750–770 nm (and the concentration of the reduced chromophore) decreases. It is interesting to compare the responses of the monolayer to the first, second, and third exposures to laser radiation. The first exposure to radiation with a wavelength of 670 nm causes no substantial changes in the 670 nm absorption band (curve 2) as compared to that of the unexposed monolayer (curve 1). It is only the subsequent two exposures that result in the intensification of the 670 nm band (curves 3 and 4, respectively). An entirely different effect is observed to occur in the complex absorption band in the region of 745–780 nm. The first exposure (curve 2) brings about a sharp change in both the intensity and shape of the complex band profile, practically no changes taking place as a result of the second and third exposures (curves 3 and 4, respectively), which is very like a saturation effect. Two bands, one with $\lambda=745$ and a shoulder at $\lambda=755$ nm and the other with $\lambda=775$ nm, are clearly defined in the absorption spectra of the exposed monolayer (curves 2–4 in Fig. 7(a)). The shape of the band points to the presence of two conformers in the hypothetical reduced chromophore.

The exposure of the cellular monolayer to laser light with $\lambda=820$ nm (Fig. 7(b)) was also observed to cause changes in the absorption bands in the vicinity of 670 and 775 nm. Recall that the action spectra feature a band at around 825 nm, which is supposedly associated with the oxidized chromophore Cu_A (Fig. 2). The exposure of the monolayer to light with $\lambda=820$ nm was carried out in the same way as in the case of laser light with $\lambda=670$ nm in the preceding series of experiments. The sample was exposed for 10 s to the radiation at a dose of 6.3×10^3 J m^{-2} once (curve 2), twice (curve 3), or three times (curve 4). Following the first exposure (curve 2), a sharp increase of absorption is observed to occur in the band near 670 nm (and a correspondingly sharp reduction of absorption in the band near 775 nm) in comparison with the intact monolayer (curve 1). The second (curve 3) and the third (curve 4) exposures cause no sharp changes in absorption, which is likely due to an equilibrium being established between the oxidized and reduced forms of the chromophore Cu_B.

Clearly manifest in Fig. 7(b) (curves 2–4) are two bands, one with $\lambda_1=750$ nm and the other with $\lambda_2=770$ nm, as was also the case with irradiation at $\lambda=670$ nm (Fig. 7(a)). One can therefore state with a certain caution that radiation with a wavelength of 670 or 820 nm has most likely no effect on the chromophore reduction mechanism itself. In both cases ($\lambda=670$ nm and $\lambda=820$ nm), irradiation reduces the absorption and the concentration of the reduced form of the chromophore and gives rise to its two conformably ordered forms.

On the contrary, the behavior of the 670 nm band in the case of exposure to laser light with $\lambda=820$ nm (Fig. 7(b)) differs drastically from that in Fig. 7(a) (i.e., in the case of irradiation at $\lambda=670$ nm). The very first exposure to light with $\lambda=820$ nm increases the intensity of the 670 nm band (curve 2 of Fig. 7(b)). Following the second exposure (curve 3), this band changes but little, and practically no changes are observed to occur following the third exposure (curve 4) (something like saturation takes place).

Thus, the light wavelength and the number of exposures have different effects on the supposedly oxidized form of the chromophore with the absorption maximum at $\lambda=670$ nm. Radiation with $\lambda=820$ nm oxidizes the chromophore Cu_B more strongly in the first exposure (i.e., oxidation proceeds in a stepwise manner), whereas radiation with $\lambda=670$ nm has practically no effect on the oxidized form of Cu_B in the first exposure (Fig. 7(a)). It is only after the second and third exposures that the concentration of the oxidized form of the chromophore increases, no saturation being reached in our experiments.

The 718 nm band suffers virtually no changes following exposure to laser light differing in wavelength. It should be noted that this band is not manifest in the action spectra (Fig. 2). The absence of changes in absorption following exposure to radiation with two wavelengths (Fig. 7(a,b)) also seems quite logical and gives reason to believe that the chromophore absorbing in this region takes no part in the photoregulation process responsible for the action spectrum.

Taken together, the terminal respiratory chain oxidases in eukaryotic cells (cytochrome *c* oxidase) and in prokaryotic cells of *Echerichia coli* (cytochrome *bd* and *bo* complexes [69]) are believed to be photoacceptor molecules for red-to-near-IR radiation. In the violet-to-blue spectral region, flavoproteins (e.g., NADH-dehydrogenase [11,52]) are also among the photoacceptors as well as terminal oxidases. It is suggested that the photoacceptor is not fully reduced or oxi-

dized terminal oxidase, but one of its intermediate forms (a so-called mixed valence oxidase), which has not yet been identified.

3. Primary mechanisms

The primary mechanisms of light action on the photoacceptor molecules have not yet been established. Below, four main possibilities discussed in the literature so far are considered.

First, it has been suggested that one possible process involves an acceleration of electron transfer in the respiratory chain due to a change in the redox properties of the carriers following photoexcitation of their electronic states [4,11]. A similar principle governs the function of photosynthetic reaction centers [70] and is involved in many so-called 'blue light responses' [71,72]. It is well known that electron excitation changes the redox properties of molecules [73]. The results of recent measurements ([68], Fig. 7) as well as the discussion from Section 2 allow one to propose that the photoexcitation of certain reaction centers in the cytochrome *c* oxidase molecule (like Cu_A and Cu_B or hemes *a* and a_3) or in cyt *bd* and *bo* complexes of *Escherichia coli* [69] influences the redox state of these centers, and consequently, the rate of the electron flow in the molecule. It was suggested, for example, that irradiation shifts the cytochromes to more reduced forms, which naturally promote electron transport [74]. Recently, the redox absorbance changes of the respiratory chain components of *Escherichia coli* were measured following He–Ne laser irradiation. A dose of 4.3×10^4 J m^{-2} led to partial oxidation of cyt *b* and cyt *d*, while flavoproteins were found to be slightly reduced [75]. Recent developments clearly show that photoinduced electron-transfer reaction can initiate the folding of protein, as it was demonstrated for reduced cytochrome *c* [76,77]. The thermodynamic analyses of Pascher et al. [76] suggest that many redox-active proteins will be amenable to a protein-folding trigger based on electron-transfer chemistry.

Secondly, during light excitation of electronic states, a noticeable fraction of the excitation energy is inevitably converted to heat, which causes a local increase in the temperature of the absorbing chromophores [78]. Any appreciable time- or space-averaged heating of the sample can by prevented by controlling the irradiation intensity and dose appropriately. However, there is still the possibility of localized transient heating of absorbing chromophores. The local transient rise in temperature of absorbing biomolecules may cause structural (e.g., conformational) changes and trigger biochemical activity (secondary dark reactions) such as activation or inhibition of enzymes.

To evaluate the contribution of local transient heating of light-absorbing microregions to biochemical activity, *Escherichia coli* [79] and HeLa cells [80] were irradiated using femtosecond laser pulses ($\lambda = 620$ nm, $\tau_p = 3 \times 10^{-13}$ s, $f = 0.5$ Hz, $E_p = 1.1 \times 10^{-3}$ J cm^{-2}, $I_{av} = 5.5 \times 10^{-4}$ W cm^{-2}, $I_p = 10^9$ W cm^{-2}) as well as c.w. laser radiation ($\lambda = 632.8$ nm, $I = 1.3$ W cm^{-2}). The irradiation dose required to produce a similar biological effect (an increase in the clonogenic activity of irradiated cells compared with the nonirradiated control) was found to be a factor of about 10^2–10^3 lower for pulsed radiation than for c.w. radiation. The minimum size of the microregions transiently heated by irradiation with femtosecond laser pulses was estimated at about 10 Å, which corresponds to the size of the chromophores of the suggested photoacceptors, the respiratory chain components [79].

The effects of slight local heating and a substantial local temperature gradient [78] occur for both c.w. and femtosecond pulses. The difference is that the density of such local heating sites in the case of c.w. radiation is extremely low because of the low radiation intensity. This density is lower than the density of the absorbing chromophores by a factor of approximately 10^7–10^{10}, depending on the relaxation time and excitation cross section of the chromophores. In the case of femtosecond pulses, the chromophores become excited, which causes a stronger biological response.

Therefore, it can be concluded that higher than average local transient heating of microregions with a size characteristic of absorbing chromophores is possible. The evaluation of the transient heat intensity of these microregions and the diffusion of heat from them requires a more detailed analysis. One should note here that the above considerations involved individual cells. The problem of local heating of absorbing chromophores takes on entirely new dimensions and significance when whole tissues are irradiated. Some considerations of this problem are found in Refs. [78,81].

Thirdly, the principal use of oxygen in a respiratory chain involves its four-electron reduction to water. In normal metabolic processes as well as in a number of nonenzymatic biological reactions both univalent and bivalent reductions of molecular oxygen also occur (Fig. 8). For example, free

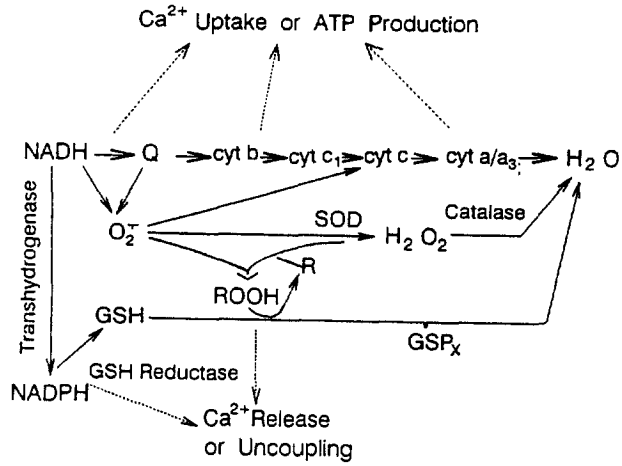

Fig. 8. The connection of antioxidant enzymes to electron transport, oxidative phosphorylation, and Ca^{2+} flux. Abbreviations: GSH, glutathione; GSPx, glutathione peroxidase; SOD, superoxide dismutase; Cat, catalase; R, reduced lipid; ROOH, lipid peroxide (after [82]).

radicals like $OH^{\bullet}$ (hydroxyl) and $O_2^{\bullet -}$ (superoxide) appear as a result of one-electron reduction. It has been shown that in mitochondrial electron transport the superoxide radical is produced, and the production of $O_2^{\bullet -}$ as well as the product of its dismutation, H_2O_2, primarily depend on the metabolic state of the mitochondria [82]. By activating electron flow in the respiratory chain, one can also expect increasing superoxide anion, $O_2^{\bullet -}$, production. Some data in the literature also suggest that besides the above-mentioned method of generating $O_2^{\bullet -}$, there is another NADH-related way to generate $O_2^{\bullet -}$ in the mitochondria [83].

It has been demonstrated experimentally that mitochondria possess a mechanism for the reabsorption of $O_2^{\bullet -}$, and $O_2^{\bullet -}$ may be a source of electrons for the oxidative phosphorylation of ADP under physiological conditions [84]. Experimental data show that liver mitochondrial ATP synthesis can be inhibited and promoted by the UV generation of $O_2^{\bullet -}$ [85]. Recall also that even a small increase in $O_2^{\bullet -}$ concentration (and subsequently, an increase in the concentration of the product of its dismutation, H_2O_2) in a cell results in multiple secondary responses, such as an increase in $[Ca^{2+}]_i$ and pH_i (alkalization), release of arachidonate, activation of Na^+/H^+ antiport and Ca^{2+} ATPase, and alteration of Na^+–Ca^{2+} exchange [86–88]. Both $O_2^{\bullet -}$ and H_2O_2 are considered to be oxidizers [89], and, for example, very low concentrations of H_2O_2 can cause activation of lymphocytes [90]. Many of these reactions also occur in irradiated cells as with visible-to-near-IR radiation will be considered in Section 4.

Experiments measuring the luminol-amplified chemiluminescence of murine splenocytes after near-IR irradiation [91] do not exclude the possibility of increased $O_2^{\bullet -}$ also by mitochondria. A comparison of the action spectrum of chemiluminescence stimulated after irradiation with various wavelengths of light and the absorption spectrum of the cytochrome *c* oxidase indicated some similarity between these two. As the absorption band of cytochrome *c* oxidase at 830 nm is thought to be due to its copper component [50], and the chemiluminescence emitted by mitochondria is believed to be copper dependent [85], one should consider the possibility that some low-power laser effects can be related to increased $O_2^{\bullet -}$ production. Recent experimental results [92–94] support the hypothesis of $O_2^{\bullet -}$ and H_2O_2 participation in photosignal, the transduction chain leading to an increase of proliferation. This possibility, discussed first in Ref. [91], becomes more serious if we take into account the fact that H_2O_2, the product of $O_2^{\bullet -}$ dismutation, is involved in a complex set of reactions linking the H_2O_2 production in mitochondria to the regulation of cellular metabolism [82]. As shown Ref. [82] and also in Fig. 8, many antioxidant enzymes are involved in this link. One of the enzymes taking part in the pathway is catalase. Catalase has been shown to be involved in the increase in protein synthesis induced by He–Ne laser light in yeast cells [95]. In that study, the activity of catalase was measured immediately after the irradiation of *Torulopsis sphaerica* and the amount of synthesized protein was tested 18 h later. The activity of catalase as well as the amount of synthesized protein were increased in the irradiated cells. The inhibition of catalase activity by 3-amino-1,2,4-triazole at the moment of irradiation suppressed protein synthesis. At the same time, the protein synthesis was not affected by 3-amino-1,2,4-triazole in non-irradiated cells. Possible specific links between the increase in catalase activity and protein synthesis in irradiated cells were discussed in Ref. [95]. So, a mechanism involving H_2O_2 as a secondary messenger might also be involved. It is also quite possible that generated reactive oxygen species like $O_2^{\bullet -}$ or even H_2O_2 simply modulate the redox activity of mitochondria and/or the redox state of the cell. In other words, the first and third mechanisms discussed above can provide a similar result.

Fourthly, certain photoabsorbing molecules like porphyrins and flavoproteins (some respiratory chain components belong to these classes of compounds) can be reversibly converted to photosensitizers [96,97]. Based on monochromatic visible-light action spectra for DNA synthesis in HeLa cells, and spectroscopic data for porphyrins and flavins, a hypothesis was advanced that the absorption of light quanta by these molecules is responsible for the generation of singlet oxygen, $^1O_2$ [39]. Chemically active $^1\Delta_g$ and $^1\Sigma_g^+$ states of oxygen with energies of 1.0 and 1.5 eV might play an active mediator role in achieving the biological effects of irradiation. This possibility has been considered for some time as a predominant suppressive reaction during irradiation of cells with high doses and intensities of light [2,11]. The possible role of singlet oxygen in low-power laser effects was discussed in several other papers (e.g., [98,99]). However, experiments demonstrating the generation of singlet oxygen in cells or tissues after irradiation as well as examination of the possible secondary (dark) biochemical reactions are to date non-existent. Fig. 9 presents two principal pathways for $^1O_2$ generation discussed in the papers cited above. The pathway on the left includes strong permitted transitions and for this reason is highly probable (classical photodynamic mechanism). This pathway for low-power laser effects was discussed in Refs. [11,39,99]. Conversely, the pathway on the right, showing direct excitation of the oxygen molecule, which was discussed in Ref. [98], has extremely low probability due to forbidden transitions.

The effects of light on respiration are oxygen dependent and prevented by anaerobiosis; it is generally believed that photodynamic reactions promoted by certain respiratory chain components (flavins, hemes and Fe–S centers) are associated and occur in aerobic conditions [96,97,100,101]. Irradiating yeasts in aerobic and anaerobic conditions with an He–Ne laser increased protein synthesis (which was measured as the final photobiological macroeffect) in both cases, the only difference being the dose range [102]. This finding indicates that at least in this particular case, the $^1O_2$-connected mechanism is not involved. On the other hand, in some other cases extremely small amounts of $^1O_2$ could cause some change in the redox status of a cell.

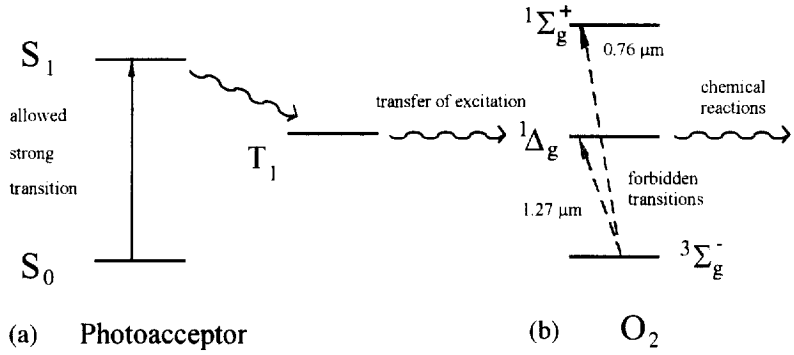

Fig. 9. Two principal ways for generation of singlet oxygen in a cell: (a) photodynamic action; (b) direct excitation of triplet oxygen. Details can be found in the text.

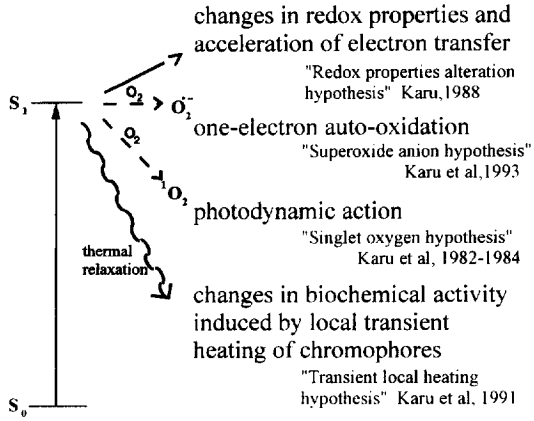

Fig. 10. Possible primary reactions with a photoacceptor molecule after promotion to excited electronic states. This theoretical scheme does not mean that all relevant reactions occur from the first singlet state.

Taken together, there is certainly more than one reaction involved in the primary mechanisms of low-power laser effects. The four types of possible reactions discussed above are summarized in Fig. 10. There are no grounds to believe that only one of these processes occurs when a cell is irradiated. An important question for the future is which of these reactions is responsible for a certain low-power laser effect. However, recent experimental results of measurements of redox absorbance changes of living cells after irradiation [68,75] clearly indicate that a mechanism based on changes in redox properties of terminal enzymes of respiratory chains might be crucial.

4. Secondary mechanisms

Sections 2 and 3 considered photoacceptor molecules and possible primary reactions occurring under irradiation. On the other hand, it is well known that many biochemical reactions in cells occur hours and even days after the irradiation procedure. Usually, the irradiation lasts on a time scale of seconds and minutes. The biological responses of cells occurring later, when the radiation is switched off, are called secondary reactions. The specificity of light action is believed to be due to the absorption of quanta by a photoacceptor, cytochrome *c* oxidase molecule (Section 2). Also, a flavoprotein like NADH-dehydrogenase has been discussed as a possible photoacceptor for blue and red light [4,11].

If the photoacceptors are located in the mitochondria (Section 2), then how are the primary reactions occurring in the respiratory chain (Section 3) connected with DNA synthesis in the nucleus? A scheme presented in Fig. 11(b) can explain this. This scheme, originally proposed in Ref. [11], is based on the fact that a redox chain such as the respiratory chain is capable of controlling cellular homeostasis. The photoexcitation induces changes in cytochrome *c* oxidase (Section 3) and/or in flavinic components of the chain (e.g., NADH-dehydrogenase [4,11]); this event can in turn cause other redox changes and modulations of biochemical reactions through a photosignal transduction and amplification chain. The photosignal transduction and amplification chain leads to a photobiological macroeffect, such as increased proliferation (marked by DNA synthesis in Fig. 11(b), (c)).

We proposed that the absorption of light quanta by the respiratory chain components causes a short-term activation of the respiratory chain and oxidation of the NADH pool [11]. It is known that oxidation of the NADH pool leads to changes in the redox state of both mitochondria and cytoplasm [103,104]. The activation of the electron-transport chain also results in an increase of proton motive force (pmf, $\Delta\mu_H{}^+$), electrical potential of mitochondrial membrane ($\Delta\psi$), ATP pool, and acidification of the cytoplasm. It has been confirmed experimentally that this is also true in the case of respiratory chain activation by irradiation: changes in pmf, $\Delta\psi$, and ΔpH as well as extrasynthesis of ATP have been achieved by irradiating mitochondria with a He–Ne laser [24]. By irradiating cells with wide-band visible light of $\lambda > 400$ nm, the enhancement of the activity of ATP-synthase was observed [105]. The ATP level was also found to be raised after irradiation of whole cells: human lymphocytes at 820 nm [35], R3230AC adenocarcinoma cells at 630 nm [29], and HeLa cells at 632.8 nm [32]. Irradiating human lymphocytes with a He–Ne laser also caused changes in the ultrastructure of mitochondria, such as the formation of giant mitochondria, which was interpreted by the authors as an intensification of the energy metabolism [33]. A perturbation of the mitochondrial energy production was measured by

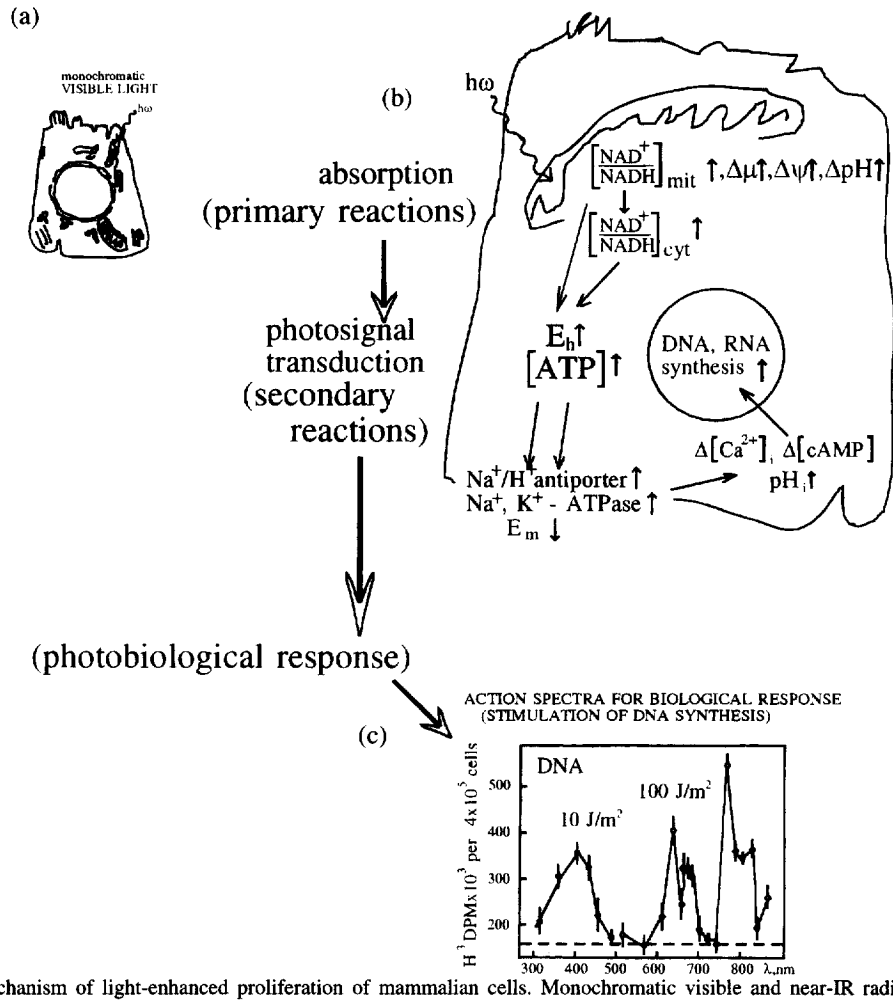

Fig. 11. A possible mechanism of light-enhanced proliferation of mammalian cells. Monochromatic visible and near-IR radiation initially absorbed by mitochondria (a) eventually causes stimulation of DNA synthesis (c) after numerous intervening dark reactions called the photosignal transduction and amplification chain (b). Explanation in the text.

rhodamine 123 uptake when human oral fibroblasts were irradiated at 812 nm [36].

The acidification of the cytoplasm (rise of intracellular H^+ concentration), caused by the activation of the respiratory chain, controls allosterically the activity of the Na^+/H^+ antiporter situated in the cytoplasmic membrane [106]. This enzyme plays a key part in the alkalization of the intracellular medium. A short-term increase in the intracellular pH (ΔpH_i) is one of the necessary components involved in the transmission of mitogenic signals in the cell [106–108].

Recall that a jump in intracellular pH (pH_i) after irradiation has been measured experimentally. pH_i is a parameter of cellular homeostasis that is closely connected with the cellular redox state. These experiments proved that pH_i was increased due to irradiation with red light by 0.20 units in mammalian cells [109] and 0.32 units in *E. coli* [110]. The conclusion of these works is that by means of irradiation it is indeed possible to shift the pH_i and overall redox state of cells into a more oxidized direction, as suggested by the scheme in Fig. 11(b).

In a eukaryotic cell, a change in the redox state of the mitochondria causes changes in the redox state of the cytoplasm or, in other words, changes the overall redox state of a cell. In Fig. 11(b), this event is marked by the NAD/NADH couple. More exactly, the overall redox state of the cell represents the net balance between stable and unstable reducing and oxidizing equivalents in dynamic equilibrium and is determined by three couples: NAD/NADH, NADP/NADPH, and GSH/GSSG (GSH = glutathione). These pairs are mutually dependent, and altering the ratio in one couple causes changes in the others. The pH_i is also closely connected to these ratios.

There is also an indirect indicator that He–Ne laser irradiation alters the redox state of cells. It is known that any shift in the redox state and metabolic activity of a cell will necessarily alter its radiosensitivity, adaptive response to radiation or other forms of endogenous or exogenous free-radical toxicity [111]. In experiments when the γ-irradiated monolayer of HeLa cells was irradiated with a He–Ne laser at various time intervals before exposure to ionizing radiation, the viability of the cells previously irradiated with a laser increased significantly as compared with γ-irradiated cells [112].

The Na^+/H^+ antiporter is not the only cell membrane enzyme to participate actively in the photosignal transduction and amplification chain. Other ion carriers such as Na^+, K^+-ATPase and enzymes controlling cAMP level in a cell are also activated. The activation of the Na^+, K^+-ATPase following He–Ne laser irradiation of human erythrocytes [113] and diode laser ($\lambda = 830$ nm) irradiation on a rat saphenous nerve [114] has been established. Experimental data on changes in cellular cAMP level after irradiation with light of various wavelengths provide reason to believe that the action of light on proliferation may be connected with the regulation of cell metabolism via cAMP [64].

There is far more than one secondary cellular response to irradiation connected with the plasma membrane. The depolarization of spontaneously active neurons in subesophageal ganglia of *Helix pomatia* occurred after irradiation with a He–Ne laser [115]. The intracellular Ca^+ concentration was found to rise during the first few minutes after He–Ne laser irradiation of human lymphocytes [116] and neutrophils [117], as well as of bovine sperm cells [94,118] and rat Schwann cells [65]. Depending on the time elapsed after irradiation and the wavelength used, irradiation increased both the cell–cell and cell–glass adhesion [41]. Direct measurement of ionic currents through the plasma membrane of both excitable (cardiomyocytes, neurons) and nonexcitable (glial) cells using the patch-clamp technique under He–Ne laser irradiation showed the activation of background channels, probably ATP-dependent K^+-channels or Ca^+-dependent K^+-channels [119]. First, the ATP dependence of the light-sensitive background single-channel currents supports the scheme of the photosignal transduction chain under discussion (Fig. 11(b)). What is more, in the same series of experiments it was established that the light-sensitive ion currents were recorded only in a cell-attached configuration of the patch pipette, i.e., in conditions of cellular integrity, but not in a whole-cell configuration. Indeed, the cascade of biochemical reactions (photosignal transduction and amplification chain) depends on the cellular homeostasis and can occur only in conditions of cellular integrity.

The photosignal transduction and amplification chain in its part from plasma membrane to nucleus is not specific for light signals but also includes a standard way of controlling cell proliferation (cAMP level, changes in intracellular content of H^+, K^+, Na^+, Ca^{2+}). This is a complicated area in cell biology and the regulation mechanisms are not completely clear. The interested reader is referred to Refs. [106–108, 120–125]. The alteration of the cellular homeostasis parameters leads to a parallel shift of different reactions, and it is not easy to establish the causal relationships.

In Fig. 11(b), two principal regulation paths by light were suggested. The first is the existence of a connection between the light-activated redox functions of mitochondria via light absorption by chromophores or generation of reactive oxygen species, changes in redox state of cytoplasm, the depolarization of cellular membrane and Ca^{2+} influx, and the increase of pH_i (alkalization of cytoplasm). The second is the photoacceptor control over the level of intracellular ATP. As we know, even small changes in ATP level can alter the cellular metabolism significantly [126].

5. Concluding remarks

Biological responses of cells to visible and near-IR (laser) radiation occur due to physical and/or chemical changes in photoacceptor molecules, components of respiratory chains like NADH-dehydrogenases, and cytochrome *c* oxidase. As a result of the photoexcitation of electronic states, the following physical and/or chemical changes can occur: alteration of redox properties and acceleration of electron transfer, changes in biochemical activity due to local transient heating of chromophores, one-electron auto-oxidation and $O_2^{\cdot -}$ production (and subsequent production of H_2O_2), and photodynamic action and $^1O_2$ production. It is not excluded that different reaction pathways can provide the same result: a change in mitochondrial redox activity.

The primary physical and/or chemical changes induced by light in photoacceptor molecules are followed by a cascade of biochemical reactions in the cell that do not need further light activation and occur in the dark (photosignal transduction and amplification chains). These reactions are connected with changes in cellular homeostasis parameters. The crucial step here is thought to be an alteration of the cellular redox state: a shift towards oxidation is associated with stimulation of cellular vitality, and a shift towards reduction is linked to inhibition. It was shown that cells with a lower than normal intracellular pH, in which the redox state was shifted in the reduced direction, were more sensitive to the stimulative action of light as compared to cells in which the respective parameters were optimal or near optimal. Recall here a change in action spectrum recorded after a decrease of partial pressure of oxygen before irradiation (Fig. 4(a), curve 3) and as well as the results of a set of experiments where the irradiation effects were modified by various chemicals [4]. The shift in pH_i of *Escherichia coli* cells due to He–Ne laser irradiation was measured experimentally [110]. Also, in a 'light only' control experiment of photodynamic therapy, the tissue pH was raised ($\Delta pH = 0.2$–0.3) after irradiation with light at 630 nm [109]. A transient increase in fluorescence (a change of redox state of flavin cofactors to the oxidized direction) was recorded by measuring the Raman spectra (excitation at 514.5 nm) of synchronous *Escherichia coli* [127]. Fluorescent measurements allowed proliferating and nonproliferating cell populations to be discriminated [128]. This result indicates that the cellular proliferation rate is tightly connected with NAD/NADH ratio (and cellular redox potential, respectively).

It was proposed in Section 3 that positive effects of irradiation connected with improvement of cell viability and metabolism are due to a shift in the overall cellular redox state to a more oxidized direction. The question is whether

such a phenomenon is known in cell biology. The answer is positive, as will be summarized briefly below.

First, it has already been mentioned above that any shift in the redox state of a cell will necessarily alter its radiosensitivity. Some methods of radioprotection are based on this fact [129]. The fact that the preirradiation of cells with a He–Ne laser decreased their cytotoxic response to γ-radiation was taken as an additional proof of the theory that irradiation shifts the cellular redox state [112].

Secondly, besides radiomodification, a modulation of hypoxia-induced cell injury is supposed to occur after the intracellular redox state is affected [130]. It is known that one of the factors contributing to hypoxic injury is a reductive stress created by an accumulation of reducing equivalents [131]. It is also known that chemicals which decrease the lactate/pyruvate ratio and oxidize cellular NADH prevent hypoxic injury and normalize the NAD/NADH ratio. In the case of hepatocytes, chemicals acting in this way were the following: the glycolytic nutrients fructose, dihydroxyacetone, and glyceraldehyde; the intermediate metabolites oxalacetate and acetoacetate; the artificial electron acceptors methylene blue and dichlorophenolindophenol [130]. The same authors conclude that the susceptibility of cells to hypoxic injury depends more on the cells' capacity to maintain the redox homeostasis and much less on the cells' capacity to maintain their energy status. Recent results of Zhu et al. [132] confirm the suggestion made above that laser radiation can decrease hypoxic injury and reductive stress. In this work laser radiation of 660 nm improved the functional preservation of isolated rat heart [132].

One should here set methylene blue apart from the chemicals tested in Ref. [130]. This is because methylene blue was also tested in our experiments. The treatment of cells in the dark with this artificial electron acceptor significantly stimulated DNA synthesis, i.e., methylene blue in the dark affected the cells in a similar way to He–Ne laser irradiation [4]. One should recall that methylene blue has been used in medical and laboratory practice during the last century for various purposes. The following are only a few examples: for pain relief [133] and treating malaria [134]; for fertilization of marine eggs [135]; to stimulate oxygen consumption [136]; to improve the therapeutic index of toxic oxidative drugs like doxorubicin and other anthracylins and to prevent ethanol-induced hypoxic injury [137]; to protect from sublethal radiation damage [138]. In most of these cases the action mechanism of methylene blue is believed to be due to its affect on the concentration of intracellular reducing agents.

Thirdly, the experiments described by Keyse and Tyrrell [139,140] support the suggestion about the crucial role of redox changes in alterations of cellular metabolism. It has been demonstrated that agents which reduce the level of available glutathione in human skin fibroblasts, i.e., shift the cellular redox state to a more oxidized direction, also induce heme oxygenase (a 32 kDa stress protein). In other words, it appears that the level of heme oxygenase is fully regulated by the redox state of the cell [141]. Among the agents causing this effect were UV-A radiation, visible light at 405 nm, hydrogen peroxide, cadmium chloride, iodoacetamine, and menadione [139,140].

Fourthly, cellular activation events occurring in T lymphocytes and monocytes and mediated through translocation of the transcription factor NF-κB are believed to be dependent on the redox state of these cells [142]. It appeared that a basal redox equilibrium tending toward oxidation was a prerequisite for full activation of T lymphocytes (J. Jhan) and U937 monocytes; both constitutive activation as well as that induced by mitogenes was inhibited or even canceled by treatment of cells with reducing agents or antioxidants [143–145]. For example, a reducing agent, cysteine, canceled blast transformation of lymphocytes [144], and decreased HIV replication in parallel with decreased NF-κB binding activity [146]. Recall that in our experiments treatment with cysteine canceled early transcriptional activation of cells irradiated with He–Ne laser or activated with PHA, and the effect was dependent on cysteine concentration [147].

Fifthly, one should keep in mind that many agents which induce cell apoptosis (programmed cell death) are either oxidants or stimulators of cellular oxidative metabolism; various antioxidants can prevent factor-deprived immunocompetent cells from undergoing apoptosis [148]. In this connection, recall that when the proliferation of HeLa cells was stimulated by He–Ne laser radiation, the number of cells decreased drastically at the end of the logarithmic phase of growth [149]. Whether this was connected with apoptosis or happened for some other reason should be clarified.

So, it seems quite reasonable to conclude that one of the secondary responses of a cell to irradiation proposed in Section 4, namely a shift in redox potential of a cell, is not a specific cellular response characteristic only for visible-light irradiation, but a rather widespread cell response to various exogenous factors. In all examples discussed above, oxidation is required at some point(s) of signal transduction.

Cells with a lower than normal pH_i, where the redox state is shifted in the reduced direction, are considered to be more sensitive to the stimulative action of light than those with the respective parameters being optimal or near optimal. This circumstance explains the possible variations in observed magnitudes of low-power laser effects. Light action on the redox state of a cell via the respiratory chain also explains the diversity of low-power laser effects. Beside explaining many controversies in the field of low-power laser effects (i.e., the diversity of effects, the variable magnitude or absence of effects in certain studies), the proposed redox-regulation mechanism may be a fundamental explanation for some clinical effects of irradiation, for example, the positive results achieved in treating indolent wounds, chronic inflammation, and ischemia, all characterized by acidosis and hypoxia. The problems of controversies as well as limits and limitations are considered in detail in Ref. [9].

One unsolved mystery integral to low-power laser effects is the following. It is rather well documented that more than one of the cellular functions (e.g., the replicative, motile, and

secretory functions of fibroblasts or keratinocytes) can be influenced by radiation at the same wavelength. Even more interesting is that when one of these cellular functions is altered by irradiation, the others remain unchanged [9]. This finding dearly indicates the existence of some unknown regulatory mechanism establishing priorities in a cell. Identifying this priority regulation system (i.e., the preferential utilization of light-generated proton motive force, ATP, etc.) will have profound importance for future studies of low-power laser effects.

Finally, a remark about the reliability of low-power laser effects should be made. Years ago, a rather usual perception existed that laser biostimulation occurred in Eastern but not Western laboratories. At that time, the actual situation was indeed rather close to this. Very few Western scientists took laser biostimulation seriously and even fewer performed experiments. However, lately there have been changes in this attitude, and a number of well-designed experiments have been performed on various cells. These data are summarized in Ref. [9].

In some cases the effects of visible and near-IR light on various cellular functions have been described in papers not dealing with the issue of laser biostimulation at all (e.g., [23,109,150]). This circumstance gives a higher reliability to those results for the field of low-power laser effects.

Alternatively, these findings clearly indicate that the responsiveness of mammalian cells to various wavelengths of monochromatic visible and near-IR radiation can be used to understand some physiological processes (e.g., adaptation). Laser biostimulation can be only one field where the cellular sensitivity (which seems to be increased in injured or otherwise stressed systems) to monochromatic visible and near-IR radiation is used. In the paper by Albrecht-Büehler [150] the phenomenon of fibroblast phototaxis (pseudopodia moving towards a monochromatic light source) was described. Kato et al. [23] suggested that mitochondria in a special part of bird brain could work as photoreceptors for a photobiological process relating to gonadal growth.

To complete the present paper, two remarks from a classical paper by Smith [151] should be remembered. First, just because humans cannot see through the human body does not mean that the human body is opaque to all wavelengths of light. Secondly, since light (especially red light) can penetrate deep into human tissues, and since absorbed light can cause photochemistry, it is appropriate to be concerned with the biological consequences of such absorbed light in the tissues of man.

References

[1] T.I. Karu, Photobiological fundamentals of low-power laser therapy, IEEE J. Quantum Electron. QE-23 (1987) 1703–1717.

[2] T.I. Karu, Photobiology of low-power laser effects, Health Phys. 56 (1989) 691–704.

[3] K.S. Smith, The photobiological basis of low-level laser radiation therapy, Laser Therapy 3 (1991) 19–25.

[4] T.I. Karu, Photobiology of Low-Power Laser Therapy, Harwood, London, 1989.

[5] M. Belkin, B. Zaturunsky, M. Schwartz, A critical review of low energy laser bioeffects, Lasers Light Ophthalmol. 2 (1988) 63–71.

[6] D.M. Harris, Laser biostimulation: review and hypothesis, Laser Topics 10 (3) (1988) 9–14.

[7] M.W. Berns, J.S. Nelson, Laser applications in biomedicine. Part I: biophysics, cell biology, and biostimulation, J. Laser Applic. 1 (1988) 34–39.

[8] J.R. Basford, Low-energy laser therapy: controversies and new research findings, Lasers Surg. Med. 9 (1989) 1–5.

[9] T. Karu, The Science of Low Power Laser Therapy, Gordon and Breach, London, 1998.

[10] T.I. Karu, Stimulation of metabolic processes by low-intensity visible light: a scientific basis for biostimulation, in: M. Wolbsrsht (Ed.), Laser Application in Biology and Medicine, vol. 5, Plenum, New York, 1991, pp. 1–47.

[11] T.I. Karu, Molecular mechanism of the therapeutic effect of low-intensity laser radiation, Lasers Life Sci. 2 (1988) 53–74.

[12] T. Karu, Interaction of monochromatic visible light and near infrared radiation with cells: currently discussed mechanisms, SPIE Proc. 2391 (1995) 576–586.

[13] T. Karu, Effects of visible radiation on cultured cells, Photochem. Photobiol. 52 (1990) 1089–1099.

[14] T. Karu, Effects of visible (laser) radiation on cultured cells, in: S. Martelluci, A.N. Chester (Eds.), Laser Systems for Photobiology and Photomedicine, Plenum, New York, 1991, pp. 89–113.

[15] O. Tiphlova, T. Karu, Action of low-intensity laser radiation on *Escherichia coli*, CRC Crit. Rev. Biomed. Eng. 18 (1991) 387–412.

[16] T. Karu, Activation of metabolism of nonphotosynthesizing microorganisms with monochromatic visible (laser) light: a critical review, Lasers Life Sci. 7 (1996) 11–34.

[17] T. Karu, Derepression of the genome after irradiation of human lymphocytes with He–Ne laser, Laser Therapy 4 (1992) 5–24.

[18] T.I. Karu, Derepression of genome and alteration of DNA template activity in nonproliferation cells by means of UV and visible laser radiation. in: A.M. Verga-Scheggi, S. Martellucci, A.N. Chester, R. Pratesi (Eds.), Biomedical Optical Instrumentation and Laser-Assisted Biotechnology, Kluwer, Dordrecht, 1996, pp. 3–32.

[19] T. Oshiro, R.G. Calderhead, Low Level Laser Therapy, Wiley, Chichester, 1988.

[20] T. Oshiro, R.G. Calderhead, Progress in Laser Therapy, Wiley, Chichester, 1991.

[21] R.G. Calderhead, K.C. Moore, A. Nagasawa, Low Reactive-Level Laser Therapy: Practical Application, Wiley, Chichester, 1991.

[22] G.D. Baxter, Therapeutic Lasers. Theory and Practice, Churchill and Livingstone, Edinburgh, 1994.

[23] M. Kato, K. Shinizawa, S. Yoshikawa, Cytochrome oxidase is a possible photoreceptor in mitochondria, Photobiochem. Photobiophys. 2 (1981) 263–269.

[24] S. Passarella, F. Casamassima, S. Molinari, D. Pastore, E. Quagliariello, I.M. Catalano, A. Cingolani, Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by He–Ne laser, FEBS Lett. 175 (1984) 95–99.

[25] S.A. Gordon, K. Surrey, Red and far-red light action on oxidative phosphorylation, Radiat. Res. 12 (1960) 325–339.

[26] N.L. Vekshin, G.P. Mironov, Flavin-dependent oxygen uptake in mitochondria under illumination, Biophysics (Moscow) 27 (1982) 537–539.

[27] S. Passarella, E. Perlino, E. Quagliariello, L. Baldassarre, I.M. Catalano, A. Cingolani, Evidence of changes induced by He–Ne laser irradiation in the biochemical properties of rat liver mitochondria, Bioelectrochem. Bioenerg. 10 (1983) 185–198.

[28] S. Passarella, A. Ostuni, A. Atlante, E. Quagliariello, Increase in the ADP/ATP exchange in the rat liver mitochondria irradiated in vitro by He–Ne laser, Biochem. Biophys. Res. Commun. 156 (1988) 978–986.

[29] R. Hilf, R.S. Murant, U. Narayanan, S.L. Gibson, Relationship of mitochondrial function and cellular adenosine triphosphate levels to hematoporphyrin derivative-induced photosensitization in R3230AC mammary tumors, Cancer Res. 46 (1986) 211–217.
[30] Y. Morimoto, T. Arai, M. Kikuchi, S. Nakajama, H. Nakamura, Effect of low-intensity argon laser irradiation on mitochondrial respiration, Lasers Surg. Med. 15 (1994) 191–199.
[31] W. Yu, J. Naim, M. McGowan, K. Ippolito, R. Lanzafame, Photomodulaton of oxidative metabolism and electron chain enzymes in rat liver mitochondria, Photochem. Photobiol. 66 (1997) 866–871.
[32] T. Karu, L. Pyatibrat, G. Kalendo, Irradiation with He–Ne laser increases ATP level in cells cultivated in vitro, J. Photochem. Photobiol. B: Biol. 27 (1995) 219–223.
[33] L.E. Bakeeva, V.M. Manteifel, E.B. Rodichev, T.I. Karu, Formation of giant mitochondria in human blood lymphocytes after He–Ne laser irradiation, Molec. Biol. (Moscow) 27 (1993) 608–617.
[34] V. Manteifel, L. Bakeeva, T. Karu, Ultrastructural changes in chondriome of human lymphocytes after irradiation with He–Ne laser: appearance of giant mitochondria, J. Photochem. Photobiol. B: Biol. 38 (1997) 25–30.
[35] K.E. Herbert, L.L. Bhusate, D.L. Scott, C. Diamantopoulos, D. Perrett, Effect of laser light at 820 nm on adenosine nucleotide levels in human lymphocytes, Lasers Life Sci. 3 (1989) 37–46.
[36] H. Loevschall, D. Arenholdt-Bindslev, Low level laser therapy effect on mitochondrial rhodamine 123 uptake in human oral fibroblasts, Laser Therapy 6 (1994) 30–31.
[37] J.J. Anders, V. Mysliwiec, S. Preston, Low power laser irradiation alters the fluorescence intensity of rhodamine 123 labeled mitochondria in human fibroblasts, Lasers Surg. Med., Suppl. 7 (1995) 7–8 (abstr. 30).
[38] K.M. Hartmann, Action spectroscopy, in: W. Hoppe, W. Lohmann, H. Marke, H. Ziegler (Eds.), The Biophysics, Springer, Heidelberg, 1983, ch. 3.2.7, pp. 115–144.
[39] T.I. Karu, G.S. Kalendo, V.S. Letokhov, V.V. Lobko, Biostimulation of HeLa cells by low intensity visible light. II. Stimulation of DNA and RNA synthesis in a wide spectral range, Nuovo Cimento D 3 (1984) 309–318.
[40] T.I. Karu, G.S. Kalendo, V.S. Letokhov, V.V. Lobko, Biostimulation of HeLa cells by low intensity visible light. III. Stimulation of nucleic acid synthesis in plateau phase cells, Nuovo Cimento D 3 (1984) 319–325.
[41] T.I. Karu, L.V. Pyatibrat, G.S. Kalendo, R.O. Esenaliev, Effects of monochromatic low-intensity light and laser irradiation on adhesion of HeLa cells in vitro, Lasers Surg. Med. 18 (1996) 171–177.
[42] G. Wilkinson, R.D. Gillard, J.A. McCleverty (Eds.), Comprehensive Coordination Chemistry, vols. 1–7, Pergamon, Oxford, 1987.
[43] H. Siegel (Ed.), Metal Ions in Biological Systems, vols. 1–13, Marcel Dekker, New York, 1971–1981.
[44] M. Hughes, Coordination compounds in biology, in: G. Wilkinson, R.D. Gilland, J.A. McCleverty (Eds.), Comprehensive Coordination Chemistry, vol. 6, Pergamon, Oxford, 1987, pp. 541–753.
[45] M. Gouterman, Optical spectra and electronic structure of porphyrins and related rings, in: D. Dolphin (Ed.), The Porphyrins, vol. 3, Academic Press, New York, 1978, pp. 1–165.
[46] B.J. Hathaway, Copper, in: G. Wilkinson, R.D. Gillard, J.A. McCleverty (Eds.), Comprehensive Coordination Chemistry, vol. 5, Pergamon, Oxford, 1987, pp. 534–774.
[47] R. Lontie (Ed.), Copper Proteins and Copper Enzymes, vols. 1–3, CRC Press, Boca Raton, 1984.
[48] G. Spiro (Ed.), Copper Proteins, Wiley-Interscience, New York, 1981.
[49] D. Karlin, J. Zubieta (Eds.), Copper Coordination Chemistry, Academic Press, New York, 1983.
[50] M. Brunori, B. Chance (Eds.), Cytochrome Oxidase: Structure, Function and Physiopathology, Ann. N.Y. Acad. Sci., vol. 550, 1988.
[51] T. Tsukahara, H. Aoyma, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Hoh, R. Nakashima, R. Yano, S. Yoshikawa, Structures of metal sites of oxidized bovine heart cytochrome *c* oxidase at 2.8 Å, Science 269 (1995) 1069–1074.
[52] T. Tsukahara, H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Hoh, R. Nakashima, R. Yano, S. Yoshikawa, The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å, Science 272 (1996) 1136–1144.
[53] S. Iwata, C. Ostermeir, B. Ludwig, H. Michel, Structure of 2.8 Å resolution of cytochrome *c* oxidase from *Paracoccus denitrificans*, Nature 376 (1995) 660–669.
[54] R. Gennis, S. Ferguson-Miller, Structure of cytochrome *c* oxidase, energy generator of aerobic life, Science 269 (1995) 1063–1064.
[55] A. Sucheta, K.E. Georgiadis, O. Einarsdottir, Mechanism of cytochrome *c* oxidase-catalyzed reduction of dioxygen to water: evidence for peroxy and ferryl intermediates at room temperature, Biochemistry 36 (1997) 554–565.
[56] G.T. Babcock, M. Wikström, Oxygen activation and the conservation of energy in cell respiration, Nature 356 (1992) 302–309.
[57] M.I. Verkhovsky, J.E. Morgan, M. Wikström, Redox transitions between oxygen intermediates in cytochrome *c* oxidase, Proc. Acad. Sci. USA 93 (1996) 12235–12339.
[58] R.A. Capaldi, Structure and function of cytochrome *c* oxidase, Annu. Rev. Biochem. 59 (1990) 569–596.
[59] G. Palmer, T. Antalis, T. Babcock, L. Garcia-Iniguez, M. Tweedle, L.J. Wilson, L.T. Vickery, Electronic states of heme in cytochrome oxidase, in: T.P. Singer (Ed.), Mechanisms of Oxidizing Enzymes, vol. 1, Elsevier, Amsterdam, 1978, pp. 221–238.
[60] G. Palmer, Current issues in the chemistry of cytochrome *c* oxidase, J. Bioenergetics Biomembr. 25 (1993) 145–151.
[61] T.I. Karu, N.I. Afanasyeva, Chytochrome oxidase as primary photoacceptor for cultured cells in visible and near IR regions, Dokl. Akad. Nauk. — Dokl. Biochem. (Moscow) 342 (1995) 693–695.
[62] T.I. Karu, V.S. Letokhov, V.V. Lobko, Biostimulation of HeLa cells by low-intensity visible light. IV. Dichromatic irradiation, Nuovo Cimento D 5 (1985) 483–496.
[63] D. Pastore, V.A. Petragallo, M. Greco, E. Marra, S. Passarella, The effect of He–Ne laser light on the mitochondrial cytochrome *c* oxidase, in: G. Galletti, L. Bolognani, G. Ussia (Eds.), Laser Application in Medicine and Surgery, Monduzzi Editore, Bologna, 1992, pp. 259–263.
[64] T.I. Karu, G.G. Lukpanova, I.M. Parkhomenko, O.A. Tiphlova, Effect of irradiation with monochromatic visible light in cAMP content in Chinese hamster fibroblasts, Nuovo Cimento D 9 (1987) 1245–1251.
[65] H.H.F.I. van Breugel, C. Engels, P.R. Bär, Mechanism of action in the laser induced photobiomodulation depend on the wavelength of the laser, Lasers Surg. Med., Suppl. 5 (1993) 9 (abstr. 36).
[66] P. Nicholls, W.B. Elliot, The cytochromes, in: A. Jacobs, M. Worwood (Eds.), Iron Biochemistry and Medicine, Academic Press, New York, 1974, pp. 221–272.
[67] H.H.F.I. van Breugel, P.R. Dop Bär, Power density and exposure time of He–Ne laser irradiation are more important than total energy dose in photobiomodulation of human fibroblasts in vitro, Laser Surg. Med. 12 (1992) 528–537.
[68] T.I. Karu, N.I. Afanasyeva, S.F. Kolyakov, L.V. Pyatibrat, Changes in absorption spectra of monolayer of living cells after irradiation with low intensity laser light, Dokl. Akad. Nauk. — Dokl. Biophys. (Moscow) 360 (1998) 267–270 (also: SPIE Proc. 3198 (1997) 2–11).
[69] N.I. Afanasyeva, T.I. Karu, O. A Tiphlova, Oxidases *bd* and *bo* as primary photoacceptors when *Escherichia coli* cells are irradiated with monochromatic visible (laser) radiation, Dokl. Akad. Nauk. — Dokl. Biochem. (Moscow) 345 (1995) 404–406.
[70] Govindjee (Ed.), Photosynthesis, vol. 1, Academic Press, New York, 1982.

[71] H. Senger (Ed.), Blue Light Effects in Biological Systems, Springer, Berlin, 1980.
[72] H. Senger (Ed.), Blue Light Effects in Biological Systems, Springer, Berlin, 1984.
[73] R.A. Marcus, N. Sutin, Electron transfers in chemistry and biology, Biochim. Biophys. Acta 811 (1985) 265–322.
[74] F. Adar, T. Jonetani, Resonance Raman spectra of cytochrome oxidase. Evidence for photoreduction by laser photons in resonance with the Soret band, Biochem. Biophys. Acta 502 (1978) 80–86.
[75] A. Dube, P.K. Gupta, S. Bharti, Redox absorbance changes of the respiratory chain components of *E. coli* following He–Ne laser irradiation, Lasers Life Sci. 7 (1997) 173–180.
[76] T. Pasher, Y.P. Ghesick, J.R. Winkler, H.B. Gray, Protein folding triggered by electron transfer, Science 271 (1996) 1558–1560.
[77] C.-K. Chain, J. Hofrichter, W.A. Eaton, Optical triggers of protein folding, Science 274 (1996) 628–629.
[78] V.S. Letokhov, Effects of transient local heating of spatially and spectrally heterogeneous biotissue by short laser pulses, Nuovo Cimento D 13 (1991) 939–948.
[79] T.I. Karu, O.A. Tiphlova, Yu.A. Matveyets, A.P. Yartsev, V.S. Letokhov, Comparison of the effects of visible femtosecond pulses and continous wave laser radiation of low average intensity on the clonogenicity of *Escherichia coli*, J. Photochem. Photobiol. B: Biol. 10 (1991) 339–344.
[80] T. Karu, Local pulsed heating of absorbing chromophores as a possible primary mechanism of low-power laser effects, in: G. Galletti, L. Bolognani, G. Ussia (Eds.), Laser Applications in Medicine and Surgery, Monduzzi Editore, Bologna, 1992, pp. 253–258.
[81] T.I. Karu, T. Ryabykh, V.S. Letokhov, Different sensitivity of cells from tumor-bearing organisms to continuous-wave and pulsed laser radiation ($\lambda = 632.8$ nm) evaluated by chemiluminescence test: III Effect of dark period between pulses, Lasers Life Sci. 7 (1997) 141–156.
[82] H.J. Forman, A. Boveris, Superoxide radical and hydrogen peroxide in mitochondria, in: A. Pryor (Ed.), Free Radicals in Biology, vol. 5, Academic Press, New York, 1982, pp. 65–90.
[83] H. Nohl, A novel superoxide radical generator in heart mitochondria, FEBS Lett. 214 (1987) 269–273.
[84] K. Mailer, Superoxide radical as electron donor for oxidative phosphorylation of ADP, Biochem. Biophys. Res. Comm. 170 (1990) 59–64.
[85] L.F. Dmitriev, M.V. Ivanova, I.I. Ivanov, Liver mitochondrial ATP synthesis can be promoted by UV generation of superoxide, Biol. Membr. (Moscow) 7 (1990) 961–965.
[86] J.G. Murphy, T.W. Smith, J.D. Marsh, Mechanisms of reoxygenation-induced calcium overload in cultured chick embryo heart cells, Am. J. Physiol. 254 (1988) H1133–H1141.
[87] M. Shibanuma, T. Kuroki, K. Nose, Superoxide as a signal for increase in intracellular pH, J. Cell. Physiol. 136 (1988) 379–383.
[88] M. Kaneko, P.K. Singal, N.S. Dhalla, Alteration in heart sarcolemmal Ca^{2+}-ATPase and Ca^{2+}-binding activities due to oxygen free radicals, Basic Res. Cardiol. 85 (1990) 45–54.
[89] J.K. Bannister, G. Rotilio, Aspects of the structure, function, and application of superoxide dismutase, CRC Crit. Rev. Biochem. 22 (1987) 111–179.
[90] A.F. Agro, P. Conti, M.G. Cifone, P.U. Angeletti, C. Rotilio, Effect of hydrogen peroxide on lymphocytes, in: W. Bannister, V. Bannister (Eds.), Biological and Superoxide Clinical Aspects of Dismutase, Elsevier, Amsterdam, 1980, pp. 276–280.
[91] T. Karu, T. Andreichuk, T. Ryabykh, Changes in oxidative metabolism of murine spleen following diode laser (660–950 nm) irradiation: effect of cellular composition and radiation parameters, Lasers Surg. Med. 13 (1993) 453–462.
[92] A. Callaghan, C. Riordan, W.S. Gilmore, I.A. Mcintyre, J.A. Allen, B.H. Hannigan, Reactive oxygen species inducible by low-intensity laser radiation after DNA synthesis in the haemopoietic cell line U937, Lasers Surg. Med. 19 (1996) 201–206.
[93] N. Grossman, N. Schneid, H. Reuveni, S. Halevy, R. Lubart, 780 nm low power diode laser irradiation stimulates proliferation of keratinocyte cultures: involvement of reactive oxygen species, Lasers Surg. Med. 22 (1998) 212–218.
[94] N. Cohen, R. Lubart, S. Rubinstein, H. Breitbart, Light irradiation of mouse spermatozoa: stimulation of in vitro fertilization and calcium signals, Photochem. Photobiol. 68 (1998) 404–413.
[95] T.I. Karu, T.S. Lyapunova, N.A. Pomoshnikova, The activation of yeast metabolism with He–Ne laser radiation. IV. Relationship between the activity of catalase and protein synthesis, Lasers Life Sci. 5 (1993) 251–258.
[96] A.C. Giese, Photosensitization of organisms with special reference to natural photosensitizers, in: F. Hillenkampf, R. Pratesi, C. Sacchi (Eds.), Lasers in Biology and Medicine, Plenum, New York, 1980, pp. 299–314.
[97] J.D. Spikes, Photosensitization, in: K.C. Smith (Ed.), The Science of Photobiology, 2nd ed., Plenum, New York, 1989, pp. 79–111.
[98] V.P. Danilov, S.D. Zakharov, A.V. Ivanov, Photodynamic injury of cells in red and IR absorption peaks of endogeneous oxygen, Dokl. Akad. Nauk SSSR (Moscow) 311 (1990) 1255–1258.
[99] H. Friedman, R. Lubart, J. Laulicht, S. Rochkind, A possible explanation of laser-induced stimulation and damage of cells cultures, J. Photochem. Photobiol. B: Biol. 11 (1991) 87–95.
[100] C.S. Kim, J. Jung, Inactivation of the respiratory chain in plant mitochondria by visible light: the primary target for photodamage and endogeneous photosensitizing chromophores, J. Photochem. Photobiol. B: Biol. 29 (1995) 135–139.
[101] B.L. Epel, Inhibition of growth and respiration by visible and near ultraviolet light, in: A.C. Giese (Ed.), Photophysiology, vol. 8, Academic Press, New York, 1973, pp. 209–229.
[102] T.I. Karu, E.V. Kutomkina, N.A. Pomoshnikova, The activation of yeast metabolism with He–Ne laser radiation. III. Protein synthesis in *Saccharomycodes ludwigii* grown in aerobic and anaerobic conditions, Lasers Life Sci. 5 (1993) 259–266.
[103] H.K. Krebs, R.L. Veech, Regulation of the redox state of the pyridine nucleotides in rat liver, in: H. Sund (Ed.), Pyridine Nucleotide-Dependent Dehydrogenases, Springer, Berlin, 1970, pp. 413–434.
[104] E. Kohen, C. Kohen, J.G. Hirschberg, A.W. Wouters, B. Thorell, H.V. Westerholff, K.K.N. Charyulu, Metabolic control and compartmentation in single living cells, Cell Biochem. Funct. 1 (1983) 3–16.
[105] O.S. Nedelina, O.N. Brzevskaya, L.P. Kayushin, Redox regulation in ATP synthesis, Biophysics (Moscow) 30 (1985) 179–191.
[106] J. Pouyssegur, A. Franchi, G.L. Allemain, S. Paris, Cytoplasmic pH, a key determinant of growth factor-induced DNA synthesis in quiescent fibroblasts, FEBS Lett. 90 (1985) 115–119.
[107] T.R. Hesketh, J.R. Moore, J.D.H. Morris, M.V. Taylor, J. Rogers, G.A. Smith, J.C. Metcalfe, A common sequence of calcium and pH signals in the mitotic stimulation of eukaryotic cells, Nature 313 (1985) 481–484.
[108] W.H. Moolenaar, Regulation of cytoplasmic pH by Na^+/H^+ exchange, Trends Biochem. Sci. 11 (1986) 141–143.
[109] H. Chopp, Q. Chen, M.O. Dereski, F.W. Hetzel, Chronic metabolic measurement of normal brain tissue response to photodynamic therapy, Photochem. Photobiol. 52 (1990) 1033–1036.
[110] T.R. Quickenden, L.L. Daniels, L.T. Byrne, Does low-intensity He–Ne laser radiation affect the intracellular pH of intact *E. coli* cells?, in: S.L.Jacques, A. Katzir (Eds.), Laser–Tissue Interaction VI, SPIE Proc., vol. 2391, 1995, pp. 535–545.
[111] C.L. Greenstock, Radiation-induced aging and induction and promotion of biological damage, in: J.E. Johnson, D. Harman, R. Walford J. Miguel (Eds.), Radiation, Free Radical Damage, and Aging, Alan Liss, New York, 1986, pp. 197–230.
[112] T. Karu, L. Pyatibrat, G. Kalendo, Irradiation with He–Ne laser can influence the cytotoxic response of HeLa cells to ionizing radiation, Int. J. Radiat. Biol. 65 (1994) 691–697.

[113] A.M. Moroz, Na, K,-ATPase activity after the laser irradiation, Ukr. Biochem. Zh. 55 (1983) 674–676.
[114] Ch. Kudoh, K. Inomata, K. Okajama, M. Motegi, T. Ohshiro, Effects of 830 nm GaAlAs diode laser radiation on rat saphenous nerve K^+, Na^+, ATPase activity: a possible pain attenuation mechanism examined, Laser Therapy 1 (1989) 63–68.
[115] P. Balaban, R. Esenaliev, T. Karu, E. Kutomkina, V. Letokhov, A. Oraevsky, N. Ovcharenko, He–Ne laser irradiation of single identified neurons, Lasers Surg. Med. 12 (1992) 329–337.
[116] T. Karu, N. Smolyaninova, A. Zelenin, Long-term and short-term responses of human lymphocytes to He–Ne laser radiation, Lasers Life Sci. 4 (1991) 167–178.
[117] H. Loevschall, O.B. Scharft, B. Foder, D. Arenholdt-Bindslev, Effects of low level laser irradiation on cytosolic Ca^{2+} in human neutrophils in vitro, Lasers Life Sci. 8 (1998) 127–136.
[118] R. Lubart, H. Friedman, M. Sinyakov, N. Cohen, H. Breitbart, Changes in calcium transport in mammalian sperm mitochondria and plasma membranes caused by 780 nm radiation, Lasers Surg. Med. 21 (1997) 493–499.
[119] T. Karu, A. Kurchikov, V. Letokhov, V. Mokh, He–Ne laser radiation influences single-channel ionic currents through cell membranes: a patch-clamp study, Lasers Life Sci. 7 (1996) 35–48.
[120] H.L. Boynton, J.F. Whitfield, The role of cyclic AMP in cell proliferation: a critical assessment of the evidence, in: P. Greengard, G.A. Robinson (Eds.), Advances in Cyclic Nucleotide Research, vols. 1–5, Raven Press, New York, 1983, pp. 195–294.
[121] C.D. Cone, Unified theory on the basic mechanism of normal mitotic control and oncogenesis, J. Theor. Biol. 30 (1971) 151–181.
[122] J.G. Kaplan, Membrane cation transport and the control of proliferation of mammalian cells, Annu. Rev. Physiol. 40 (1978) 19–41.
[123] E. Rozengurt, Early signals in the mitogenic response, Science 234 (1986) 161–166.
[124] E. Rozengurt, S. Mendoza, Monovalent ion fluxes and the control of cell proliferation in cultured fibroblasts, Ann. N.Y. Acad. Sci. 339 (1980) 175–190.
[125] D. Hülser, W. Frank, Stimulating von Kulturen embryonaler Rattenzellen durch eine Protein-Fraktion aus fötalem Kälberserum. I Elektrophysiologische Messungen an den Oberflächenmembranen, Z. Naturforsch. 266 (1971) 1045–1048.
[126] G.C. Brown, Control of respiratory and ATP synthesis in mammalian mitochondria and cells, Biochem. J. 284 (1992) 171–182.
[127] S.P. Layne, I.J. Bigio, A.C. Scott, P.S. Lomdahl, Transient fluorescence in synchronously dividing *Escherichia coli*, Proc. Natl. Acad. Sci. USA 82 (1985) 7599–7603.
[128] J.C. Zhang, H.E. Savage, P.G. Sacks, Th. Delohery, R.R. Alfano, A. Katz, S. Schantz, Initiate cellular fluorescence reflects alterations in cellular proliferation, Lasers Surg. Med. 20 (1997) 319–331.
[129] J.E. Johnson, R. Harman, R. Walford, J. Miguel, Radiation, Free Radical Damage, and Aging, Alan Liss, New York, 1986.
[130] S. Khan, P.J. O'Brien, Modulating hypoxia-induced hepatocyte injury by affecting intracellular redox state, Biochim. Biophys. Acta 1269 (1995) 153–161.
[131] B. Chance, Cellular oxygen requirements, Fed. Proc. Fed. Am. Soc. Exp. Biol. 16 (1957) 671–680.
[132] Q. Zhu, J. Wei, X. Jang, G.L. Hichks, R.J. Lanzafame, T. Wang, Photo-irradiation improved functional preservation of the isolated rat heart, Lasers Surg. Med. 20 (1997) 332–339.
[133] P. Ehrlich, A. Leppmann, Über schmerzstillende Wirkung des Methyleneblau, Dtsche Med. Wochenschr. 16 (1890) 493–494.
[134] P. Gutman, P. Ehrich, Über die Wirkung des Methyleneblau bei Malaria, Berliner Klin. Wochenschr. 28 (1891) 953–956.
[135] M.M. Brooks, Mechanism of fertilization of eggs, Fed. Proc. Fed. Am. Soc. Exp. Biol. 2 (1943) 6–8.
[136] R.K. Mishra, H. Passow, Induction of intracellular ATP synthesis by extracellular ferricyanide in human red blood cells, J. Membr. Biol. 1 (1969) 214–224.
[137] W.J.M. Hrushesky, R. Olshefski, P. Wood, S. Meshnick, J.W. Eaton, Modifying intracellular redox balance: an approach to improving therapeutic index, Lancet 8428 (1985) 565–567.
[138] S.O. Chung, S.Y. Nam, The radioprotective effect against gamma irradiation of methylene blue in the rat with reference to serum enzymes and pancreatic protein fractions examined by isoelectric focussing, J. Radiat. Res. 16 (1975) 211–223.
[139] S.M. Keyse, R.M. Tyrrell, Both near ultraviolet radiation and the oxidizing agent hydrogen peroxide induce a 32-kDA stress protein in normal human skin fibroblasts, J. Biol. Chem. 262 (1987) 14821–14825.
[140] S.M. Keyse, R.M. Tyrrell, Heme oxygenase is the major 32-kDA stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite, Proc. Natl. Acad. Sci. 86 (1989) 99–103.
[141] R.M. Tyrrell, Activation of mammalian expression by the UV component of sunlight — from models to reality, Bioessays 18 (1996) 139–148.
[142] F.J.T. Staal, M. Roederer, L.A. Herzenberg, L.A. Herzenberg, Intracellular thiols regulate activation of nuclear factor κB and transcription of human immunodeficiency virus, Proc. Natl. Acad. Sci. USA 87 (1990) 9943–9948.
[143] N. Israël, M.-A. Gougerot-Pocidalo, F. Aillet, J.-L. Verelizier, Redox status of cells influences constitutive or induced NF-κB translocation and HIV long terminal repeat activity in human T lymphocytes and monocytic cell lines, J. Immunol. 149 (1992) 3386–3393.
[144] A. Novogrodski, Lymphocyte activation induced by modifications of surface, in: S. Rosenthal (Ed.), Immune Recognition, Academic Press, New York, 1975, pp. 43–61.
[145] A. Novogrodski, A. Ravid, A.L. Rubin, K.H. Stenzel, Hydroxyl radical scavengers inhibit lymphocyte mitogenesis, Proc. Natl. Acad. Sci. USA 79 (1982) 1171–1177.
[146] M. Roederer, F.J.T. Staal, P.A. Raju, S.W. Ela, L.A. Herzenberg, L.A. Herzenberg, Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine, Proc. Natl. Acad. Sci. USA 87 (1990) 4884–4889.
[147] G.E. Fedoseyeva, N.K. Smolyaninova, T.I. Karu, A.V. Zelenin, Human lymphocyte chromatin changes following irradiation with a He–Ne laser, Lasers Life Sci. 2 (1988) 197–205.
[148] T.M. Buttke, P.A. Sandstrom, Oxidative stress as a mediator of apoptosis, Immunol. Today 15 (1994) 7–10.
[149] T.I. Karu, G.S. Kalendo, V.V. Lobko, L.V. Pyatibrat, Kinetics of tumour HeLa cells growth under subcultiration after irradiation by low-intensity red light at the stationary growth phase, Eksp. Onkologiya (Kiev) 6 (1) (1984) 60–63.
[150] G. Albrecht-Büehler, Surface extensions of 3T3 cells towards distant infrared light sources, J. Cell Biol. 114 (1991) 494–502.
[151] K.C. Smith, Common misconceptions about light, in: R. Pratesi, C. Sacchi (Eds.), Lasers in Photomedicine and Photobiology, Springer, Berlin, 1980, pp. 23–25.