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Invited Review

Mitochondrial Signaling in Mammalian Cells Activated by Red and Near-IR Radiation

Tiina I. Karu*

Institute of Laser and Information Technologies, Russian Academy of Sciences, Troitsk, Moscow Region, Russia

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ABSTRACT

Mitochondrial signaling is an information channel between the mitochondrial respiratory chain and the nucleus for the transduction signals regarding the functional state of the mitochondria. The present review examines the question whether radiation of visible and near-IR (IR-A) radiation can activate this retrograde-type cellular signaling pathway. Experimental data about modulation of elements of mitochondrial retrograde signaling by the irradiation (mitochondrial membrane potential $\Delta\Psi_m$, reactive oxygen species ROS, Ca^{2+}, $NO^{\bullet}$, pH_i, fission-fusion homeostasis of mitochondria) are reviewed. The terminal enzyme of the mitochondrial respiratory chain cytochrome *c* oxidase is considered as the photoacceptor. Functions of cytochrome *c* oxidase as a signal generator as well as a signal transducer in irradiated cells are outlined.

INTRODUCTION

Mitochondria are at the center of many diverse cellular functions as integrators of signals between the organelle and the nucleus. Recent work has uncovered an impressive number of extramitochondrial factors that regulate the expression of nuclear genes for mitochondrial proteins. However, relatively little is known as to how mitochondria send signals to the nucleus and how the nucleus controls the expression of individual genes. One pathway of communication in cells from mitochondria to the nucleus that influences many cellular activities under both normal and pathophysiologic conditions is the mitochondrial retrograde signaling (reviews: 1,2). This recently discovered signaling is a signaling pathway opposite to a common and well-defined pathway transforming information from the nucleus and cytoplasm to the mitochondria. The retrograde signaling sends information back to the nucleus about changes in the functional state of the mitochondria. The most investigated mitochondrial retrograde signaling pathways so far are those in the budding yeasts *Saccharomyces cerevisiae* (review: 3) and plant cells (review: 4). Mechanisms of mitochondrial-nuclear crosstalk are also described in mammalian cells: myocytes (5) and cancer cells (review: 6). It appeared that mitochondria have, alongside their well-known functions, also a function of the retrograde regulation.

In the present review we will concentrate our attention on the initial parts of the mitochondrial (probably retrograde) signaling pathways. The aim of this review was the following. A signaling pathway in mammalian cells under irradiation with monochromatic and quasimonochromatic light in visible and near-IR region has been known for some time as the photosignal transduction and amplification chain or cellular signaling cascade (7, review: 8) but has not been explored. Now, with the discovery of the mitochondrial retrograde signaling phenomenon, a possible retrograde pathway activated by light has gained new attention.

The review analyzes comparatively characteristics typical of the mitochondrial retrograde signaling with the respective characteristics under irradiation. The characteristics under examination are mitochondrial membrane potential ($\Delta\Psi_m$), mitochondrially generated reactive oxygen species (ROS), Ca^{2+} concentration in a cell ($[Ca^{2+}]_i$) and in mitochondria ($[Ca^{2+}]_m$), free radical $NO^{\bullet}$, intracellular pH (pH_i) and parameters connected with mitochondrial biogenesis.

HOW THE DISCOVERY OF MITOCHONDRIAL (RETROGRADE) SIGNALING IN IRRADIATED CELLS HAPPENED

Various cellular responses to visible and IR-A radiation have been studied for decades in connection with investigating the mechanisms of low power laser therapy (laser phototherapy, photobiomodulation). Photobiomodulation uses monochromatic and quasimonochromatic light in the optical region of ~600–1000 nm from lasers and light-emitting diodes to treat in a nondestructive and nonthermal fashion various soft tissue and neurologic conditions (reviews: 9,10). Nowadays it is thought that this kind of treatment is based on the ability of light to alter cell metabolism as a result of its being absorbed by mitochondria (7, review: 8) and cytochrome *c* oxidase in particular (11–14).

The existence of a cellular signaling pathway—mitochondria → cytoplasm → (plasma membrane → cytoplasm) → nucleus—was proposed as far back as 1988 (7, review: 8). The reason for suggesting the existence of such a cellular signaling pathway (then named photosignal transduction and

*Corresponding author email: tkaru@isan.troitsk.ru (Tiina I. Karu)

amplification chain) was simple. It appeared that the action spectra for increase in DNA and RNA synthesis rate can be recorded when cultured cells are irradiated with radiation in the 300–860 nm region. The nucleus does not have chromophores absorbing in this region. Secondly, the data gathered by this time certainly demonstrated that the photoacceptors are located in the respiratory chain. So, it was then a logical way to suppose the existence of a cellular signaling cascade between organelles. In 2004, a novel mitochondrial-signaling pathway in mammalian cells activated by red and near-IR radiation was discovered experimentally (15). It was shown recently by Schroeder *et al.* (16) that IR-A radiation (760–1440 nm), in contrast to UV, elicits a retrograde signaling response in normal human skin fibroblasts.

One widely used model system for exploring the mechanisms of mitochondrial retrograde signaling is proliferation. It is experimentally proved in this model that mitochondria have the capacity to communicate with the rest of the cell and send signals to the nucleus (review: 2). The experimental data demonstrate that this signaling can be mediated *via* $\Delta\Psi_m$, generation of ROS, changes in Ca^{2+} flow, $NO^{\bullet}$ binding to cytochrome *c* oxidase, to name the most important characteristics (review: 2). Changes in the proliferation of mammalian cell cultures are rather well studied after irradiation with light of different wavelengths, doses and intensities (reviews: 9,17,18). The initial phase of the proliferation, adhesion of cells to a matrix, as well as DNA and RNA synthesis rate are the cellular responses studied most systematically.

The analysis of the action spectra of DNA and RNA synthesis rate in the wavelength range 330–860 nm allowed to conclude that cytochrome *c* oxidase, the terminal enzyme of the mammalian respiratory chain, is the responsible photoacceptor (11,12). The bands in the action spectra of DNA and RNA synthesis stimulation were identified by analogy with the metal-ligand system absorption spectra characteristic of visible-to-near-IR spectral range (review: 11). This analysis allowed to conclude that all bands in the action spectra (one maximum at 400 nm with the edge of the envelope near 450 nm and two series of doublet bands in the 620–680 and 760–895 nm range with well-pronounced maxima near 620, 680, 760, and 825 nm) may be related to the cytochrome *c* oxidase. Bands at 404–420, 680 and 825 nm were attributed to a relatively 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 were supposed to belong to a relatively reduced form of the enzyme (review: 11). Exact peak positions for every action spectrum can be found in (19). Later, these action spectra were compared with absorption spectra of cell monolayers in the 600–860 nm region (12,20). Comparison between the absorption and action spectra provided evidence that all bands present in the action spectra were present in the absorption spectra of cellular monolayers as well (12). Cellular signaling pathways between mitochondria, plasma membrane, and the nucleus were explored on the cell adhesion model (15,21–27).

Figure 1 presents a putative schematic of mitochondrial retrograde signaling activated with irradiation in visible and IR-A regions. This schematic was first proposed in 1988 (7, review: 8) and later supplemented with new experimental

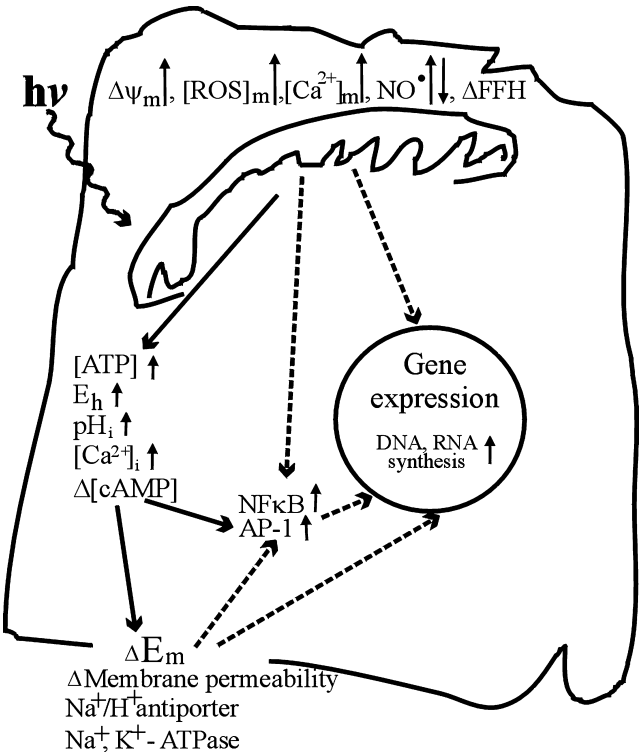

Figure 1. A schematic explaining putative mitochondrial retrograde signaling pathways after absorption visible and IR-A radiation (marked *hν*) by the photoacceptor, cytochrome *c* oxidase. Arrows ↑ and ↓ mark increase or decrease in the values, brackets [] mark concentration. ΔFFH = changes in mitochondrial fusion-fission homeostasis; AP-1 = activator protein-1; NF-κB = nuclear factor kappa B. Experimentally proved (→) and theoretically suggested (⇢) pathways are shown.

data (11,18). Some new modifications are also included in the present schematic in Fig. 1.

Irradiation of mammalian cells causes an upregulation of various genes. The upregulation of genes and increase in DNA and RNA synthesis rate are marked in Fig. 1 in the nucleus of the cell. The cDNA microarray technique was used for studying human fibroblasts irradiated at 628 nm. The gene expression profiles studied (9982 in summary) revealed that 111 genes of 10 function categories were upregulated (28). Let us note that among these 10 function categories, seven were directly or indirectly involved in cell proliferation. The other three function categories upregulated were genes related to transcription factors, immune/inflammation and cytokines as well as some genes not identified. The schematic in Fig. 1 is explained below in the text.

One has to emphasize that the responses of mammalian cells to visible and near-IR radiation as well as the sensitivity of mitochondrial respiratory chain components to this radiation have never gained serious attention of photobiologists as that of functional photoacceptors, such as chlorophyll and rhodopsin. However, fragmentary knowledge gathered so far forces one to ask whether the photosensitivity of some enzymes of the mitochondrial respiratory chain may have a physiologic significance in spite of the complete adaptation of living systems to photons as a natural external factor. It was suggested as early as 1981 that photosensitivity might be a common mitochondrial property in higher animals and could

have physiologic significance under certain conditions, *e.g.* exposure to orange-red light and high ADP levels (29).

Mitochondrial retrograde signaling has been considered as a cellular stress response (reviews: 2,3) as well as an adaptive response (review: 1). It has been also suggested that the action of visible and near-IR radiation on mammalian cells and tissues can be described within the framework of the adaptation syndrome, which means that responses have much in common, regardless of the type of irritant (30). When the action of a physical factor is increased (*e.g.* its intensity, dose), cyclic changes occur in the metabolic activity of mitochondria (31). First, a stimulation characterized by a threshold and phase of increase occurs. After a strict maximum and a phase of decrease, the control level is reached and then an inhibition is recorded. Specific characteristics exist relating to the photobiologic character of the light action (30). These are the absorption of light quanta by the photoacceptor molecules and the presence of cellular signaling pathways, mitochondrial retrograde signaling included. In other aspects like manifestation of bell-shaped dose and intensity dependences, the light action characteristics respond to general concepts of adaptation syndrome (32) and are generalized in pharmacology as Arndt-Schulz law.

$\Delta\Psi_M$ SIGNALING

The mitochondrial respiratory chain produces energy, which is stored as an electrochemical gradient called mitochondrial membrane potential ($\Delta\Psi_m$). This electrochemical gradient consists of an electrical transmembrane potential and a proton gradient (ΔpH) and drives the synthesis of ATP. Mitochondrial retrograde signaling was initially defined by altered $\Delta\Psi_m$ (review: 1). Later, other characteristics like changes in the concentration of mitochondrial ROS and Ca^{2+} were introduced. Below, we will consider every parameter separately. One has to emphasize that there is a delicate balance between positive and negative influence of every mitochondrial signaling element ($\Delta\Psi_m$, $[Ca^{2+}]_i$, ROS) to signaling pathways (reviews: 2,3). A detailed analysis of this balance goes beyond the framework of this review.

It is well documented in the models of isolated mitochondria and whole cells that monochromatic radiation in visible and IR-A region can affect $\Delta\Psi_m$ and stimulate ATP synthesis. The illumination of isolated rat liver mitochondria increased ATP synthesis and the consumption of O_2 (29,33,34). Irradiation with light at wavelengths of 415 nm (29), 602 nm (35), 632.8 nm (34), 650 and 725 nm (33) enhanced ATP synthesis. Light at wavelengths of 477 and 554 nm (29) 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 (35,36). Irradiation with light at 632.8 nm increased $\Delta\Psi_m$ and ΔpH (37,38), caused changes in mitochondrial optical properties, modified some NADH-linked dehydrogenase reactions (37) and increased the rate of ADP/ATP exchange (39). Mitochondrial RNA and protein synthesis (40), replication of mitDNA (41) as well as both cytosolic and mitochondrial protein syntheses (42,43) were stimulated by He-Ne laser irradiation. He-Ne laser irradiation increased not only O_2 uptake by cytochrome *c* oxidase but also electron transfer and proton pumping activity of this enzyme (44). In the case of state 4 respiration, the 351 and 458 nm laser irradiation accelerated oxygen consumption by rat liver mitochondria; such acceleration was not observed with 514.5 nm irradiation. On the contrary, in the case of state 3 respiration, the 514.5 nm laser irradiation activated oxygen consumption by mitochondria. Activation did not occur with 458 nm irradiation but 351 nm irradiation reduced oxygen consumption in respiration state 3 (45). Irradiation at 660 nm increased state 3 oxygen consumption at coupling II and III sites, as well as increasing the respiratory control ratio (46).

At the level of a single cell and during real-time recording, a maximal increase in mitochondrial membrane potential 30% of its basal value was observed at 2 min after a 15 s irradiation at 647 nm (about 165 mV compared with the control value of 140 mV). Then $\Delta\Psi_m$ decreased gradually back to the basal level 4 min later (47). $\Delta\Psi_m$ fluctuations have been associated with the opening of the mitochondria permeability transition pore (MPTP). Alexandratou *et al.* (47) suggested that the MPTP operates in the low conductance state in the case of low power laser irradiation.

It is known that the treatment of cells with mitochondria-specific ionophores (uncoupling agents) that abolish the obligatory linkage between the electron transport chain and the oxidative phosphorylation induces alterations in $\Delta\Psi_m$ followed by upregulation of a number of genes in the nucleus (review: 1). Using the model of the increase in the number of attached cells, the treatment of cell suspension before irradiation at 820 nm with ionophore dinitrophenol as well as respiratory chain inhibitors rotenone (the inhibitor of complex I in the respiratory chain) and azide (inhibitor of complex IV) strongly modified the light effect. These chemicals that acted on the respiratory chain but at different points eliminated the attachment stimulation induced by the irradiation (21). This finding shows that the plasma membrane could be involved in retrograde mitochondrial signaling pathways (Fig. 1). Treating isolated mitochondria with rotenone and complex III inhibitor antimycin A as well as with oligomycin (inhibitor of ATP synthase) or with various uncoupling agents abolished any increase in $\Delta\Psi_m$ induced by He-Ne laser irradiation (34).

Increase and decay of $\Delta\Psi_m$ as well as changes in cytokine gene expression and subcellular localization of promyelocytic leukemia protein were measured after irradiation of human keratinocytes with light at 780 nm (48). The $\Delta\Psi_m$ was increased immediately after the end of irradiation and then subsequently decayed within 200 min by an exponential curve. Following irradiation, the expression of interleukin-1L, interleukin-6 and keratinocyte growth factor genes were transiently upregulated. The expression of the proinflammatory gene interleukin-1β was suppressed. The subnuclear distribution of promyelocytic leukemia protein (a cell-cycle checkpoint protein) was altered from discrete domains to its dispersed form within less than 1 h after irradiation. The results of Gavish *et al.* (48) show the connection between a short-time activation of the mitochondrial photoacceptor and secondary cellular responses after mitochondrial retrograde signaling (gene activation marked in Fig. 1).

A connection between primary events in mitochondria and following retrograde signaling is also seen in data published in Hu *et al.* (38). He-Ne laser irradiation of melanoma cells induced immediately an increase in $\Delta\Psi_m$ and ATP concentration *via* enhanced cytochrome *c* oxidase activity and later

promoted phosphorylation of Jun N-terminal kinase (JNK) and activator protein-1 (AP-1) expression besides inducing an increase in cell proliferation. Irradiation-induced cell proliferation was significantly abrogated by the addition of chemicals that decreased $\Delta\Psi_m$ or inhibited JNK (38). This investigation binds together light action upon the photoacceptor (putatively cytochrome *c* oxidase), modulation of mitochondrial respiratory chain activity and cell proliferation *via* AP-1. All these events are shown in Fig. 1.

ROS SIGNALING

Recent evidence highlights a specific role of mitochondrial ROS in redox cellular signaling and in mitochondrial retrograde signaling in particular. It appeared that mitochondrial ROS are not just damaging by-products of respiration, but an important factor in cellular signaling (49,50), including retrograde mitochondrial signaling (4,51). Primary ROS made by mitochondria is superoxide anion ($O_2^{\bullet-}$), which is converted to H_2O_2 either by spontaneous dismutation or by the enzyme superoxide dismutase (SOD). The main source of $O_2^{\bullet-}$ in mitochondria is the ubisemiquinone radical intermediate ($QH^{\bullet}$), formed during the Q cycle at the Q_0 site of complex III of the respiratory chain (52,53). Flavin mononucleotide group of complex I of the respiratory chain is also a source of $O_2^{\bullet-}$. It is known that ROS generation site in complex I liberates $O_2^{\bullet-}$ predominantly to the mitochondrial matrix space and that in complex III liberates it with preference to the cytosolic space (52).

Broadband radiation (760–1440 nm) induced the formation of mitochondria-derived ROS ($[ROS]_m$) in cultured human dermal fibroblasts. Increase in $[ROS]_m$ caused an increase in intracellular redox potential (E_h in Fig. 1). It was suggested on the basis of these measurements that mitochondrial retrograde signaling pathways are at work (16). The formation of mitochondrial $O_2^{\bullet-}$ was found to be relevant for matrix metalloproteinase-1 (MMP-1) upregulation that needed a functional mitochondrial respiratory chain. In summary, the experiments performed in Schroeder *et al*. (16) demonstrated that the respiratory chain activity was a determinant of light response.

Mitochondrial ROS generation (Fig. 1) has been measured for investigating the mechanisms of low power laser therapy (reviews: 11,18). One has to underline the complex work of Alexandratou *et al*. (47). In their experiments, ROS generation was monitored alongside modulation of $\Delta\Psi_m$, intracellular pH (pH_i) and ($[Ca^{2+}]_i$) after irradiation at 647 nm on the single-cell level in real time scale. A 15 s irradiation evoked a progressive accumulation of H_2O_2 during 8 min after the irradiation (47). Let us note that both stimulation and inhibition of the respiratory chain can result in enhanced ROS generation (53).

A series of experiments was performed to investigate possible signaling pathways between mitochondria and plasma membrane in the irradiated cells. The number of cells attached to a glass matrix was increased and then decreased by a bell-shaped curve when the cells were irradiated at 820 nm (a band in the action spectrum of cell adhesion increases) (54). The treatment of the cells with ROS scavengers (antioxidants) before irradiation decreased the number of cells stimulated to attach by the irradiation, but did not influence the basal level (21). Mannitol, melatonin, ethanol and ascorbic acid were used in these experiments. Let us look closer at the action of melatonin, because its action was studied most extensively: first, dependent on its concentration (both in physiologic and pharmacologic concentration ranges) and secondly, dependent on the wavelength of radiation used (25). Pineal gland hormone melatonin is a significant scavenger of free radicals. The antioxidative action mechanism of melatonin is believed to be different from the action of antioxidants such as vitamin C and E or glutathione (55). Melatonin as a highly lipophilic small molecule quickly reaches the mitochondria and can have a direct action on respiratory chain carriers (56). Melatonin regulates the mitochondrial redox state, as it was shown experimentally for brain and liver mitochondria (57).

Melatonin modified the action spectrum in the IR-A region only. In this particular region, the peak at 831 nm, which is characteristic of the light action spectrum of cell attachment stimulation, was absent in the spectrum recorded in the presence of melatonin. The band in the action spectra at 820–830 nm is thought to belong mainly to oxidized Cu_A chromophore of cytochrome *c* oxidase (11,12). Other peak positions in the light action spectrum at 618, 668 and 751 nm were not altered in the presence of melatonin (25). The elimination of the peak with maximum near 830 nm demonstrates that the Cu_A chromophore of cytochrome *c* oxidase (this chromophore gives around 85% of absorption in this region [58]) becomes more reduced in the presence of melatonin. It is known that the peak at 820–830 nm disappears with the reduction of cytochrome *c* oxidase (58).

In another series of experiments, the oxidative agents H_2O_2 (in a low concentration range) and methylene blue increased cell adhesion in the dark (21). The magnitude of this effect was comparable to the magnitude of the effect of radiation at $\lambda = 820$ nm at an optimal dose (21). Methylene blue added to the cells in the dark also caused stimulation of DNA synthesis at a percentage comparable with the stimulation caused by He-Ne laser radiation. Methylene blue acts as electron shuttle to oxygen that bypasses cytochrome *c* oxidase. Both chemicals cause a shift in cellular redox potential (E_h) to the relatively oxidized direction (18).

An involvement of the plasma membrane in signal transduction between mitochondria and nucleus is demonstrated by patch-clamp studies of ionic currents through the plasma membrane under He-Ne laser radiation (59). No laser light effects were found in patch configurations where the disruption of the plasma membrane and cellular homeostasis occurred (voltage-activated whole-cell, inside-out and double whole-cell recordings). Only in the case of cell-attached recordings, *i.e*. in conditions of cellular integrity, the background single-channel currents through the plasma membrane were sensitive to He-Ne laser radiation. This finding proved to be valid in the case of both excitable (neurons, cardiomyocytes) and nonexcitable (glia) cells. Although the nature of the light-sensitive background channels was not studied in detail, these channels were thought to be ATP-dependent K^+ channels and Ca^{2+}-dependent K^+ channels (59). The ATP dependence of light-sensitive currents through the plasma membrane supported the suggestion about mitochondrial origin of the photoacceptor. This study demonstrated that cellular signaling pathways beginning from the mitochondria should be at work for

recording currents through the plasma membrane under irradiation. Otherwise, the light-sensitive currents could be recorded in all patch configurations. Involvement of the plasma membrane in retrograde mitochondrial signaling is also marked in Fig. 1. The permeability of the plasma membrane to precursors of DNA and RNA synthesis was increased in irradiated cells (60). An oscillatory increase in E_m (plasma membrane electrical potential) has been recorded together with an increase in $[Ca^{2+}]_i$ (61).

Two circumstances considering complex changes in the mitochondrial characteristics of retrograde signaling should be underlined. First, there exists a unique mitochondrial oscillator that depends on oxidative phosphorylation, ROS and permeability of mitochondrial inner membrane ion channels (61,62). It was hypothesized that the balance between $O_2^{\bullet-}$ efflux through inner membrane anion channels and the intracellular ROS scavenging capacity plays a key role in the oscillation mechanism. Synchronized whole cell oscillations in mitochondrial metabolism which were triggered by a local release of ROS by a single local laser flash after a delay of ~40 s in more than 70% of the mitochondrial population are thought to be at work in all types of cells that contain mitochondria (63). Oscillations in $[Ca^{2+}]_i$ after irradiation at 647 nm (47) are described in the next part of this review.

Secondly, spatiotemporally synchronized whole cell oscillations in mitochondrial metabolism were initiated only after a specific threshold level of mitochondrially produced ROS was exceeded, and they did not involve the MTPT or $[Ca^{2+}]_i$ overload. A local release of ROS by ~1% of mitochondria triggered the oscillations throughout the entire volume of the cell (63). One has to note that the strict threshold characterizes dose and intensity dependences of various cellular bell-shaped responses to the irradiation (reviews: 7,9,18).

CA^{2+} SIGNALING

Mitochondria play an important role in calcium storage and Ca^{2+} homeostasis, sequestering these ions upon their release from endoplasmic reticulum or following increased Ca^{2+} uptake across the plasma membrane. These organelles can release their Ca^{2+} to increase its local concentrations in subcellular regions to activate different processes, including mitochondrial proliferation and mitochondrial retrograde regulation in response to accumulation of ROS (50). There appears to be a multifactor cross-talk among Ca^{2+}, $\Delta\Psi_m$ and ROS, centered on the mitochondrion. Remarkable that this "love–hate triangle" (53) is at work both in excitable and nonexcitable cells (63). Various agents that uncouple respiration and oxidative phosphorylation cause increase in concentrations of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) and upregulation of a number of genes (review: 1). It is known that the uncoupling of respiration and ATP synthesis not only alters $\Delta\Psi_m$ but also affects mitochondrial Ca^{2+} ($Ca^{2+}{}_m$) accumulation and ROS generation (53). A sustained three- to eight-fold increase in steady-state $[Ca^{2+}]_m$ as a part of retrograde signaling leads to the activation of calcineurin, which in turn activates nuclear factor kappa B (NF-κB) and other signaling pathways (review: 1) (Fig. 1).

An increase in $[Ca^{2+}]_i$ has been, for a long time, one of the early events measured after the irradiation of various types of cells (latest review: 18). Insofar as we are dealing with a photobiological phenomenon, every modulation in $[Ca^{2+}]_i$ or $[Ca^{2+}]_m$ can occur only after absorbing the light by a photoacceptor. This circumstance, however, is often forgotten when discussing the effects of Ca^{2+} in irradiated cells. $[Ca^{2+}]_i$ was first measured after irradiation of human lymphocytes with a He-Ne laser (64) and later in sperm cells (65,66) and isolated mitochondria (67,68), hepatocytes (68), nerves (69) and mast cells (70) after irradiation with light of various wavelengths. After laser irradiation of mast cells with light at 405 nm, $[Ca^{2+}]_i$ was increased, followed by histamine release (70). The increase in mitochondrial membrane potential $\Delta\Psi_m$ caused a stimulation of c-fos gene expression in a Ca^{2+}-dependent manner (42).

The irradiation of hepatocytes with a He-Ne laser caused an increase in $[Ca^{2+}]_i$ and the increase in cell membrane potential (E_m) correlated with it. The increases of both parameters took place in an oscillatory manner (61). Laser irradiation at 647 nm triggered recurrent spikes in $[Ca^{2+}]_i$, measured at the single cell level. An immediate increase in $[Ca^{2+}]_i$ was observed reaching a peak at about 60 s after laser stimulation for 15 s. The $[Ca^{2+}]_i$ level then returned to the basal value. After 240 s, without any further stimulation, a second global Ca^{2+} oscillation occurred and after 320 s, the cell returned to its initial resting $[Ca^{2+}]_i$ level (47).

$NO^{\bullet}$ SIGNALING

Nitric oxide ($NO^{\bullet}$) is considered as an important intramitochondrial signaling molecule, which modulates mitochondrial respiration by direct binding to cytochrome *c* oxidase (71,72). This cGMP-independent (cGMP is cyclic quanosine monophosphate) action of $NO^{\bullet}$ is known to induce the production of ROS by mitochondria and trigger redox signaling (72). It is supposed that $NO^{\bullet}$ produced in mitochondria under physiologic conditions may induce mitochondrial retrograde signaling through altered $\Delta\Psi_m$ (review: 1).

$NO^{\bullet}$ is a free radical generated in biologic systems by nitric oxide synthases (NOS). Mitochondria have their own NOS (mtNOS), the enzyme activity of which is localized in the mitochondrial inner membrane (73). It was hypothesized that the inhibition (or modulation) of mitochondrial respiration by $NO^{\bullet}$ may represent a novel biochemical pathway regulating the supply of O_2 and energy to tissues under dynamic conditions (72).

Cytochrome *c* oxidase, which is considered as the photoacceptor in photobiomodulation (11–13), shares control over ATP synthesis with several other components of the oxidative phosphorylation machinery. Even under metabolic demand, cytochrome *c* oxidase rarely has more than 20% of the total control over ATP synthesis (74). This means that $NO^{\bullet}$-mediated changes in O_2 consumption can occur without significant effects on ATP synthesis rate. Brookes *et al.* (74) proposed that this property of oxidative phosphorylation has an important implication for mitochondrial ROS generation and cellular redox signaling. This is because the inhibitor of cytochrome *c* oxidase $NO^{\bullet}$ not only affects O_2 consumption but also affects the redox status of the respiratory chain and thus its $O_2^{\bullet-}$ generation. Therefore, a window exists at which a low level of $NO^{\bullet}$ can regulate mitochondrial ROS generation without affecting ATP synthesis.

Donors of $NO^{\bullet}$ added to the cellular suspension before irradiation at 820 nm eliminated in a concentration-dependent way the radiation-induced increase in the number of cells attached to the glass matrix (23,27). Dependence of $NO^{\bullet}$-mediated effects on the wavelength used for irradiation (action spectra) can be found in previous works (15,21,24). These experiments demonstrated the existence of a light-activated mitochondrial cellular signaling pathway. It appeared that cytochrome *c* oxidase can act as a signal generator after the absorption of light quanta as an analysis of various action spectra evidenced (11,12). However, cytochrome *c* oxidase can act also as a signal transducer as demonstrated by the experimental results using $NO^{\bullet}$ donors on the cell attachment model (15,23,26). One can suggest that $NO^{\bullet}$, a physiologic inhibitor of cytochrome *c* oxidase that binds to its catalytic center, dissociates from the catalytic center when the enzyme is reduced by irradiation. This event could transiently relieve a block in cytochrome *c* oxidase that causes a reversal of signaling consequences (Fig. 1).

CHANGES IN FISSION–FUSION HOMEOSTASIS OF MITOCHONDRIA

Mitochondria have the capacity to communicate with the nucleus also by the structural changes in the organelle itself, *e.g.* by changes in fission–fusion homeostasis in a dynamic mitochondrial network (review: 2). It appeared that the morphology of the mitochondria is an element in mitochondria–nuclear communications. Mitochondria are not static; they are in constant movement within cells, and numerous fusion and/or fission events take place. These fusion and fission events are accompanied by variations in mitochondrial size, number and mass, which are triggered by a variety of physiologic stimuli. In metabolically active cells, mitochondria make up ~40% of the cytoplasm. Mitochondria replicate during cell proliferation by a mechanism of recruitment of new proteins, which are added to preexisting subcompartments (75).

Coordinated changes in fission–fusion homeostasis of mitochondria are a part of the complex of communications during retrograde signaling (review: 2). The dynamic nature of mitochondria might protect them by ensuring that regional changes in membrane potential ($\Delta\Psi_m$) are always transient. A key messenger to activate the mitochondrial biogenesis program in various cell types is $NO^{\bullet}$ *via* $NO^{\bullet}$-cytochrome *c* oxidase system. $NO^{\bullet}$-induced mitochondrial biogenesis plays an important role in regulation of cellular metabolism operating a sensitive feedback system—a retrograde response that enables the cell to compensate for defects in electron transport chains (75).

Light effects on mitochondrial ultrastructure are investigated only to some extent. Mitochondrial ultrastructure was changed after the irradiation of isolated rat liver organelles with a He-Ne laser under experimental conditions in which ATP extrasynthesis took place. In addition to the well-known orthodox, condensed and swollen mitochondrial conformations, previously unknown bipartite mitochondria with different degrees of attenuation in their mid regions, and small atypical mitochondria were observed (76). Small atypical mitochondria were characterized biochemically (77).

The experiments performed with yeast cells (78,79) allowed to conclude that a short-time He-Ne laser irradiation (λ = 632.8 nm) influenced in a dose-dependent manner the mitochondrial ultrastructure alongside the changes in cell proliferation rate at least for seven successive generations. A short-time irradiation of cells with doses that stimulated the respiratory activity caused changes in ultrastructure of the giant mitochondrion and mitochondria–endoplasmic reticulum associations in progeny cells. Irradiation in doses suppressing the respiratory activity caused damaging effects on mitochondria of the cells of successive generations (78,79). The mechanisms of mitochondrial biogenesis in irradiated cells as well as the ways in which progeny cells respond to maintain mitochondrial functions is a research field of the future. This problem is especially interesting taking into account that the mitochondrion has its own DNA that is inherited maternally. Recall here that He-Ne laser radiation was found to cause activation of mtDNA replication (41). It is also worthy to mention that yeasts, which do not depend on their mitochondrially produced ATP to survive, actually live longer when retrograde signaling is active (51). The mitochondrial retrograde response links metabolism with chromatin-dependent gene activation and genome stability in yeast aging (80).

SIGNALING EVENTS OCCURRING IN THE CYTOPLASM

It was suggested after the first recordings of light action spectra of increase in DNA and RNA synthesis rate that the photoexcitation of a photoacceptor in mitochondria is the event that initiates a cascade of redox changes and modulations of biochemical reactions in a cell (7). This photosignal transduction chain (or cellular signaling in contemporary language) was supposed to lead to a proliferation increase. In this cascade, a transient modulation of cellular redox potential (E_h in Fig. 1) was thought to be the crucial element of cellular signaling (7, review: 8). Indeed an increase in E_h in irradiated cells has been measured recently (16). It was suggested that irradiation also causes a transient increase in pH_i (alkalization) (7,81). pH_i is the parameter tightly connected with cellular redox potential. Indeed, the pH jump was soon measured after He-Ne laser irradiation in prokaryotic cells of *Escherichia coli* (82) and later, in human fibroblasts (47). Measurements at the single fibroblast level demonstrated that the alkalization of 0.21 ± 0.08 units occurred 6 min after a 15 s irradiation at 647 nm. After 15 min, the pH_i returned to its resting level (47). This result demonstrates that a transient alkalization occurs as a part of retrograde cellular signaling from mitochondria to the nucleus. It was found in the same work that the Na^+/H^+ antiporter did not participate as a regulator mechanism in the alkalization of pH_i after laser stimulation. Also, no dissipation of cellular pH gradient occurred (47).

Thiol-reactive chemicals (glutathione, 2-mercaptoethanol, cysteine) eliminated the increase in the number of attached cells induced by irradiation at 820 nm. Treatment of the cells with glutathione disulfide, hydroquinone and copper sulfate eliminated light-induced attachment to the glass matrix only at certain light doses (22, review: 18). These experiments showed that fluctuations in cellular redox balance were involved in the enhancement of cell attachment induced by irradiation. It also

allowed suggesting that mitochondrial retrograde signaling pathways are influenced by intracellular redox balance. However, one should not forget the complex nature of cellular signaling pathways when discussing the experiments with chemicals. For example, adding mercaptans and cupric ions to cells causes not only changes in cellular redox balance but also triggers large and rapid Ca^{2+} release from sarcoplasmic reticulum vesicles (83). Recent measurements showed that IR-A radiation significantly shifted the equilibrium of reduced and oxidized glutathione in cells to the oxidized direction, *i.e.* to an increase in E_h. This event was due to the increased ROS generation by mitochondria (16) (Fig. 1).

A redox-sensitive transcription factor, NF-κB is considered to be one of the key regulatory molecules in oxidative stress-induced cell activation (84), including retrograde mitochondrial signaling (85). ROS, in particular H_2O_2, are implicated in the activation of NF-κB and the degradation of its inhibitor IκB. Inhibitors of the respiratory chain rotenone and antimycin inhibited pharmacologically induced IκB degradation indicating that this ROS had mitochondrial origin (86). It is believed that activation of NF-κB by mitochondrial retrograde signaling involves a novel mechanism, distinct from the known activation mechanisms by cytokines and chemokines (review: 1).

Involvement of the redox-sensitive transcription factor NF-κB and another transcription factor AP-1 in cellular signaling in irradiated cells has been suggested theoretically (Fig. 1) (review: 11). First experimental data are now available. The formation of osteoclast cells was stimulated by irradiation of osteoclast precursor cells at $\lambda = 810$ nm. This activation occurred *via* RANK (receptor activator of NF-κB) expression (87). He-Ne laser radiation ($\lambda = 632.8$ nm) influenced the NF-κB signaling pathways and inducible NOS expression in an experimental model of muscle trauma (88). An involvement of AP-1 (38) was already noted in the section $\Delta\Psi_m$ Signaling of the present review. Changes in cyclic adenosine monophosphate can be found in (89), and the data about involvement of Na^+/H^+ antiporter and Na^+,K^+-ATPase elsewhere (15,26). I would like to emphasize that the connections between cytoplasmic events in Fig. 1 are still very putative.

CONCLUDING REMARKS

There is every reason to believe on the basis of experimental data gathered so far that mitochondrial retrograde signaling, a recently discovered cellular signaling pathway, is at work also in the irradiated cells. Modulation of retrograde mitochondrial signaling elements like $\Delta\Psi_m$, $[ROS]_m$ and $[Ca^{2+}]_m$ in irradiated cells is rather well documented. In addition, the responses to the irradiation occurring in the nucleus (increase in DNA and RNA synthesis rate, expression of genes of various function categories) are definitely documented. However, the pathways of light signal transduction between these two ends are still rather obscure.

Direct targeting of drugs to mitochondria has long been the vision of pharmacologists. New therapeutic strategies in connection with mitochondrial signaling elements $\Delta\Psi_m$, ATP and ROS have been highlighted recently (53). These strategies concern cardio- and neuroprotection as well as ischemic preconditioning. One has to note that laser irradiation has successfully been used in all these cases not only in animal models but also clinically (latest review: 18). Laser irradiation may have some limitations in clinical practice due to limited penetration depth into tissues. On the other hand, by this method, possible deleterious side effects of mitochondrially targeted drugs can be avoided. Recent experimental data (13,90–92) demonstrate that laser phototherapy may facilitate recovery from retinal injuries and other ocular diseases wherein mitochondrial dysfunction is postulated to play a role. A more detailed understanding of the role of mitochondria and especially retrograde mitochondrial signaling mechanisms in irradiated cells is needed for further development of laser phototherapy.

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