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Mitochondrial Reticulum in Skeletal Muscles: Proven and Hypothetical Functions

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Abstract—The mitochondrial reticulum of skeletal muscles has been characterized in the 1970-80s. It has been suggested and then proven its role is delivering energy in a form of transmembrane potential on the mitochondrial inner membrane throughout the cell volume, followed by ATP synthesis by the mitochondrial ATP synthase. However, the data on the mitochondrial ultrastructure still remains a subject to criticism. To exclude the possibility of artifacts caused by the sample preparation for electron microscopy, we compared the structure of mitochondria in the ultrathin sections of muscle fibers observed by electron microscopy and in intact fibers stained with a membrane potential-dependent dye and visualized by confocal microscopy. The comparison was carried out for mice and naked mole rats known for their superior longevity. The obtained results confirmed previous findings regarding the structure of mitochondrial reticulum. A model suggesting the functioning of giant mitochondria as intracellular structures preventing tissue hypoxia was proposed.

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Keywords: mitochondria, ultrastructure, reticulum, membrane potential, hypoxia, oxygen transport, mouse, naked mole rat

INTRODUCTION

According to the concepts established based on the chemiosmotic theory by Peter Mitchell, mitochondrial energetics is determined by the intramitochondrial coupling of the oxidation of respiratory substrates and ATP generation [1-3]. The energy generated by the activity of mitochondrial proton pumps (complexes I, III, and IV) is stored in two forms: the difference in the hydrogen ion concentrations on both sides of the inner mitochondrial membrane (IMM) (ΔpH; the exterior being more acidic than the matrix) and the difference in the electrical charges in these compartments (negative charge of the matrix side). This energy, in particular its electrical form (ΔΨ), is used for the rotation of a portion of the ATP synthase complex with the generation of ATP [4, 5]. Later, it was shown that mitochondria also use potassium energetics, in which the transport of potassium ions through the ATP synthase complex also controls the synthesis of ATP [6-9]. The main component in both types of mitochondrial energetics is the mitochondrial membrane potential created by the respiratory chain [10].

The maintenance of the optimal balance between the energy production and expenditure is a rather significant issue [11], especially under stress conditions, which requires increased ATP production

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in order to adequately supply the entire cell volume with oxidative substrates and oxygen and/or with ATP to power endergonic reactions. The primary supplier of oxygen and nutrient substrates is the circulatory system that delivers them to tissues. An imbalance created when the consumption of oxygen and substrates exceeds their delivery to compartments located downstream in the circulation system results in ischemia/hypoxia. When a quick switch to glycolysis is impossible (even if the efficiency of glycolysis as an energy-producing mechanism is low), both hypoxia and ischemia can have fatal consequences. This imbalance can be avoided by reducing the dependence on the oxidative energy sources, i.e., by delivering ATP evenly throughout the tissue. There are several possible scenarios providing an adequate ATP supply to the entire cellular volume. In one of them, ATP is produced by the mitochondria located near the source of oxygen and oxidation substrates (blood capillaries), after which it diffuses into the tissue. However, this diffusion is limited by the existence of various intracellular protein and lipid structures that act as a barrier to ATP diffusion, which prevents the cells from maintaining a balance between the energy intake and expenditure within the entire cell volume, especially under stress conditions.

Another possibility for providing energy to all cellular compartments can be realized if the cells located between the blood capillaries are crossed by a continuous, gap-free mitochondrial network with a preferential location of proton pumps at the sources of substrates and oxygen and with a uniform distribution throughout the network of ATP synthase complexes ready to provide ATP synthesis at any moment. In this case, the network will be energized by the activity of proton pumps located near the capillaries, while ATP can be produced at any site within the cell volume due to the uniform distribution of $\Delta\Psi$ (equipotentiality) along the entire length of the mitochondrial network. This second scenario, was theoretically justified by V. P. Skulachev in 1969 for the IMM and other coupling membranes (e.g., chloroplast and bacterial ones) and allowed to formulate the theory of coupling membranes as “electrical cables” used for the rapid and efficient transfer of electrical energy in the cell [12]. In particular, it was suggested that IMMs act as intramitochondrial “electrical wires” for providing an adequate supply of ATP to the cellular volume.

It should be admitted that before the development of mitochondria visualization methods using fluorescent probes, the optical limitations of conventional light microscopy together with a rapid development of electron microscopy, had led to the loss of the intuitive perception of the three-dimensional structure of mitochondria based on two-dimensional electron microscopy image. A significant progress has been achieved when the scientists started to use serial ultrathin sections for the three-dimensional reconstruction of cells and most importantly, the mitochondria. A breakthrough was the use of this approach for the reconstruction of the mitochondrial network in the diaphragm (striated) muscle, which revealed that in rat diaphragm muscle fibers, the bulk of mitochondrial material was located in a plane perpendicular to the long axis of the muscle fiber, in the isotropic zones on both sides of the Z-line in a form of layers consisting of extended, branched mitochondria. Accordingly, in each muscle fiber, the number of such mitochondrial layers was equal to the number of Z-lines multiplied by two. All these numerous layers were interconnected and formed a single mitochondrial system represented by the vertical rows of mitochondria running along the myofibrils. This structure of the mitochondrial apparatus was named the mitochondrial reticulum [13]. However, in the diaphragm muscles of rat embryos and neonatal rats, the entire mitochondrial system was represented by small, single, non-branching, elongated mitochondria located along the myofibrils [14]. In 1978, compelling arguments were obtained in support of the theory of mitochondria functioning as intracellular electrical cables. Based on the idea that IMMs act as mitochondrial electrical cables extending over long distances without breaks, it has been found that mitochondrial branches contact through the osmiophilic electron-dense junctions, while the IMMs themselves do not form physical contacts with each other [13]. Such electron-dense junctions have been found in abundance in rat cardiomyocytes, suggesting that the mitochondrial reticulum in cardiac cells is formed by multiple clusters of mitochondrial branches connected by the mitochondrial junctions [15].

In 1986-1988, the theory of mitochondria as electrical cables has been experimentally confirmed, first, for the filamentous mitochondria of fibroblasts and then for the mitochondria of neonatal cardiomyocytes formed by establishing the contacts between the mitochondrial clusters [16, 17]. It was shown that local deenergization of mitochondria led to the depolarization of the entire mitochondrial cluster, including its connections formed by the junctions, thus indicating the possibility of electrical communication between the IMMs. It was suggested that the junctions can be in the “on” or “off” state, depending on the need for a certain size of the equipotential mitochondrial cluster. Several decades later, similar conclusions were made when the three-dimensional organization of striated muscle cells was assessed with modern methods, using the concept of mitochondria as extended power plant [18, 19]. However, despite the obtained evidence, there is still an occasional criticism

of the methodology employed to assess the mitochondrial reticulum organization in skeletal muscles, mostly, of procedures used for the sample preparation in electron microscopy. Hence, this required the use of other methods, whose results could be compared with the electron microscopy data. Such arguments have become the basis for our study, in which we compared the mitochondrial organization in vital skeletal muscle sections observed by confocal microscopy with the images obtained by conventional electron microscopy. The muscles were isolated from mice and naked mole rats, as the latter have a superiorly long lifespan associated, in part, with the structural and functional characteristics of their mitochondria [20-23].

MATERIALS AND METHODS

Laboratory animals. *Mice*. Male 2.5-month-old C57Bl/6 mice (n = 5) were housed in individually ventilated cages (IVCs), with a 12/12 light/dark cycle at 20-24°C with ad libitum access to food and water. Safe BK 8/15 wood chips (JRS, Germany) were used as bedding.

Naked mole rats (*Heterocephalus glaber*). Two groups of naked mole rats (6-week- and 7-year-old) were used in the experiments; each group contained four animals. Given that the lifespan of a naked mole rat is ~30 years, a 7-year-old animal is approximately equivalent to a young mouse assuming that the mouse lifespan is ~1.5-2 years. Naked mole rats were taken from the colonies maintained in plexiglass mazes at the Belozersky Institute of Physico-chemical Biology, Lomonosov Moscow State University kept at 26–29°C and relative humidity of 60-80%. Food was ad libitum and included sweet potatoes, carrots, apples, fennel, cereals with vitamins and minerals and oatmeal.

Visualization of muscle vital sections by confocal microscopy. *Quadriceps femoris* muscles were collected from the animals anesthetized with 2.5% isoflurane using a SomnoSuite® system (Kent Scientific Corporation, USA) and sacrificed by decapitation. The samples were placed in the incubation medium (DMEM/F12 without sodium bicarbonate; PanEco, Russia) to remove blood, after which they were embedded in low-melting-point agarose (Thermo Fischer Scientific, USA). Sections (70-100 μm thick) were prepared with a Leica VT-1200s vibratome (Leica Biosystems, UK), washed with the incubation medium, and incubated for 30 min with 200 nM tetramethylrhodamine ethyl ester (TMRE), Thermo Fisher Scientific). All procedures were performed at 25°C. Mitochondria in the TMRE-loaded muscle sections were visualized using an LSM 710 inverted laser confocal microscope (Carl Zeiss, Germany) with excitation at 543 nm and emission >560 nm.

Electron microscopy. Excised muscle tissue samples were fixed with 3% glutaraldehyde (Sigma-Aldrich, USA) in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4°C and then with 1% osmium tetroxide for 1.5 h, dehydrated in a series of increasing ethanol concentrations of 50, 60, 70, 80, and 96% (70% ethanol contained 1.4% uranyl acetate (Serva, Germany) to enhance the contrast) and embedded in Epon812 epoxy resin. A series of sequential ultrathin sections were prepared using a Leica ultramicrotome (Leica Biosystems). Visualization was performed with a JEM1400 electron microscope (JEOL, Japan) equipped with a QUEMESA camera (Olympus, USA) at an accelerating voltage of 100 kV and beam current of 65 μA. The images were processed using the software provided by the manufacturer (EMSIS GmbH, Germany).

RESULTS

Figure 1a shows an image taken from the skeletal muscle fiber (*m. quadriceps*) section from a 3-month-old mouse obtained by confocal microscopy. The sections were treated for 20-30 min with the ΔΨ-dependent probe TMRE for 20-30 min immediately after the animal had been sacrificed, which made possible the detection of energized mitochondria due to the difference in the mitochondrial fluorescence intensity relative to the cytoplasm. The use of the fluorescent dye allowed to observe in real-time both the morphology of the mitochondrial apparatus (all cellular structures that fluoresced were mitochondria) and the level of mitochondrial activity (the higher the TMRE fluorescence intensity, the higher the mitochondrial energization). The entire cross-sectional area of the muscle fiber was packed with a dense network of branched, energized mitochondria. The obtained image of the mitochondrial system structure in live skeletal muscle fibers fully corresponded to the ultrastructure of the mitochondrial reticulum revealed by transmission electron microscopy (Fig. 1b).

Using the same approach, we analyzed the structure of the mitochondrial apparatus in the skeletal muscles (*m. quadriceps*) from naked mole rats aged 6 months and 7 years. Figure 2a shows that unlike the muscle fibers of mice, the muscle cells of mole rats lacked the mitochondrial network, and their mitochondria are distributed rather randomly.

These data are fully consistent with the assumption that the mitochondrial reticulum is absent in skeletal muscle fiber of naked mole rat. Even by the age of 7-11 years, when the organization of the mitochondrial apparatus is permanently established, it differs from that in mice [23]. It should be noted once again that the lifespan of naked mole rats reaches 30 years, and an animal at the age of 7-11

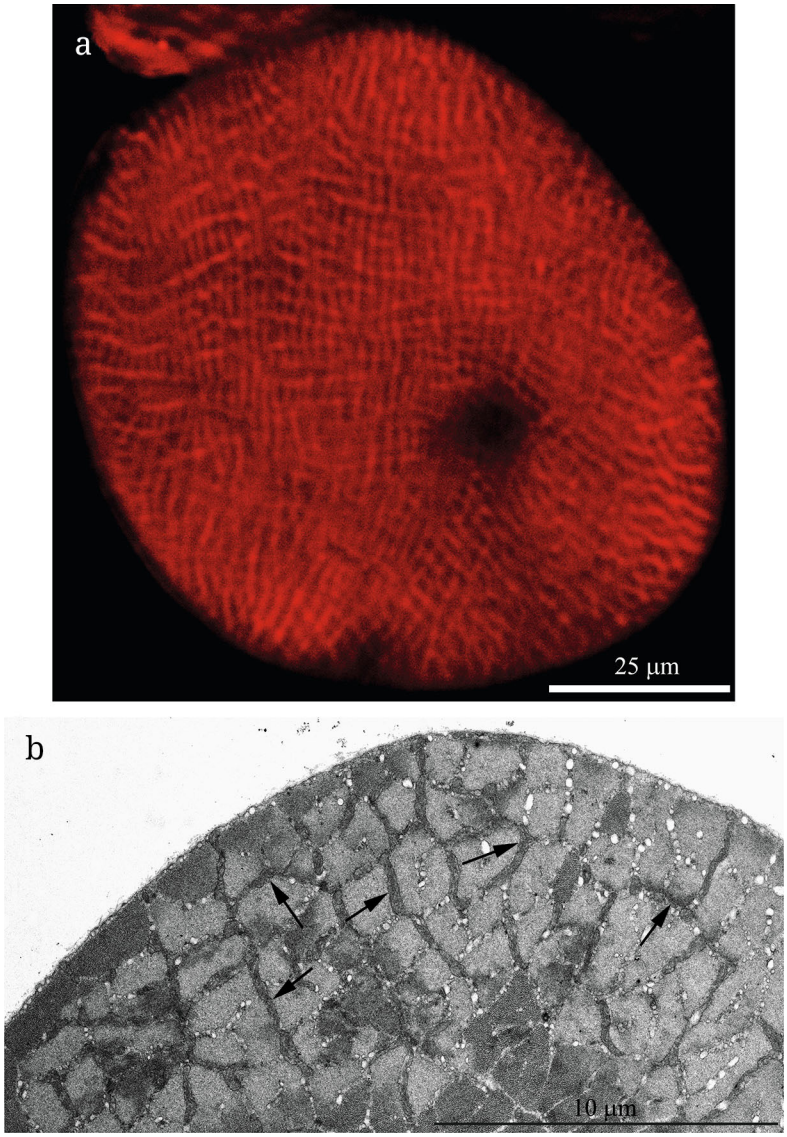

Fig. 1. Comparison of the mitochondrial apparatus in the skeletal muscle fiber (*m. quadriceps*) of a 2.5-month-old mouse revealed by confocal microscopy and transmission electron microscopy: a) confocal microscopy of a TMRE-loaded vital section of skeletal muscle fiber; b) electron microscopic image of a fixed section through the muscle fiber. The mitochondrial reticulum in panel b (dark profiles; arrows indicate mitochondrial elements of the reticulum) forms a single mitochondrial network organized by the branched mitochondria occupying the entire sectional area of the muscle fiber, similar to that revealed by confocal microscopy. Both images clearly demonstrate a developed network of the mitochondrial reticulum formed by a system of thread-like extended mitochondria located in the isotropic region of the muscle fiber.

is approximately equivalent to a sexually mature mouse, whose lifespan is ~1.5-2 years.

Therefore, the use of intact tissue and its examination immediately after sampling (i.e., without fixation, dehydration, or freezing), allowed us to observe the actual structure of the skeletal muscle mitochondrial apparatus in a form of reticular (continuous) and non-reticular (discontinuous) mitochondrial structures.

To obtain more a convincing and unambiguous evidence of the absence of mitochondrial reticulum in the skeletal muscles of naked mole rats, we analyzed the mitochondrial profiles in a series of six sequential ultrathin sections observed by electron microscopy (Fig. 3). Unlike in mice and rats, the mitochondrial structures in naked mole-rats did not form a single network (reticulum), but were represented by fragments that did not contact each other. This characteristic mitochondrial organization was observed in the muscle cells of both young (6-month-old) (Fig. 4) and mature (7-year-old) animals.

DISCUSSION

The presence of a branched and morphologically homogeneous mitochondrial network in rat striated

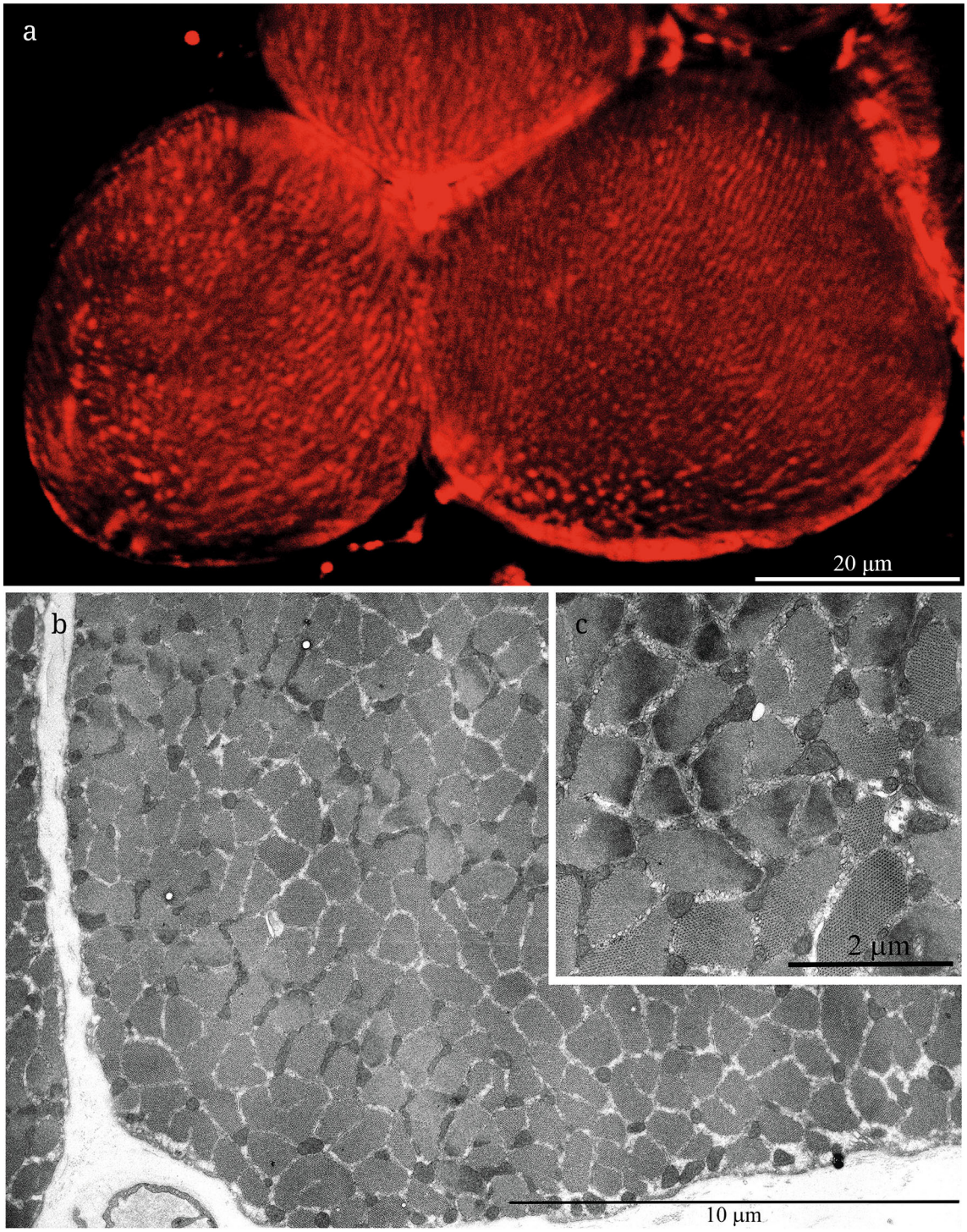

Fig. 2. The structure of mitochondrial population in the skeletal muscle (*m. quadriceps*) of a 7-year-old naked mole rat: a) confocal microscopy image of the muscle fiber vital cross-section showing the absence of mitochondrial network and a chaotic distribution of individual mitochondria; b) transmission electron microscopy image of the cross-section through the muscle fiber isotropic zone showing small single mitochondria (dark profiles indicated by arrows); c) enlarged image of the fixed muscle fiber cross-section.

muscle was first described in 1978 [13] based on analysis of electron microscopy images. However, sample preparation for electron microscopy includes fixation, staining, and dehydration steps, so there is always a concern that these procedures may alter the actual morphology existing in an intact tissue. Therefore, it is advisable to confirm the electron microscopy data using other methods excluding the influence

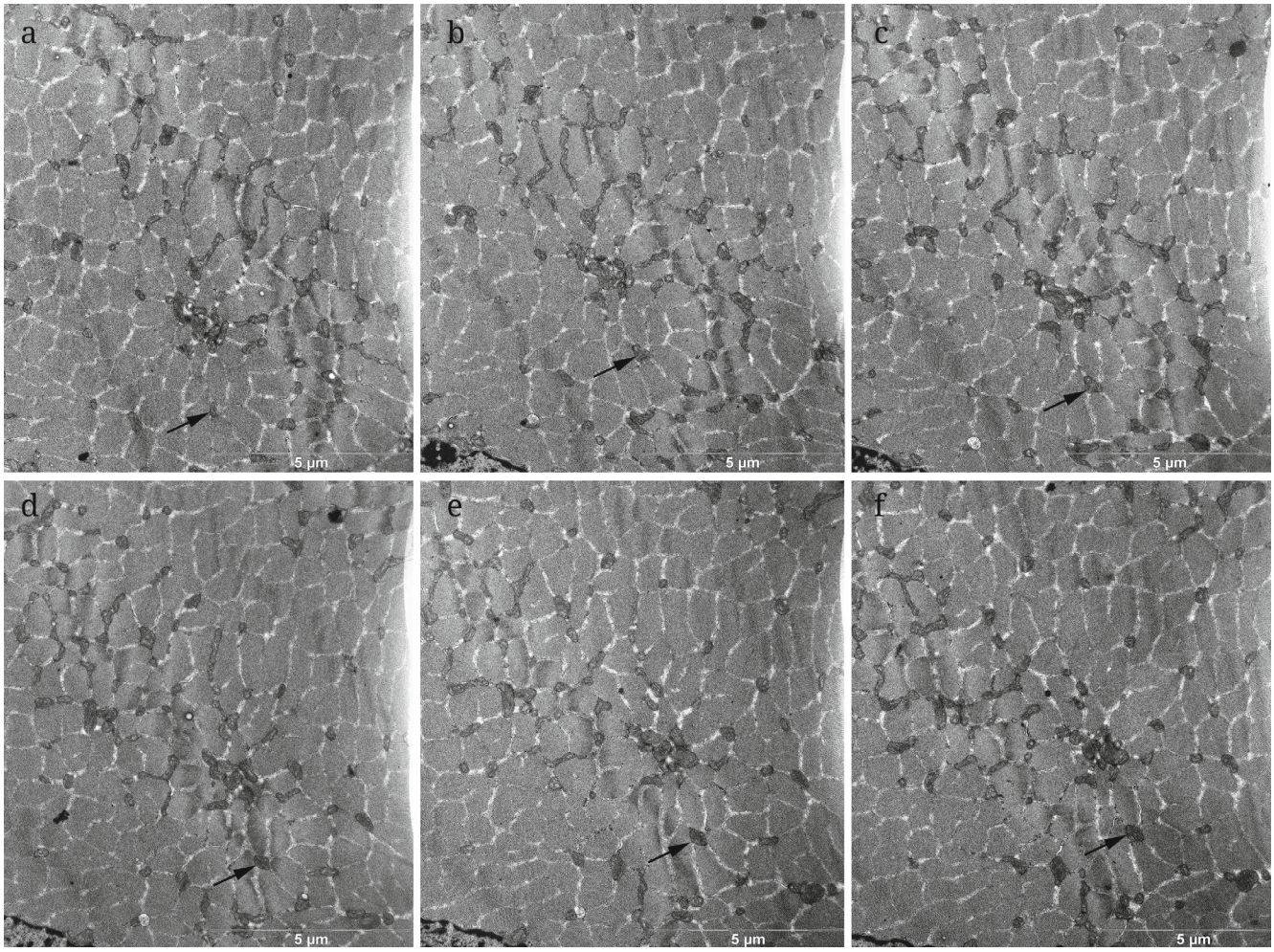

Fig. 3. Ultrathin serial sections (a-f) of a skeletal muscle fiber isotropic region from a 7-year-old naked mole rat containing single, small, unbranched mitochondria that do not form a mitochondrial reticulum. Section thickness, ~700-900 Å; total thickness of the examined portion of the fiber, ~5 μm; arrow indicates the same mitochondrion in each image for orientation along the sections.

of chemical agents used for sample fixation and of other treatment procedures, including dehydration.

Another issue that requires consideration is whether the mechanism of structural unification of mitochondria into a single network is universal. It is obvious that the formation of such mitochondrial network can be advantageous if it leads to a more or less uniform delivery to cellular compartments of the membrane potential formed in the mitochondria, ATP, heat, reactive oxygen species (ROS), and products of mitochondrial synthesis (e.g., steroid hormones), as well as removal of nitrogen metabolites through the synthesis of urea and implementation of other, non-energetic functions [24]. On the other hand, such unification of mitochondria is possible only under conditions that are “comfortable” for the cell, because even a local disruption in the mitochondrial structure integrity can lead to the energetic death of entire mitochondrial system [16, 17]. Therefore, the fragmentation of mitochondrial reticulum may be more rational under the action of pathogenic factors [25-31]. In this case, even if individual components of the mitochondrial reticulum are damaged, there is a chance for the network restoration due to proliferation of undamaged elements after removal of the deleterious factor. Here, we demonstrated that in the skeletal muscle of naked mole rat, the mitochondrial structure is represented by individual mitochondria, while in the same muscles of mice and rats, the mitochondria form a reticular structure. Moreover, this unification of mitochondria occurs at the early stages of postnatal development, since no mitochondrial reticulum was detected in the muscles of rat embryos and newborn pups [14]. In naked mole rats, the mitochondrial population is represented by individual mitochondria at all studied stages of postnatal development [23]. This is consistent with the concept proposed by V. P. Skulachev on the neoteny as a characteristic feature of naked mole rats [32]. In any case, with the reticulum fragmentation, all possible hypothetical advantages

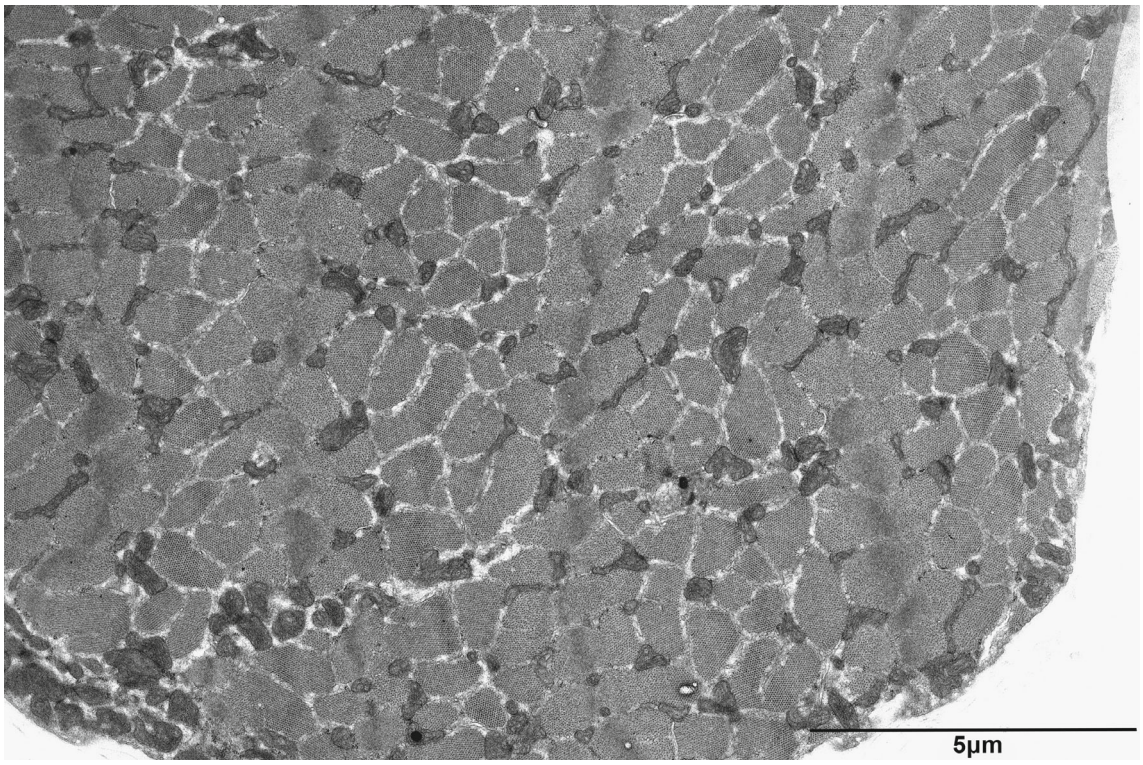

Fig. 4. Electron microscopy image taken from the ultrathin section of *quadriceps femoris* of a 6-month-old naked mole rat.

of cooperation of individual mitochondria disappear, as well as the danger of simultaneous damage to the entire mitochondrial population. Such danger may arise due to the unexpectedly high levels of oxidative stress observed in the tissues of naked mole rats [33-35]. It is known that the process of fragmentation (fission) of mitochondria occurs primarily under conditions of increased ROS generation [31]. However, the differences in the three-dimensional organization of the mitochondrial population do not exclude the possibility of mitochondria performing vital functions not associated with energetic functions [24].

It is generally accepted that the primary function of mitochondria is energy production; mitochondria are one of the key elements in maintaining the balance between the expenditure and production energy in the cells. A disruption of this balance leads to the energy crisis and can be fatal for any cell, organ, or organism [11].

Another function of mitochondria, which for a long time had remain unobvious, is adjustment of their three-dimensional structure and ultrastructure to the cell's immediate needs, which have led to the emergence of the term "mitochondrial morphofunction" [36]. The use of modern microscopy methods has made it possible to describe in detail the structural elements of mitochondria and their dynamics, which have been linked to the functional characteristics of these organelles [37].

Early attempts to find the associations between the functional states of mitochondria *in vitro* (proposed by Chance and Williams [38, 39]) and their structure resulted in the description of different structural states, such as orthodox, twisted, swollen, and condensed [40-45]. However, very few studies attempted to find this correspondence for the *in situ* or *in vivo* results [46, 47]. Later, these studies were criticized; in particular, the critics required to re-evaluate the IMM architecture based on the data obtained by methods other than electron microscopy [48-50]. A comprehensive description of changes in the internal structure of mitochondria and its response to various challenges needs more time. However, such studies can be significantly hindered by the heterogeneity of the three-dimensional structure and ultrastructure of mitochondria [51-55], together with the proven functional specialization of the mitochondrial population members in the cell [56]. The heterogeneity and specialization of mitochondria are even more complicated by the contribution of mitochondrial quality control [57], whose integral elements are mitochondrial fusion and symmetric and asymmetric fission [25-31]. Visually detectable disruptions of the mitochondrial structure can be a significant indicator of the mitochondrial state and indicate profound changes in the mitochondrial functioning, including formation of pathological cell phenotypes. In this case, the energy production aspect may recede into

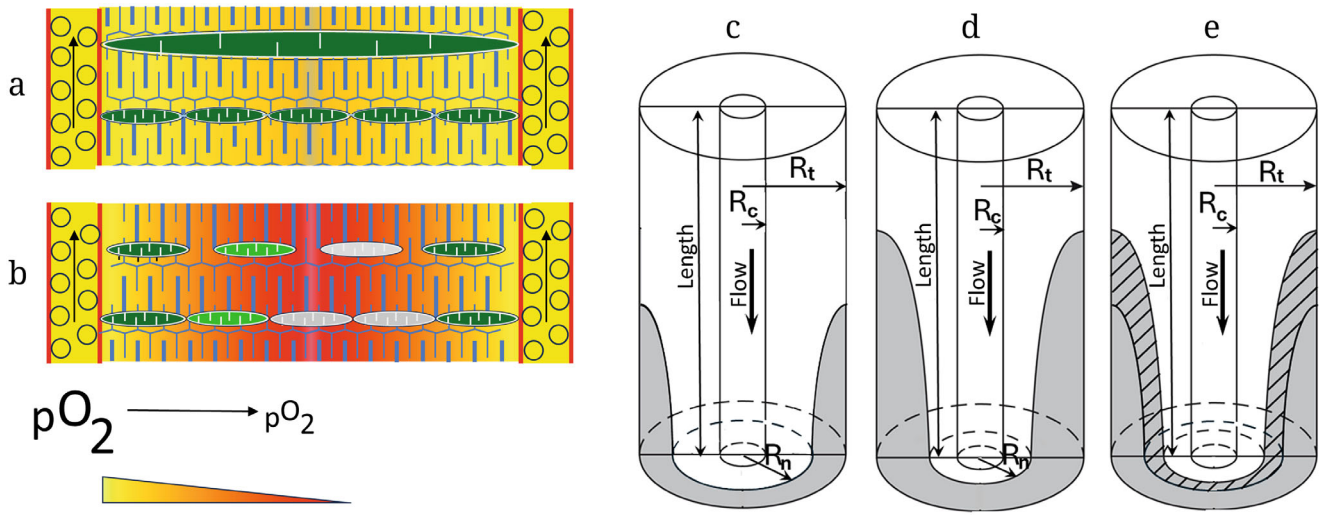

Fig. 5. Proposed model of the influence of muscle cell mitochondrial reticulum on the redox potential and oxygen partial pressure (pO_2) across the tissue. a) Less steep oxygen gradient along the distance from the blood capillary (limited by red lines) when the mitochondrial reticulum is organized as a continuous mitochondrial tree (upper part) or chains of mitochondria connected into an equipotential unit by electrically permeable inter-mitochondrial junctions (lower part; junctions are shown in pink). The value of the membrane potential value in the mitochondrial matrix (dark green color in all mitochondria) is the same over all mitochondrial chain. b) Steeper oxygen gradient when the mitochondrial tree has been broken into fragments or in the absence of electrical communication between the mitochondria connected end to end. The changes in color from yellow to red with the increasing distance from the capillary indicate the degree of tissue oxygenation. The values of mitochondrial membrane potential are directly proportional to the intensity of green color in the mitochondrial matrix; c-e) Krogh's model describing changes in pO_2 in muscle tissue during physical exercise in the case of the giant mitochondrion functioning as an oxygen supplier (c) and in its inability in the case of mitochondrial fragmentation (d). Panel d shows the superposition of the two models for a qualitative representation of the volumetric advantage (indicated by the shaded area), i.e., an increase in the volume fraction of normoxic tissue and decrease in the oxygen gradient in the longitudinal and transverse directions relative to the capillary. Rt, radius of tissue with pO_2 values not limiting the rate of tissue respiration; Rc, radius of the capillary; Rn, radius of normoxic tissue along the length of the capillary. The direction of blood flow is shown with a downward arrow

the background and reflect changes in the functioning of mitochondria as important source of alternative non-energetic functions [24].

Despite all these problems, the existence of a three-dimensional mitochondrial structure ranging from single rounded and small fragments to extensive branched mitochondrial networks, has been fully proven [58-67]. It should be noted that the mitochondrial reticulum in a form of extended and often branched structures is characteristic of cells under relatively comfortable conditions. In these cells, energy expenditure is initially high or can increase acutely depending on the situation. This is what explains the existence of a single, complexly organized mitochondrial reticulum in muscle cells, which are distinguished by their high metabolism and ability to mobilize in response to incoming challenges. In this regard, the organization of mitochondria in muscle tissue differs from that in other cells, where mitochondria sometimes form a network that breaks into fragments under unfavorable conditions. No visually similar fragmentation of the mitochondrial reticulum in muscle cells has been demonstrated, although based on the presence in the muscle mitochondria of proteins responsible for the mitochondrial fission and fusion (e.g., fis-1, drp1, Mfn2 [68, 69]) it has been claimed that it also happens in these cells. The changes in the three-dimensional organization of mitochondria from individual mitochondrial filaments to a widely branched mitochondrial network filling the entire cell volume in the ontogenesis were observed only for the diaphragm muscle [13, 14]. An important element in the organization of this network in mature muscle cells is intermitochondrial junctions [15], which can be electrically conductive [17] and, presumably, can be in the "off" position, although this remains to be proven. However, it can be assumed that transmission of electrical signal through the mitochondria over a distance can be blocked by the fragmentation of the mitochondrial reticulum, e.g., through the rupture of existing intermitochondrial junctions [28, 31]. Our study proves the existence of an extended mitochondrial reticulum in muscle cells based on a comparison of data obtained by two different methods (electron and laser confocal microscopies) and demonstrates the variability of mitochondrial reticulum in the same type of cells in animals belonging to the same group (rodents).

In a recent publication [70], we hypothesized that the mitochondrial reticulum has functions other than acting as an electrical cable [70]. We proposed that the presence of a single equipotential mitochondrial network significantly equalizes both the redox potential and energy availability to cellular compartments throughout the cell. This might be the case at the steady-state levels of energy intake and utilization in the absence of oxidative stress. However, in the presence of oxidative stress, mitochondria can break apart, and each mitochondrial fragment can have its own redox environment. The relationship between the potential on the IMM and redox potential can be quite complex due to the fact that the overall redox potential in the tissue will be in equilibrium with the mitochondrial NADH/NAD ratio, which should theoretically be high in a tissue hypoxic region (because of the absence of respiration and resulting NAD reduction). However, hypoxia can lead to the increased ROS generation with the possibility of ROS-induced ROS release in some mitochondria with a depleted redox buffer [71-73]. As a result, cells in potentially hypoxic tissue regions can have a highly heterogeneous pattern of membrane potential values in individual mitochondrial fragments and different redox potential values around them. This picture may change if we assume the role of giant mitochondria as oxygen conductors. The main argument in favor of this assumption is a higher solubility of oxygen in the lipid environment compared to the aqueous medium surrounding the phospholipid membranes. The continuity of phospholipid membranes, which is observed in the case of extensive branching of the mitochondrial network, can provide oxygen delivery throughout the tissue volume. This continuum can also be extended to the organization of the entire mitochondrial reticulum by combining individual mitochondrial clusters via intermitochondrial contacts, which is typical for striated muscles, in particular, cardiomyocytes [13-15, 17]. Considering a high osmiophilicity (lipophilicity) of mitochondrial junctions, one can also assume the possibility of high oxygen solubility in these contacts, leading to a relative continuum of oxygen content throughout the mitochondrial network. Figure 5 presents a model explaining a possible role of a giant mitochondrial network as an intracellular system enabling an anti-hypoxic defense. An important component of this model is the previously discussed Krogh cylinder model [74] that allows to calculate the possibility of existence of a hypoxic tissue volume when moving deeper into the tissue and along the flow from the blood capillary delivering oxygen [75, 76]. If a mitochondrial network can serve as an oxygen carrier, this will allow the tissues (especially those under heavy loads, for example, during active muscular work) to have an oxygen gradient that would be less steep compared to that in the tissue containing a network disintegrated into individual mitochondria. Of course, this model needs experimental support.

CONCLUSION

The presence of mitochondrial reticulum in skeletal muscle can be considered proven. The three-dimensionality of this reticulum is achieved by the connection of individual mitochondria by electrically permeable junctions throughout the cell's volume, which allows the mitochondrial structure to completely encompass the entire volume of muscle cell. Given a high metabolic activity of the working muscle, this reticulum-like organization of mitochondria likely better meets the metabolic demands. Based on this reasoning, it was hypothesized that the electrical activity of mitochondria could support these demands due to the potential equipotentiality of the mitochondrial network, ensuring uniform energy delivery to all cellular compartments. The experimentally confirmed concept of the mitochondrial reticulum functioning as a branched electrical cable is complemented by the hypothesis that this reticulum can also ensure a uniform distribution of the redox potential and molecular oxygen throughout the cell. This may be an important factor in the cell metabolic homeostasis, in which mitochondria play a key role.

Abbreviations

IMM	inner mitochondrial membrane
ROS	reactive oxygen species
TMRE	tetramethylrhodamine ethyl ester

Contributions

L.E.B. supervised electron microscopy studies; V.B.W., I.M.V., and Ch.M.E. conducted electron microscopy studies; V.A.P. performed laser confocal microscope experiments; L.D.Z. and S.D.Z. developed the antihypoxic model of extended mitochondria involvement and edited the manuscript; D.B.Z. developed the concept, supervised the study, and wrote the manuscript.

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Ethics approval and consent to participate

All procedures involving animals complied with the ethical standards of the institution where the research was conducted and approved legal acts of the Russian Federation and international organizations. Animal

study protocols were reviewed and approved by the Ethics Committee of the Belozersky Research Institute of Physicochemical Biology in accordance with the Federation of European Laboratory Animal Societies (FELASA) guidelines.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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