Milestones of World Life Science
Mitochondriology and Cell Energy Supply

One of the most important aspects of modern biochemistry is the detailing of the mechanisms of cell energy supply. Mitochondria have for a long time been considered the “power plant” of cellular metabolism. In 1961, Peter Mitchell, who was later awarded the Nobel Prize for his discovery, published in *Nature* a revolutionary paper [1] that laid the groundwork for the chemiosmotic theory. According to this theory, the electrochemical potential of protons ($\Delta \mu_H^+$) plays a critical role in energy production by mitochondria. During respiration, protons are transferred from the mitochondrial matrix to the intermembrane space to form a potential on the inner mitochondrial membrane. This respiration-generated potential is used by $H^+$-ATP synthase to convert ADP to ATP. Mitchell also assumed that uncoupling agents (such as dinitrophenol), which suppress the synthesis of ATP, do not inhibit ATP synthase directly, but their action is a result of the dissipation of the membrane potential - the driving force of ATP synthesis. This was a revolutionary interpretation of events at that time. It aroused great interest among leading scientists, many of whom expressed skepticism and offered their own hypotheses on mitochondrial ATP synthesis. A valuable contribution to the development of modern mitochondriology was made by the outstanding Russian biochemist V.P. Skulachev. In this issue, the Editorial Board has decided to devote the “Forum” section to a brief description of the major milestones in the development of this fascinating field of research.

In 1965, V.P. Skulachev established the world’s first department of bioenergetics as a part of the Interdepartmental Laboratory of Molecular Biology and Bioorganic Chemistry of the Moscow State University (now the Belozersky Institute of Physico-Chemical Biology) organized by A.N. Belozersky. It was then that investigation of the coupling role of the proton potential in oxidative phosphorylation started on the basis of the chemiosmotic theory. In 1967, V.P. Skulachev, together with E.A. Lieberman, obtained one of the first experimental proofs for the Mitchell’s theory [2]. Using various protonophores, the two found a correlation between stimulation of the respiration of mitochondria, oxidizing succinate, and the proton conductivity of the lipid bilayer membranes. Such organic ions were found. The tetraphenylphosphonium cation ($\text{TPP}^+$) and tetraphenylborate anion ($\text{TPB}^-$), different in regard of a central atom that is positively charged in TPP+ and negatively charged in TPB-, appeared to be the most effective.

Employing these compounds revealed that mitochondria, energized by ATP or substrate oxidation, can accumulate cations, and that submitochondrial particles can accumulate anions [3]. Besides the importance of the results obtained in that work, the term “protonophore” was introduced to the scientific literature for the first time, which is still successfully used today. The publication generated great interest in the scientific community, and, in recognition of its importance, the developed ions were called, at the suggestion of the famous American biochemist Professor David Green, “Skulachev’s ions” ($\text{Sk}^+$ and $\text{Sk}^-$, cations and anions, respectively) [4]. For this work, V.P. Skulachev was awarded the State Prize of the USSR in 1975.

In the 1990s, X. Wang in a series of publications [5–7] demonstrated that mitochondria are involved in induction of apoptosis – programmed cell death that plays an important role in the development of the organism and the pathology of many diseases. Cytochrome $c$, a key molecule of the respiratory chain, was found to be able, under certain conditions, to leave the mitochondria and trigger apoptosis, upon association with other molecules into the apoptosome complex.
in the cytosol. Long before Wang’s publications, during the investigation of massive cell death under the influence of ionizing radiation, suppression of oxidative phosphorylation in mitochondria isolated from radiosensitive tissues (thymus and spleen) was established. However, in mitochondria isolated from radioresistant liver tissue oxidative phosphorylation was not altered by radiation [8, 9]. Such changes in oxidative phosphorylation could be detected as early as 30–60 min after total-body x-ray exposure of rats to relatively low doses (50–100 cGy) [10]. Suppression of oxidative phosphorylation in radiosensitive tissues correlated with the formation of the so-called pyknotic nuclei [11]. Furthermore, mitochondria isolated from radiosensitive tissues after irradiation contained a smaller amount of cytochrome c compared to mitochondria from radioresistant tissues [12]. Later, radiation damage was found to lead to reduced binding of cytochrome c to the inner mitochondrial membrane, and it was established that addition of exogenous cytochrome c can stimulate oxidative phosphorylation in mitochondria isolated from the radiosensitive tissues of irradiated rats [9, 13]. The mechanism underlying this phenomenon was established only in 2005 [14]. It is necessary to note that the mechanisms of radiation-induced cell death were extensively studied in the Soviet Union. Soviet researchers postulated and proved that radiation death of lymphoid cells is an example of a broader biological phenomenon – programmed cell death [15, 16] – which was recognized by the world community [17].

Simultaneously with Wang’s studies, G. Kroemer demonstrated that a decline in the mitochondrial membrane potential is one of the key events that trigger cell death [18]. V.P. Skulachev became interested in this phenomenon, viewing it from a different perspective. He decided to search for a relationship between programmed cell death and aging. Back in the late 19th century, A. Weismann suggested a hypothesis that in the heart of death caused by aging is the evolutionary-developed adaptive mechanism [19]. Based on this hypothesis, V.P. Skulachev proposed that the altruistic death of individuals, as an adaptive mechanism, could be beneficial to other groups of organisms in the environment. He first coined the term “phenoptosis” and explained it as a mechanism of ridding the community of undesirable elements [20]. The simplest example of phenoptosis can be observed in bacteria. Altruistic programmed death in these organisms is necessary for: (a) preventing the expansion of phage infection in the bacterial population; (b) eliminating cells whose genome or other key systems are damaged; and (c) optimizing the number of bacterial cells in the environment [21]. Later, the phenomenon of phenoptosis was described in yeast, where phenome-dependent death was suppressed by protein synthesis inhibitors and was recognized as programmed death [22]. Phenoptosis examples were also described in some higher organisms [21, 23]. However, the molecular mechanisms that trigger the phenomenon remain unknown.

The aging process should be more important than acute phenoptosis in the evolution of permanently reproducing organisms, because the function of aging-dependent phenoptosis is to reduce the number of individuals in the population of long-living predecessors, thereby stimulating evolution. In other words, slow phenoptosis enhances this process [24]. What can be the regulators of age-dependent phenoptosis? V.P. Skulachev suggested that telomere shortening could underlie this mechanism (a similar mechanism was predicted by the Russian researcher A.M. Olovnikov many years ago [25]). Unfortunately, it remains unknown whether telomere shortening determines the lifespan of a multicellular organism as a whole or whether it applies only to its specific cell systems.

In recent years, several fundamental observations have been published that have tried to establish a relationship between aging and death of the organism at the molecular level. For example, the lifespan of animals expressing and not expressing the p66shc protein, which is involved in the regulation of the level of reactive oxygen species, has been demonstrated to differ by 30% [26]. Furthermore, although no tumor growth has been observed in mutant mice with an increased activity of the tumor suppressor p53, their lifespan is shorter than that of wild-type mice [27]. These two proteins are actively involved in the regulation of apoptosis. Based on these observations, V.P. Skulachev suggested that the lifespan could be regulated through a coordinated activation of p53 and suppression of the p66shc protein. Time will tell whether it will be possible to reproduce this quite logical assumption in real life [23].

Based on the growing body of data on the mechanisms of programmed cell death and the phenoptosis phenomenon, V.P. Skulachev put forward the idea of the “samurai rule” in biology. This rule can briefly be formulated as follows: “it is better to die than to be wrong.” He wrote in one of his outstanding articles: “Any biological systems, ranging from organelles to the whole body, have a self-destruction program. This suicidal mechanism is activated when the system is dangerous to coexistence with other systems in the biological hierarchy” [21].

The development of science provides interesting examples of in-
terdisciplinary, pioneering breakthroughs based on the efforts of researchers from different countries who often only get to know each other personally after publishing their own seminal observations. The story told today is a clear example of this. V.P. Skulachev met with Peter Mitchell at the FEBS Congress in Warsaw only in 1966. From then on, an enduring friendship developed between the two, based on common interest in mitochondriology. This area of knowledge remains far from exhausted, and it is particularly rewarding to know that Russian researchers were among its originators.

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REFERENCES