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SEEDS SOUNDING THE ENERGY ALARM

What do biological powerhouses look like? How do they work? Why does generating a single "dose of energy" demand a highly complicated process? Why do seeds age? Answers to all these questions are to be found in one of the most complex cellular organelles: the mitochondrion.

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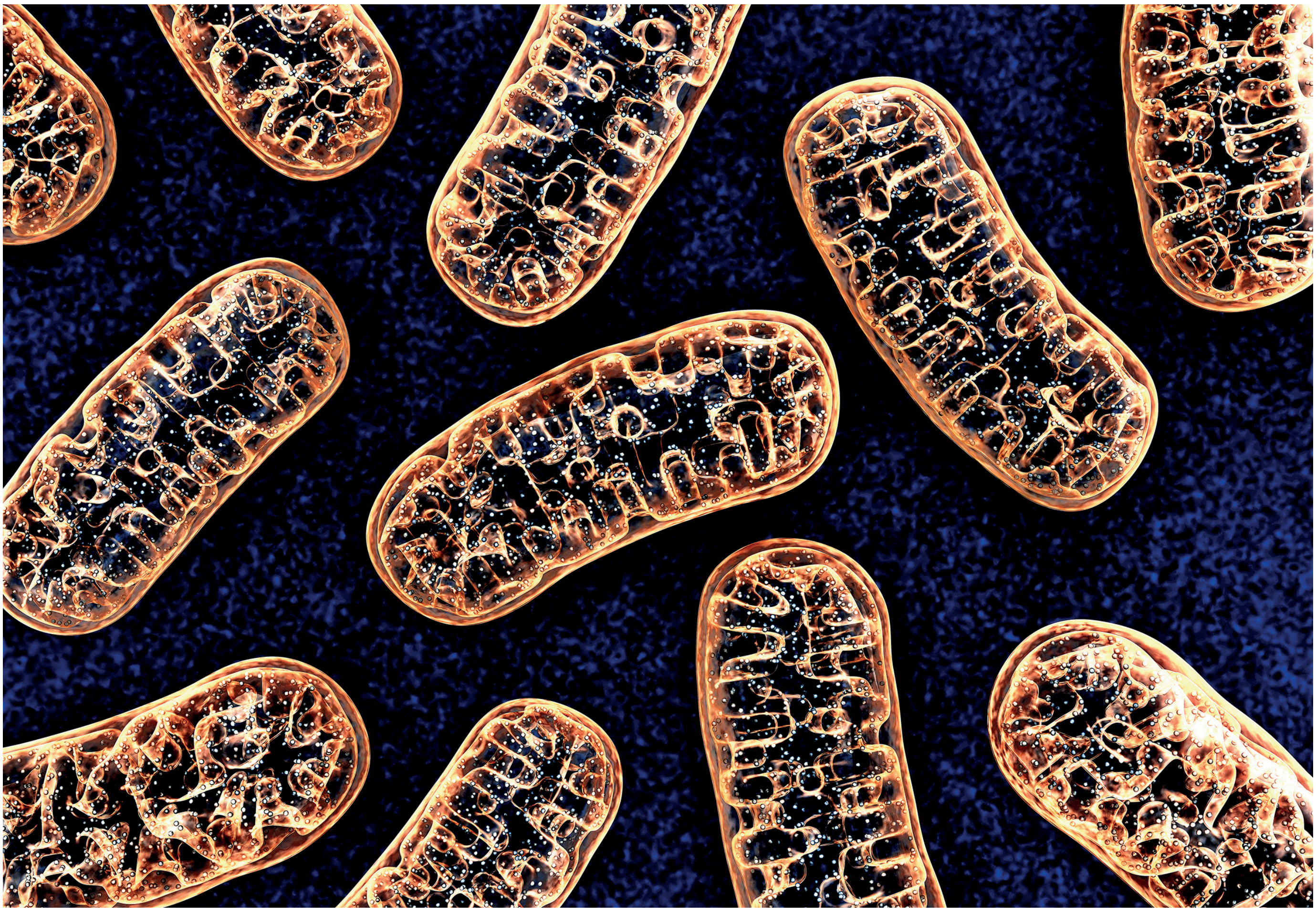

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In view of their crucial role in energy production, mitochondria are often described as "powerhouses" of the cell. Analysis of the genes coded for in the mitochondrial DNA (mtDNA) indicates that these organelles are descended from a common ancestor – an α-proteobacterium known as the proto-mitochondrion. These findings underpin what is called the endosymbiotic theory, which posits that about 2 billion years ago an anaerobic cell took in a smaller, oxygen-requiring proteobacterium, forming a symbiotic relationship. The "alliance" so forged enabled the anaerobic cell to switch over to an oxygen-related metabolism, allowing it to produce much more energy than before. In the course of evolution, that proto-mitochondrion evolved into a cellular organelle that is today the energy center of the eukaryotic cell – the mitochondrion. In the face of climate change, however, it turns out that these microscopic "powerhouses" may be in serious jeopardy – especially the mitochondria in seed cells.

Structure of mitochondria

The existence of mitochondria was first discovered in the muscle cells of insect wings, by the Swiss scholar Rudolf Albert von Kölliker. We now know that a vast majority of eukaryotes have mitochondria in their cells. These mitochondria are round- or oval-shaped and measure about 2–8 μm in length and about 0.5 μm in diameter. They are surrounded by outer and inner membranes, separated by an intermembrane space. The inside of the mitochondrion is filled with the matrix, which is an aqueous solution of proteins and metabolites used by the organelle in the process of cellular respiration. The inner membrane is the energy center of the mitochondrion. The surface of the inner membrane folds deep into the matrix. These folds, called cristae, increase the surface area in which the production of energy-carrying molecules of adenosine triphosphate (ATP) takes place. These molecules can be described as molecular batteries because they transport and store the energy needed for cells to function. This energy is stored in chemical bonds and released when these bonds are broken. It is in the mitochondrial cristae that the enzymes associated with what is called the electron transport chain (or the respiratory chain) are embedded. The mitochondria of cells that have different functions differ in terms of the number of cristae, depending on energy needs. Likewise, the number of organelles depends on the type of the organism and the cell. A typical cell contains several hundred to several thousand of these organelles, which divide independently of the cell (Photo 1).

The DNA found inside a cell may take on various forms. In most cases, DNA has the form of a double-stranded linear molecule, but there are exceptions to this rule. In bacterial cells, the genome is circular. Mitochondria also have their own genome, separate from other organelles. For reasons related to their prokaryotic origin, this genome has the form of a circular nucleoid (mtDNA) in which some of the proteins and the RNAs associated with their functioning are encoded. As the proto-mitochondrion evolved into a cellular organelle, the mitochondrial genome underwent numerous modifications, such as the transfer of genes from the proto-mitochondrion to the nucleus of the cell and *vice versa*. Interestingly, animal and plant mitochondrial genomes differ in their structure. In animals, the mitochondrial genome is typically a simple and small circular DNA molecule. The mtDNA of plants, however, has not been studied as well, for reasons related to significant variations in its structure, length, and organization. For the sake of simplicity, the plant genome is pictured as a circular molecule containing all the genetic information of the mitochondrion, which is called the master chromosome.

The functions of mitochondria

The main role that mitochondria play is to produce energy for the cell. But they also have other tasks, which include the regulation of the membrane potential, apoptosis (programmed cell death), and redox regulation.

The production of energy in the form of ATP, known as oxidative phosphorylation, takes place in the inner mitochondrial membrane, where the enzyme proteins of the electron transport chain are embedded. The electron transport chain comprises five major enzymes: NADH-coenzyme Q reductase (complex I), succinate dehydrogenase (complex II), coenzyme Q-cytochrome *c* reductase (complex III), cytochrome *c* oxidase (complex IV), and ATP synthase.

The essence of oxidative phosphorylation lies in transporting electrons through successive elements of the electron transport chain. This occurs through a series of oxidation and reduction ("redox") reactions. The electrons are supplied by two chemical compounds that are formed elsewhere in the cell,

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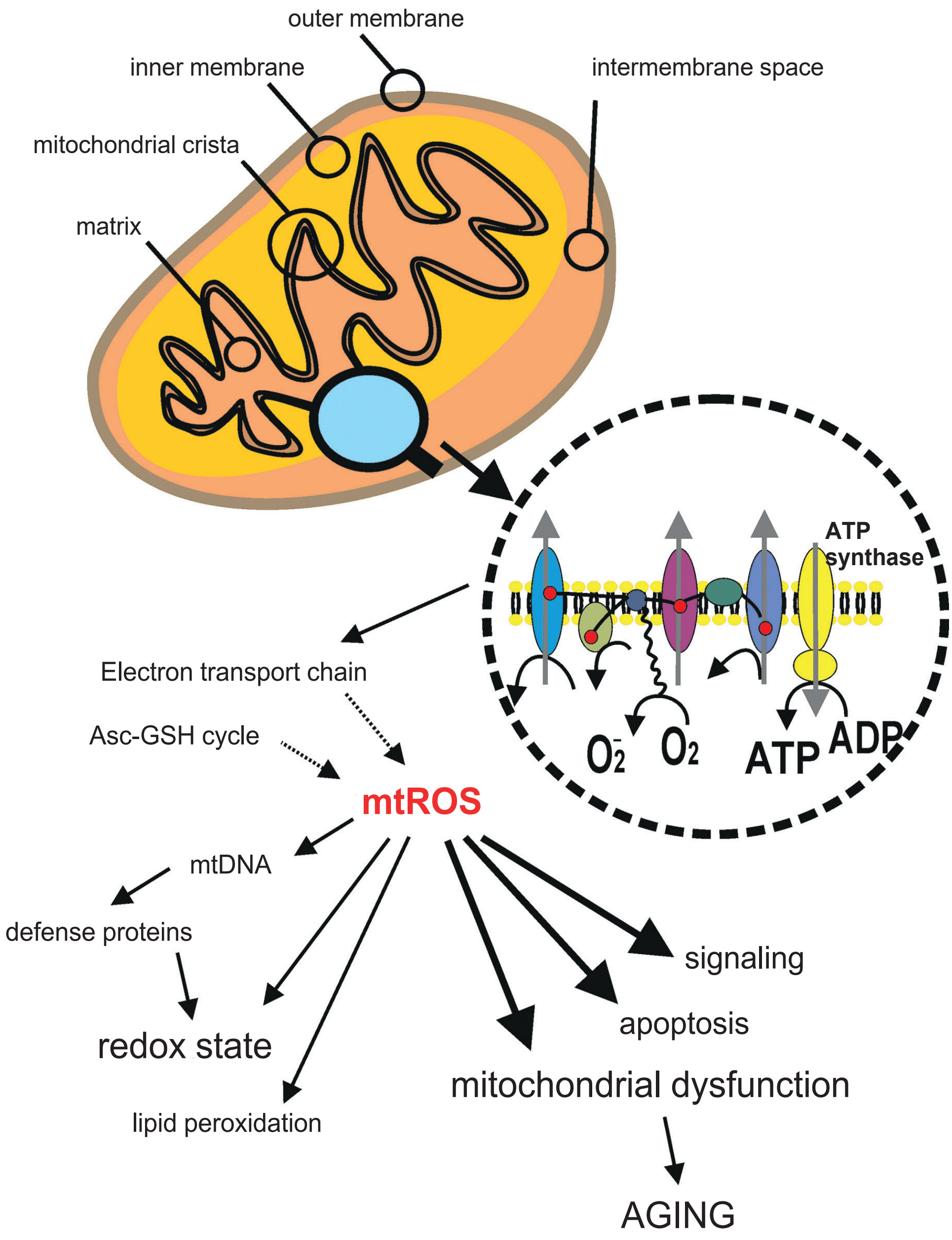

Fig. 1
The relationship between oxidative phosphorylation in mitochondria and the production of reactive oxygen species (mtROS) and their impact on changes in metabolism and the induction of seed aging

namely the reduced forms of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). The energy contained in the electrons of these compounds is the driving force behind oxidative phosphorylation. The transport of these electrons by the protein complexes of the electron transport chain produces the protonmotive force, causing protons to be pumped out of the mitochondrial matrix into the intermembrane space. Then, under the pressure of protons flowing back into the mitochondrial matrix, ATP synthase synthesizes ATP molecules (Fig. 1).

An important role in oxidative phosphorylation is played by oxygen molecules, which are the final acceptors of the electrons in the electron transport chain. Because of its affinity for electrons, molecular oxygen produces a significant amount of thermodynamic drive in the electron transport chain. Partial reduction leads to the formation of hazardous compounds called reactive oxygen species (ROS). They are considered the main instigators of cellular aging – including in the cells of seeds, which are usually associated with a state of dormancy and limited metabolism. Nevertheless, in the course of the long-term storage of seeds, their cells may experience increased ROS production during metabolic changes associated with respiration, which negatively affect their genetic material and the nutrients they store. Mitochondrial DNA is neither protected in any way by protein membranes, nor bound to stabilizing proteins, called histones, which makes it more vulnerable to damage.

Although the mitochondria serve as powerhouses for cell growth and metabolic activity, they are also one of the major sites of ROS production, formed during electron transport through the complexes in the mitochondrial electron transport chain. Superoxide anion radical ($O^{2\bullet -}$) is a precursor of most ROS and a mediator in oxidative chain reactions. It can be formed in the electron transport chain as a result of one-electron reduction occurring with flavins (such as vitamin B_2, which is involved in oxidation and reduction processes) or ubiquinone (mainly known as coenzyme Q_{10} in the cosmetics industry). Superoxide anion radical is formed in complex I, complex II, and complex III (Fig. 1). A balance between the generation of ROS and their removal by a specialized defense system enables uninterrupted energy production in seed cells.

What threats do tree seeds face nowadays?

Trees are long-lived organisms – species exhibiting the greatest longevity, such as the Rocky Mountain bristlecone pine (*Pinus aristata* Engelm.), may live as long as 5,000 years. Such a long lifespan is inevitably linked to continual exposure to stress stimuli at different stages of the plant's development. Even seeds still maturing on the branches of their parent tree experience stresses of varying type and intensity. The increasing frequency of extreme weather events, such as droughts and heat waves, is the result of factors including high CO_2 concentrations in the Earth's atmosphere. In 2015, CO_2 levels exceeded 400 ppm (parts per million) for the first time since measurements began in 1958, when it was 315 ppm (as a point of comparison, note that in 2021 the CO_2 concentration is already 416 ppm). This has serious implications, not only for species' ability to colonize new areas, but also for the quality of seeds, which ensure the sustainability of forests.

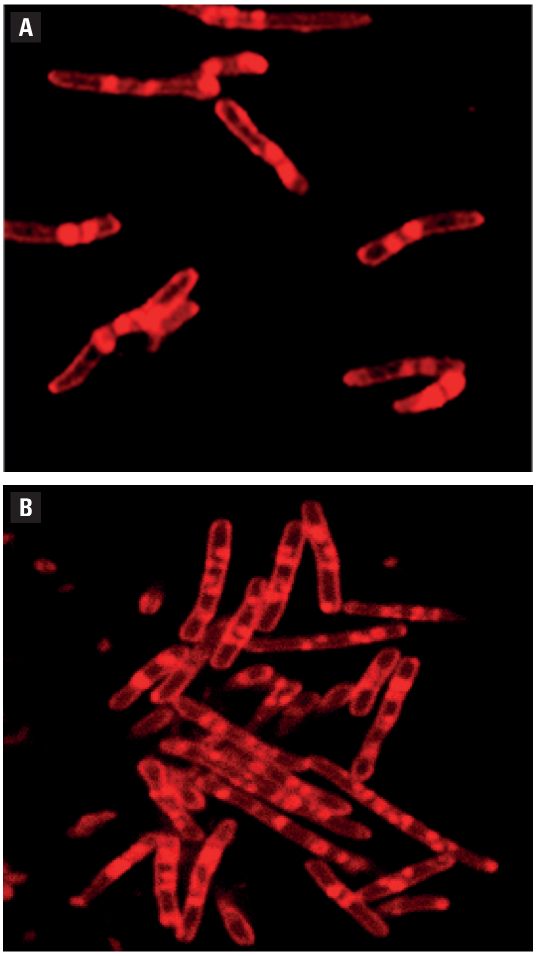

Photo 1
Microscopic observations of mitochondria in beech seeds showed that their ultrastructures change gradually during the aging process
A. Mitochondria isolated from stored seeds are smaller, occurring singly or in small clusters
B. Mitochondria in fresh seed cells are much larger, found in large clusters, elongated in shape, and evidently retain their metabolic activity

Over the past decades, many surprising observations have been made, especially in the case of species that show cyclical seed production patterns, such as the beech and oak. Such trees recognize an increase in air temperature as a signal to start increased seed production the next season – this next year is then called a seed year. As a result of the rises in average temperatures now being recorded every year, trees are constantly trying to prepare for a seed year. However, the species that produce relatively heavy seeds, such as acorns and beechnuts, need up to eight years to regather the energy necessary for the production of many flowers and fruit. Consequently, seeds are produced every year, but there are far fewer of them, and most do not have a complete embryo – they are "empty" seeds. Not so long ago, however, such trees had a number of years to regenerate between seed years.

Seeds raising the alarm: the initiation of aging

How can we prevent the loss of seed material, which is crucial for forest sustainability? We need to continuously collect and store it in very specific and species-dependent ways, which poses a considerable challenge. All this is due to the molecules we mentioned earlier, namely reactive oxygen species. Studies show that a reduction in the viability of seeds stored for several years is closely linked to an increased concentrations of ROS and the products of the oxidation of storage materials in the form of lipids. This means that the plant material has aged. But what do we even mean by the "aging" of seeds? After all, we associate seeds primarily with the very beginning of new life.

Respiration and other metabolic processes are controlled by the oxidation-reduction potential (the oxidation and reduction systems within the cell), also known as the "redox" state. The matrix of a mitochondrion abounds in a range of defensive compounds involved in this regulation. This system includes many compounds. The most important of these are manganese superoxide dismutase (Mn-SOD), the ascorbate-glutathione cycle (Asc-GSH), catalase, and the

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Empty seeds can be observed with growing frequency, such as in the case of sycamore maple trees (*Acer pseudoplatanus* L.)

proteins of peroxiredoxins (including peroxiredoxin IIF, important for defining physiological differences between drought stress-resistant and drought stress-sensitive seeds) and thioredoxin. In normal conditions, the level of ROS molecules is effectively regulated, and its slight fluctuations are involved in intercellular communication, acting as transmitters of physiological responses to various phenomena. However, under the influence of such stress factors as drought or heat, ROS are accumulated in excessive amounts and become toxic despite the fact that intracellular messengers play an important role. This happens because ROS have powerful oxidizing properties and therefore change the redox state in different parts of the cell from reducing to oxidizing. They oxidize lipids, which are the main components of cell membranes, including mitochondrial membranes (Photo 1). In so doing, they disrupt the structure of cell membranes (biological membranes), causing changes in their selective permeability and the activity of the proteins bound to them, including antioxidant enzymes. We can determine these changes, for example, by measuring the efflux of electrolytes from the inside of cells into the water in which the tissue being examined has been placed. A state in which the cell cannot detoxify harmful molecules fast enough is called oxidative stress. Its level is determined using lipid oxidation products, typical of tissues under oxidative stress.

Prof. Denham Harman's free radical theory posits that aging is the accumulation of oxidative damage over time, which gradually increases the risk of death in all living organisms. Seen from this perspective, the aging process directly affects the efficiency of mitochondria and their ability to produce energy. Examples may include elm seeds, in which as many as 48 mitochondrial proteins change in the course of aging. Moreover, these changes have been shown to be related to electron transport and the Krebs cycle, which are processes that form the basis of aerobic respiration. An excess of ROS in mitochondria causes mtDNA damage, which makes it very difficult to preserve genetic information. In turn, the lack of genome integrity impacts negatively on seed viability. All these events taken together translate into the progressive destruction of genetic material and the inhibition of metabolic processes. The cells of aging seeds are unable to produce energy and store it in the form of ATP in amounts enabling the initiation of germination.

For these reasons, we can say that stress caused by changes in climate conditions or improper seed storage leads to the activation of a specific type of intracellular signal – an energy deficit alarm. This signal affects the cell's ability to produce respiration-related mitochondrial proteins such as ATP synthase. Cutting off the supply of metabolic energy needed to maintain the reducing environment in the cell ultimately determines its fate. The cell declares that it is "time to die." In turn, the more cells die, the more "aged" (i.e. unable to germinate) the seed becomes.

Light at the end of the tunnel

Although the aforementioned processes taking part in seeds may have serious consequences, nature has provided seeds with a defense mechanism that protects them from aging. Certain chemical compounds can "switch off" the energy alarm. One of them is proline, an amino acid that has been so far studied intensively in cereal species and other crop plants. Proline accumulation increases in response to environmental stress factors, for example during drought. Proline has the ability to stabilize cell membranes and proteins, which helps reduce water loss and increase the activity of the antioxidant system, causing ROS molecules to be removed more efficiently. For this reason, this interesting amino acid could indicate the level of damage caused by oxidative stress. In addition, it could be used to monitor the conditions of storing seeds to reduce the possibility of oxidative stress in their cells as much as possible. The commercial use of proline may not only protect seeds from aging, but also improve the efficiency of producing seedlings that would be better adapted to changing stress conditions. There is every indication, therefore, that the future will bring an effective solution to the problem of aging – if not for us humans, then at least for seeds! ■