AGEING AND REACTIVE OXIGEN SPECIES: THE FREE RADICAL THEORY OF AGEING

The Free Radical Theory of Ageing

Ageing is an intrinsic biological process characterized by the gradual decline in cellular and molecular function over time. It involves disruptions in normal cellular mechanisms, the buildup of damaged molecules and toxins, changes in gene expression, and weakened immune and stress responses. Although extensively studied, the fundamental mechanisms underlying ageing remain incompletely understood. According to the **Free Radical Theory of Ageing** (**FRTA**), one contributing factor to ageing is the accumulation of molecular damage caused by **Reactive Oxygen Species** (**ROS**) produced during normal metabolic processes.

1. Sources of Reactive Oxygen Species and their consequence

Mitochondria are the primary source of ROS in cells, although they are not the sole contributors. Electrons moving through the mitochondrial electron transport chain can interact with oxygen, forming superoxide ($O_2^{\cdot-}$), a reactive free radical. This superoxide is then converted into hydrogen peroxide (H_2O_2) and oxygen by the enzyme superoxide dismutase (SOD). Hydrogen peroxide can also be produced in peroxisomes and has the capacity to traverse cellular membranes, reaching the cytoplasm. When H_2O_2 encounters iron ions (Fe^{2+}), it can generate hydroxyl radicals ($HO^{\cdot}$), which are highly reactive. The reactivity of these radicals enables them to damage essential biomolecules like DNA, proteins, and lipids by stealing electrons, leading to oxidative stress.

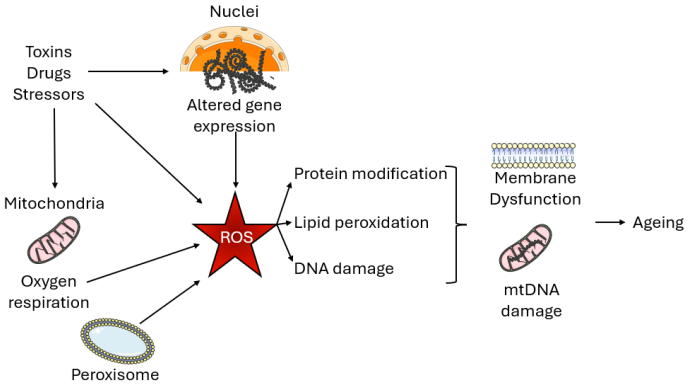
DNA damage caused by ROS can disrupt genetic integrity, potentially resulting in double-strand breaks and genomic instability. Proteins exposed to ROS may undergo structural modifications that impair their functions, while lipids can suffer peroxidation, causing cell membrane dysfunction. These types of oxidative damage are known to increase with age, making them important biomarkers of the ageing process.

2. The link between ageing and ROS

Support for the FRTA comes from observations linking ROS levels, oxidative damage, and longevity. Such findings include: (1) increased ROS production and oxidative damage with age, (2) progressive mitochondrial dysfunction in ageing cells, (3) heightened ROS production upon disruption of electron transport chain components, and (4) the association of oxidative stress with various age-related diseases.
To explore the relationship between ROS and ageing, researchers have used comparative biology approaches, analysing ROS levels and oxidative damage across species with varying lifespans. These studies often reveal a negative correlation between lifespan and both ROS levels and oxidative damage. The relationship between antioxidant levels and lifespan is less clear, as antioxidant expression is regulated in response to ROS presence. Elevated antioxidant levels may signify higher ROS generation rather than enhanced longevity.

3. Hallmarks of cellular ageing

Certain biomolecules, such as mitochondrial DNA (mtDNA) and membrane fatty acids, display strong correlations with lifespan. Due to its proximity to mitochondrial ROS production sites, mtDNA is particularly vulnerable to oxidative damage, accumulating deletions over time. This vulnerability results from elevated oxidative attack rates rather than defective repair mechanisms. Additionally, the degree of fatty acid unsaturation in cellular and mitochondrial membranes is negatively associated with lifespan. Highly unsaturated fatty acids, with unstable electrons at double bonds, are more prone to oxidative damage.



4. Measuring ROS in ageing process

Although comparative studies provide valuable insights into the relationship between ROS and ageing, they primarily establish correlations rather than causation. Experimental manipulation of ROS levels is necessary to directly assess their impact on lifespan. However, measuring ROS in living systems poses significant challenges due to their short lifespan and high reactivity. Fluorescent probes are commonly used for ROS detection

through oxidation of the probes, but their accuracy is often questioned due to nonspecific redox reactions by different oxidants or lack of sensitivity to stimuli such as local O_2 levels and pH, that influence the signal of the probe. In addition, these probes tent to leak out from cells, reducing the sensitivity of the signal sensed and their use over long period of experimentation.

To address these limitations, technologies like the **CytoCHECK SPAchip® OHrad ROS Single-Detection Kit** have been developed. This tool employs **suspended planar array chip** (**SPAchip®**) technology to monitor intracellular hydroxyl radicals dynamically without altering the cell's physiological or metabolic state. The SPAchip® system overcomes challenges such as pH sensitivity, lack of selectivity and probe cytotoxicity. The fluorescent probes immobilized on their surface enable long-term monitoring of ROS levels in living cells and do not permeate out of the cell with time, potentially throughout their lifespan.

6. Conclusions

Aging is a complex biological process intricately linked to oxidative stress and the activity of reactive oxygen species. While the FRTA provides a foundational framework for understanding the role of ROS in cellular damage and age-related decline, emerging perspectives highlight the dual nature of ROS as both harmful and beneficial under different conditions.

Advances in comparative studies and experimental techniques have deepened our understanding of oxidative damage and its correlation with lifespan, emphasizing the importance of maintaining balanced ROS levels and exploring biomarkers such as mtDNA and membrane fatty acid composition.

Notably, the development of innovative tools like the **CytoCHECK SPAchip® OHrad ROS Single-Detection Kit** represents a significant breakthrough in ROS research. By enabling precise, dynamic, and non-disruptive monitoring of intracellular hydroxyl radicals, SPAchip® technology addresses longstanding challenges associated with ROS detection, such as pH sensitivity, probe cytotoxicity, and ROS specificity This advancement allows researchers to study ROS dynamics and their effects on aging more effectively, offering new opportunities to uncover causative relationships and develop targeted interventions to mitigate the impacts of oxidative stress on cellular health and longevity.

CytoCHECK SPAchip® OHrad ROS Single-Detection kits by A4cell is novel fluorescence assay for the detection of intracellular ROS in live cells, which allows for extended periods of experimentation

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