Seeking insights into ageing through yeast mitochondrial electrophysiology

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Abstract

During ageing, mitochondrial membrane potential, a key indicator for bioenergetics of cells, depolarizes in a wide range of species – from yeasts, plants to animals. In humans, the decline of mitochondrial activities can impact the high-energy-consuming organs, such as the brain and heart, and increase the risks of age-linked diseases. Intriguingly, a mild depolarization of mitochondria has lifespan-extending effects, suggesting an important role played by bioelectricity during ageing. However, the underpinning biophysical mechanism is not very well understood due in part to the difficulties associated with a multiscale process. Budding yeast *S. cerevisiae* could provide a model system to bridge this knowledge gap and provide insights into ageing. In this perspective, we overview recent studies on the yeast mitochondrial membrane electrophysiology and ageing and call for more electrochemical and biophysical studies on ageing.

Keyword

Ageing; Mitochondrial membrane potential; Yeast; Electrophysiology

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Main text

Further to being a model system for complex fundamental biological processes in eukaryotic organisms, the budding yeast *Saccharomyces cerevisiae* has been established in the last few decades as a powerful model system for studying ageing [1]. Even though some characteristics of yeast ageing are specific to this organism, many of the modulators for vertebrates lifespans, such as calorie restriction, the nutrient-sensing signalling pathway Target Of Rapamycin (TOR) and sirtuins, are conserved from yeast to human [1,2]. In fact, *S.*

cerevisiae was the fast model system revealing the molecular mechanisms by which calorie restriction extends lifespan [3]. Building on this finding, it is now evident that calorie restriction is the most ubiquitous approach for extending lifespan in a wide range of species including humans [4]. *S. cerevisiae* is particularly useful because the process of ageing is heterogeneous and multiscale, especially regarding time; *i.e.* a long-time-scale process like ageing emerges from faster dynamic molecular processes such as genomic instability, cellular senescence and mitochondrial dysfunction [5]. Their relatively short lifespans, compared to the other model organisms for ageing research, such as *Mus musculus* (mice), *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (worm), enable bridging the differences in time scales, while also characterizing cell-cell heterogeneity [6]. In a typical cultivation condition, *S. cerevisiae* has a replicative lifespan (RLS) of ~24 buds, although it varies between strains [1]. RLS is defined as the number of buds produced by a single mother cell and it is a model of mitotically active cells. Another aspect of yeast ageing is the chronological lifespan (CLS) , which is a model of post-mitotic cells. CLS is defined as the survival time of cells in the stationary phase (non-dividing cells); it is generally measured by colony forming units (CFU) after 3 days of the start of the culture (when the majority of cells stops dividing)[7].

Among the classic theories on cellular ageing are the programmed ageing theory and free-radical theory. The programmed ageing theory proposes that ageing is driven by genetic pathways where sequential switching on and off of certain genes occur over time. The free-radical theory proposes that free radicals like superoxide, cause accumulating damage to DNA and proteins and impair cell function [8]. While the free-radical theory is consistent with many observations, some findings appeared to contradict this theory [9]. Accounting for these apparent contradictions, the damage-accumulation theory considers, not only oxidative damages but also cumulative damage such as translational errors and transcriptional heterogeneity. Accumulation of oxidatively damaged proteins, extrachromosomal ribosomal DNA circles and defective mitochondria, in parallel to dysregulated nutrient sensing, are aspects of the ageing process [12]. While an increasing amount of evidence suggests that these aspects are interconnected, *e.g.* the emerging impact that defective mitochondria has on stem-cell pool decline during ageing [10], a comprehensive understanding of the mechanisms underpinning the ageing process is not yet achieved.

During ageing, mitochondrial ATP production —a process, driven by an electrochemical potential gradient of proton [11]— declines in yeast, as well as in mammalian cells [10,12–15]. In animals, mitochondrial dysfunction, in part due to changes in mitochondrial DNA (mtDNA) integrity and production of reactive oxygen species (ROS), appears to be associated with

age-related disabilities and diseases, such as reduced heart function, neurodegeneration and even cancer progression [16]. Intriguingly, naturally long-lived animals, like naked mole rat and long-living bats, possess a mechanism to sustain a mild depolarized mitochondrial membrane potential during life [17] (Figure 1). These results suggest that there is a fundamental link between mitochondrial activity and ageing. Multiscale analysis bridging the fast-time-scale activities of mitochondria (*e.g.* ATP synthesis) and the long-time-scale process of ageing is a key to elucidate this link. Given that ageing-associated mitochondrial dysfunction is observed in yeasts [12–14], *S. cerevisiae* could provide a useful model system for investigating the dynamic crosstalk between fast and slow dynamics and elucidate the underpinning mechanisms of age-linked mitochondrial function loss. With the hope of facilitating more research into this direction, this perspective overviews *S. cerevisiae* studies highlighting the fundamental link between ageing and bioelectricity - more specifically, mitochondrial membrane potential.

Mitochondrial membrane depolarizes during ageing

Analyzing the function and structure of mitochondria by measuring oxygen consumption in yeasts, Volejnikova *et al.* demonstrated that respiration increases in the first four days, and decreases over the remaining period [12]. During the course of a 10-day experiment, the routine oxygen consumption by uncoupled respiration increases from ~10 to ~30 pmol O_2/s·10^6 cells during the first four days, which then decline to near zero on day 10. This change in oxygen consumption is accompanied by the fragmentation of mitochondria and depolarization of their inner membrane, which may reflect the cell cycle arrest in the G1 phase. Coinciding with the start of oxygen consumption decline, the membrane potential sharply depolarizes after day 4. Prior to the mitochondrial depolarization during ageing, the vacuolar pH increases [13]. By performing a screen for genes whose overexpression prevented mitochondrial dysfunction, Hughes *et al*. identify VMA1 and VPH2, genes that are required for the function of the vacuolar H^+-ATPase, which acidifies the vacuole. Overexpression of VMA1 and VPH2 not only prevents the age-associated vacuolar acidity decline but also inhibits mitochondrial membrane depolarization and contributes to lifespan extension by also maintaining adequate amino acid import by the vacuole.

The age-linked mitochondrial dysfunction and depolarization are also associated with genome instability and increased loss of heterozygosity (LOH) [14]. Deletion of the mtDNA induces a reduction in respiration capacity, depolarization of the inner membrane and defective synthesis or transport of Iron-Sulfur-Clusters (ISC), core components of several proteins including those responsible for DNA repair. One mitochondrial function, besides energy production, is synthesis and export of ISC, and Veatch *et al*. report that more than respiration capacity, the mitochondrial inner membrane potential is essential for correct

production and transport of ISC. While fluorescent reporters for mitochondrial membrane potential demonstrate the depolarization, quantitative measurements of mitochondrial membrane potential are yet to be done. The biophysical mechanism by which mtDNA degradation, calorie restriction and vacuolar pH increase induce mitochondrial membrane depolarization is still not entirely clear.

Mild mitochondrial depolarization as a possible lifespan-extending mechanism

A mildly depolarized mitochondrial membrane, while keeping all of this organelle's functionalities, appears to have an anti-ageing effect in *S. cerevisiae* —similar to the way in naturally long-lived organisms [17]. *S. cerevisiae* cells cultivated in caloric restriction conditions, an intervention capable of increasing lifespan, displayed reduced mitochondrial membrane potential. Among cells cultivated on non-restrictive conditions, those that display depolarized membrane potential have a longer lifespan, compared to the cells with more polarized potential [18]. Miceli *et al*. also demonstrate this correlation between mildly reduced mitochondrial membrane potential and extended longevity [19]. Through activation of the retrograde process, inter-organelle signalling that modulates nuclear gene expression, cells that had their mtDNA deleted, display reduced mitochondrial membrane potential and longer lifespan. In the cells with deleted mtDNA, extra-chromosomal rDNA circle (ERC) is augmented. And, in a trend similar to that observed by Veatch *et al*. [14], an increase in mitochondrial membrane potential by overexpression of *ATP1-111*, encoding a hyperactive F_1 ATP synthase, leads to the levels of ERC comparable to wildtype cells. Interestingly, reduction of mitochondrial membrane potential by deletion of *COX4,* which encodes a subunit of cytochrome oxidase, leads to a longer lifespan, while maintaining the cells mtDNA and keeping ERC similar or lower to that of wildtype cells [19]. Exposing yeast cells to the uncoupler dinitrophenol (DNP), which is capable of depolarizing mitochondria [20], decreases ROS, and increases yeast chronological lifespan [21]. However, whether the effect of DNP treatments causes mitochondrial depolarization is not yet demonstrated directly.

Interventions for increasing lifespan reduce mitochondrial membrane potential in yeast

Interventions capable of increasing lifespan, like caloric restriction, induce a mild depolarization of the mitochondria. As demonstrated by Delaney *et al*. [18], cultivation of yeast cells in caloric restriction reduces mitochondrial membrane potential, as does buffering of cultured media, another intervention capable of extending lifespan in yeast. Furthermore, it has been observed that yeast cells receiving lithocholic bile acid (LCA) delays ageing process and, for young cells, LCA lowers mitochondrial membrane potential and sustains it in *S. cerevisiae* [22,23]. It is worth noting that the pathways modulated by the addition of LCA

do not overlap with nutrient-sensing signalling pathways like TOR and cAMP/protein kinase A (cAMP/PKA), as LCA is able to extend the lifespan of yeast cells cultivated in calorie-restriction conditions. The mitochondrial membrane depolarization and anti-ageing effects, caused by the administration of LCA, also involve the activation of the retrograde signalling pathway, which is in agreement with a previous study [19].

Inhibiting the TOR signalling pathways results in polarized mitochondrial membrane potential [24]. Pan *et al*. show that the mutant yeast strain Δ*tor1*, lacking a gene encoding a protein subunit unique to TORC1, has higher respiration activity, a more polarized mitochondrial membrane potential and a longer chronological lifespan compared to isogenic wild type control. However, when entering the stationary phase, Δ*tor1* cells have reduced ROS production and reduced mitochondrial membrane potential to the levels below wild type, which promoted a longer lifespan [24]. Adding to the complexity of how the modulation of mitochondrial electrophysiology affects longevity, supplementing DNP to wild-type cultures increases CLS, as also observed by Barros *et al.* [21], but the same treatment with Δ*tor1* cultures severely impairs CLS extension. Δ*tor1* cells have a metabolism shifted towards respiration, in comparison to wild type, which might provide an explanation to why dissipating mitochondrial membrane potential in Δ*tor1* cells by the addition of DNP does not extend CLS, but impairs its extension. The facts that Δ*tor1* cells received DNP in the first 24 hours of cultivation and were transferred to the media lacking this compound might have had an impact on the observed results, as the CLS extension for WT cells was observed in culture with DNP for the whole cultivation period (growth and stationary phases).

Future perspectives: is there a bioelectric code of ageing?

As outlined above, qualitative evidence demonstrates both that mitochondrial depolarization is a characteristic of ageing, and that a sustained mildly depolarized mitochondria throughout life extends longevity. Evidence also highlights the association between metabolism and the ageing process, and how changes in metabolic pathways can impact lifespan, emphasizing how cell metabolism is an interconnected network, linking metabolic states (fermentative or respiratory in yeast), electron flow (membrane potential) and the ageing process [18,24,25]. However, it is not clear yet if mitochondrial depolarization is a consequence of ageing or if it is a compensatory mechanism to regulate the accumulation of damage (*e.g.* a mildly depolarized mitochondrial membrane potential decreases ROS production [17,24]). Some hallmarks of ageing are considered as compensatory or antagonistic responses to damage [5]. Even though it is still an open question if this is the case for mitochondrial membrane potential, it could provide an explanation of why mitochondrial depolarization is observed in old cells and as a lifespan-extending strategy. Thus, an

important point for investigation is whether bioelectrical dynamics has a causal (or, circular causal [26]) effect on ageing, or if it is correlational. If it is causal, decoding the fast and slow dynamics of mitochondrial membrane potential may offer new biomedical applications for ageing. For example, dynamically controlling the permeability of ion channels may allow accelerating or slowing ageing. To this end, it is interesting that the permeability of voltage-dependent anion channel (VDAC) is modified during ageing in *Drosophila* and in rat cardiomyocytes [27,28]. Another important regulator of permeability in mitochondrial is the Permeability Transition Pore (mPTP), which has its activity altered during ageing in the brain and heart. mPTP opening can be altered by variations in membrane potential [29], hence being an additional target for dynamic control as a means to modulate lifespan.

Another interesting point is that ageing is generally associated with reduced regenerative capacity. Intriguingly, it has been shown that the plasma membrane potential and ionic changes can act as a signal for regeneration [30]. The possible bioelectric code for reprogramming cancer and ageing has been also discussed [31]. However, a thorough multiscale understanding of bioelectrical dynamics during ageing is still lacking. To this, a multiscale computational model integrating biophysical dynamics of the plasma and mitochondrial membrane potential is needed. Towards this, we believe that *S. cerevisiae* could be an ideal model system for biophysical experimental characterization due to their short lifespan and our ability to control their growth and manipulate their genes and genomes. Furthermore, yeast could be useful for a high-throughput investigation of different drugs on mitochondrial activity and membrane potential regarding their impacts on ageing, which may allow drug repurposing for anti-ageing and healthspan extending interventions. Among several potential drug candidates for screening, one worth mentioning is metformin, a drug used to treat diabetes, but which also provides mitochondrial protective properties [27,32].

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Figure 1. Membrane potential and lifespan

Recent evidence shows that mitochondrial membrane potential is inversely correlated to lifespan, to an extent that does not impair energy production. Yeast cultivated in lifespan extending calorie restriction conditions, have a mildly depolarized mitochondrial membrane potential, while yeast cells cultivated in regular conditions have a more polarized mitochondria and shorter lifespan. Long-lived bat and naked mole rat, naturally longevous organisms, have a mildly depolarized membrane potential sustained through life. Mice, which have a 10-fold shorter lifespan, loses its mild mitochondrial depolarization early in life.