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# Emerging role of mitochondrial calcium levels in cellular senescence and in switching cell fates

Céline Margand<sup>1,2,\*</sup>, Pablo Morgado-Cáceres<sup>3,4,\*</sup>, Ulises Ahumada-Castro<sup>5,\*</sup>, J. César Cárdenas<sup>3,4,\*</sup>, Nadine Martin<sup>1,2,\*</sup>, David Bernard<sup>1,2,+</sup>

<sup>1</sup>Cellular Senescence, Cancer & Aging team, Cancer Research Center of Lyon, INSERM U1052, CNRS UMR 5286, Centre Léon Bérard, Université Claude Bernard Lyon 1, South-ROCK Consortium, Institut Convergence Plascan, 69008 Lyon, France

<sup>2</sup>Equipe Labellisée la Ligue Contre le Cancer, Lyon, France

<sup>3</sup>Center for Integrative Biology, Faculty of Sciences, Universidad Mayor, Santiago 8580745, Chile.

<sup>4</sup>Geroscience Center for Brain Health and Metabolism, Santiago 8580745, Chile.

<sup>5</sup>Facultad de Odontología, Universidad San Sebastián, Bellavista, Santiago, Chile.

\* Share equal contribution

+ Corresponding author: david.bernard@lyon.unicancer.fr

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## **Abstract**

Manipulating senescent cells by eliminating them or by modifying their activity has attracted huge interest to delay or even treat many age-related diseases and to improve healthy aging. Mitochondria and in particular their calcium levels have emerged as key regulators of cellular senescence, cell death and the balance between the two, and may constitute targets for novel strategies to stifle senescent cell viability or their properties.

### **Emerging role of mitochondria in regulating cellular senescence**

Cellular senescence is a specific cell state resulting in a stable cell cycle arrest, a complex senescence associated-secretory phenotype (SASP), including proinflammatory and profibrotic mediators, growth factors and/or proteases, and in expression of cell surface proteins that modulates immune activity. Senescent cells are characterized by a senescence-associated  $\beta$ -galactosidase activity (SA- $\beta$ -Gal activity), elevated levels of cyclin-dependent kinase inhibitors (CDKI) p16 and p21, increased DNA damage and reactive oxygen species (ROS) production or/and resistance to apoptosis. Cellular senescence is induced by many signals, such as developmental signals, oncogenic stress, DNA damage, ROS, and metabolic stresses. Timely regulated senescence exerts positive effects on health in different contexts including embryonic development, wound healing or to protect from tumor formation. However, senescent cells accumulate in aging and in disease contexts, and they display deleterious effects. Indeed, pharmacological or genetic elimination of senescent cells improve aging by decreasing many age-related diseases in mouse pre-clinical models, and by increasing healthspan and lifespan of mice. Cellular senescence is therefore considered to be a druggable hallmark of aging, though the mechanisms of its development are not fully understood <sup>1</sup>.

Perturbing mitochondrial activity by pharmacological or genetic tools has been shown to induce premature senescence. More recently mitochondria have been proposed to play a key role in the regulation of physiological cellular senescence, in particular by Correia-Melo et al. Indeed, they showed that removing mitochondria of senescent human cells largely inhibits many hallmarks of senescent cells, including ROS production, SA- $\beta$ -Gal activity, p21 and p16 accumulation as well as SASP production <sup>2</sup>. Other studies have described various mitochondrial alterations occurring in senescent cells. These alterations include an increase in mitochondrial mass, a reorganization of the mitochondrial network toward a more fused and elongated organization, an impairment of mitophagy, a reduction in mitochondrial potential, a change in electron transport chain (ETC) activity, a reduction in the efficacy of ATP production and an increase in the production of mitochondrial ROS. The latter seem to be functionally important in mediating senescence by inducing molecular damage in different macromolecules, in particular nuclear DNA, promoting the DNA damage response (DDR) and cell cycle arrest, as well as stimulating the NF- $\kappa$ B activity, an important regulator of the SASP <sup>1</sup>. Other mechanisms, including recently described mitochondrial permeabilization and release of mitochondrial DNA and mitochondrial dsRNA, could stimulate cytosolic sensors also known to promote SASP production<sup>3</sup>. In the last few years, an increase in calcium ( $\text{Ca}^{2+}$ ) levels and in particular in mitochondria has been reported in senescent cells <sup>4</sup>. Here, we discuss the importance of mitochondrial  $\text{Ca}^{2+}$  levels in regulating different cell fates (viability, cell death or induction of senescence) and in contributing to cell fate decision, orienting cells towards senescence or apoptosis, which could be critical for manipulating senescent cells to improve aging.

### **Mitochondrial calcium is required for mitochondrial activity and cell survival**

Ca<sup>2+</sup> fluxes and levels are tightly regulated within cells and in the different subcellular compartments and organelles. Indeed, differences ranging in 1000-fold in Ca<sup>2+</sup> concentrations are observed between compartments displaying the highest concentrations (endoplasmic reticulum, ER) and the lowest concentrations (nucleus or cytosol) in cells (Figure 1, left panel). This finely regulated Ca<sup>2+</sup> concentration plays a pivotal role in coordinating cellular workload and energy production by regulating the activity of key proteins and enzymes involved in mitochondrial metabolism. For instance, several enzymes of the tricarboxylic acid cycle (TCA) require Ca<sup>2+</sup> to be enzymatically active, and Ca<sup>2+</sup> is thus essential for maintaining overall cellular metabolic and bioenergetic homeostasis (Figure 1, left panel). Mitochondrial Ca<sup>2+</sup> levels are adjusted pending on the level of mitochondrial activity, in particular for ATP production. When needed, Ca<sup>2+</sup> can be released from ER, the cellular Ca<sup>2+</sup> reservoir, through Inositol 1,4,5-Trisphosphate (IP3) Receptors (ITPRs or IP3Rs) and transferred to mitochondria through Voltage Dependent Anion Channels (VDACs) at the outer mitochondrial membrane and through mitochondrial Ca<sup>2+</sup> uniporter (MCU) at the inner mitochondrial membrane. IP3R-mediated Ca<sup>2+</sup> release is a critical mechanism to favor Ca<sup>2+</sup> transfer to the mitochondria and to control mitochondrial functions, bioenergetics and cellular homeostasis (Figure 1, left panel). Besides channel activity, transfer of Ca<sup>2+</sup> from the ER, through IP3Rs, to the mitochondria, through VDAC and MCU, is facilitated by physical contacts between these organelles at mitochondria-ER contact sites (MERCs) which promote physical proximity between these channels. MERCs maintain ER and mitochondria at a ~10-40 nm distance, which allows mobile IP3Rs to concentrate in and to participate in the formation of these sites, thus facilitating Ca<sup>2+</sup> transfer and bioenergetics, and preventing its dilution in the cytosol <sup>5</sup>. Decreasing the transfer of Ca<sup>2+</sup> to the mitochondria leads to bioenergetic stress, characterized by the activation of AMPK and autophagy as survival mechanisms <sup>6</sup>. In addition of Ca<sup>2+</sup> influx,

mitochondrial  $\text{Ca}^{2+}$  level is also regulated by mechanisms controlling its exit by transient opening of the mitochondrial permeability transition pore (mPTP) and/or activity of the  $\text{Ca}^{2+}$  exchanger NCLX, also named Solute Carrier Family 8 Member B1 <sup>7</sup>. Hence, the tight control of mitochondrial  $\text{Ca}^{2+}$  levels is essential for cellular bioenergetics and their decrease cause bioenergetic stress and activation of survival pathway <sup>6</sup> or cell death in some circumstances <sup>8</sup>.

### **A peak of mitochondrial calcium promotes apoptotic cell death**

Although mitochondrial  $\text{Ca}^{2+}$  is necessary for cell metabolism and survival, its accumulation can lead to cell death. As previously discussed, intracellular  $\text{Ca}^{2+}$  levels must be tightly and spatially controlled inside the different compartments of the cell. In this context and aside from the role of  $\text{Ca}^{2+}$  in mitochondrial metabolism, mitochondria have also evolved as an essential sensor and modulator of cytosolic  $\text{Ca}^{2+}$  levels. Through its proximity with the ER within MERCs, mitochondria can rapidly sense cytosolic  $\text{Ca}^{2+}$  hotspots stemming for instance from the opening of IP<sub>3</sub>Rs and then act as a spatiotemporal buffer that shapes cytosolic  $\text{Ca}^{2+}$  signals, sequestering and releasing  $\text{Ca}^{2+}$  when needed to avoid toxic mitochondrial  $\text{Ca}^{2+}$  accumulation. Nevertheless, if this protective mechanism is saturated and mitochondrial  $\text{Ca}^{2+}$  levels increase above a threshold, cells can die. Indeed, mitochondrial  $\text{Ca}^{2+}$  overload in the mitochondrial matrix triggers the sustained opening of the mPTP. Consequently, mitochondria undergo rapid swelling upon osmotic pressure, rupture of the outer mitochondria membrane, a drop in mitochondrial membrane potential and depletion of ATP production which, in turn, enable the leakage of proapoptotic factors, including cytochrome c, ultimately causing apoptosome formation and eventually cell death. Beyond mitochondrial swelling, Hwang et al. proposed that mitochondrial  $\text{Ca}^{2+}$  also triggers mPTP opening through its binding to cardiolipin in the inner mitochondrial membrane leading to disassembly of ETC complex II,

which results in ROS production. These Ca<sup>2+</sup>-induced ROS could subsequently promote mPTP opening and the release of proapoptotic factors, ultimately leading to apoptosis<sup>9</sup>. Cytochrome c released at early stage of apoptosis is proposed to bind to IP3Rs and to further elicit Ca<sup>2+</sup> release from the ER, and increase Ca<sup>2+</sup> levels in the cytosol and in the mitochondria, thus amplifying and propagating mitochondrial permeability. This in turn stimulates the release of cytochrome c and other proapoptotic factors from the mitochondria across the whole cell, resulting in cell death. Mitochondrial Ca<sup>2+</sup> accumulation thus constitutes a critical signal promoting mitochondrial permeability, release of proapoptotic factors and ultimately leading to cell death (Figure 1, right panel).

#### **Increased mitochondrial calcium contributes to the induction of cellular senescence**

Although a massive rise in Ca<sup>2+</sup> in mitochondria triggers cell death, it has been shown in the last decade that increased mitochondrial Ca<sup>2+</sup> levels can also induce an alternative cell fate, namely cellular senescence. Ca<sup>2+</sup> concentrations were reported to increase in the cytosol and also in the mitochondria<sup>4</sup> of cells undergoing senescence cells. Increase in mitochondrial Ca<sup>2+</sup> is critical for senescence induction as it was shown that decreased levels of IP3Rs, that release Ca<sup>2+</sup> from the ER, as well as decreased levels of the MCU, that permits entry of Ca<sup>2+</sup> into the mitochondrial matrix, inhibit the establishment of cellular senescence<sup>10</sup>. Consistently, MERCS may be more abundant in cells undergoing senescence *in vitro* and *in vivo*, where they could participate in inducing senescence by facilitating mitochondrial Ca<sup>2+</sup> accumulation. Indeed, forcing MERCS by constitutively expressing a synthetic linker bridging ER and mitochondria induces premature senescence<sup>11</sup>. Though the precise mechanisms are still largely unclear, this increased mitochondrial Ca<sup>2+</sup> results in mitochondrial depolarization and the production of ROS. This in turn induces DNA damage and provokes the downstream events required for the

establishment of senescence: proliferation arrest and SASP production<sup>13,14</sup>. Whether blocking efflux of mitochondrial  $\text{Ca}^{2+}$  will lead to accumulation of mitochondrial  $\text{Ca}^{2+}$  and then induce a premature senescence is rather unknown. Nevertheless, cyclosporine A, which among others can decrease the efflux of mitochondrial  $\text{Ca}^{2+}$ <sup>7</sup>, can induce premature senescence<sup>12</sup>.

We can also speculate that the mitochondrial  $\text{Ca}^{2+}$  increase observed in senescent cells contributes to their survival. Indeed, inducers of senescence, such as UVB, can induce mitophagy, which can be triggered by mitochondrial  $\text{Ca}^{2+}$  increase. In that case, mitophagy contributes to the survival of senescent cells<sup>13</sup>.

Together, these results demonstrate that an increased mitochondrial  $\text{Ca}^{2+}$  can promote cellular senescence, as well as, in some contexts, cell death. These observations then raise the question of how cell fate is directed towards cell death or cellular senescence upon mitochondrial  $\text{Ca}^{2+}$  increase (Figure 1, middle panel).

### **Changes in mitochondrial calcium levels switch cell fate between death and senescence**

Recent publications support that a key driver in cell fate decision and even in switching cell fate between cell death and cellular senescence could be the overload level in mitochondrial  $\text{Ca}^{2+}$ . Indeed, Madreiter-Sokolowski et al reported that endothelial senescent cells display increased MERCS and ER-mitochondrial  $\text{Ca}^{2+}$  fluxes, probably boosting mitochondrial respiration. However, this adaptation also increases susceptibility to  $\text{Ca}^{2+}$  overload-induced death. Resveratrol, a natural polyphenol, induces a further increase in mitochondrial  $\text{Ca}^{2+}$  in senescent cells, resulting in the death of senescent cells and offering the first senolytic strategy around this phenomenon<sup>14</sup>. A proper balance in mitochondrial  $\text{Ca}^{2+}$  levels also depends on its release through specific mechanisms, either transient opening of the mPTP or activity of NCLX  $\text{Ca}^{2+}$  exchange protein. Protasoni et al. recently identified that senescent cells

transiently release mitochondrial  $\text{Ca}^{2+}$  through the transient opening of mPTPs, which mitigate mitochondrial  $\text{Ca}^{2+}$  overload in senescent cells. Blocking the release of mitochondrial  $\text{Ca}^{2+}$  in senescent cells by inhibiting mPTP activity, through the genetic decrease of its key component cyclophilin D or pharmacologically, or by blocking NCLX leads to a further increase in mitochondrial  $\text{Ca}^{2+}$  and in cell death <sup>7</sup>. These two studies advocate for further increasing mitochondrial  $\text{Ca}^{2+}$  levels in senescent cells as a treatment to induce senolysis and improve age-related diseases. The higher sensitivity of senescent cells when compared to non-senescent cells to increased mitochondrial  $\text{Ca}^{2+}$  influx and/or to decreased efflux is potentially due to their higher basal level of mitochondrial  $\text{Ca}^{2+}$  <sup>10,11</sup>. Future works will be required to clarify whether increasing mitochondrial  $\text{Ca}^{2+}$  could constitute a generalizable nontoxic senolysis strategy and if so through which type of cell death.

Could reducing mitochondrial  $\text{Ca}^{2+}$  load in cells on the path to cell death change their fate and force their entry into senescence? This question was recently tackled by Nehme et al, who used a model of cell death induced by exposure to ROS and demonstrated that this cell death is mediated by the poly (ADP-ribose) polymerase 1 (PARP1) and subsequent accumulation of mitochondrial  $\text{Ca}^{2+}$ . Importantly, they showed that inhibiting PARP1 prevented mitochondrial  $\text{Ca}^{2+}$  overload-induced death and shifted cell fate towards senescence. In kidney ischemia-reperfusion injury models, PARP inhibition was reported to reduce cell death, promote transient senescence at injury sites, and improve recovery <sup>15</sup>. These studies support that the level of mitochondrial  $\text{Ca}^{2+}$  overload is critical to determine whether cells will senesce or die.

### **Concluding remarks**

It is now clear that mitochondrial  $\text{Ca}^{2+}$  is critical in determining different cell fates. Mitochondrial  $\text{Ca}^{2+}$  increases in several models of senescence tested supporting that this

increase could constitute a novel hallmark of senescent cells, even if we cannot rule out that in some models of cellular senescence, cell types or in response to some senescence inducers, that may not be the case. It will be critical to better characterize the kinetics of mitochondrial  $\text{Ca}^{2+}$  accumulation in senescent cells and whether this increase is sustained in late stages of senescence. The recent publications discussed above suggest that modulating mitochondrial  $\text{Ca}^{2+}$  levels could lead to a switch in cell fate. Further increasing mitochondrial  $\text{Ca}^{2+}$  accumulation in senescent cells might thus constitute a novel strategy to eliminate senescent cells and improve age-related diseases <sup>7</sup>, but this strategy can be risky as it might promote senescence of initially non-senescent cells. Decreasing mitochondrial  $\text{Ca}^{2+}$  to decrease senescence might also be a valuable strategy to improve aging <sup>11</sup>. Moreover, decreasing mitochondrial  $\text{Ca}^{2+}$  accumulation may prevent cell death and lead to cell survival in a senescent state and improve some diseases <sup>15</sup>, supporting that in some contexts it is better to have senescent cells than no cells (Figure 1).

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### **Competing interests**

The authors declare that they have no competing interests.

## Author contribution

All the authors contributed to writing individual parts. DB conceptualized this article, modified and compiled all the contributions. All the authors revised and finalized the manuscript. CM drew the figures with the help of PMC.

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We would like to apologize for all the relevant articles in that topic that cannot be cited because of space limitation.

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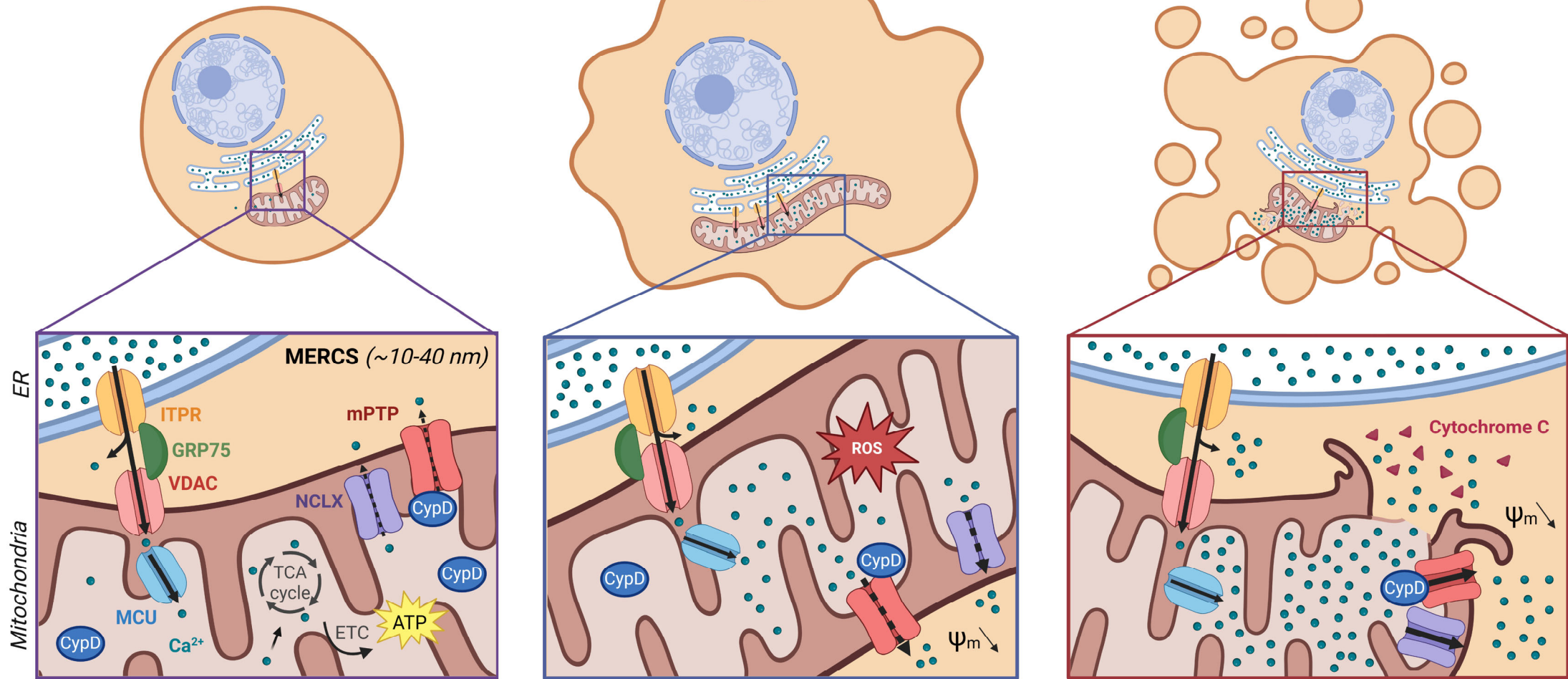
## Figure Legend

**Figure 1.** Emerging role of mitochondrial  $\text{Ca}^{2+}$  levels in switching cell fates. Mitochondrial  $\text{Ca}^{2+}$  levels depend on  $\text{Ca}^{2+}$  influx (ITPR/VADC/MCU) or efflux (mPTP or NCLX). At steady state, mitochondrial  $\text{Ca}^{2+}$  ensures basal mitochondrial activity including TCA cycle, ETC and ATP production. Mitochondrial  $\text{Ca}^{2+}$  increase contributes to cellular senescence through mitochondrial depolarization and ROS production. In this context, a mechanism called  $\text{Ca}^{2+}$  flickering occurs through transient NCLX and/or CypD-dependent mPTP opening to limit mitochondrial  $\text{Ca}^{2+}$  levels and then sustain the survival of senescent cells. Beyond a threshold, mitochondrial  $\text{Ca}^{2+}$  overload drives persistent mPTP opening, which induces mitochondrial permeability and subsequent release of cytochrome c and other pro-apoptotic factors leading to apoptosis or to other form of cell death. The double arrows represent the shift from one cell fate to another that can occur when mitochondrial  $\text{Ca}^{2+}$  concentrations are manipulated, representing potential emerging senolytic or senomorphic strategies to impact senescence-related diseases and aging. ER: endoplasmic reticulum; MERCS: mitochondria-ER contact sites; ITPR: inositol 1,4,5-triphosphate receptor; GRP75: glucose regulated protein 75; VDAC: voltage-dependent anion channel; MCU: mitochondrial calcium uniporter; CypD : cyclophilin D; mPTP: mitochondrial permeability transition pore; NCLX: mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  exchanger; ETC: electron transport chain; ROS: reactive oxygen species; TCA: tricarboxylic acid cycle;  $\Delta\psi_m$ : mitochondrial membrane potential. This figure has been created with BioRender.com.

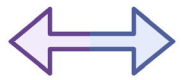
# PROLIFERATIVE / QUIESCENT STATE

# CELLULAR SENESCENCE

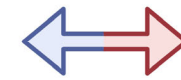
# CELL DEATH



Basal Ca<sup>2+</sup>-dependent bioenergetic metabolism



Ca<sup>2+</sup> load increase limited by transient mPTP opening



Ca<sup>2+</sup> overload-induced mitochondrial permeability through persistent mPTP opening