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SHORT COMMUNICATION

Mitochondrial communication in the context of aging

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Abstract
Mitochondria constantly contribute to the cell homeostasis and this, during the lifespan of a cell, takes its toll. Indeed, the functional decline of mitochondria appears correlated to the aging of the cell. The initial idea was that excessive production of reactive oxygen species (ROS) by functionally compromised mitochondria was the causal link between the decline of the organelle functions and cellular aging. However, in recent years accumulating evidence suggests that the contribution of mitochondria to cellular aging goes beyond ROS production. In this short review, we discuss how intracellular signalling, specifically the cAMP-signalling cascade, is involved in the regulation of mitochondrial functions and potentially in the processes that link mitochondrial status to cellular aging.

Keywords cAMP · Signalling · Mitochondria · Aging

Introduction

Eukaryotic cells originated from the endosymbiosis between two prokaryotes, one of which evolved in the mitochondrion. A crucial event during their co-evolution was the transfer of genetic material from the endosymbiont (likely an α-proteobacterion) to the host (likely an archaeon). By transferring part of their genetic material to the nucleus, mitochondria consolidated their connection to the host; however, at the same time, this event required the development of mechanisms that allowed the two symbionts to exchange information on their status and their needs. As a result, mitochondria became major signalling hubs, transmitting and receiving adaptive regulatory signals that evolved to control a wide range of cellular functions.

The establishment of such a sophisticated communication centre, however, requires the coordinated action of many components and, consequently, it is not failproof. In fact, deregulated communication between mitochondria and host cell has major consequences and is an underlying factor of many pathophysiological conditions, including the process of aging.

The free radical theory of aging, dating back to the 1950s, postulated that the generation of ROS, the waste products of mitochondrial oxidative phosphorylation, induces the accumulation of damaged proteins, lipids and DNA, which over time causes aging. Although mitochondrial dysfunction is associated with the onset of age-related diseases, and over time damaged mitochondria with increased ROS production accumulate, extensive experimental work has challenged the causal relationship between ROS and aging. The most compelling evidence against the free radical theory is that antioxidants fail to extend the lifespan of model organisms or to show beneficial effects on age-related diseases in humans. Moreover, mice with increased ROS do not display accelerated aging but, on the contrary, moderately increases in ROS promote healthy aging and increase lifespan in yeast, *C. elegans* and mice. This evidence led to a change in perspective on the role of mitochondrial ROS signalling in aging.

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Currently, it is well accepted that excessive mitochondrial ROS levels can cause molecular damage and accelerate aging, however, the importance of these molecules for the physiology of the cell is also recognized. Indeed, during stress, ROS are crucial for activating a hormetic response that induces the antioxidant defence pathways which collectively result in an adaptive response to maintain homeostatic cellular functions and, ultimately, promote longevity [1, 2]. Based on this view, aging is now recognized as a complex and multifactorial process, during which several mitochondria-driven processes are affected, including mitophagy (the mitochondrial quality control), mitochondria-to-nucleus retrograde signalling [3] and mitochondrial metabolism [4].

Mitochondria and second messengers

For long time mitochondria have been regarded solely as bioenergetic and biosynthetic sites; however, beyond their metabolic functions these organelles possess signalling functions, critical to prevent divergences between their performing effectiveness and the metabolic demand of the cell [5]. The classic example of how mitochondria are integrated into cellular signalling is calcium (Ca^{2+}) handling. These organelles have developed sophisticated molecular machineries that allow them to both uptake and release Ca^{2+} and consequently use this second messenger as a means of bidirectional information exchange with the host cell [6]. Contrary to Ca^{2+} that can readily reach the mitochondrial matrix, the other main second messenger, cyclic AMP (cAMP) cannot cross the inner mitochondrial membrane (IMM) and its actions in the matrix depend exclusively on its production in situ. Thanks to this peculiarity mitochondria can uncouple cAMP signals coming from the host cell and signals generated domestically.

From a functional point of view, cAMP acting at the outer mitochondrial membrane (OMM) has been connected to the regulation of several processes, such as mitochondrial protein import, apoptosis, autophagy, mitophagy and mitochondrial dynamics [7]. On the contrary, for long time the leading view was that cAMP had no role in the mitochondrial matrix, a belief based on its expected impermeability across the IMM (due to its negative charge) and on the absence of canonical targeting sequences in the known cAMP effectors. However, 10 years ago Acin-Perez and colleagues [8] suggested that extramitochondrial cAMP is unable to cross the IMM, but it can be generated in the matrix by a local soluble adenylyl cyclase (sAC), in response to bicarbonate derived from the carbon dioxide produced by the Krebs cycle. According to their model, a cAMP-driven signalling cascade is contained in the mitochondrial matrix, encompassing sAC, a cAMP-degrading phosphodiesterase and protein kinase A (PKA). Later, in two independent studies [9, 10], we used cAMP-sensitive FRET-based sensors targeted to the matrix and confirmed that cytosolic cAMP cannot cross the IMM, except, notably, during mitochondrial permeability transition (MPT) [10]. In addition, we showed that intramitochondrial cAMP production can be driven by increases in matrix Ca^{2+} levels [9], suggesting an interconnection between cAMP and Ca^{2+} signalling within the mitochondria. An unexpected result of our study was that a matrix-targeted FRET reporter of PKA-dependent phosphorylation was unable to detect any PKA activity in response to increases in matrix cAMP levels [10]. These data challenged the idea that PKA is the effector responsible for the actions of cAMP in the matrix, but also opened the possibility that this cascade may rely on other cAMP-sensitive effectors. In line with this idea another cAMP effector, exchange protein activated by cAMP 1 (EPAC1), has been recently proposed by several independent studies [11] as the main cAMP effector inside mitochondria. However, the existence and the identity of a cAMP-dependent kinase present in the mitochondrial matrix has not been unequivocally excluded yet.

Contrary to the matrix, FRET experiments using a PKA-dependent phosphorylation sensor evidenced high PKA activity at the OMM. In experiments where cAMP and PKA-dependent phosphorylation were simultaneously measured at the OMM and in the cytosol of living cells, we found no difference in cAMP between these two domains. However, in response to similar cAMP levels the activity of PKA at the OMM was significantly different, both in terms of entity (stronger) and timing (persisted longer), from that measured in the cytosol. We established that this differential regulation was independent of PDEs and depended on lower phosphatase activity at the OMM compared with the cytosol. From a functional point of view, due to the limited phosphatase access, the OMM harbours higher PKA-dependent phosphorylation, which is observed even at basal conditions, and perturbing this equilibrium in favour of dephosphorylation leads to severe mitochondrial fragmentation [12].

Based on these data, signalling through cAMP at the mitochondria appears unique in its strict compartmentalization. Indeed, cAMP generated at the plasma membrane and in the cytosol, by both extracellular (through transmembrane ACs) and intracellular (through sAC) signals, reaches the mitochondrial surface, but it is excluded from the mitochondrial core, whereas a separate pool of cAMP is generated in the matrix, in response to local metabolic cues. Currently, the sole link between these hermetic cAMP pools appears to be the moving Ca^{2+}, which regulates sAC as well as the MPT, during which the IMM forms pores allowing non-specific, bidirectional molecular fluxes. As Ca^{2+} is taken up and released by mitochondria, the low conductance permeability transition pore (PTP) appears to flicker between open and closed state [13]. In case of mitochondrial Ca^{2+} overload, the PTP is strongly activated, the mitochondrial

membrane potential is lost and cytochrome c release from mitochondria leads to apoptosis. Hence, MPT can be the irreversible phenomenon that drives cell death, or, through its flickering, PTP can act as a transient 'safety valve', allowing mitochondria to limit stress-induced damage.

Considering that during MPT activation cAMP is free to diffuse into and out of the mitochondrial matrix [8], it is tempting to hypothesize a functional significance for this transient cAMP exchange between mitochondria and host cell. For example, cytosolic cAMP entering the matrix during transient permeability events might have a role in the downstream recovery of these organelles. On the other hand, MPT would provide the means for mitochondria to generate a cAMP signal towards the cell; one could imagine that during PTP flickering cAMP contained in the matrix can reach the OMM-tethered signalling machinery, impinging on mitochondrial dynamics and, eventually, on mitophagy. Hence cAMP could serve as an alarm message between mitochondria and the rest of the cell. Along this line, it is also tempting to speculate that, whatever the cAMP source, the low phosphatase activity recently highlighted at the OMM could be instrumental to maximally protect mitochondria from mitophagy and from unwanted fragmentation, which could lead the cell to a bioenergetic crisis. It is reasonable to hypothesize that the more active cAMP-PKA axis could confer to the OMM the enhanced ability to "sense" cAMP alarm signals, in order to summon the appropriate players for facing the emergency. Indeed, cAMP is involved in the regulation of mitochondrial dynamics at different levels and, interestingly, its actions are consistently beneficial both for mitochondria and the host cell. Activation of PKA at the OMM is a well-recognised pro-survival signal, while, thanks to the cAMP/PKA axis, mitochondria elongate to escape unnecessary degradation. In addition, it has been shown that the cAMP/PKA axis opposes non-selective mitophagy, and it has been suggested that it may participate in the redistribution of damaged mitochondria from the axons to the soma of neurons, hence facilitating their degradation [14].

cAMP signalling and aging

Aging can be defined as the transition from a fully functional to a failing-to-function network of hierarchically wired and interdependent subsystems, i.e. organs, tissues, cells and organelles. The breakdown of one subsystem, such as an organelle, can trigger a domino cascade which may lead to the sequential collapse of other subsystems and finally compromise the function of the network. Or, on the contrary, the homeostatic nature of wiring can provide the means of compensation for the decline of one subsystem. This highlights the importance of well-functioning interconnections and signalling between subsystems in the aging process.

In recent years, an increasing number of studies have focused on the activation of cAMP signalling as an alternative strategy to treat age-related cognitive deficits. This idea stems from a long-line of studies that established the importance of cAMP signals in the mechanisms of memory consolidation, and from more recent ones indicating altered (generally decreased) cAMP signalling and CREB function associated with either physiological brain aging and neurodegenerative diseases. However, CREB can be activated by multiple pathways besides the cAMP/PKA-axis, including Ca^{2+}/CaMKII/IV and the MAPK/ERK pathway. In addition, the molecular mechanisms and effectors through which cAMP exerts its effects on memory are not unequivocally established and are matter of intense research. For instance, while cAMP-dependent activation of PKA and EPAC is beneficial for hippocampal memory consolidation, increases in cAMP levels in other brain regions have opposite effects on working memory. In line with this, recent studies performed in aging humans and animal models point to specific changes in several players of the cAMP signalling cascade and unveil that age-dependent cAMP signalling dysregulation is associated with specific brain regions and, in some cases, with specific subcellular compartments [15]. This suggests that both therapeutic efficacy and side effects of cAMP-centric treatments most likely will depend on the topology of the treatment administration, both at the tissue (brain region) and cell level (subcellular compartment). Thus, a better understanding of the subcellular distribution and dynamics of cAMP microdomains and of their alteration and dysregulation during the aging processs will pave the way to novel therapeutic lines against age-related diseases.

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Compliance with ethical standard

Conflict of interest On behalf of all authors, the corresponding authors state that there is no conflict of interest.

Statement of human and animal rights In the original work by the authors all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Informed consent Not applicable.

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