Defining the mechanisms underpinning the mitochondrial cAMP signalling

Giulietta Di Benedetto¹², Konstantinos Lefkimmiatis²³, Tullio Pozzan,¹

¹CNR-Istituto di Neuroscienze, sezione di Padova, Padova
²VIMM (Veneto Institute of Molecular Medicine), Padova
³Università degli Studi di Pavia, Pavia

Our main interest focuses on cAMP signalling, in particular that involving mitochondria.

To study cAMP and PKA activity dynamics we developed genetically encoded FRET-based sensors, sensitive to variations in cAMP or in PKA-dependent phosphorylation. We target such sensors to different subcellular compartments, e.g. plasma membrane (PM), endoplasmic reticulum (ER), nucleus and different mitochondrial subcompartments, i.e. matrix, inner and outer mitochondrial membranes (IMM and OMM), inter-membrane space (IMS). In a recent manuscript we used OMM-targeted sensors for cAMP and PKA phosphorylation, and found that in spite of indistinguishable cAMP rises between the bulk cytosol and OMM, the latter displayed high and sustained PKA-dependent phosphorylation, due to limited access of phosphatases[1,2]. Currently, we are generating CaMKII phosphorylation-sensitive sensors targeted to the OMM, to explore whether phosphatases shape in time and space the actions of kinases other than PKA. In addition, we investigate PKA-dependent phosphorylation in different intracellular sites, such as ER and PM, to study whether phosphatase-dependent regulation is unique to OMM or it extends to the vicinity of other membranes as well. Finally, we are generating cAMP- and PKA phosphorylation-sensitive sensors targeted in the IMS, to unveil cAMP and PKA activity dynamics in this mitochondrial compartment, where they are completely unexplored.

Previously, by exploiting a matrix-targeted FRET-based sensor, we showed that cAMP can be synthesized in the matrix (mt-cAMP) in response to raises in local [Ca²⁺], and that increases in mt-cAMP ameliorate the efficiency of ATP production. These data suggested that the processes that modulate the generation of ATP in response to nutrient availability and cellular demand, are at least partially regulated by mt-cAMP[3]. These findings opened a number of questions regarding the effectors, the targets and the regulatory proteins of the cAMP signalling within the matrix, and the existence of additional functions regulated by mt-cAMP. For example, the domain function and regulation of the mitochondria-targeted isoform(s) of the soluble adenyl cyclase (sAC), the matrix cAMP-generating enzyme, are still poorly known. Although sAC has been detected in the mitochondrial matrix, it is still unknown which isoform enters mitochondria, and under which conditions. To gain insights on these aspects, we set up a split-GFP-based tool. We generated a non-fluorescent GFP 1-10 fragment targeted selectively to the mitochondrial matrix, IMS and OMM, while the full length sAC was fused to GFP β-Strand 11. Upon complementation of the GFP β-Strand 11 with the GFP 1-10 moiety, GFP fluorescence is regained indicating the presence in the same domain of the bait and target protein. We found that FL-sAC can be detected at all mitochondrial subcompartments, and that is found more frequently in the matrix upon glucose starvation. To determine the domains responsible for the mitochondrial targeting of sAC and its regulation by starvation, we are generating a series of sAC deletion mutants fused to GFP βS11. We also employed CRISPR/Cas9 technology and generated rodent and human sAC-deficient lines, currently under characterization. These lines will allow us to study the cellular effects of mitochondrial and cytosolic sAC isoforms, and to dissect the functional relevance of different sAC domains.
Based on our findings and published evidences we formulated the overarching hypothesis that mt-cAMP could impinge on the regulation of metabolic flexibility (MF). Mitochondria are emerging determinants in MF regulation, which involves the control of mitochondrial biogenesis and activity, resulting in the fine-tuning of oxidative metabolism. Interestingly, cAMP is involved in both bacteria and eukaryotes in the regulation of the response to fasting. Our preliminary data indicate that nutrients differentially modulate cAMP in the mitochondrial matrix. We are thus currently testing the hypothesis of the existence of a feedback loop where nutrient availability could regulate mt-cAMP level, which, in turn, would participate in the processes underpinning MF through its action on mitochondrial metabolism.

References:

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Contacts: giulietta.dibenedetto@cnr.it
Website(s): http://www.in.cnr.it/index.php/it/9-people/63-giulietta-dibenedetto http://www.biomed.unipd.it/people/dibenedetto-giulietta/ https://orcid.org/0000-0002-1489-3896

Other: