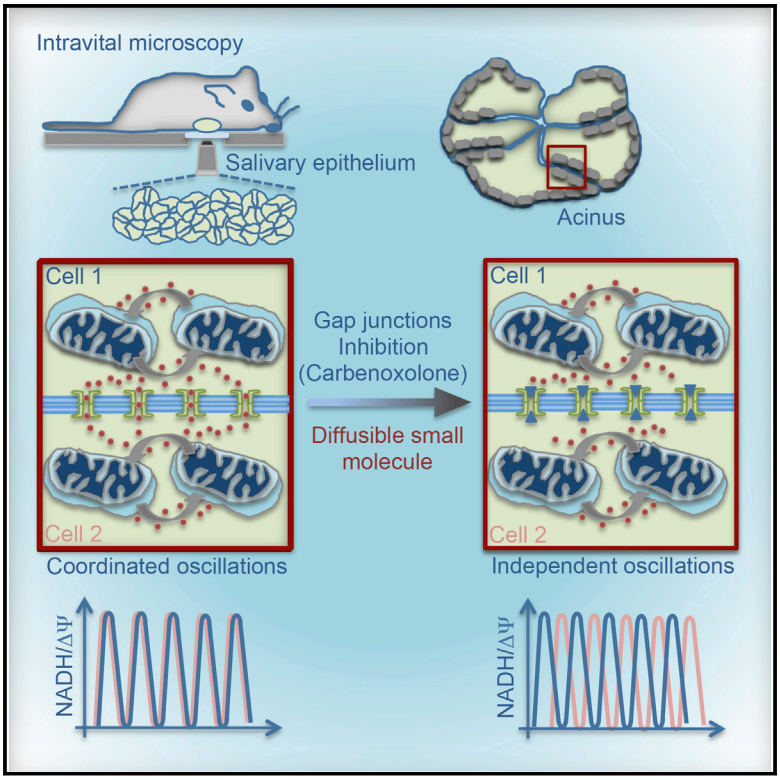
Report

Cell Reports

In Vivo Tissue-wide Synchronization of Mitochondrial Metabolic Oscillations

Graphical Abstract



Highlights

In live animals, mitochondria undergo rapid and constitutive metabolic oscillations

Mitochondria self-organize and are functionally linked in supracellular networks

Gap junctions control the coordination of mitochondrial activity across the tissue

Authors

Natalie Porat-Shliom, Yun Chen, ..., Andrius Masedunskas, Roberto Weigert

Correspondence

weigertr@mail.nih.gov

In Brief

Porat-Shliom et al. examine mitochondrial metabolism in live animals. They find that NADH levels and mitochondrial potential undergo spontaneous, rapid, and highly coordinated oscillations that are maintained by gap junction activity.

Porat-Shliom et al., 2014, Cell Reports 9, 514–521
October 23, 2014 ©2014 The Authors
http://dx.doi.org/10.1016/j.celrep.2014.09.022

OPEN ACCESS
CellPress

Cell Reports
Report

In Vivo Tissue-wide Synchronization of Mitochondrial Metabolic Oscillations

Natalie Porat-Shliom,[1] Yun Chen,[2] Muhibullah Tora,[1] Akiko Shitara,[1] Andrius Masedunskas,[1] and Roberto Weigert[1,*]
[1]Intracellular Membrane Trafficking Unit, Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, 30 Convent Drive 303A, Bethesda, MD 20892-4340, USA
[2]Laboratory of Pathology, National Cancer Institute, National Institutes of Health, 10 Center Drive 6B17, Bethesda, MD 20892, USA
*Correspondence: weigertr@mail.nih.gov
http://dx.doi.org/10.1016/j.celrep.2014.09.022

SUMMARY

Little is known about the spatiotemporal coordination of mitochondrial metabolism in multicellular organisms in situ. Using intravital microscopy in live animals, we report that mitochondrial metabolism undergoes rapid and periodic oscillations under basal conditions. Notably, mitochondria in vivo behave as a network of functionally coupled oscillators, which maintain a high level of coordination throughout the tissue via the activity of gap junctions. These findings reveal a unique aspect of the relationship between tissue architecture and self-organization of mitochondrial metabolism in vivo.

INTRODUCTION

Mitochondria provide the energy required to sustain biological processes by producing ATP through oxidative phosphorylation. The metabolic activity of mitochondria within a cell is the result of the coordination of several highly dynamic processes characterized by complex temporal patterns, which can display dynamic instability (Berridge and Rapp, 1979; Iotti et al., 2010). For example, studies in fermenting yeast, isolated cardiac cells, pancreatic islets, and exocrine glands acini have shown that oxidative phosphorylation exhibits oscillatory behaviors with periods spanning from seconds to minutes upon stimulation (Aon et al., 2003; Bruce et al., 2004; Danø et al., 1999; Kopach et al., 2011; Lloyd et al., 2012; Luciani et al., 2006; Voronina et al., 2002). Metabolic oscillations have been proposed to be either intrinsic to mitochondria (Vergun et al., 2003) or secondary to oscillations in other cellular processes such as glycolysis (Danø et al., 1999), intracellular Ca^{2+} levels (Voronina et al., 2002), or plasma membrane potential (Berridge, 2008; Bertram et al., 2007). In other systems they have been linked to increased levels of reactive oxygen species (ROS) produced in the mitochondrial matrix (Aon et al., 2003). Moreover, it was recently proposed that mitochondria within a cell behave as a network of coupled oscillators under pathological conditions and suggested that the communication among mitochondria is mediated by diffusible cytosolic messengers, such as ROS released from the mitochondrial matrix, which synchronize the energy status of the whole population of mitochondria (Aon et al., 2004, 2006; Kurz et al., 2010).

However, whether mitochondrial metabolic oscillations occur in live mammalian tissues in situ under physiological conditions, how they are regulated, and how they are spatially and temporally coordinated on a tissue scale have not been investigated. Here, by using intravital two-photon (2P) microscopy, we show that metabolic oscillations occur in the salivary gland epithelium of living rats under basal conditions and that their characteristics and response to stimulation differ from what previously reported for ex vivo model systems. Indeed, the oscillations were altered not by manipulating cellular cytoplasmic Ca^{2+} but rather by interfering with mitochondrial ROS production, suggesting that they are driven by metabolism rather than receptor-mediated signal transduction. Notably, we find that the ability of mitochondria to self-organize extends beyond individual cells and encompasses larger multicellular units within the tissue. Finally, we show that gap junction activity is important to maintain the synchronization of the mitochondrial metabolic activity.

RESULTS

In order to measure the metabolic activity of the mitochondria in vivo, we used intravital 2P microscopy that enables imaging of biological processes at cellular and subcellular levels in live animals (Weigert et al., 2013). As a model organ, we used the salivary glands (SGs) in live rats (Figure 1A), which are ideal to perform in vivo imaging since the motion artifacts due to heartbeat and respiration can be significantly reduced without compromising organ physiology (Masedunskas et al., 2011; Masedunskas and Weigert, 2008). As a reporter of the metabolic activity, we used NADH, a substrate of complex I that has the advantage of emitting upon 2P excitation without the need for exogenous labeling (Kasischke et al., 2004; Vishwasrao et al., 2005; Zipfel et al., 2003) (Figure 1A). The SGs were imaged 20–30 μm from the surface of the organ. At this depth, the tissue is composed primarily of acini, the main secretory units of the glands (Figure 1A), and NADH can be efficiently excited using minimal laser power, thus minimizing tissue photodamage (Figure S1). Although NADH is present both in the cytoplasm and in the mitochondrial matrix, most of the detected endogenous emissions originated from the latter, as shown by the overlap with mitochondrial markers (Figure S1). When the SGs epithelium was imaged in time-lapse mode by using a fast scanning speed, we measured periodic oscillations of the NADH emissions acquired from areas of the tissue encompassing 80–100