World Mitochondria Society

12th World Congress on

TARGETING MITOCHONDRIA

October 27-29, 2021

Best Mitochondria Image

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Nominated...

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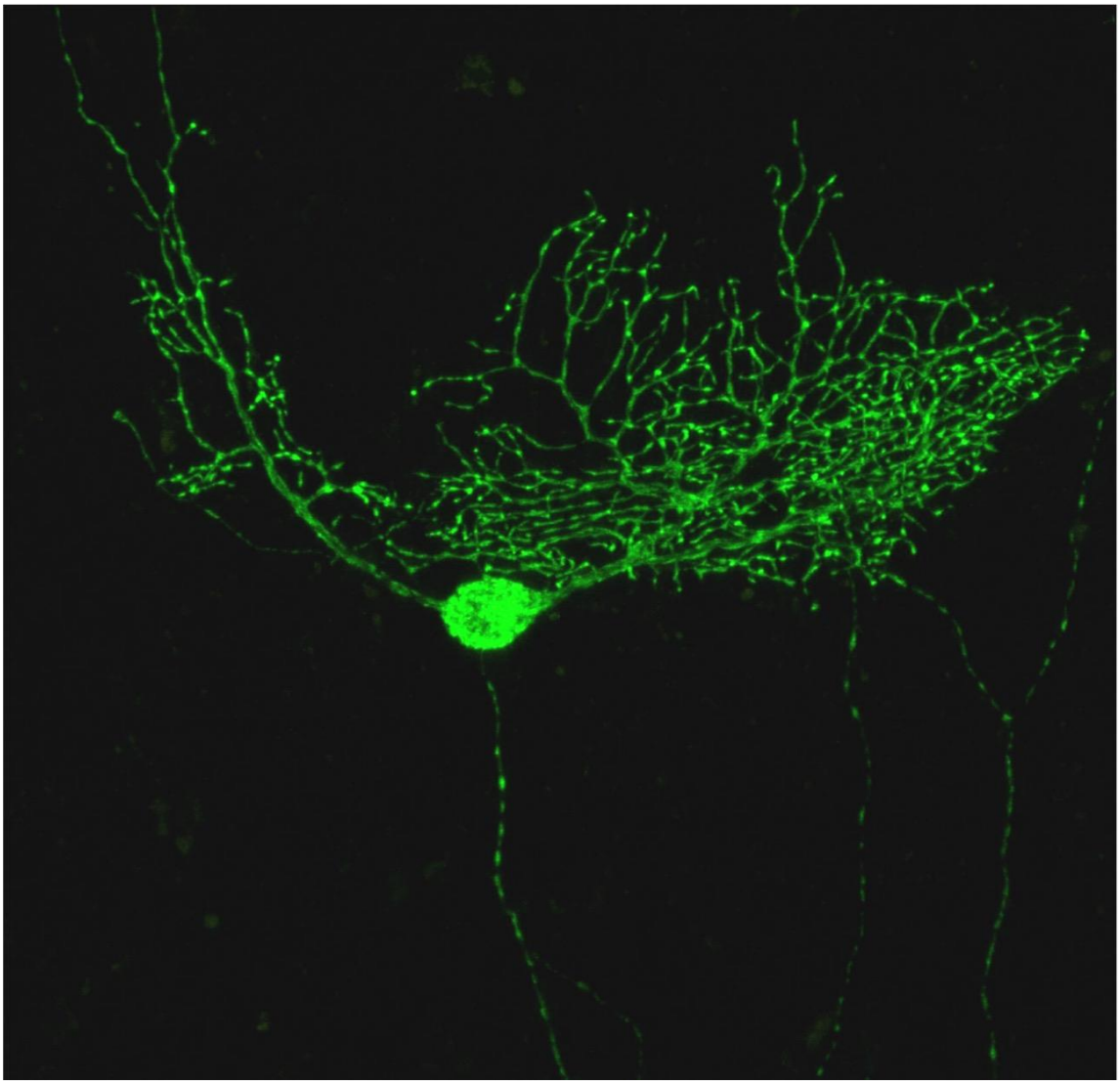
Simon Licht-Mayer, PhD

Institute for Molecular Biotechnology (IMBA)

Vienna, Austria



The image depicts a live image of the mitochondrial network in a Purkinje cell, including the cell body, axon and huge dendritic tree. This was taken during live imaging of an organotypic cerebellar slice culture (mouse), two weeks after transfection with a Lentivirus encoding for mEOS2 protein targeted to mitochondria.

Study Context:

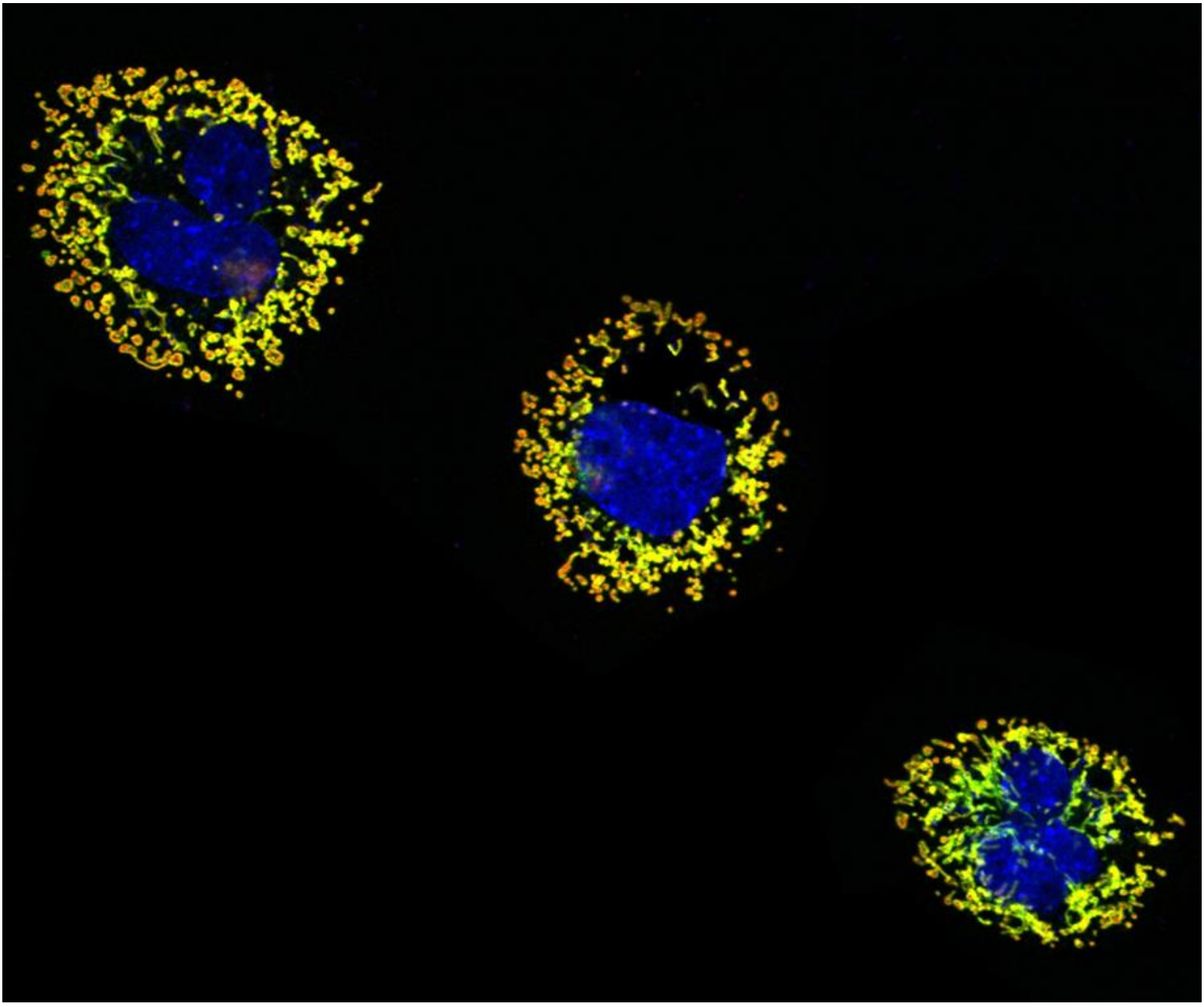
The aim was to make use of the photoconvertible mEOS2 protein to study mitochondrial movement dynamics of different mitochondrial populations after photoconversion by using different compounds targeting the BH4 pathway.

Sara Benhammouda

University of Quebec at Trois-Rivieres (UQTR),

Canada

Neuro-N2A Cells



Confocal microscopy image showing three cells of Neuro-N2A WT line made after staining with the specific marker for mitochondrial outer membrane TOM20 (green), HSP70 (red) and DAPI for nucleus (blue) to analyze the mitochondrial structure.

Study Context

Effect of Mutations in the TBC1D24 Protein Associated with Doors Syndrome on Mitochondrial Dynamics.

Mutations in the TBC1D24 protein are involved in several hereditary neurological diseases including DOORS syndrome. This disease is characterized by deafness at birth, malformation of the nails, defective formation of certain bones and intellectual disability. It can also be associated with epilepsy. The variety of phenotypes associated with these mutations, as well as the limited information we have on their exact role, make it difficult to develop treatments for the management of diseases with which TBC1D24 has been associated. At the molecular level, TBC1D24 is thought to play a role in vesicular transport. However, recent data suggests that TBC1D24 also regulates mitochondrial function. In order to define the role of TBC1D24 in the regulation of mitochondrial structure and function, we used DOORS syndrome patient's fibroblasts. As mitochondria are regulated by dynamic changes in their architecture, we first measured the effect of mutations in TBC1D24 on mitochondria length and connectivity. Our results showed that mutations in the TBC1D24 protein.

Bhargavi Duvvuri

Division of Rheumatology, Department of Medicine,

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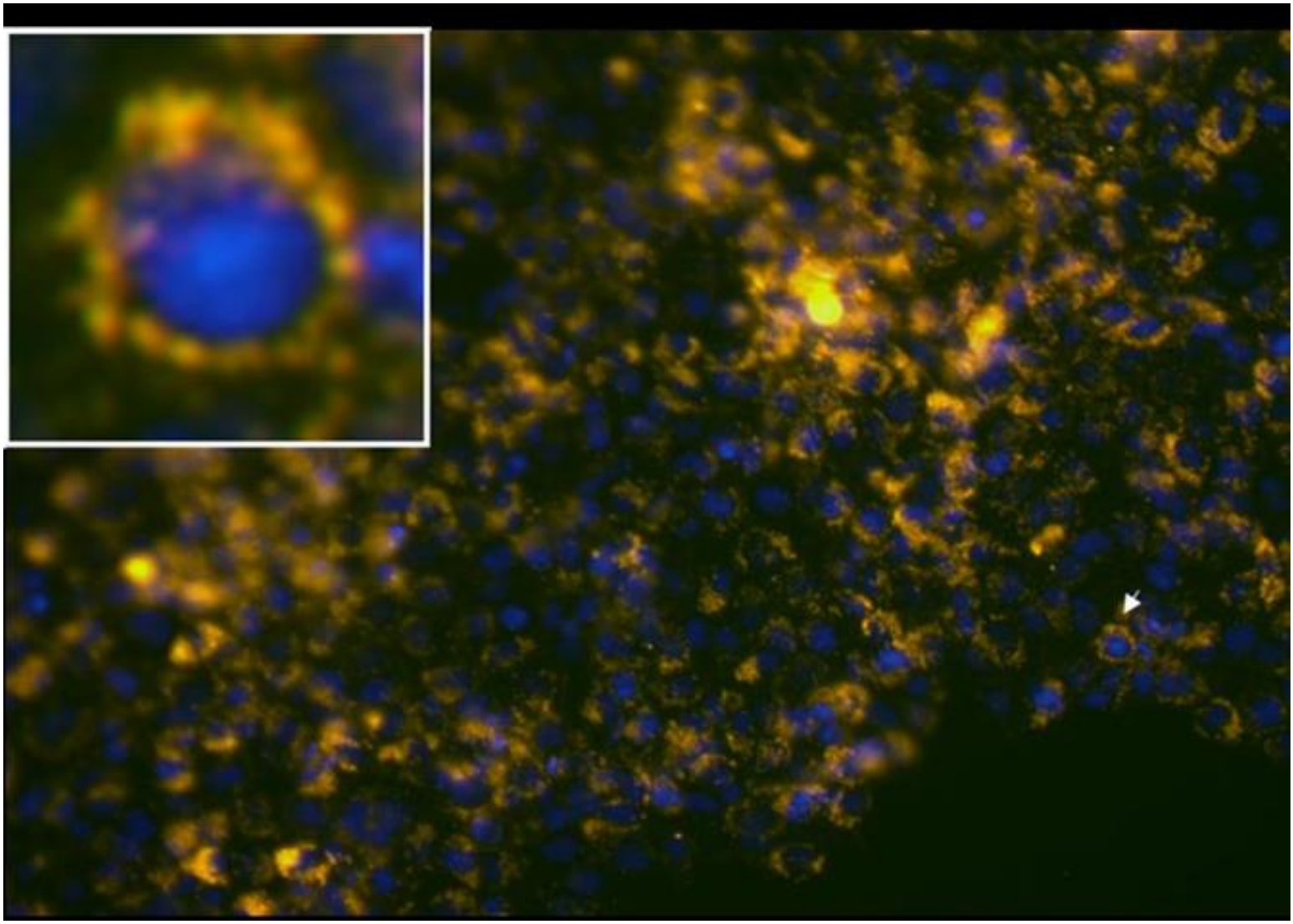
Calcification of Mitochondria



Cells from RH30 cells (a skeletal muscle cell line) are treated over night with calcium phosphate medium and then stained for the following markers to identify the formation of calcium phosphate complexes of hydroxy apatite in nature within mitochondria, using fluorescent microscopy. Briefly, following markers are used: Nuclei (Blue, DAPI), Mitochondria (Red, Anti-ToM22), and Calcium phosphate complexes (Green, Osteoimage); Yellow (Overlap of red and green stains) corresponding to calcified mitochondria in the context of skeletal muscle cells.

Study Context

Cross Talk of Mitochondrial Calcification and Inflammation in Human Skeletal Muscle Cells: Implications for Calcinosis

Juvenile dermatomyositis (JDM), a systemic vasculopathy, is the most common inflammatory myopathy of childhood, though still rare, with an incidence of 3.2 cases/million children/year in the United States. One of the debilitating manifestations of JDM is calcinosis, an abnormal accumulation of calcium salt crystals in soft tissues like skeletal muscle, skin, and blood vessels, which is reported in 30% of JDM patients. However, the mechanisms remain poorly understood, thus impeding the development of therapeutic interventions. Based on the clinical data from patients with JDM and given the role of mitochondria in cellular calcium handling, we hypothesized the role of mitochondria in the calcification of human skeletal muscle cells.

To elucidate the mechanisms of skeletal muscle calcification and, more importantly, the role of mitochondria in such a process, we treated cells of the skeletal muscle cell line with a calcium phosphate medium. We then imaged the formation of calcium phosphate complexes in mitochondria in the context of the calcifying human skeletal muscle cell. This observation of the formation of calcium phosphate complexes in mitochondria led us to establish a novel link between skeletal muscle calcification and inflammation, characteristic features of JDM patients and further expand on drivers of mitochondrial calcification. induce mitochondrial structural alterations in the primary fibroblasts of patients with DOORS syndrome. However, the results are variable possibly because of mutations which are genetically different, and / or because of the genetic background of each patient. So, we analyzed the mitochondrial structure in neuronal lines that are more pathologically related: Neuro-2a WT and Neuro-2a with TBC1D24 bi-allelic mutations introduced by CRISPR / Cas9. For this, we labeled the mitochondria with the TOM20 antibody and the nucleus with Dapi. Results showed that for the N2a WT line, most of the cells have normal mitochondria, while knocking out TBC1D24 in the N2a cell line induces mitochondrial structure alterations.

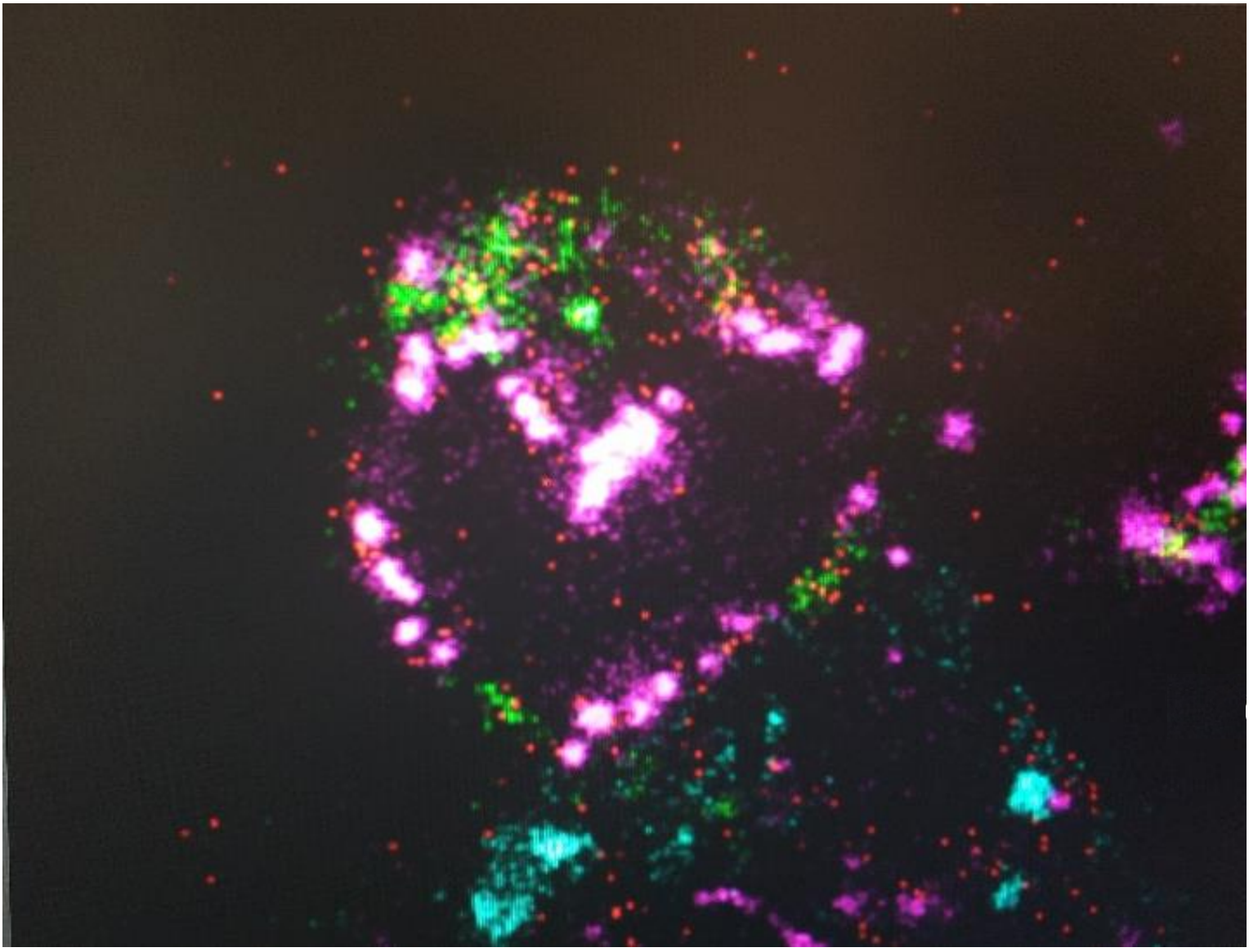
Our results therefore suggest that mutations in the TBC1D24 protein responsible for the appearance of DOORS syndrome induce mitochondrial structural alterations in the primary fibroblasts of patients with this rare genetic disease as well as in Neuro-2a TBC1D24-KO neuronal lines.

Nikita Nikiforov

Laboratory of Angiopathology,

Russia

Science is Romantic



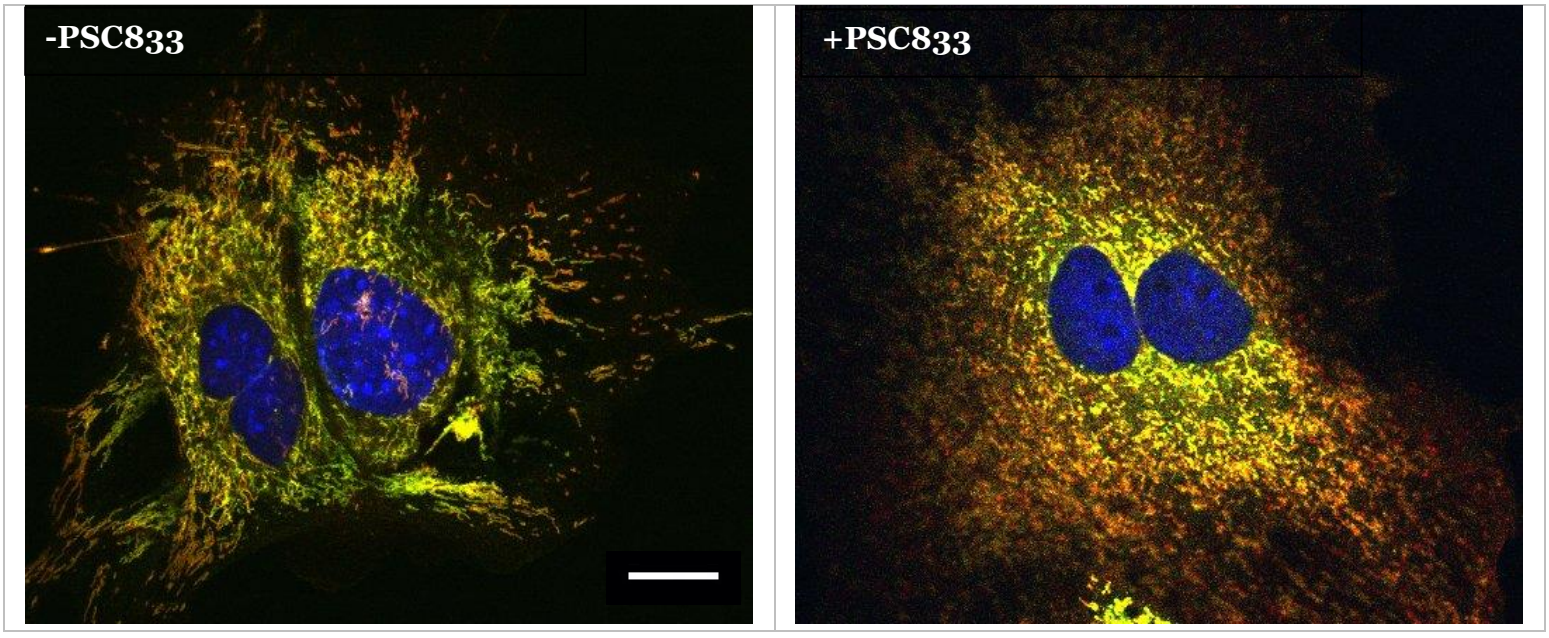
Here, you can see the human monocyte derived macrophage stained with Mitotracker Green (green) and potential dependent Mitotracker Orange (purple). Green pixels (without purple staining) indicate dysfunctional mitochondria with decreased inner membrane.

Dalileh Nabi, PhD

PostDoc researcher in Dr. Brayboy Lab/ Charité

Universitätsmedizin Berlin, Germany

MitUniverse



Assessment of PSC833 induced mitophagy in Mito-QC primary fibroblasts. Representative widefield fluorescence images of fibroblasts expressing the mito-QC reporter. Mitophagy was stimulated with 10 µM PSC833 for 16 h and the number of mitolysosomes were quantified subsequently using Fiji mito-QC counter. Note also the increase of fission events in the trated group. Scale bar = 20 µm.

Study Context

The mito-QC reporter consists of a fluorescent pH-biosensor system of a tandem mCherry-GFP tag attached to an outer mitochondrial membrane protein FIS1 (amino acids 101–152) (McWilliams et al., 2016; Allen et al.,2013). This assay is based on the acid-labile properties of GFP and mCherry, as while GFP fluorescence becomes quenched in the lysosome, the mCherry signal does not (Mizushima et al., 2010; Allen et al., 2013). Hence, mitophagy can be quantified by the increase in the number and size of mCherry-only foci that corresponds to mitochondria delivered to lysosomes.

Here we use the mito-QC reporter mice to assess mitophagy in mouse primary fibroblasts isolated from the reporter mouse. Fibroblasts were treated with 10 µM MDR-1 inhibitor, PSC833, for 16hr and the mitophagy was subsequently measured by using the Fiji macro mito-QC counter developed by Montava-Garriga et al in 2020. Briefly, the macro creates a new image by dividing the intensity of each pixel in the mCherry channel by the equivalent in the GFP channel from the source image. The resulting ratio image enables us to highlight the red foci, or mitolysosomes, which appear as high mCherry/GFP intensity ratio puncta.

Our preliminary data show that inhibiting MDR-1 increases significantly the number of mitolysosomes per total cell area by almost 2 folds in the ctrl cells compared to the treated one. Moreover, the treated fibroblasts present a significantly bigger mitolysosomes foci size compared to the controls.

Bianka Porubská

Department of Cell Biology, Faculty of Science,

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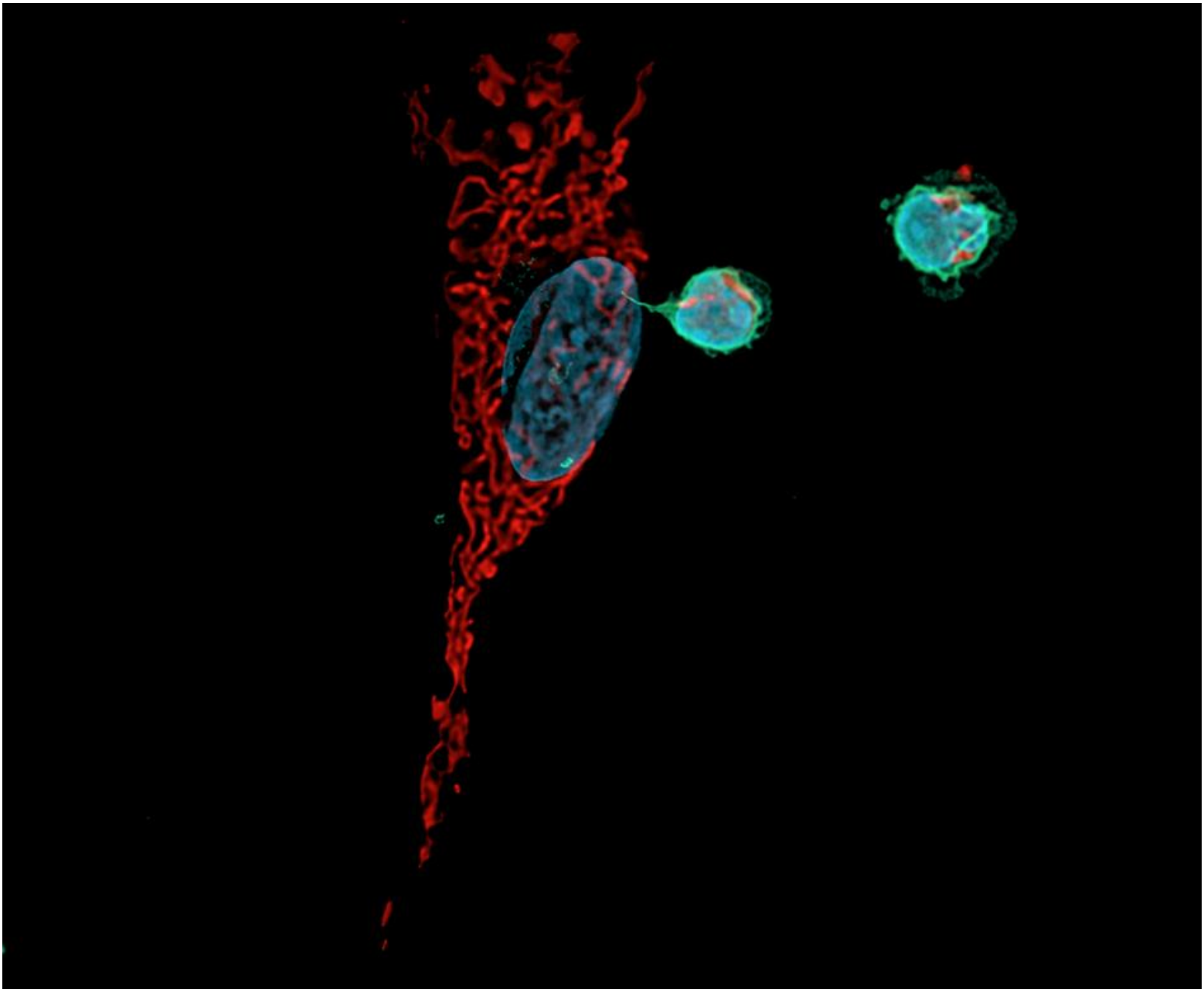
Prague

A Gift



Sertoli cell transferring MitoTrackerCMXROS (red) mitochondria to immune cell (CD45, green), Nuclei DAPI (blue).

Study Context

Our study aims to uncover immunomodulatory properties of Sertoli cells connected to the mitochondrial transfer and the metabolic changes within immune cell populations. It is well known that simple metabolic rewiring of the immune cell is and can be behind the phenotypic and functional changes and of immune cells.

Our aim is to recognize those changes in immune cells after mitochondrial transfer (from Sertoli cells) and assign them precisely to their actions. Wider context of the study is to compare Sertoli cells to Mesenchymal stromal cells regarding the ability to transfer mitochondria and what would be the consequence (difference) of receiving the mitochondria from either Sertoli or Mesenchymal stromal cells, since they are fairly similar in immunomodulation.

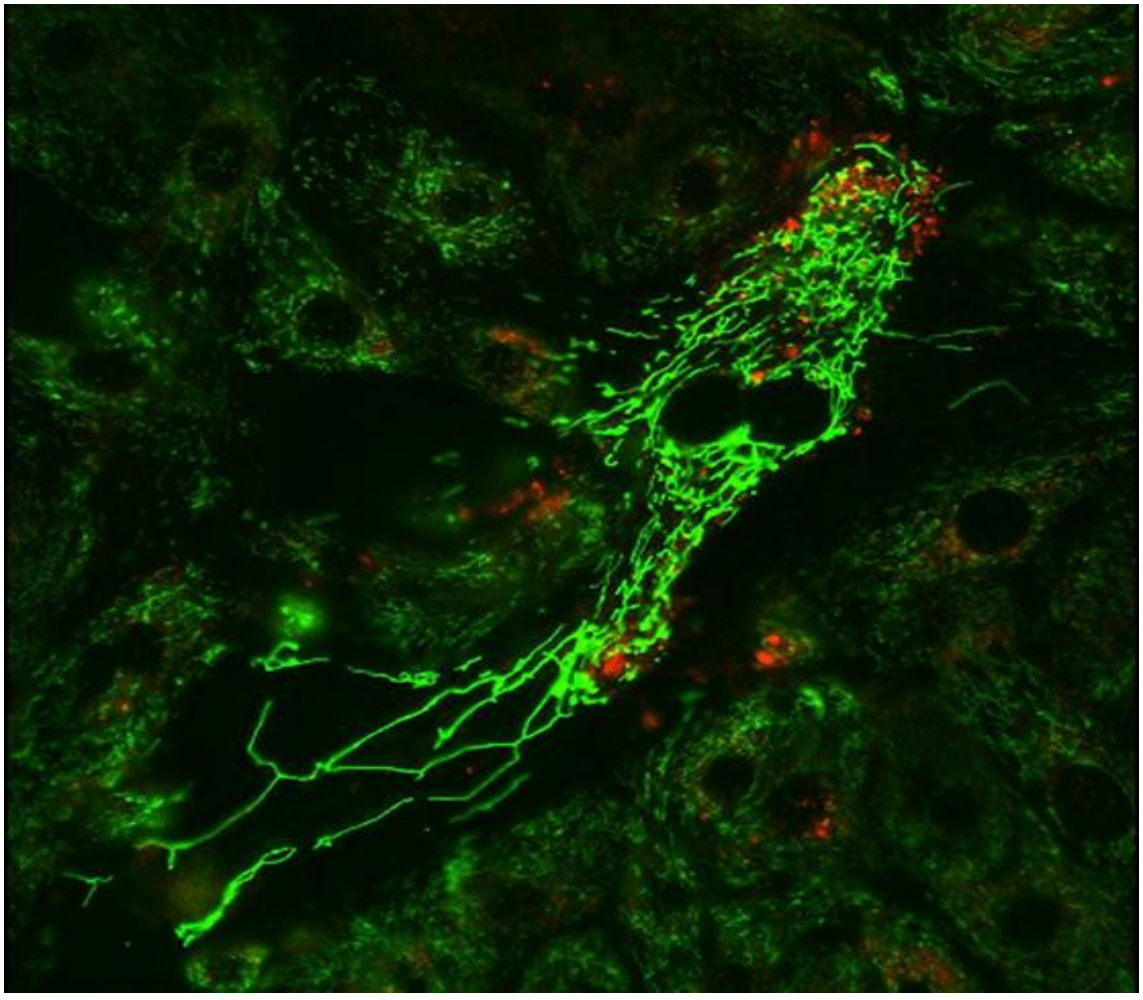
In the future, we would like to move towards therapeutic potential of Sertoli cells regarding suppression of inflammation in vivo.

Adelheid Weidinger, PhD

in cooperation with **Asmita Banerjee, PhD**, and **Naimeh Hashemi, PhD**

Ludwig Boltzmann Institute for Traumatology.
The Research Center in Cooperation with Austrian Cluster for Tissue Regeneration, Vienna, Austria

Midnight Mitochondrial Ghost



Human amniotic epithelial cells incubated with Fe-nanoclusters by Laser-scanning confocal microscope. Cells and nanoparticles were incubated for 2 h at 37 °C, 5 % CO_2.

Study Context

The human amniotic membrane is of foetal origin and protects and nourishes the foetus during pregnancy. To investigate the impact of nanoparticles on these foetal cells, we incubated freshly isolated human amniotic cells with Fe-nanoclusters. Some of the cells reacted in a peculiar way when they came into contact with these nanoparticles.

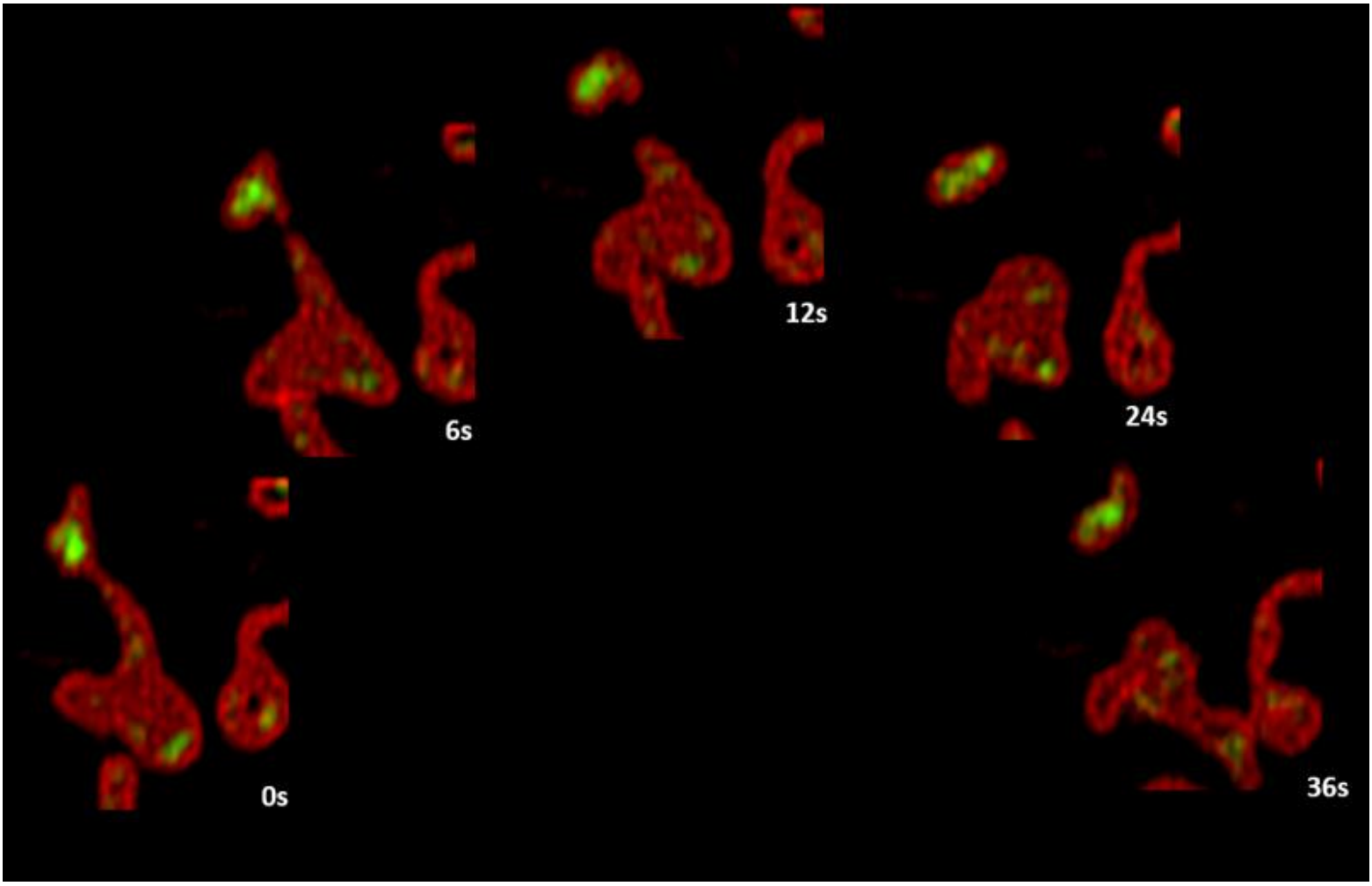
That day, it was way past midnight, this mitochondrial ghost appeared under our laser-scanning confocal microscope. Intrigued, we searched for more, and indeed, one mitochondrial ghost after the other appeared. Nanoparticles of natural, but also, and especially of artificial origin are found almost everywhere in our environment. The impact of nanoparticles on the development of foetuses, however, is mostly not known. The readiness by which the nanoparticles were taken up by the amniotic cells, and the morphological changes the mitochondria went through, made us wonder. The idea for a new project was born, thanks to our midnight mitochondrial ghost.

Josefa Macuada Alvarado

Pontificia Universidad Católica de Chile,

Chile

Reshaping Together



Mitochondria (OMP25TM-mCherry) and mitochondrial nucleoids (Tfam-GFP) move alongside during a mitochondrial fission and reshaping event in a WT MEF cell. Nucleoid displacement and dynamic clustering ("reshaping") can be seen after fission and as the mitochondrion change shape. Live-cell imaging was performed every 3s in a high-resolution confocal microscope Zeiss LSM 880 with Airyscan detection.

Study Context

We study mitochondrial nucleoids in live WT MEF cells to understand how they distribute and move when mitochondrial cristae are intact and how nucleoid dynamics can be affected in cells with abnormal cristae, a pathological hallmark found in different mitochondrial diseases such as ADOA. ADOA (Autosomal Dominant Optic Atrophy) is a leading cause of inherited blindness, where ~60% of the patients are heterozygous for OPA1 mutations. ADOA patients-derived fibroblasts and muscle biopsies have shown cristae abnormalities and mtDNA integrity defects and deficiency.

We want to understand if nucleoid dynamics are affected after the expression of ADOA-causing OPA1, which could help us find the source of the mtDNA abnormalities found in the patients. Our current working model is MEF WT acutely-expressing ADOA-causing OPA1 cDNA. Live-cell imaging data of mitochondria and nucleoids allowed us to identify single-organelle nucleoid displacement and clustering events as exemplified in the picture. We are currently developing a semi-automated method to describe mitochondrial and nucleoid velocity vectors at a population level.

Soheila Rezaei Adariani

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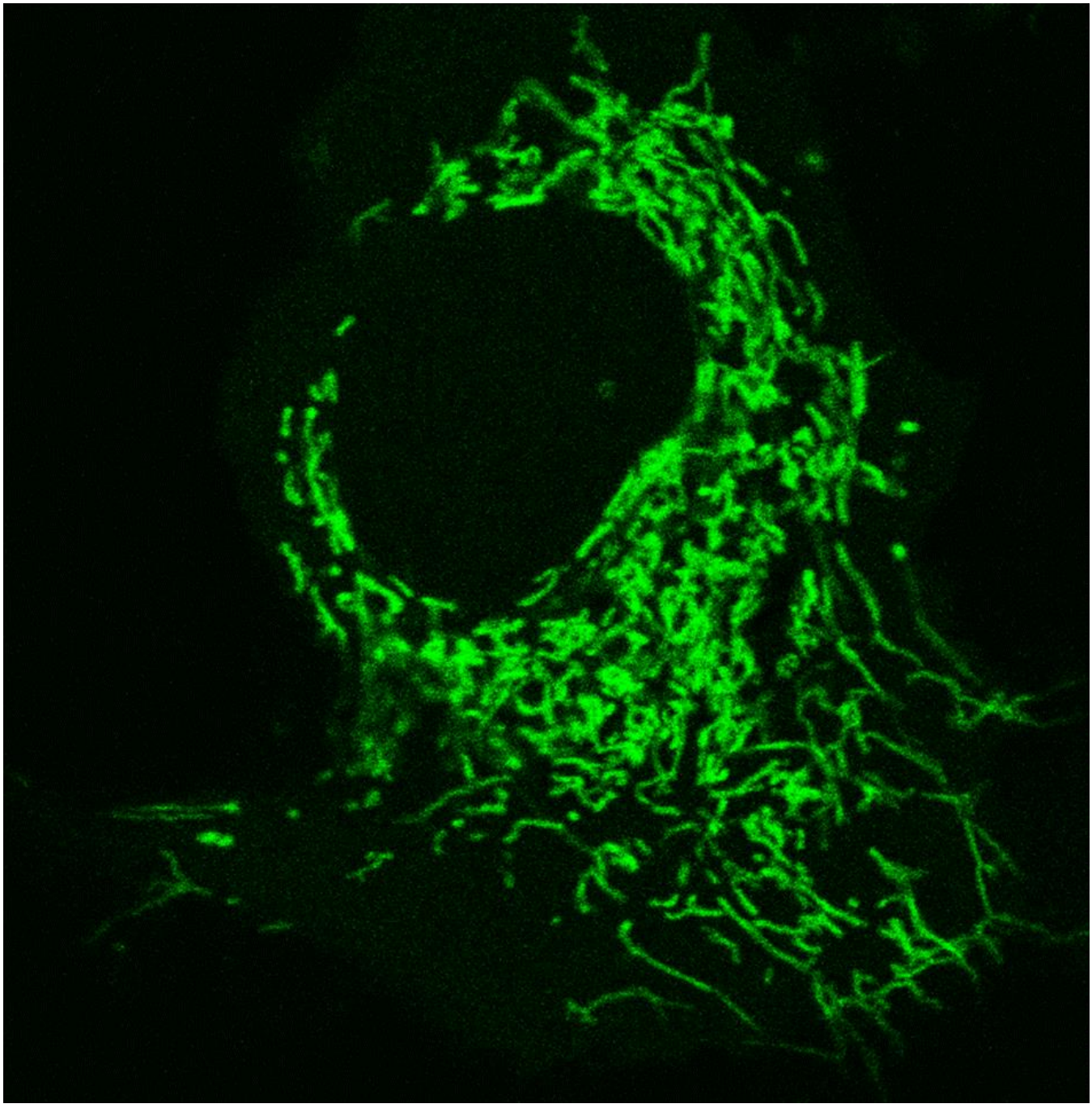

Monitoring of Mitochondria morphology. U2OS cells were transfected with cell light Mitochondria-GFP Bacmam (24h) and after that, cells were objected to the confocal microscopy.

Bojjibabu Chidipi, PhD

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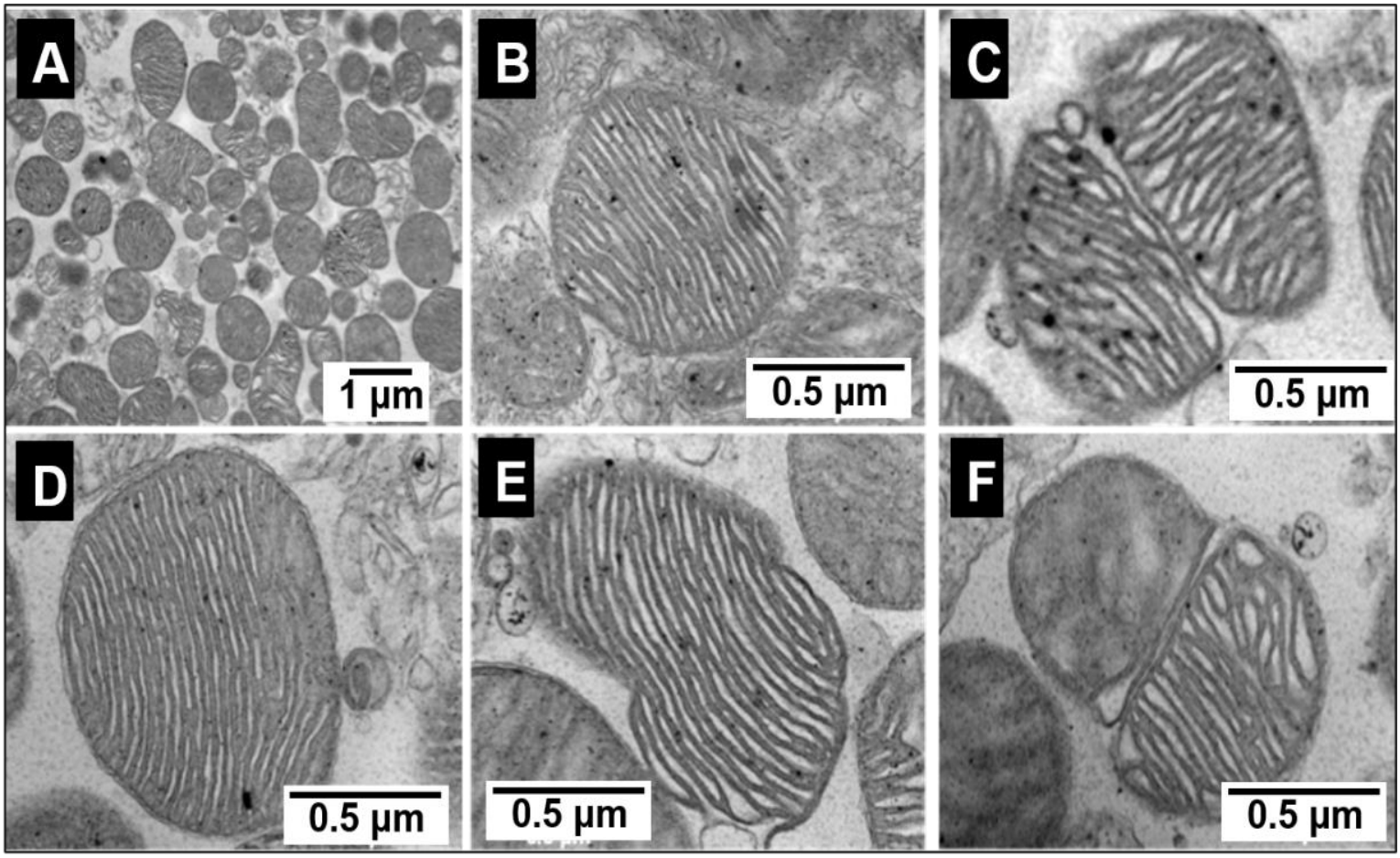
Mitochondrial Fusion and Fission Events



Mitochondrial fusion and fission events. Mitochondria are isolated from C57BL/6J mice heart tissue, imaged by Transmission Electron Microscope. (A and B) Single healthy mitochondria at 10000x and 80000x magnification, (C) two mitochondria ready to fuse (just before fusion), (D) elongated mitochondrion after fusion, (E) during the fission event, and (F) separated unhealthy part by fission.

Study Context

So far, we have only imaginary cartoon images of Mitochondrial fusion and fissions, and there is no real mitochondrial fusion and fission detection. We successfully detected mitochondrial fusion and fission in WT mice heart using a Transmission electron microspore.

This method could help scientists to evaluate fusion and fission in their studies and could be one of the parameters for assessing fusion and fission events.

Method:

We isolated live mitochondria from the heart by the sucrose-percoll gradient method using ultracentrifugation. Mitochondria were fixed in 2.5% of glutaraldehyde and dehydrated with a graded series of ethanol (35%, 50%, 70%, and 95% in water) and 100% dry acetone. Polymerized samples were sliced by a diamond knife and transferred to copper grids. Ultrathin sections were stained with lanthanum nitrate and uranyl acetate and imaged with the JEOL JEM-1400 TEM.