Application Note
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Mitochondrial motility in budding yeast

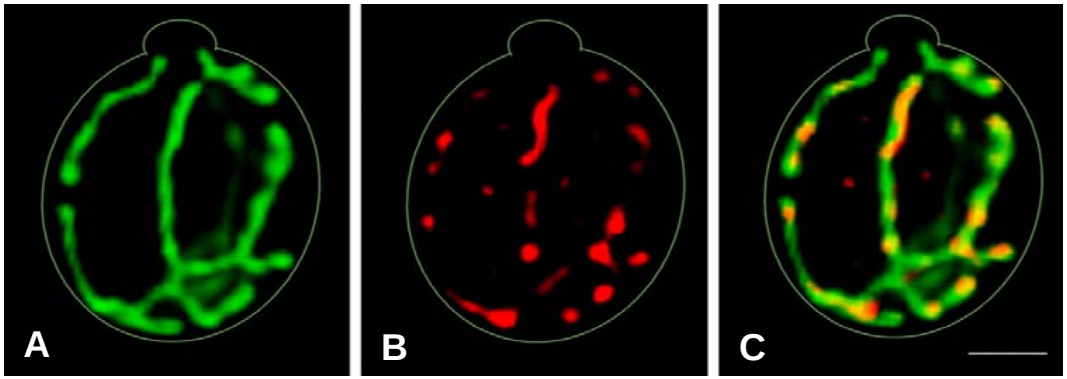

Fig 1: ***Volocity*** 2D projections of 3D rendered yeast cells labeled for mitochondria (green) and Jsn1p (red) Image C: overlay of image A and B showing colocalization of Jsn1p with mitochondria (Bar, 1µm)

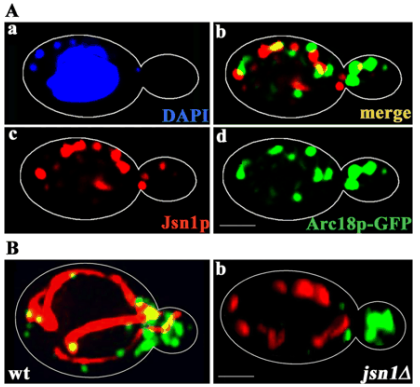

Fig 2: ***Volocity*** 2D projections of 3D rendered yeast cells

Dr Liza Pon and colleagues from the Department of Anatomy and Cell Biology at Columbia University in New York are currently working on mitochondria, which are essential organelles that must be produced from pre-existing mitochondria. Despite their major importance in energy mobilization and biosynthesis of molecules, little is known about their inheritance during cell division. Transfer of the nucleus from mother to daughter cell is known to be cytoskeleton-dependent and the researchers in Dr Pon's lab have found that mitochondrial movement during yeast cell division is similar. Their work is now focused on studying the role of the actin cytoskeleton in mitochondrial motility in budding yeast, in order to provide more evidence on the organelle inheritance mechanisms, which are essential for accurate cell division and function.

In the work described here, the researchers were interested in Jsn1p and Arp2/3, two mediators that are potentially involved in actin nucleation and mitochondria motility. Arp2/3 is a complex that localizes in specific cellular areas of budding yeast cells, but the mechanism by which Arp2/3 is targeted within the cell is not well understood. Jsn1p protein is known to bind to Arp2/3 complex and to the mitochondrial outer membrane, which suggests that Jsn1p could have a role in recruiting Arp2/3 to mitochondria. If this hypothesis is right, Jsn1p should localize to mitochondria. In order to test this, they used yeast expressing mitochondria-tagged GFP to label the mitochondrial network (Fig. 1A) and Jsn1p was visualized by indirect immunofluorescence (Fig 2B). 25 Z-sections with a 0.2µm Z-step were acquired through the cell using a standard fluorescence microscope controlled by ***Openlab*** and data sets were then directly imported into ***Volocity***. To remove out-of-focus light in each image section, the award winning iterative restoration algorithm in ***Volocity Restoration*** was applied to the data volumes. The deconvolved images were then 3D rendered using ***Volocity Visualization***. The deconvolved 3D rendered volumes provided improved spatial resolution and by viewing the volumes from all directions, the researchers were able to show that Jsn1p does in fact colocalize with mitochondria (Fig 1C).

The images in Fig. 2 are also ***Volocity*** 2D projections of 3D rendered yeast cells, showing colocalization of Jsn1p (Fig. 2 Ac) with mitochondria-associated-GFP-Arp2/3 complexes (Fig. 2 Ab). Deletion of Jsn1p (Fig. 2Bb) resulted in fragmentation of mitochondria and a decrease of GFP-Arp2/3 that colocalized with mitochondria. Both results show that Jsn1p colocalizes with mitochondria-associated-Arp2/3 complexes and is required for localization of Arp2/3-GFP to mitochondria.

By combining the use of ***Openlab*** and ***Volocity***, Dr Pon and colleagues have clearly shown that Jsn1p has physical interactions with mitochondria associated Arp2/3 complex and that it contributes to physical and functional association of the Arp2/3 complex with mitochondria.

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