Mitochondrion 26 (2016) 33–42

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Review

Analysis of mitochondrial structure and function in the *Drosophila* larval musculature

Zong-Heng Wang [a], Cheryl Clark [b], Erika R. Geisbrecht [a,b,*]

[a] Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri, Kansas City, MO 64110, United States
[b] Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506, United States

ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form 18 November 2015
Accepted 19 November 2015
Available online xxxx

Keywords:
Drosophila
Mitochondria
Larval musculature

ABSTRACT

Mitochondria are dynamic organelles that change their architecture in normal physiological conditions. Mutations in genes that control mitochondrial fission or fusion, such as *dynamin-related protein* (*Drp1*), *Mitofusins 1* (*Mfn1*) and *2* (*Mfn2*), and *Optic atrophy 1* (*Opa1*), result in neuropathies or neurodegenerative diseases. It is increasingly clear that altered mitochondrial dynamics also underlie the pathology of other degenerative diseases, including Parkinson's disease (PD). Thus, understanding mitochondrial distribution, shape, and dynamics in all cell types is a prerequisite for developing and defining treatment regimens that may differentially affect tissues. The majority of *Drosophila* genes implicated in mitochondrial dynamics have been studied in the adult indirect flight muscle (IFM). Here, we discuss the utility of *Drosophila* third instar larvae (L3) as an alternative model to analyze and quantify mitochondrial behaviors. Advantages include large muscle cell size, a stereotyped arrangement of mitochondria that is conserved in mammalian muscles, and the ability to analyze muscle-specific gene function in mutants that are lethal prior to adult stages. In particular, we highlight methods for sample preparation and analysis of mitochondrial morphological features.

Contents

Abbreviations: ATP, adenosine triphosphate; AEL, after egg laying; CMT2A, Charcot–Marie–Tooth Type 2A; ConA, concanavalin A; Drp1, dynamin-related protein; DOA, dominant optic atrophy; GFP, green fluorescent protein; IFM, indirect flight muscle; IMF, intermyofibrillar; IMM, inner mitochondrial membrane; LRRK2, leucine-rich repeat kinase 2; L3, third instar larvae; ΔΨm, mitochondrial membrane potential; mtDNA, mitochondrial DNA; Mfn1, mitofusin 1; Mfn2, mitofusin 2; Opa1, optic atrophy 1; OMM, outer mitochondrial membrane; PD, Parkinson's disease; PINK1, *PTEN-induced kinase*; *S2*, Schneider 2 cells; S2R+, S2 receptor plus cells; SS, subsarcolemmal; TEM, transmission electron microscopy; 3-D, 3-dimensional.

* Corresponding author at: Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS 66506, United States.
E-mail address: geisbrechte@ksu.edu (E.R. Geisbrecht).

http://dx.doi.org/10.1016/j.mito.2015.11.005
1567-7249/

1. Introduction

Mitochondria have a long and storied history. The first observations that describe these intracellular organelles date back to the beginning of the 1840s (Aubert, 1852; Butschli, 1871; Flemming, 1882; Henle, 1841; Kolliker, 1856, 1888). For over 150 years, the field of 'mitochondriology' has focused on multiple facets of mitochondrial composition and function. Only since the 1970s has the focus to mitochondrial fission/fusion and biogenesis shed new light on the role of these powerhouse organelles in biology (Ernster and Schatz, 1981). Most of the adenosine triphosphate (ATP) within cells is generated by mitochondria, and muscle cells in particular, require abundant amounts of ATP to carry out mechanically and energetically demanding functions, including muscle contraction, ion transport, protein synthesis, and other general metabolic roles (Nunnari and Suomalainen, 2012). The diversity in mitochondrial morphology, which varies widely in size from small, individual mitochondrion to highly interconnected, tubular networks, is influenced by regulated fission and fusion events that are collectively referred to as 'mitochondrial dynamics.' Multiple factors influence the ability of cells to rapidly adapt to intracellular or extracellular cues to regulate mitochondrial morphology, including cell type, organism, and environmental stressors (Rafelski, 2013).

1.1. The delicate balance between fission and fusion determines mitochondrial fate

The opposing processes of fission and fusion determine the architecture of the mitochondrial network within cells and influence the balance between organelle life and death. Fusion events are thought to bolster fitness, permitting normal and slightly damaged organelles to intermingle mitochondrial DNA (mtDNA), membrane components, and metabolic enzymes (Twig and Shirihai, 2011). While many of the signals that promote organelle fusion are not yet defined, it is clear that polarized mitochondria with normal or slightly reduced inner membrane potentials ($\Delta\Psi m$) are competent to fuse with other mitochondria (Legros et al., 2002; Mattenberger et al., 2003). Recent findings also suggest that fusion mechanisms are maintained in mitochondria undergoing macroautophagy, thus enabling a constant supply of ATP during nutrient starvation (Gomes et al., 2011; Rambold et al., 2011).

Healthy mitochondria also undergo fission, for example, to segregate mitochondria into daughter cells during cell division (Mishra and Chan, 2014). In contrast, potentially dysfunctional mitochondria that have decreased or complete loss of $\Delta\Psi m$ cannot fuse with other mitochondria and are fated to undergo fission, resulting in fragmented, depolarized mitochondria (Ishihara et al., 2003; Legros et al., 2002; Malka et al., 2005). This feature ensures that healthy mitochondria do not fuse with damaged organelles. Typically, these smaller, depolarized mitochondria are selectively targeted for mitophagy, a selective autophagic process that eliminates damaged mitochondria (Gomes and Scorrano, 2013; Shirihai et al., 2015; Tanaka et al., 2010; Twig and Shirihai, 2011).

Numerous proteins located in the outer (OMM) or inner (IMM) mitochondrial membranes are required for mammalian mitochondrial fission or fusion. The predominant regulator of mitochondrial fission is dynamin-related protein 1 (Drp1). Drp1 is an evolutionarily conserved GTPase that shares structural similarity to dynamin and is located predominantly in the cytoplasm (Bleazard et al., 1999; Smirnova et al., 2001). Upon signals that induce fission, Drp1 translocates to mitochondria and oligomerizes to form rings around the OMM that eventually results in the division into two separate organelles (Ingerman et al., 2005; Smirnova et al., 2001). Core proteins essential for mitochondrial fusion include the dynamin-like GTPase OMM components Mitofusins 1 (Mfn1) and 2 (Mfn 2), and the IMM protein Optic atrophy 1 (Opa1).

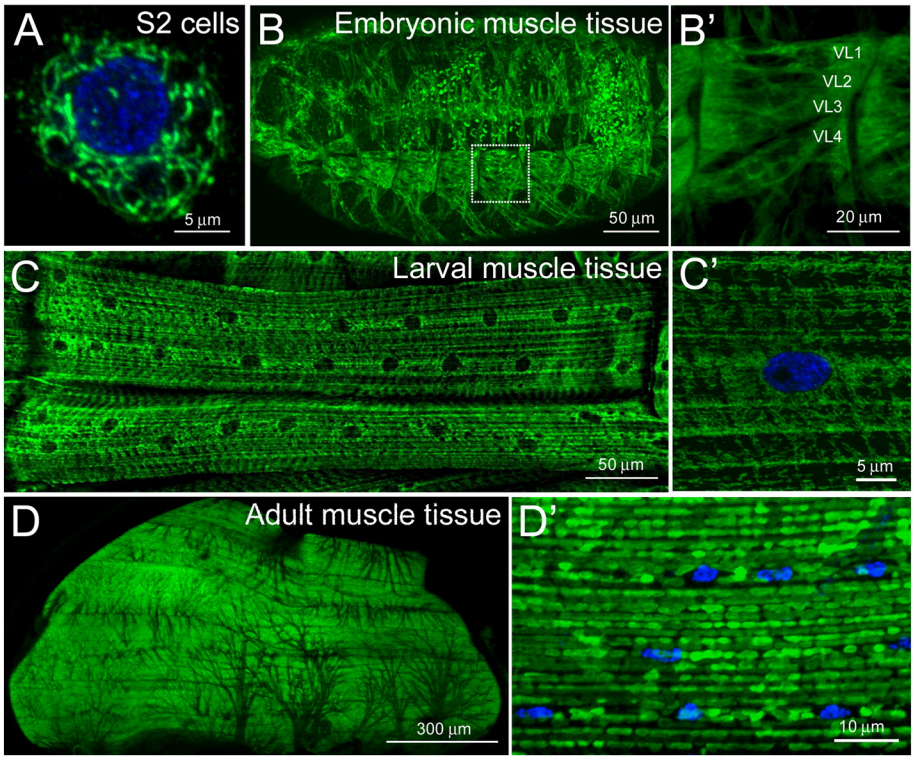

Fig. 1. Mitochondrial distribution in *Drosophila* muscle tissues. (A–D′) Confocal micrographs of mitochondria immunostained with an antibody against the IMM ATP synthase complex (green) in the indicated cell types. Nuclei are stained with DAPI (blue). (A) An assortment of mitochondrial shapes is observed in S2 cells plated on ConA. (B, B′) Mitochondria are abundant and uniformly distributed in embryonic muscle tissue (B). High magnification of the ventral longitudinal muscles 1–4 (also designated 6, 7, 12, and 13) shows an overall ubiquitous mitochondrial distribution with a slight accumulation at the myofiber ends (B′). (C, C′) In the contractile muscles of the third instar larvae, ventral longitudinal muscles 1 and 2 (12 and 13) accumulate around nuclei and adopt an overall striated appearance (C). Tubular mitochondria can be viewed at the surface of the muscle (C′). Mitochondria appear homogeneous in the adult thoracic flight muscle (D) and adopt a round appearance at higher magnification (D′). All images were acquired using a Zeiss 700 confocal microscope and processed using ImageJ and Adobe Photoshop software. Scale bars are indicated.