Published in final edited form as:

Microsc Res Tech. 2013 December ; 76(12): 1213–1216. doi:10.1002/jemt.22287.

Division of Mitochondria in Cultured Human Fibroblasts

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Abstract

Ovate mitochondria in cultured human fibroblasts divide by pinching. In the process, as observed by transmission electron microscopy, a deep incisure of the surface membranes separates the organelle into two lobes connected by a slender isthmus. A single element of smooth endoplasmic reticulum (SER) invariably accompanies each incisure, extending deep into the cleft. When the ingrowing membranes meet and fuse with the antipodal membranes, fission occurs. Elongated mitochondria that give no indication of division often are cloaked by a single, continuous cistern of SER.

Keywords

mitochondrial division; mitochondrial pinching; fission; fusion; fibroblasts

INTRODUCTION

Fibroblasts are the mainstay of fibrous connective tissue in all metazoans—they produce both the fibers and the matrix of this tissue. As do all other eukaryotic cells, fibroblasts depend on mitochondria to furnish the requisite energy for their survival and function. In cultured fibroblasts, the mitochondria have variable morphology, although there is a tendency for them to assume an elongated configuration: organelles as long as 26 μm have been reported by Armchenkova et al. (1988) and 46 μm have been described by Lea et al. (1994); such long mitochondria measure $\sim 0.2 \mu\text{m}$ in width (Pham et al., 2004). In addition, circular and saccular forms are present (Goldstein et al., 1984). Examination of living

fibroblasts has revealed that their mitochondria are structurally dynamic, changing their morphology in response to prevailing conditions (Pham et al., 2004) such as the stage of the cell cycle (Kennady et al., 2004). In tissue culture, human fibroblasts can survive up to 50 generations (Hayflick and Moorhead, 1961). Obviously, with each new generation, the number of mitochondria per cell would be halved as would be the mitochondrial mass unless there existed a means for their biogenesis. The pathway and mechanism(s) of mitochondrial division have assumed center stage in investigations of this critical organelle. This study was undertaken to determine the pathway(s) for mitochondrial fission in fibroblasts. The morphology of mitochondrial division in cultured human fibroblasts is described herein.

MATERIALS AND METHODS

All research involving human subjects for this study was conducted with written approval by the Case Institutional Review Board. Informed written consent was obtained from patients and all clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. Skin specimens were obtained by punch biopsy of normal occipital scalp from patients seen at the clinics of the University Hospitals of Cleveland.

Fibroblasts were cultured from these biopsies according to Laboratory Procedures for Human Cell Culture, EuroBioBank, 2004. Briefly, newly harvested scalp biopsies were placed in Dulbecco's Modified Eagle's Medium (DMEM) containing gentamicin (Life Technologies Corporation, Grand Island, NY). Tissue was rinsed with Hank's Balanced Salt Solution (HBSS), minced, and resuspended in 3mL complete medium and transferred to a Falcon T25 flask. The flasks were placed in a 37°C, 10% CO₂ incubator. By day 7, cell outgrowth had occurred. The medium was carefully removed by suction and 3 mL fresh medium was added. After 2–3 weeks, the fibroblasts were subcultured; the cells were trypsinized (7–10 min at 37°C), centrifuged (10 min at 1000 rpm), the pellet resuspended, and cells transferred to new T75 flasks.

For transmission electron microscopic analysis, cells were seeded onto a sterilized Aclar Embedding Film (Electron Microscopy Sciences, PA), lining the wells in a 6-well cell culture cluster, placed in a 37°C, 10% CO₂ incubator and allowed to reach ~80% confluence. After the cells had reached this level of confluence, the Aclar sheets with their attached cells were immersed in fixative. The initial fixative was 2.5% glutaraldehyde in cacodylate buffer (pH 7.3). The specimen was postfixed in ferrocyanide-reduced 1% osmium tetroxide (Karnovsky, 1971). After a soak in acidified uranyl acetate (Tandler, 1990), the specimen was dehydrated in ethanol, passed through propylene oxide, and embedded in Poly/Bed (Polysciences, PA). Sections were cut in a horizontal plane parallel to that of the Aclar film to provide panoramic views of the fibroblasts and to ensure that structures in different cells were observed in equivalent subcellular sites. Thin sections were stained first with acidified uranyl acetate in 50% methanol (Tandler, 1990), then with the triple lead stain of Sato as modified by Hanaichi et al. (1986). These sections were examined in a JEOL 1200 EX electron microscope (Tokyo, Japan).

RESULTS

The cultured fibroblasts matched previous ultrastructural descriptions of such cells (Movat and Fernando, 1962) in that they had a prominent rough endoplasmic reticulum and a number of scattered dictyosomes (Fig. 1). Mitochondria were variable in form, ranging from elongated (sometimes highly branched) to oblate spheroidal. The most striking of these latter organelles were those undergoing division by the process known as “pinching” (Bleazard et al., 1999; Ingeman et al., 2005). Such mitochondria were deeply incised to a point where their prospective daughter organelles were connected only by a slender isthmus. These pinching organelles invariably featured a single element of smooth endoplasmic reticulum (SER) that closely paralleled their surface and extended deeply into the notch (Fig. 2). Figure 3a–d is a gallery of dividing mitochondria that are close to completion of the process. Under ordinary circumstances, mitochondria repel one another because of their identical surface charge (Nelec et al., 1982). The only exception to this general rule is in striated muscle cells where they are physically entrapped by the contractile apparatus so that they actually abut one another. In the fibroblasts, completion of the pinching maneuver leaves the two newly independent organelles still in juxtaposition to one another. Figure 4 shows two newly “hatched” mitochondria that have not yet moved apart; they remain partially shrouded by the same SER cistern. Because the mitochondria shown in Figure 4 give no sign of fusion (*vide supra*), it may be inferred that these organelles are the recent offspring of organelle division and that they will soon separate.

In addition to conventionally sized mitochondria, the fibroblasts contained very long mitochondria, which we called ‘string mitochondria’ in the context of mouse skeletal muscle (Fujioka et al., 2013) where they reached lengths of over 6.25 μm , but rarely were more than 1 μm in width. For the most part, the string mitochondria had relatively straight surfaces, although a few had very shallow surface indentations. Sparse elements of endoplasmic reticulum (ER) were closely apposed to most of the string mitochondria; a few of the latter were cloaked by a single continuous cistern of SER (Fig. 5). The mitochondrial surface structure had no bearing on the location of the associated ER: some were apposed to straight outer membranes, some to slightly inflected membranes. None of these string mitochondria appeared to be involved in fission.

DISCUSSION

This study upholds the supposition that fibroblast mitochondria replicate by division to maintain the energetic requirements of their host cells. Moreover, it reveals the morphology behind replication of this organelle. In a word, fibroblast mitochondria divide by pinching. This is accomplished by a deep incursion of the limiting membranes into the body of the mitochondrion, until the prospective mitochondrial progeny are connected by a slender isthmus. When the ingrowing membranes finally make contact with their opposite numbers, scission ensues.

Of course, two mitochondria in close juxtaposition could be interpreted as a harbinger of fusion. It should be pointed out that there are very few studies of the morphological stages of this process. The only study that unequivocally shows the early steps of fusion is that of the

formation of hepatic megamitochondria resulting from riboflavin deficiency (Tandler et al., 1968). Here, the fusing organelles are characterized by surface irregularities, i.e., one organelle forms a protrusion that inserts into an appropriately shaped invagination of the surface of the conjoint mitochondrion. The only other cells in which mitochondrial fusion has been ultrastructurally documented are those undergoing spermiogenesis in the testes of many types of insects (for example, Pratt, 1968; Phillips, 1970; Fawcett, 1981). In such cells, the nebenkern, a large mitochondrial organelle, is formed by the complex interlocking of mitochondria; again, this is not simple side-by-side fusion. Because propinquitous mitochondria lack surface irregularities, it seems reasonable to exclude fusion as a result.

In every case, the incisures are accompanied by an element of ER. Friedman et al. (2012) hold that it is the juxtaposition of ER to a mitochondrion slated for division that is a key stage in the fission process. Their study was based on high-voltage electron microscopy-tomography reconstruction of two yeast mitochondria, followed by confocal microscopy of living Cos cells using colocalization of fluorescent probes. In an electron microscope study of rat cardiomyocytes, Fujioka et al. (2012) illustrated at high resolution a characteristic relationship in rat cardiomyocytes between SER and mitochondria undergoing pinching. Although in fibroblasts the mitochondrially involved ER cistern usually is smooth-surfaced, it occasionally appears to be a hybrid of rough and smooth, that is, it has ribosomes on the surface facing the cytosol, but is smooth on the side facing the mitochondrion.

In earlier stages of pinching in fibroblasts, the ER cisterns, hybrid or smooth, maintain a close relationship with the mitochondrial outer membrane. This association of the two organelles probably is stabilized by bridges that might consist of mitofusin-2, a dynamin-related protein that has been identified in mammalian cells (de Brito and Scorrano, 2008, 2009) or of molecules similar to ERMES complex consisting of Mdm10, Mdm12, Mdm34, and Mmm1 that are found in yeast (Kornmann et al., 2009; Kornmann and Walter, 2010). This intimate contact of ER and mitochondria may be necessary to direct proteins, lipids, and calcium to specific sites on the surface of the latter, especially where pinching is taking place (Fujioka et al., 2012; Westermann, 2011).

In the aforementioned cardiac study, Fujioka et al. (2012) described a second modality of mitochondrial fission, namely, partition formation. Because mitochondrial proteins are encoded by nuclear genes (Herrmann et al., 2012) and the number of these organelles per cell is controlled by the nucleus (Rapaport, 2003), Fujioka et al. (2012) posited that at least two separate nuclear genes are involved in directing the choice of pathways that lead to organelle division. Despite assiduous search, we could not identify a single example of partitioned mitochondria in the fibroblasts, a finding foreshadowed by studies of dividing giant liver mitochondria. Hepatic megamitochondria induced by riboflavin deficiency were returned to normal dimensions strictly by partition formation (Tandler et al., 1969), whereas those induced by cuprizone intoxication were restored to normality exclusively by pinching (Tandler and Hoppel, 1973). Thus it is apparent that even though the genes that control mitochondrial fission co-exist in the same nuclei, the activation of either or both depends on an as yet unidentified signal. In human fibroblasts, only the pinching pathway is operative.

Acknowledgments

This work was supported in part by NIH grant R01 AR056245 to Dr. Karnik.

The authors thank Dr. Jason Mears for his helpful comments. Kiet Luc provided expert technical assistance.

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Fig. 1. Survey electron micrograph of a typical cultured fibroblast illustrating its extensive rough endoplasmic reticulum, scattered Golgi apparatuses, and mitochondria of varying morphology. The arrow indicates a centrosome. N = nucleus.

Fig. 2.

A mitochondrion undergoing pinching. The prospective daughter organelles are still joined by a narrow neck. An element of SER extends deeply into the cleft in the organelle.

Fig. 3.

A gallery of mitochondria in the process of division by a pinching process.

Fig. 4.

Two newly formed mitochondria that remain in close juxtaposition with one another. They are still shrouded by a single element of SER. It is presumed that these organelles will shortly move farther apart because of identical surface charge.

Fig. 5.

A portion of a string mitochondrion that is closely paralleled by SER cisternae. This close relationship probably is maintained by mitofusin bridges, but such structures are not evident in our micrographs.