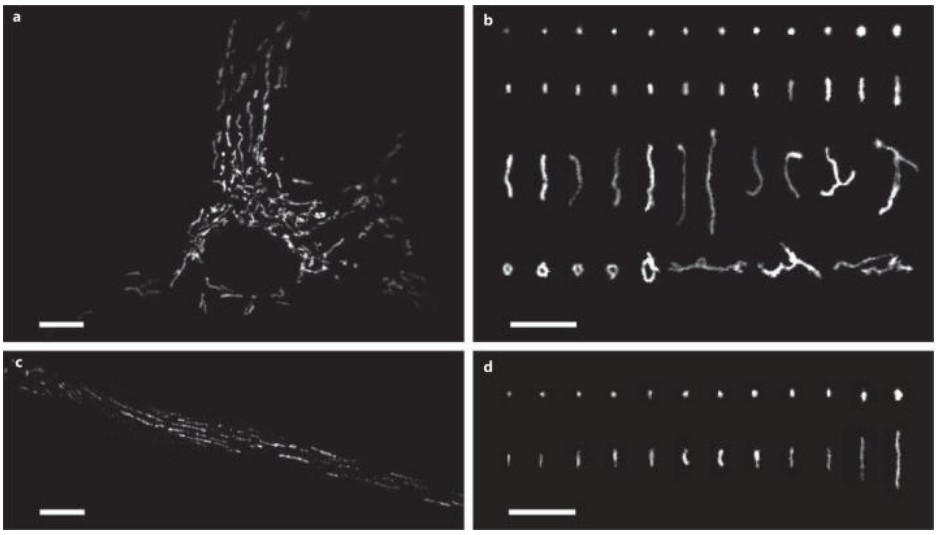
Mitochondria are always shown as ovals; in reality they come in all sorts of shapes and sizes. Why?

Sarcosomes, bioblasts, powerhouses of the cell: the variety of names that mitochondria have accumulated since their discovery in 1857 by Albert von Kolliker are recently being found almost to parallel the enormous functional versatility of these organelles – from their involvement in the oxidation of fatty acids and in signal transduction to their connection with neurodegenerative disease and even lifespan, it is becoming ever more clear how much power these organelles hold. Despite this, the study of mitochondria is still awash with lack of clarity, with recent advancements in technology such as live cell imaging stimulating much of the progress in the research around it, and perhaps one of the most debated areas this obscurity encapsulates is the organelle's morphology.

This morphology has previously been depicted most often through oval shapes, as is continuous with the ovoid mitochondria found in cells such as vascular smooth muscle cells and hepatocytes, however emerging research presents divergence from this tradition, presenting mitochondria formed as long filaments in fibroblasts, and networks of tubules in endothelium. Furthermore, differences in mitochondrial structure and function are not limited to that between different cell types, with a study in pancreatic acinar cells (exocrine cells secreting large amounts of digestive enzymes to a ductal system at the epithelial surface) uncovering three separate and unconnected groups of mitochondria.

The advent of live cell imaging has contradicted traditional views of mitochondrial shape. One structure that mitochondria have been observed in is that of a reticulum, allowing diffusion of solutes to occur more rapidly. In addition, it has been tentatively proposed that mitochondria taking the form of solitary ovals are not representative of the morphology mitochondria have been supposed to present, and rather are due either to oxidative stress causing mitochondrial networks to fragment, or due to photomicrographs capturing images only of slices of the mitochondrial reticulum, causing mitochondria to appear as punctuate structures.

Studies of mitochondrial morphology have more often been conducted in cultured cells rather than native cells. The issue with this disparity has been highlighted by a study of vascular smooth muscle cells, of which those that were cultured cells appeared to offer more mitochondrial morphological diversity than in those that are native, which rather have singular spherical or rod-like mitochondria (with native cells in most tissues being found to have these similar, punctuate mitochondria), making it unlikely that observing ovoid shapes is due to oxidative stress or difficulty in imaging. Further evidence to suggest the adherence to traditional ideas of mitochondrial shape includes that the mitochondria of native cells are not found to be electrically coupled – their membrane potential changes independently rather than as part of communal change, which would be observed in organelles forming a continuous network. Figure 1 presents images from this study of mitochondria in native and cultured cells.



a Varied arrangement of continuous mitochondria in a single cultured vascular smooth muscle cell.
b Presents diversity of mitochondrial morphology.
c Mitochondria distributed across cytoplasm of a native smooth muscle cell parallel to the cell's long axis.
d Native mitochondria presenting typical uniform phenotype.

Scale bars = 10μm

Figure 1: The phenotypes of mitochondria in native and cultured smooth muscle cells.

Despite the conflicting evidence discussed above, considering the mechanisms of mitochondrial morphology indicates that the particular function required of mitochondria prescribes their shape, which is largely regulated by the balance between mitochondrial fusion and fission. These processes are continuous in many non-dividing cells, indicating their necessity for functions other than cell division. Whilst an even balance results in a general constancy in the organelle's morphology, fusion-deficient cells often have more solitary structures due to networks breaking apart under excess fission, and fission-deficient cells contain greatly interconnected networks of mitochondrial tubules.

This concept is furthered when analysing the heterogenous distribution of these organelles: their ability to accumulate where respiration or their metabolic function is required (most predominantly, where Ca^{2+} needs to be regulated and at structures requiring high volumes of ATP to carry out their function) appears to link directly to the cytoskeletal protein transport network throughout the cytoplasm of all cells, allowing mitochondria to traverse around 80% of some cells in fifteen minutes. The cytoskeleton is comprised of microtubules and microfilaments (dynamic structures that undergo continuous assembly and disassembly), where microtubules are made of the protein tubulin, and microfilaments of the protein actin (more specifically, α-actin in mammalian muscle cells, and β- and γ-actin in mammalian non-muscle cells). Both structures are polar, where the δ+ end grows rapidly and the δ- end is positioned by the nucleus at the centrosome (an organelle that acts as a microtubule-organising centre, and is the site where microtubule growth begins, indicating its importance in causing their polarity). Allowing mitochondria to be transported across the cytoskeleton are motor proteins – namely: dyneins for retrograde transport (towards the minus end) and kinesins for anterograde transport (away from the minus end) along microtubules; myosins for transport in either direction along microfilaments – in conjunction with the adaptor complex often called Miro-TRAX, initiating a 'walking' mechanism by associating the mechanical cycle of the motor proteins with the chemical cycle of ATP hydrolysis.

It is of great importance that mitochondrial morphology and mitochondrial movement are synchronised. Most notably, mitochondrial reticulums are required, for transport, to be divided into punctuate structures by fission, highlighting a further link of the cytoskeleton with mitochondrial morphology.

The mechanisms by which fission and fusion occur mirror, to a large extent, the evolutionary history of mitochondria, which are descended from α-proteabacteria (a class of Gram-negative bacteria which are seen in an array of shapes and are found to have a range of metabolic functions) and have become integral to modern eukaryotic cells through endosymbiosis.

The mechanism of endosymbiosis is highly debated, resulting in over 20 versions of the endosymbiotic theories. Where these theories intersect, however, is that they all look to understand how mitochondria have become integral to eukaryotes, despite their origins lying in prokaryotic cells (or, more specifically, archaebacteria). Intriguingly, as no true intermediates between prokaryotes and eukaryotes have been found to exist, it appears that it is mitochondria that provide the bioenergetic means required by the complexity of eukaryotic cells. The inside-out theory of endosymbiosis by David and Buzz Baum exemplifies perhaps the most recent model for this concept: archaic prokaryotes homologous to the current eukaryotic nucleus with membrane-bound blebs (protrusions of the cell membrane due to isolated decoupling of the cytoskeleton from the plasma membrane) on the cell wall allowing proto-mitochondria to integrate into the cell. These blebs expanded around the proto-mitochondria to give rise to the cytoplasm, and the continuous gaps between the blebs formed the endoplasmic reticulum. Other instances of bleb fusion resulted in the plasma membrane. This theory illuminates the link between endosymbiosis of proteobacteria resulting in the modern mitochondria present in eukaryotic cells and the fusion and fission carried out in these organelles today.

Surviving proteobacteria, such as *Escherichia coli,* have been the subject of studies uncovering the mechanism of cell division in these archaic life forms, and perhaps the most notable protein involved in this, when applied to the context of mitochondrial morphology, is FtsZ (a homologue to eukaryotic tubulin found in prokaryotes) that forms a ring structure to aid in the cleavage of these proteobacteria into two daughter cells. This endoergonic process is supplied with GDP through the hydrolysis of GTP by FtsZ, a GTPase – and such enzymes are known to characterise the proteins involved in mitochondrial fission.

Whilst FtsZ is still present in the mechanism of cell division in lower eukaryotes, in higher eukaryotes, a large portion of the mitochondrial DNA has been passed to the nucleus (it is notable that this genomic transfer may have led to the requirement of eukaryotes to regulate the energy potential of mitochondria), thus the system of cell division in such cells now involves proteins derived from eukaryotes directly (with many of the involved proteins orthologous between the four eukaryotic kingdoms (plantae, animalia, protoctista and fungi), indicating that this change was most likely to have occurred before the evolutionary divergence of eukaryotic kingdoms around 950-1,259 million years ago). Despite there now being a lack of a direct link between higher eukaryotes and FtsZ, the system of mitochondrial

fission in eukaryotic cells shares much similarity with the process of endocytosis occurring in the membrane vesicles produced by proteobacteria, and so can provide much insight into the mechanism of mitochondrial fission.

In endocytosis of these membrane vesicles from proteobacteria, vesicles form when components that have entered the cell bud off and move into the cytosol after having been surrounded by the plasma membrane. For the vesicles to bud off, the mechanical enzyme dynamin (which, like FtsZ, is a large GTPase), utilises the release of energy from GTP hydrolysis to cause the constriction of the neck of the vesicle during budding to allow its scission from the cell. The mechanism of mitochondrial fission is not dissimilar to the process detailed above: homologues of dynamin (Drp1 in mammals and Dnm1 in yeast cells) perform the final requirements of fission by forming spirals around the sites of scission which constrict endoergonically until cleavage occurs. The importance of Dnm1 and Drp1 in specific for their role in fission is evidenced by the fission-deficiency that occurs due to mutations in these dynamin homologues, resulting in mitochondria that exist as highly reticulated tubules due to the fusion-fission balance being shifted towards fusion.

The first instance where mitochondrial fusion was observed was during a study researching spermatogenesis in *Drosophilia melanogaster* found that every punctuate mitochondrion within spermatids fuses after meiosis into just two large organelles, known as nebenkern, and that this fusion was prevented by mutation in a GTPase-coding gene located at the outer mitochondrial membrane that is colloquially known as the ‘fuzzy onions’ gene or ‘fzo’, due to the appearance of the fused mitochondria seen in the flies without this mutation. Homologues of the fzo gene are found in other types of organisms - for example, in mammals, fusion is controlled predominately by Mfn1 and Mfn2 (both of which are localised to the outer mitochondrial membrane and allow the fusion of discrete mitochondria) and OPA1 (which manages the fusion of the inner mitochondrial membrane).

Research into mitochondrial fusion occurring in mice found that deficiency of Mfn1 and Mfn2 causes death *in utero* during gestation, however, it was also observed that mice that overexpressed Mfn2 were able to indemnify deficiency of Mfn1. The link between mitochondria and a wide range of diseases presents itself within these proteins; an example being that mutations to Mfn2 has been found to cause issues with neuronal function and thus neurodegenerative diseases such as Charcot-Marie-Tooth neuropathy type 2A.

From this it is clear that emerging understanding of mitochondrial morphology does not simply translate to updating cell diagrams in biology textbooks: the impact of this organelle’s ability to shape-shift spreads from individual cells to the livelihoods of many. It is logical that shape of mitochondria (as this is largely determined by the balance between mitochondrial fission and fusion) has a significant role in prescribing cellular function: higher rates of fusion result in mitochondria that are likely to release more energy, aid in the increase of cellular proliferation and regulate more cell signalling pathways; higher rates of fission result in mitochondria that help the process of autophagy to remove damaged organelles from the cell, allow the release of cytochrome c during apoptosis and that can be motile.

Despite the advantages of both interconnected mitochondrial networks and punctuate structures, the power mitochondria have in determining cellular health can only be positively applied through balancing the causes (namely fusion and fission) of these mitochondrial shapes. Thus, variable mitochondrial morphology allows these organelles to conform to whatever roles they are required to embody and highlights that exclusively being solitary ovals in form would strip their functional versatility to the point where it is questionable as to whether they can still be considered to be mitochondria.

Bibliography:

Martin, W.F., Garg, S. and Zimorski, V. (2015). Endosymbiotic theories for eukaryote origin. *Philosophical Transactions of the Royal Society B: Biological Sciences*, [online] 370(1678), p.20140330. doi:https://doi.org/10.1098/rstb.2014.0330.

McCarron, J.G., Wilson, C., Sandison, M.E., Olson, M.L., Girkin, J.M., Saunter, C. and Chalmers, S. (2013). From Structure to Function: Mitochondrial Morphology, Motion and Shaping in Vascular Smooth Muscle. *Journal of Vascular Research*, [online] 50(5), pp.357–371. doi:https://doi.org/10.1159/000353883.

Moore, A.S. and Holzbaur, E.L. (2018). Mitochondrial-cytoskeletal interactions: dynamic associations that facilitate network function and remodeling. *Current Opinion in Physiology*, 3, pp.94–100. doi:https://doi.org/10.1016/j.cophys.2018.03.003.

Campello, S. and Scorrano, L. (2010). Mitochondrial shape changes: orchestrating cell pathophysiology. *EMBO reports*, 11(9), pp.678–684. doi:https://doi.org/10.1038/embor.2010.115.

Science in the News. (2012). *Mighty Mitochondria and Neurodegenerative Diseases*. [online] Available at: https://sitn.hms.harvard.edu/flash/2012/issue111/#:~:text=Mitochondria%2C%20often%20referred%20to%20as.

Anesti, V. and Scorrano, L. (2006). The relationship between mitochondrial shape and function and the cytoskeleton. *Biochimica Et Biophysica Acta*, [online] 1757(5-6), pp.692–699. doi:https://doi.org/10.1016/j.bbabio.2006.04.013.

Scott, I. and Youle, R.J. (2010). Mitochondrial fission and fusion. *Essays in biochemistry*, [online] 47, pp.85–98. doi:https://doi.org/10.1042/bse0470085.

Cooper, G.M. (2000). *Microtubules*. [online] www.ncbi.nlm.nih.gov. Available at: https://www.ncbi.nlm.nih.gov/books/NBK9932/#:~:text=Importantly%2C%20the%20initiation%20of%20microtubule

Nagakubo, T., Nomura, N. and Toyofuku, M. (2020) 'Cracking open bacterial membrane vesicles,' *Frontiers in Microbiology*, 10. https://doi.org/10.3389/fmicb.2019.03026.

Kruppa, A.J. and Buss, F. (2021). Motor proteins at the mitochondria–cytoskeleton interface. Journal of Cell Science, 134(7). doi:https://doi.org/10.1242/jcs.226084.

www.nature.com. (n.d.). Mitochondria, Fusion, Division | Learn Science at Scitable. [online] Available at: https://www.nature.com/scitable/topicpage/mitochondrial-fusion-and-division-14264007/#:~:text=Mitochondrial%20fusion%20is%20the%20physical [Accessed 29 Apr. 2024].

Scott, I. and Youle, R.J. (2010). Mitochondrial fission and fusion. Essays in biochemistry, [online] 47, pp.85–98. doi:https://doi.org/10.1042/bse0470085.

Westermann, B. (2012). Bioenergetic role of mitochondrial fusion and fission. Biochimica et Biophysica Acta (BBA) - Bioenergetics, [online] 1817(10), pp.1833–1838. doi:https://doi.org/10.1016/j.bbabio.2012.02.033.

Youle, R.J. and van der Bliek, A.M. (2012). Mitochondrial Fission, Fusion, and Stress. Science, 337(6098), pp.1062–1065. doi:https://doi.org/10.1126/science.1219855.

Chen, W., Zhao, H. and Li, Y. (2023). Mitochondrial dynamics in health and disease: mechanisms and potential targets. Signal Transduction and Targeted Therapy, [online] 8(1), pp.1–25. doi:https://doi.org/10.1038/s41392-023-01547-9.

Glancy, B., Kim, Y., Katti, P. and Willingham, T.B. (2020). The Functional Impact of Mitochondrial Structure Across Subcellular Scales. Frontiers in Physiology, [online] 11. doi:https://doi.org/10.3389/fphys.2020.541040.

www.sciencedirect.com. (n.d.). Mitochondrial Fusion - an overview | ScienceDirect Topics. [online] Available at: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mitochondrial-fusion.

Baum, D.A. and Baum, B. (2014). An inside-out origin for the eukaryotic cell. BMC Biology, 12(1). doi:https://doi.org/10.1186/s12915-014-0076-2.

CHARRAS, G.T. (2008). A short history of blebbing. Journal of Microscopy, [online] 231(3), pp.466–478. doi:https://doi.org/10.1111/j.1365-2818.2008.02059.x.

www.sciencedirect.com. (n.d.). Blebbing - an overview | ScienceDirect Topics. [online] Available at: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/blebbing.