Toxicology 391 (2017) 42–53

Contents lists available at ScienceDirect

Toxicology

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

Mitochondrial fusion, fission, and mitochondrial toxicity

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ARTICLE INFO

Keywords:
Mitochondrial fusion
Mitochondrial fission
Mitochondrial dynamics
Mitochondrial toxicity
Gene-environment interactions
Mitochondrial homeostasis
Mitochondrial DNA
Biomarker

ABSTRACT

Mitochondrial dynamics are regulated by two sets of opposed processes: mitochondrial fusion and fission, and mitochondrial biogenesis and degradation (including mitophagy), as well as processes such as intracellular transport. These processes maintain mitochondrial homeostasis, regulate mitochondrial form, volume and function, and are increasingly understood to be critical components of the cellular stress response. Mitochondrial dynamics vary based on developmental stage and age, cell type, environmental factors, and genetic background. Indeed, many mitochondrial homeostasis genes are human disease genes. Emerging evidence indicates that deficiencies in these genes often sensitize to environmental exposures, yet can also be protective under certain circumstances. Inhibition of mitochondrial dynamics also affects elimination of irreparable mitochondrial DNA (mtDNA) damage and transmission of mtDNA mutations. We briefly review the basic biology of mitodynamic processes with a focus on mitochondrial fusion and fission, discuss what is known and unknown regarding how these processes respond to chemical and other stressors, and review the literature on interactions between mitochondrial toxicity and genetic variation in mitochondrial fusion and fission genes. Finally, we suggest areas for future research, including elucidating the full range of mitodynamic responses from low to high-level exposures, and from acute to chronic exposures; detailed examination of the physiological consequences of mitodynamic alterations in different cell types; mechanism-based testing of mitotoxicant interactions with inter-individual variability in mitodynamics processes; and incorporating other environmental variables that affect mitochondria, such as diet and exercise.

1. Mitochondrial dynamics: fusion and fission, transport, biogenesis and mitophagy

Mitochondrial dynamics are critical in regulating morphology, number, subcellular distribution, and function. They are also critical in maintaining mitochondrial homeostasis in response to stress. The degree to which mitochondria are networked results from a dynamic equilibrium between fusion and fission, facilitated by movement of mitochondria within the cell. Similarly, the total mitochondrial content of a cell is a dynamic equilibrium between mitochondrial biogenesis (henceforth referred to as mitobiogenesis) and mitochondrial degradation, including mitophagy and other forms of mitochondrial recycling. Mitochondrial dynamics vary based on developmental stage and age, cell type, environmental factors, disease state, and genetic background. In this section, we provide a brief overview of these processes, with the goal of providing context for the subsequent sections; more detail can be found in the cited reviews. Many of the most critical proteins involved in these processes are important human disease genes; an incomplete list including the subset of these genes that is highlighted in this review is provided in Table 1. These diseases often exhibit variable severity and progression, suggesting a role for environmental factors (*i.e.*, gene-environment interactions). Altered mitochondrial dynamics and morphology also occur in a variety of other diseases (Archer, 2013; Babbar and Sheikh, 2013), although causality is less clear in those cases.

1.1. Mitochondrial fusion and fission

Mitochondria fuse in a process that requires inner- and outer-mitochondrial membrane (IMM and OMM) GTPases (Van der Bliek et al., 2014). In humans, these proteins are named Optic atrophy 1 (OPA1; IMM) and Mitofusins 1 and 2 (MFN1 and MFN2; OMM). Loss of mitofusins blocks fusion of both the OMM and IMM, while loss of OPA1 blocks fusion of the IMM, but not the OMM (Song et al., 2009). Mitochondrial fission is mediated by several proteins, but the GTPase Dynamin related protein 1 (DRP1) is the most central, or at least best understood (Chan, 2012). Fission does not require membrane potential (Twig et al., 2008a), and in fact can be triggered by low membrane potential (Section 2). The inner but not outer membrane fusion process is mitochondrial membrane potential-dependent (Van der Bliek et al.,

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http://dx.doi.org/10.1016/j.tox.2017.07.019
Received 30 March 2017; Received in revised form 10 July 2017; Accepted 31 July 2017
Available online 05 August 2017
0300-483X/

Table 1
Human disease genes involved in mitochondrial dynamics.

Human Gene	Estimated Disease Incidence	Human Disease	Function
MFN2	~1/7500	Charcot-Marie Tooth Neuropathy type 2A	Outer membrane fusion
OPA1	~1/10,000-1/30,000 (Lenaers et al., 2012)	Dominant Optic Atrophy	Inner membrane fusion
DRP1	Very rare (a few known cases) (Mishra and Chan, 2016)	Neuro-degeneration and early death	Fission
PARK2	~1/6000	Parkinson's Disease	Mitophagy
PINK1	~1/60,000	Parkinson's Disease	Mitophagy

Note that this list is not exhaustive, and in particular excludes mitochondrial biogenesis. Incidence values are as cited or estimated: CMT affects 1/2500, but MFN2 deficiency causes type IIA in 20–40% of those cases, so ~1/7500 (Cartoni and Martinou, 2009). Parkinson's Disease currently afflicts ~1/300; of which roughly 10% of cases are early-onset. PARK2 mutations account for as much as 50% (Lucking et al., 2000), and PINK1 ~5% (Bonifati et al., 2005) of early-onset cases, leading to the estimates presented.

2014), although loss of membrane potential may also lead to PARK2-mediated degradation of mitofusins, ultimately preventing fusion of mitochondria with low membrane potential with other healthier ones (Narendra et al., 2012). Regulation of mitochondrial fusion and fission has been reviewed in detail (Narendra et al., 2012; Van der Bliek et al., 2014). It should be noted that while our focus is on mitochondrial dynamics, these proteins may play roles in other cellular processes. For example, MFN2 is also involved in mitophagy (Chen and Dorn, 2013) and in tethering mitochondria both to the endoplasmic reticulum, which is important for early stages of mitochondrial fission (de Brito and Scorrano, 2008; Friedman et al., 2011), and to microtubules, permitting mitochondrial transport in neurons (Pareyson et al., 2015); OPA1 contributes to maintenance of cristae structure (Olichon et al., 2003) and may help anchor nucleoids to the IMM (Elachouri et al., 2011); and several fission proteins may also play a role in peroxisomal division (Chan, 2012).

In cell culture, fusion and fission can occur within minutes or even seconds, particularly in the case of rapid stress-induced fission or transient, partial fusion events described as "kiss-and-run" (Dalmasso et al., 2017; Duarte et al., 2012; Liu et al., 2009). At the cellular level, mixing of contents between mitochondria can occur within an hour (Youle and van der Bliek, 2012); however, there is also evidence that heterogeneous mitochondrial sub-populations persist within cells in some cases (Wikstrom et al., 2009). Genetic loss of mitochondrial fusion results in insufficient mixing of mitochondria within cells, causing dramatic mitochondrial heterogeneity in protein, mtDNA, and membrane potential (Mishra and Chan, 2016). Fusion and fission may also serve to permit subcellular specialization of mitochondria, *e.g.* such that perinuclear mitochondria function differently than axonal mitochondria (Kowald and Kirkwood, 2011). The relative rates of these two processes at any given time in specific tissues are not well understood (Mishra and Chan, 2016), but presumably act in an integrated fashion to regulate both morphology and the potential rate at which morphology can be altered. This may also relate to the rate of movement of mitochondria in the cells, estimated to be ~0.1–0.2 μm/s in the perinuclear region and up to ~0.7 μm/s in the cytosol and in axons (Dalmasso et al., 2017).

In general, it appears that more-networked mitochondria are more efficient at generating ATP, particularly by aerobic metabolism, although there are some exceptions to this (Benard et al., 2010; Correia-Melo and Passos, 2015; Mishra and Chan, 2016; Westermann, 2012; Youle and van der Bliek, 2012); there is also evidence that fusion is important for other processes, such as steroid and coenzyme Q synthesis (Duarte et al., 2012; Mourier et al., 2015b). Fusion can also be beneficial by permitting "functional complementation": if specific mitochondria carry a high level of damaged components or mutated mtDNA, the deleterious effects of these dysfunctional components may be compensated for by functional components from other mitochondria (Nakada et al., 2001; Schon and Gilkerson, 2010). The kinetics of functional complementation may be limited by the fact that mixing of OMM, intermembrane space and matrix components is faster than mixing of IMM components (including mtDNA, which is anchored in nucleoids to the IMM: (Wikstrom et al., 2009)), apparently because of cristae structure (Busch et al., 2014), the details of which remain disputed (Zick et al., 2009). Fission permits distribution of mitochondria throughout a cell (*e.g.*, transport down axons or to permit allocation prior to cell division), and facilitates apoptosis *via* release of cytochrome C under some circumstances (Mishra and Chan, 2014). Finally, fission may allow identification of dysfunctional daughter mitochondria and their subsequent removal *via* lysosomal degradation (*i.e.*, mitophagy), when combined with inhibition of fusion (which, as a mitochondrial membrane-dependent process, is inhibited in damaged mitochondria) (Mouli et al., 2009; Youle and van der Bliek, 2012).

Overall, fusion and fission maintain mtDNA copy number, integrity (*i.e.*, removal of damaged and mutated mtDNA), and distribution (Amati-Bonneau et al., 2008; Elachouri et al., 2011; Rouzier et al., 2012; Vidoni et al., 2013), yet also permit tolerance of mtDNA mutations (Kowald and Kirkwood, 2011; Lin et al., 2016), presumably *via* the processes of complementation and mitophagy as described above.

1.2. Biological variability in fusion and fission

Mitochondrial morphology is highly variable in different biological contexts, and much remains to be learned about this variability (Zick et al., 2009). We summarize some of the better-characterized patterns; a number of specific examples are reviewed by Kuznetsov et al. (2009). In stem cells, mitochondria are fragmented and spherical, predominantly perinuclearly located, and exhibit less oxidative phosphorylation, more glycolysis, low oxidative damage to macromolecules, and other functional changes (Bukowiecki et al., 2014). In dividing cells, mitochondria tend to fuse during G1-S stages, presumably to provide energy for division, and divide prior to mitosis, presumably to enable distribution into daughter cells (Mishra and Chan, 2014). Mitochondria may also exhibit tissue-specific forms and functions. For example, mitochondria in cardiomyocytes are relatively lacking in dynamics and non-networked, yet still express fusion and fission proteins which appear to have important quality-control functions (Shirihai et al., 2015); these mitochondria may have developed alternate mechanism for content exchange (Huang et al., 2013). Mitochondria in differentiating T cells undergo both biogenesis and dramatic metabolic remodeling (Ron-Harel et al., 2016). In addition, mitochondrial morphology may be altered by and influence disease processes. For instance, inhibition of mitochondrial fission can impede cancerous processes (Rehman et al., 2012; Wang et al., 2012; Zhao et al., 2013), perhaps by opposing the glycolytic and proliferative phenotypes of cancerous cells. Mitochondrial dynamics may be altered in some cell types by circadian rhythms (Manella and Asher, 2016) and as a function of age (Seo et al., 2010). Finally, recent modeling efforts suggest that low mitochondrial mass impedes production of a more-networked morphology, again illustrating the interdependence of mitochondrial parameters (Dalmasso et al., 2017).

Mitochondrial fusion and fission are regulated transcriptionally and non-transcriptionally (including proteolytic degradation and post-translational modification of proteins) by a multitude of factors, including metabolic status and energetic status, mitochondrial membrane potential, redox status, and cellular stress (Hoppins, 2014; Mishra and Chan, 2016; Toyama et al., 2016; Van der Bliek et al., 2014; Willems et al., 2015). Transcriptional regulation is relatively poorly understood, and post-translational regulation is quite complex (Dhingra and Kirshenbaum, 2014). Reported environmental regulation of mitochondrial fusion, fission and morphology are reviewed in Section 2.