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Organelles in focus

Mitochondrial diseases: *Drosophila melanogaster* as a model to evaluate potential therapeutics☆

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ABSTRACT

This article is part of a Directed Issue entitled: Mitochondrial Diseases.

1. Background

While often presented as a single entity, mitochondrial diseases comprise a wide range of clinical, biochemical and genetic heterogeneous disorders. Among them, defects in the process of oxidative phosphorylation are the most prevalent. Despite intense research efforts, patients are still without effective treatment. An important part of the development of new therapeutics relies on predictive models of the pathology in order to assess their therapeutic potential. Since mitochondrial diseases are a heterogeneous group of progressive multisystemic disorders that can affect any organ at any time, the development of various *in vivo* models for the different diseases-associated genes defects will accelerate the search for effective therapeutics.

Here, we review existing *Drosophila melanogaster* models for mitochondrial diseases, with a focus on alterations in oxidative phosphorylation, and discuss the potential of this powerful model organism in the process of drug target discovery.

Organelle facts:

- Mitochondrial diseases as a group belong to the most frequent inborn errors of metabolism.
- Patients with life-threatening mitochondrial diseases are without effective treatments.
- Mitochondrial diseases are genetically and phenotypically highly heterogeneous.
- The rarity of individual mitochondrial diseases and their phenotypic variability has greatly hampered drug development.
- Mitochondrial diseases can be modeled in *Drosophila*, an exceptionally efficient and versatile alternative animal model.
- Various *Drosophila* outcome measures can be used to screen compound collections in various models at a time.

Abbreviations: OxPhos, oxidative phosphorylation system; CI, Complex I or NADH: ubiquinone oxidoreductase; CII, Complex II or succinate: ubiquinone oxidoreductase; CIII, Complex III or ubiquinol: cytochrome c oxidoreductase; CIV, Complex IV or cytochrome c oxidase; CV, Complex V or F_0-F_1 ATP synthase; ATP, adenosine triphosphate; mtDNA, mitochondrial Deoxyribonucleic acid; ROS, reactive oxygen species; UAS, upstream activating sequence; GAL4, galactose yeast transcriptional activator; CRISP, clustered regularly interspaced short palindromic repeats; Cas9, caspase 9; TALEN, Transcription activator-like effector nucleases; tko, technical knockout; TALEN, Transcription activator-like effector nucleases; FRDA, Friedreich ataxia; NDUFS, NADH dehydrogenase (ubiquinone) Fe-S protein; MB, methylene blue.

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Mitochondrial diseases, while often regarded as a single entity, comprise a wide range of distinct clinical entities (Koopman et al., 2012). When taken as a whole, mitochondrial disorders are one of the most frequent categories of inborn errors of metabolism, with an incidence estimated of 1 in 5000 individuals (Smeitink et al., 2001). Associated with severe and an extreme variety of clinical symptoms, mitochondrial diseases can lead to substantial morbidity and premature death. There are currently no treatments available. Among mitochondrial disorders, defects in the oxidative phosphorylation (OxPhos) system are the most prevalent. This system consists of five multi-subunit complexes (CI–CV) generating the high-energy phosphate molecule adenosine triphosphate (ATP) through a series of complex biochemical processes (Distelmaier et al., 2009; Koopman et al., 2013). The subunits forming the five complexes are encoded by either the nuclear or mitochondrial DNAs (mtDNA) and are therefore potential targets to mutations in both genomes (Smeitink et al., 2001). It is still unclear how cells and tissues become dysfunctional in response to isolated or combined OxPhos deficiencies. Potential obvious cellular pathomechanisms are insufficient ATP production, increased reactive oxygen species (ROS) production, corrupted mitochondrial membrane potential, and calcium homeostasis, but also the alteration of other indispensable cellular processes such as the mitochondrial pathways of cell death (Tait and Green, 2012). All together, these cellular defects lead to devastating multisystemic symptoms affecting different organs.

Successful drug development relies, among other parameters, on a deep understanding of the underlying molecular and cellular mechanisms that can be targeted pharmacologically, and on the availability of predictive models to assay the potential responsiveness of patients to a new therapeutic approach. In this context, *in vivo* models of diseases represent critical tools to test outcome measures that are of direct relevance for complex multisystemic diseases such as mitochondrial disorders, reflecting the organizational and biological features of the different host tissues or the organism as a whole.

Testing potential treatment strategies on various *in vivo* models recapitulating the clinical and/or molecular variability observed in the different mitochondrial diseases can increase (1) the knowledge about specific mechanisms causing the different syndromes, and (2) the success rate during clinical trial phases by enabling stratification of patients based on their potential responsiveness toward a specific therapeutic.

Therefore, the development of a comprehensive collection of complementary animal models is crucial to expedite the search for new treatment for mitochondrial diseases. Since there are only a limited number of mouse models for mitochondrial diseases, the fruit fly *Drosophila melanogaster* represents an attractive alternative (Fig. 1). Here, we have reviewed the literature about existing *Drosophila* models for mitochondrial disorders, and discuss their potential use and predictive power, to evaluate new potential therapeutics as an initial step in the drug identification process.

The list of advantages to use *Drosophila* is long: a short life cycle, a high reproduction rate, easy maintenance of cultures and molecular systems with conserved cellular and physiological function, and less functional redundancies compared to mammals. Nearly 75% of disease-related genes in humans have functional orthologs in *Drosophila* (Reiter et al., 2001; Van der Voet et al., 2014). The genes encoding the OxPhos complexes are highly conserved from *Drosophila* to human (Sardiello, 2003; Jacobs et al., 2004; Tripoli et al., 2005), and so are genes encoding enzymes involved OxPhos-related processes such as ROS scavenging (Anderson et al., 2008; Angeles et al., 2014). Like in mammals, muscle fibers can be either glycolytic or oxidative (Piccirillo et al., 2014). Together, this strengthens the validity of *Drosophila melanogaster* as a model for mitochondrial disorders and more specifically for OxPhos deficiencies.

The power of *Drosophila* as a model surely resides in the massive available resources, toolboxes, and powerful genetics that enable manipulation of expression of, in principle, any gene of interest in spatiotemporal pattern of choice (Matthews et al., 2005; Dietzl et al., 2007; Venken and Bellen, 2014). Due to the advantages of *Drosophila*, many models for mitochondrial genes deficiencies can thus be generated at reasonable time and costs when compared to mammalian models. The UAS–Gal4 system developed by Brand and Perrimon in 1993 is assuredly one of the powerful tools to flexibly modulate gene expression. This system allows the expression of a transgene in a spatially and temporally controlled manner. The temperature-sensitivity of the system moreover allows, when combined with genome-wide resources of RNA interference (Dietzl et al., 2007), to decrease the level of expression of any gene in a tightly controlled manner. Therefore, while knockout or strong knockdown of a specific gene can lead to early lethality not suitable for drug testing, conditional or mild knockdown allows the investigator to tune the level of expression in order to obtain a suitable phenotype–outcome measure. Classic genetic mutants can be obtained by forward mutagenesis and complementation screening, imprecise P-element excision mutagenesis, and homologous recombination strategies. The latter can also be used to exchange wildtype proteins against their mutant versions. These methodologies, among others, have been used to target and modulate expression of mitochondrial genes in *Drosophila*. The different models reported in the literature are listed in Tables 1 and 2. It can be anticipated that recent methods for genome editing, such as the CRISPR/Cas9 system (Gratz et al., 2013) and TALENs technology enabling directed point mutations (Liu et al., 2012, 2014; Beumer and Carroll, 2014) will further facilitate generation of disease models reflecting the defects found in patients suffering from mitochondrial diseases even closer.

The power of a disease model lies in its capability to recapitulate the specific aspects of a particular human pathology, at the different level of complexity, from molecules to organism. Although the number of *Drosophila* models for mitochondrial disorders is still rather low, those already existing confirm their overall great potential. The fly mutant technical knockout (TKO) carries a point mutation (L85H) in the gene for the mitoribosomal protein S12 involved in the mitochondrial protein synthesis machinery, leading to a deficiency of mitochondrial translational capacity. The TKO flies display biochemical defects such as decreased OxPhos capacity, with reduced activity of CI, CIII, and CIV, and impaired ATP synthesis, which are key molecular features in patient suffering from mutation in the mitochondrial protein synthesis apparatus (Jacobs et al., 2004). Interestingly, the model also exhibits a defective response to sound (Toivonen et al., 2001). As sensorineural deafness is a common pathological states encounter in patients with mutations in mtDNA, the TKO fly represents a valid model for mitochondrial hearing loss.

A number of models for OxPhos complexes deficiencies have already been reported (Table 1). They often exhibit common phenotypic features, although with different levels of severity, such as shortened lifespan or lethality, developmental delay, muscle weakness, neuronal dysfunction and degeneration, seizures, and a decreased activity of the specific targeted complex, all of which are phenotypes commonly observed in patients. In *Drosophila*, multiple techniques and assays are available to assess these and related systemic defects, such as locomotors tests (*e.g.*, monitoring of activity and movements, flight, and climbing assays) (Walker et al., 2006; Fernandez-Ayala et al., 2009; Ghezzi et al., 2011), electrophysiological and morphological investigations of neurons (which due to the ease of accessibility are often applied to the *Drosophila* eye and photoreceptors as neuronal models) (Van Bon et al., 2013), and bang

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