doi: 10.26124/bec:2021-0002.r2
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Mitochondrial homeostasis in cellular models of Parkinson's Disease

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Manuscript submitted 2021-02-19, 2021-06-28 (revision 1), 2021-09-01 (revision 2)

Manuscript accepted 2021-09-27

doi:10.26124/bec:2021-0002

Reviewer 2

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Manuscript reviewed 2021-04-05 (round 1), 2021-07-20 (round 2)
doi:10.26124/bec:2021-0002.r2

*Only major points from review and responses included.

Round 1

Reviewer 2

The text may be difficult to follow for readers who are experts in mitochondria but not in neurodegenerative diseases. It could be interesting to include a scheme that conveys in a simple and quick way the general idea of this pathology. On the other hand, almost all the sections are organized as if they were a list of concepts. It could be interesting to modify the wording to facilitate the reading and not to make it tedious.

Authors

We have added Figure 1 to address this.

Reviewer 2

Since it is necessary to reduce the number of words, section 1.2, referring to mitochondrial dysfunction in the disease, would benefit greatly from a table where the genes involved in the pathophysiology are organized, indicating, for example, how they are related to mitochondrial function and how they are affected by the disease or how they favour its development. This would make it easier for readers unfamiliar with the disease to follow the list of genes mentioned in the text, as in its present form it can be somewhat monotonous.

Authors

We have included the Table 1 to address this suggestion.

Reviewer 2

In addition to the table with the genes, it could also be interesting to include a figure showing how the proteins encoded by these genes interact with each other, if applicable, and with the mitochondria or the respiratory chain. This visual aid could favour the comprehension of the text.

Authors

We have included in the Figure 2 a protein-protein interaction network to address this suggestion.

Reviewer 2

One could also reduce section 2, referring to the parameters used for the assessment of mitochondrial homeostasis, by giving a brief description and mentioning recent reviews in this regard.

Authors

We have shortened the section two as suggested.

Reviewer 2

Although mentioned in fifth place, it is for this reviewer the most important aspect of the whole text. The authors have bothered to review a multitude of studies and generate a figure from the results obtained in them to give support to what is intended to be conveyed, which would be how each of the Parkinson's disease models exposed, whether cellular or animal, can show how the pathology affects mitochondria. However, this aspect is very lacklustre because it is not presented in a coherent way, since it is presented in section 2, as what would be section 2.1. Then, and scattered throughout the text, these results are mentioned at the end of some of the sections, thus losing the perspective of the comparative analysis that is very well perceived in Figure 1, but not so in the text.

This reviewer considers that it would be much more interesting to present all the models first and, finally, to present the qualitative evaluation. This could be done, for example, by eliminating section 2.1 and creating a new section 5, explaining the objective, the method of analysis and the results obtained (Figures 1a-f). This would then make it easier to discuss the strengths and weaknesses of each model. This would easily lead to a new section 6, Prospectives.

Authors

We have considered this suggestion and restructured the text as recommended.

Round 2

Reviewer 2

Once it is defined which disease-related genes are involved in mitochondrial physiology, and the relationships between all of them are established (Table 1 and Figure 2), after the exhaustive work done by the authors to make both, what conclusions are drawn? How does what is observed in this figure relate to the importance of mitochondrial physiology and its relationship with Parkinson's disease? Does this figure then justify the need to use different cellular and/or animal models?

Authors

We appreciate this important point. The Table1 and Fig 2 highlight genetic PD factors that have a mitochondrial homeostasis role and the complexity of interactions with mitochondrial respiratory chain complexes which may impact mitochondrial physiology. This comment has now been included in the text.

Reviewer 2

The authors mention idiopathic PD. In Figure 1 they include non-modifiable risk factors (such as age and genetics) and modifiable factors, such as the environmental factors they mention (in the case of this pathology, as they themselves indicate, the use of certain pesticides is very important).

How does the fact that there are various factors that influence the development of the pathology affect the basic research studies, that is, the disease models? What effect does this have on the translation of the results to patients, especially those whose pathology is idiopathic in origin? Would a better understanding of these mechanisms and the establishment of markers (or also risk factors for PD) help to stratify these patients? This reflection may be a good starting point to justify the need for a review such as this, beyond the mere comparison between different models, as indicated by the authors in lines 94-97: To provide a platform for ongoing and future work, we have reviewed and compared studies of mitochondrial physiology in cellular PD models and compared these with animal models of PD to identify common features that may be investigated as PD risk factors.

Authors

We appreciate the reviewer's comment and we have added the appropriate discussion in Sections 1 and 6.

Reviewer 2

The other reviewer suggested the need to include clear inclusion and exclusion criteria to determine how PD models have been selected. But the authors have made this consideration only for section 5 (Qualitative analysis of mitochondrial homeostasis parameters). I believe that it would be appropriate to establish from the beginning these

inclusion and exclusion criteria for the articles selected for comment/discussion in each of the sections. This would lead to generate, after the introduction where the bases that justify this review are established, a new Methods section (which would then be section 2). In this section, in addition to including the criteria for inclusion and exclusion of the articles, authors could also indicate how Figure 2 was generated and how the qualitative study was carried out. This would also help the understanding of the text in both cases.

Authors

It is not common practice to include selection criteria for background papers used in reviews. Typically references that backup the discussion points are being employed. In many cases not all the relevant references can be included and there are numerous apologies in reviews for not including all the papers that may have been relevant to the point. The same applies to our review here and we do not consider that expanding the review with selection criteria beyond the qualitative analysis is in the scope of this work.

We have included the literature that has been used for the qualitative analysis for which we have specified the selection criteria in the text and background work that was needed to support the discussion.

The figure 2 network analysis was generated using the STRING database as specified in the text and figure caption.

The qualitative study was carried out employing a scoring system detailed in the paper which generated the data provided in the supplementary data table.

Reviewer 2

Furthermore, this section on methods better justifies the reason for the selection, since what is indicated by the authors in lines 101-104: Given the importance of consolidating and disseminating protocols for mitochondrial homeostasis dysfunction and neurodegeneration, here we have considered key parameters and common assays used to assess PD phenotypes and summarized common assays employed for these analyses, is rather vague, because at this point we do not know what they consider "key parameters", among other aspects.

Authors

We consider 'key parameters' those summarised in the text because numerous publications address them when investigating mitochondria homeostasis in health and disease particularly in PD models. – We have edited the text to make it clearer.

Reviewer 2

Regarding transgenic models, section 3.2, the authors indicate in lines 219-224: The discoveries in the genetics of PD have led to development of genetic murine models harbouring genetic modifications related to PD. However, these models do not fully recapitulate the PD characteristics and present rather mild phenotype. To complement these, other animal models of PD, particularly using Drosophila, have been successfully

employed to address mitochondrial homeostasis alongside behavioral and other mechanistic PD characteristics.

Given that the origin of PD does not necessarily have to be due to a single reason (the genetic component can be joined by the age and/or environmental component and several genetic components (possible unknown) can converge, thinking of idiopathic PD), it is impossible for this reviewer to think that a fly model is better than a mouse model.

Authors

We appreciate the reviewer's comment and want to point out that we did not state that the fly model was 'better', but just that it 'complements' other models. We hope that this reflects its use in the field.

Reviewer 2

What is clear is that these invertebrate models make it easier to elucidate mechanisms. However, at the functional level, it would be more reproducible (always with caution) to know the efficacy of possible treatments that improve, in this particular case, mitochondrial physiology, in a murine model (mammal) than in an invertebrate model. It would then be worth highlighting the advantages and disadvantages of both models, because it is easier to understand why they complement each other.

Authors

We thank the reviewer for this comment, and we have specified the contribution of the fly models to 'mechanistic' investigations in the text.

Reviewer 2

Another observation about murine models is that there are several ways of generating them and this means that there can be important differences when interpreting the results. For example, a conditional RNAi model or one generated with genes transferred through viruses (lines 233 and 262) is not the same as a knockout model (line 235) or a model of overexpression of a human gene (line 260-261); and it is important to emphasize here that the transgenic synuclein models are by overexpression of the human protein, not murine.

Authors

We have now clarified that human α-Synuclein is employed.

Reviewer 2

These differences among murine models should be made clear, as at the beginning of section 3.2, and it may even be appropriate to change the word "transgenic" in the title to a more appropriate word that encompasses the different ways of generating animal models of PD.

Finally, a conclusion/reflection on this section 3.2 is needed.

Authors

The difference among murine models is now highlighted at the end of 3.2.

Reviewer 2

Regarding section 4, cellular models, with respect to section 4.1, the authors indicate in lines 323-332: A strong advantage of these cultures is that they can be derived from transgenic animals providing homogenous genetic cellular models. However, primary neuronal cultures have multiple disadvantages including the limited number of cells that can be obtained in one preparation, while the preparation and maintenance of the cultures are not trivial. Moreover, numerous potential variations in preparation may affect the neuronal physiology, including the mitochondrial homeostasis leading to high heterogeneity in the experimental results. In addition, the primary neuronal cultures are typically derived from embryonic stage or newly born animals questioning their appropriateness for age related neurodegenerative diseases.

This reviewer does not strongly agree with all the observations. Indeed, performing primary cultures may have a number of technical disadvantages with respect to cell models derived from tumor lines, such as the SH-SY5Y mentioned in section 4.2 (although these models have their problems, also, as stated by the authors) but it is also true that at the physiological level they will more closely resemble neurons, glia or microglia. On the other hand, primary cultures can be made either from genetically modified animals (by overexpression or gene deletion) or even from wild-type animals to which, for example, fibrillar synuclein is added (ref. 44). Although it is true that they are made from cells derived from embryos or newborn animals, these models give us a lot of information on how the mitochondrial physiology, in the present case, is already affected almost from the beginning (in the genetically modified models) and could even be tissue and/or cell-dependent (as indicated in ref. 34). On the other hand, they also give us an idea of how proteins with aggregation capacity, such as synuclein, can affect this level also in wild-type models at a very early age (ref. 44). So, although the development of the disease is associated with age, from this type of models we can know that it is a cumulative effect, i.e., that there are failures from the beginning that, although initially do not show a phenotype, with the passage of time these already manifest themselves either through the years or by the accumulation of other factors (genetic and/or environmental) that help to accelerate the process.

Authors

We agree with the reviewer's comments and we have stated in the text 'Despite these drawbacks, primary neuronal cultures are a common tool for PD studies including for analysis of mitochondrial function parameters and provide valuable information on mitochondrial physiology'.

We have tried to cover pros and cons for various models and it is for the readers to assess their options.

Reviewer 2

In section 4.2, the authors consider the SY-SY5Y cell model. Why only this cell model, and are there no others? For example, models of PD with PC12 cells.

Authors

We have looked at PC12 and N2A but there were only few (2-3) studies comprising some of the studied parameters and these were not sufficient to get some meaningful analysis for each cell line.