The Influences of Mitochondrial Depolarization on Mitochondrial Network Structures

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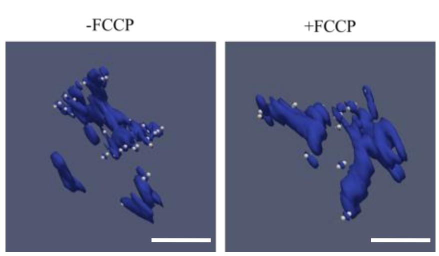
Introduction: The mitochondrial life cycle includes fission and fusion, which contribute to the dynamic morphology. Besides, mounting evidence indicates that mitochondrial fusion process avoids damaged mitochondria from fusing with others based on mitochondrial membrane potential. However, how mitochondrial damage influences mitochondrial network remains unclear. In this study, we first depolarized mitochondria by uncoupler, and investigated changes of mitochondrial 3D structure from a network point of view. Also, we have implemented an artificial neural network for automatic recognition of damaged mitochondrial network. This study provides insights into morphological response to mitochondrial damage and possible application to clinical diagnosis.

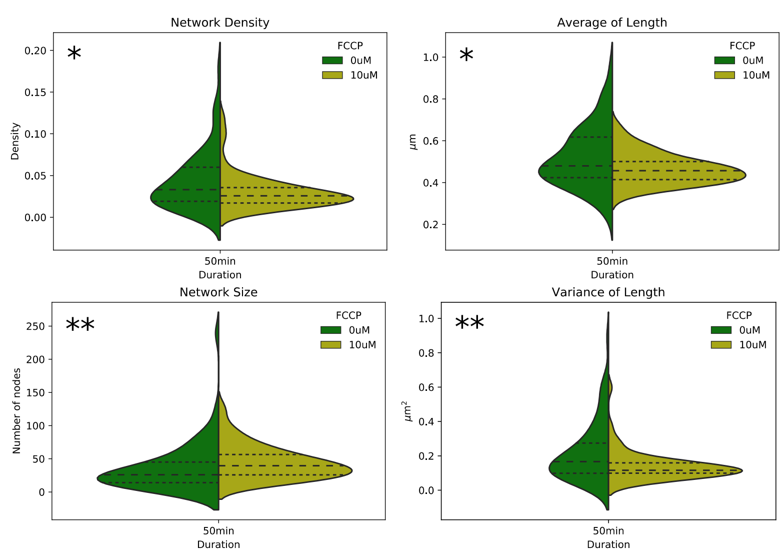
Materials and Methods: *S. cerevisiae* S288C labeled with Kgd1-GFP was used for mitochondrial imaging.
Cells were treated with 10 μM FCCP (carbonylcyanide-p-trifluorometoxyphenylhydrazon), a mitochondrial uncoupler, for 50 min to depolarize mitochondria, and the untreated cells are regarded as the control. Mitochondrial 3D images were captured by a Delta Vision microscope, and processed by MitoGraph software (Fig. 1A). We further calculated network features including network density (the ratio of actual connections to potential connections), average and variance of mitochondrial length, network size (number of nodes). The comparison for two groups was calculated by Mann-Whitney U test. Moreover, we have constructed a full-connected neural network with two hidden layers to classify mitochondrial morphology into two groups.

Results and Discussion: Network density, average and variance of mitochondrial length decrease significantly after FCCP treatment (Fig. 1B), while the network size increases significantly compared to the untreated population. The slower fusion rate of damaged mitochondria may contribute to increased nodes and decreased connections in the mitochondrial network, which result in lower network density and higher size. The fragmentation may also result in lower average and variance of mitochondrial length in the damaged network. The implemented neural network performs at 68% accuracy (Fig. 1C), applying deep learning framework may improve the performance.

(A)



(B)



(C)

		Real -FCCP	Real +FCCP
Predict	-FCCP	30%	10%
Predict	+FCCP	22%	38%

Fig. 1. (A) Mitochondrial morphology with or without FCCP treatment (10 μM). Blue part represents the mitochondrial surface, and white part represents the mitochondrial skeleton. Scale bars represent 2 μm (B) The effect of FCCP treatment to the mitochondrial network (n=126). *P < 0.05 and **P < 0.01 vs. control (Mann-Whitney U test, one-tailed) (C) Classification results. A neural network was used to classify mitochondrial status based on four mitochondrial network features (n=126).

Conclusions: The morphological changes caused by mitochondrial uncoupler includes lower density, average and variance of mitochondrial length, and higher network size. The significantly different features provide opportunities for automatic recognition of damaged mitochondrial morphology.