Development of a semi-quantitative mitochondrial protein assay

Christine Kong[1], Rebecca D. Ganetzky[2,3], Alan Cahill[2]

[1]College of Arts and Sciences, Class of 2024, University of Pennsylvania, Philadelphia PA; [2]Mitochondrial Medicine Frontier Program, Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia PA; [3]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia PA

INTRODUCTION

- Mitochondria are the most important cellular structure for energy production, regulating ATP production through oxidative phosphorylation [1]
- Mitochondrial DNA (mtDNA) is translated by dedicated mitochondrial machinery to encode 13 polypeptides which are core subunits in creating ATP [2]

Radiolabeling

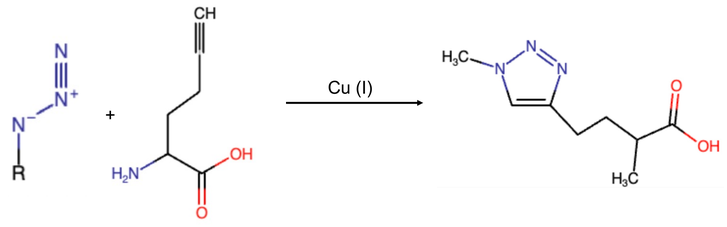
- Current method of translation analysis in mitochondria is radiolabeling of newly synthesized polypeptides in intact cells or in purified mitochondria [3]
- Toxicity and radioactive decay make radiolabeling unsuitable for long-term clinical work from an occupational health perspective

Click Chemistry

- Recently-developed fluorescent imaging approach
- This method has been previously optimized for in situ viewing of mitochondrial translation but does not allow for in-depth analysis of individual peptides [4]

The objective of this work was to adapt pre-existing methodology to create a reproducible, semi-quantitative translation assay that can characterize the amount of production of each of the 13 polypeptides encoded by mtDNA

METHODS



Azide HPG 1,4-isomer

Click Reaction

- Utilizes visualization by a copper-catalyzed cycloaddition reaction to azide-containing fluorescent dyes
- Goal is to allow cells to undergo just mitochondrial translation, using fluorescence as a proxy for translation efficacy
- In lieu of methionine, homopropargylglycine (HPG), an alkyne-containing amino acid analog of methionine will be used
- Nascent polypeptides will incorporate HPG in place of methionine [5]
- HPG can "click" to to an azide-functionalized fluorophore using a Click Chemistry Reaction Buffer Kit
- Emetine will be used to inhibit cytosolic translation
- An SDS-PAGE gel electrophoresis will be performed to identify and quantify individual proteins from translation

RESULTS

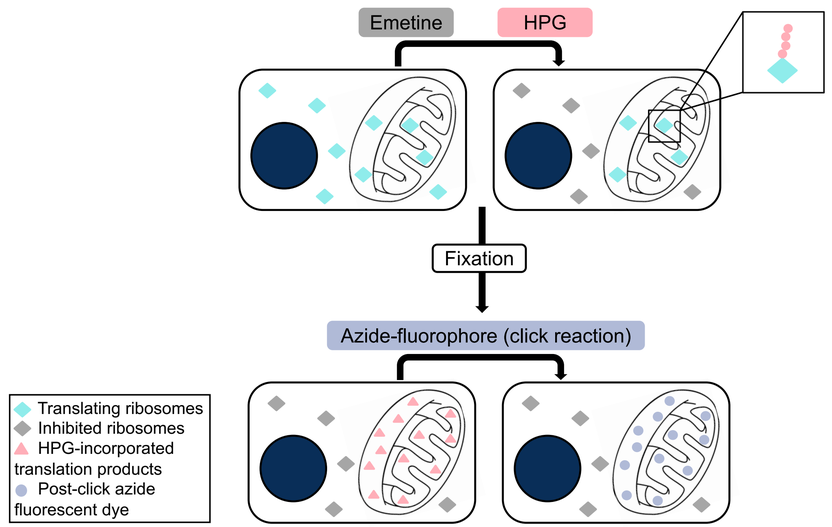


Figure 1. Schematic presentation of the approach. Cytosolic translation is inhibited with emetine, while mitochondrial ribosomes are allowed to incorporate the alkyne-containing methionine homolog (HPG) into newly synthesizes proteins. In fixed cells, HPG moieties are clicked to an azide-conjugate fluorophore through copper-catalyzed Huisgen cycloaddition (click) and can be visualized microscopically.

Cell line	Type
Q1875	Cybrid
FF3	Control
Q2096	Potential translation defect
1818	YARS2 defect

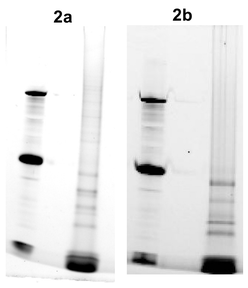


Figure 2ab. Comparison of mitochondrial translation labeling in Q1875 cells using HPG followed by click reaction with AF488-azide, in intact cells (2a) and purified mitochondria (2b). Samples were analyzed by SDS–PAGE and fluorescent gel imaging (10-20% gradient). Precision Plus Protein™ Dual Color Standards were used.

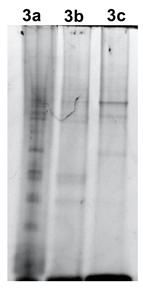


Figure 3abc. Comparison of mitochondrial translation labeling in Q1875 cells (3a), Q1875 cells treated with chloramphenicol (3b), and 1818 cells (3c) using HPG followed by click reaction with AF488-azide. Samples were analyzed by SDS–PAGE and fluorescent gel imaging (16% tricine).

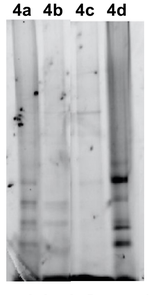


Figure 4abcd. Comparison of mitochondrial translation labeling in Q1875 cells (4a), Q1875 cells treated with chloramphenicol (4b),1818 cells (4c), and isolated mitochondria (4d) using HPG followed by click reaction with AF488-azide. Samples were analyzed by SDS–PAGE and fluorescent gel imaging (10-20% gradient).

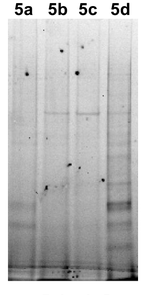


Figure 5abcd. Comparison of mitochondrial translation labeling in Q1875 cells treated with chloramphenicol (5a),1818 cells (5b), 2096 cells (5c), and 1875 cells (5d) using HPG followed by click reaction with AF488-azide. Samples were analyzed by SDS–PAGE and fluorescent gel imaging (10-20% gradient).

DISCUSSION

Streamlining the assay

- 10-20% gradient gel vs. 10% tricine gel
- Intact cells vs. isolated mitochondria
- Emetine vs. chloramphenicol as a cytosolic translation inhibitor

Difficulties

- Fibroblasts (FF3, Q2096, 1818) are more difficult to grow in quantities large enough for the click reaction
- Nonspecific molecular weight markers
- Trace amounts of cytosolic translation present
  - Interferences from cytosolic translation make it difficult to identify specific mitochondrial translation bands

Future Directions

- Incorporate FF3 cells into testing
- Streamline the the cell growth and plating/scraping process
- Mass spectrometry (MS) to quantify protein bands and label each band
  - This can definitively determine which bands are present or missing, affirming the success of the assay
- This assay can hopefully be utilized to validate diagnosis, create new potentials for evaluating treatment of mitochondrial disease, and pave the way in creating the first clinically available assay for mitochondrial translation in the United States

ACKNOWLEDGEMENTS

This research was supported by the Ernest M. Brown, Jr. College Alumni Society Undergraduate Research Grant (Center for Undergraduate Research and Fellowships) as well as the Doliner Family Fund. I would like to thank these sources of funding, as without this research would not have been possible. I would also like to thank Dr. Ganetzky and Dr. Cahill, who have been wonderful and supportive mentors. Finally, I would like to thank Sheila Clever, Irene Yee, and the members of the Falk Lab.

REFERENCES

[1] Basu, U., et al. (2020). Structure, mechanism, and regulation of mitochondrial DNA transcription initiation. *Journal of Biological Chemistry*, *295*(52), 18406-18425.
[2] Smits, P., et al. (2010). Mitochondrial translation and beyond: processes implicated in combined oxidative phosphorylation deficiencies. *Journal of Biomedicine and Biotechnology, 2010,* 1-24.
[3] Aibara, S. et al. (2020). Structural basis of mitochondrial translation. *Elife, 9,* e58362.
[4] Yousefi, R., et al. (2021). Monitoring mitochondrial translation in living cells. *EMBO Reports, 22*(4), e51635.
[5] tom Dieck., et al. (2012). Metabolic labeling with noncanonical amino acids and visualization by chemoselective fluorescent tagging. *Current Protocols in Cell Biology, 56*(1), 7-11.

EMAIL: CKONG12@SAS.UPENN.EDU