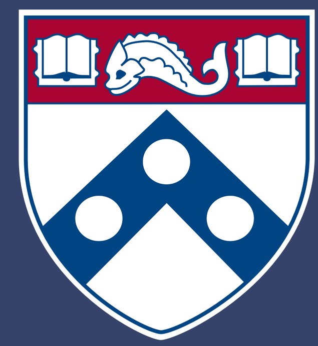

Utilizing stable isotopes to develop a quantitative mitochondrial translation assay

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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

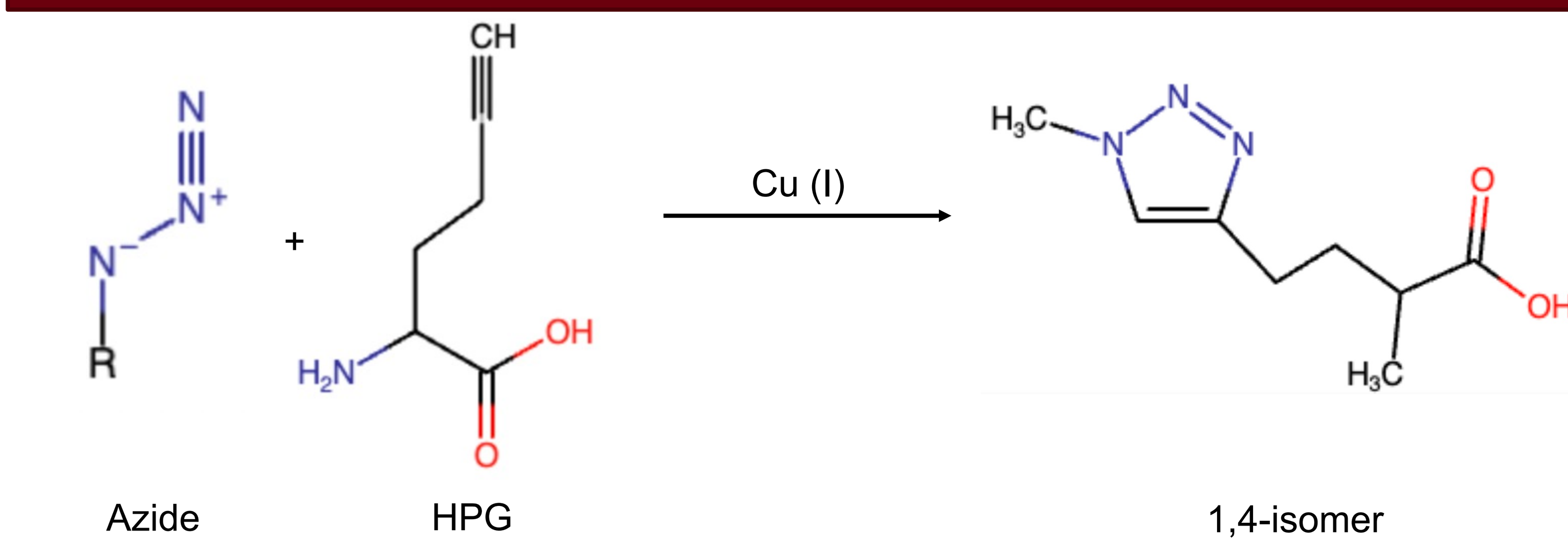

Figure 1. Visualization of the copper-catalyzed click reaction mechanism

Three wild-type cell lines, three cell line with known translation defects (*YARS2*, *MRPL3*, *MTFMT*), one cell line that had previously been found to have a translation defect with unknown genetic origin, and one cybrid cell line were utilized. Three biological replicates of each cell line were plated with methionine-free media. Emetine, a eukaryotic translation elongation inhibitor, was added to stop cytosolic translation. Then a "pulse" of alkalyne-containing methionine analogue, homopropargylglycine (HPG) was added for four hours before whole protein isolation and harvesting. Nascent polypeptides therefore incorporated HPG instead of methionine. HPG then "clicked" to an azide-functionalized fluorophore using a Buffer Kit. Therefore, fluorescence intensity in each nascent mitochondrial peptide is proportional to its mitochondrial synthesis rate during the set reaction time.

RESULTS

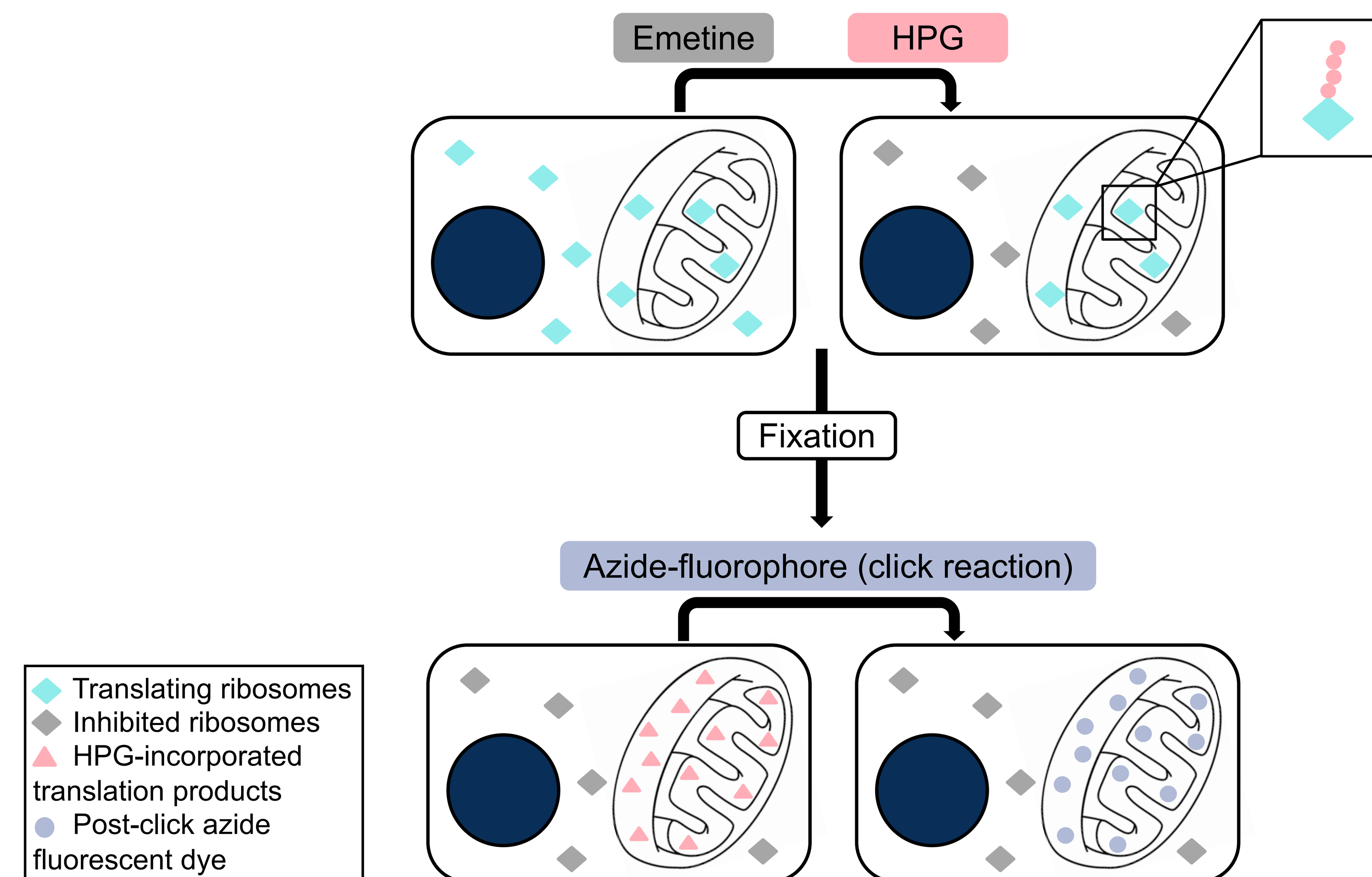

Figure 2. Schematic presentation of the approach. Cytosolic translation is inhibited with emetine, while mitochondrial ribosomes are allowed to incorporate the alkyne-containing methionine homolog (HPG) into newly synthesizes proteins. In fixed cells, HPG moieties are clicked to an azide-conjugate fluorophore through copper-catalyzed Huisgen cycloaddition (click) and can be visualized microscopically.

Cell type/mutation
Cybrid
Fibroblast control
Fibroblast control
Potential translation defect
YARS2 (mt tyrosyl-tRNA synthetase) defect
MTFMT (mitochondrial methionyl-tRNA formyl transferase) defect
MTFMT parent line (control)
MRPL3 (mitochondrial ribosomal protein L3) defect [6]

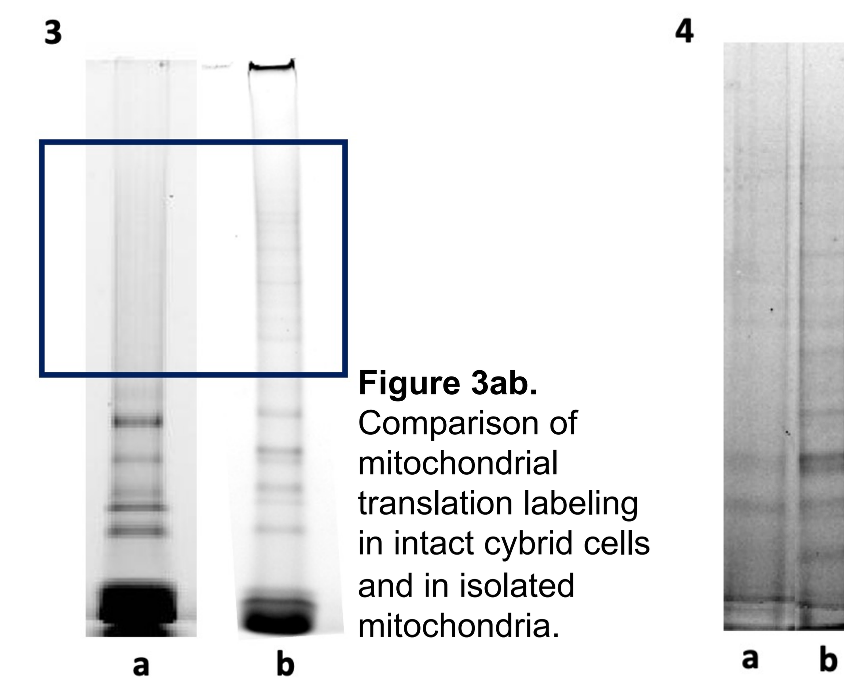

Figure 3ab. Comparison of mitochondrial translation labeling in intact cybrid cells and in isolated mitochondria.

Figure 4ab. Comparison of mitochondrial translation labeling in intact cybrid cells treated with emetine and chloramphenicol and in intact cybrid cells treated with emetine.

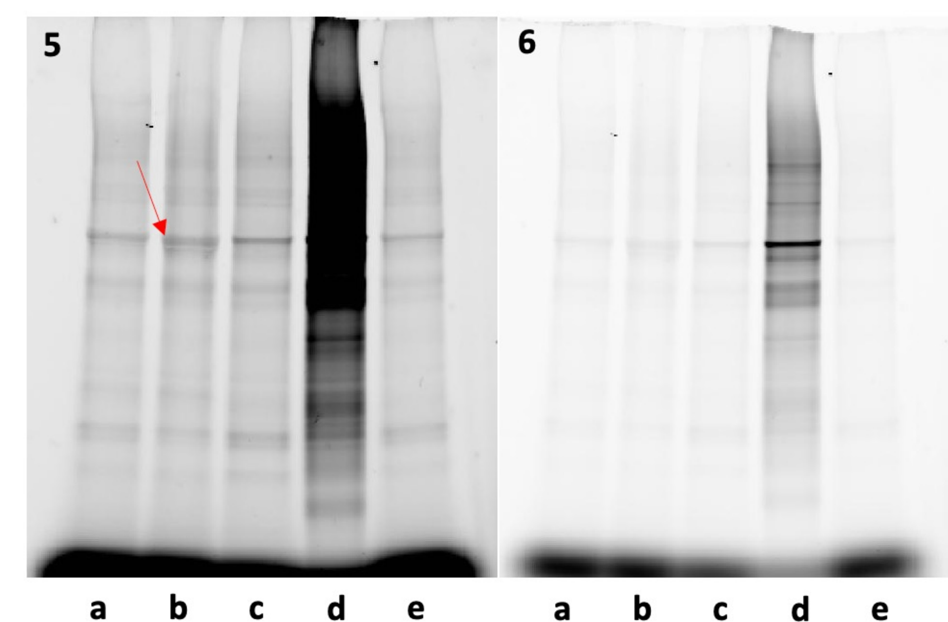

Figure 5abcde. Comparison of mitochondrial translation labeling in cells with a potential translation defect, cells with *YARS2* defect, fibroblast control cells, fibroblast control cells treated with chloramphenicol and no emetine, and fibroblast control cells treated with both emetine and chloramphenicol.

Figure 6abcde. The same image as Figure 5, but visually optimized to show the bands present in the lane with fibroblast cells treated with only chloramphenicol.

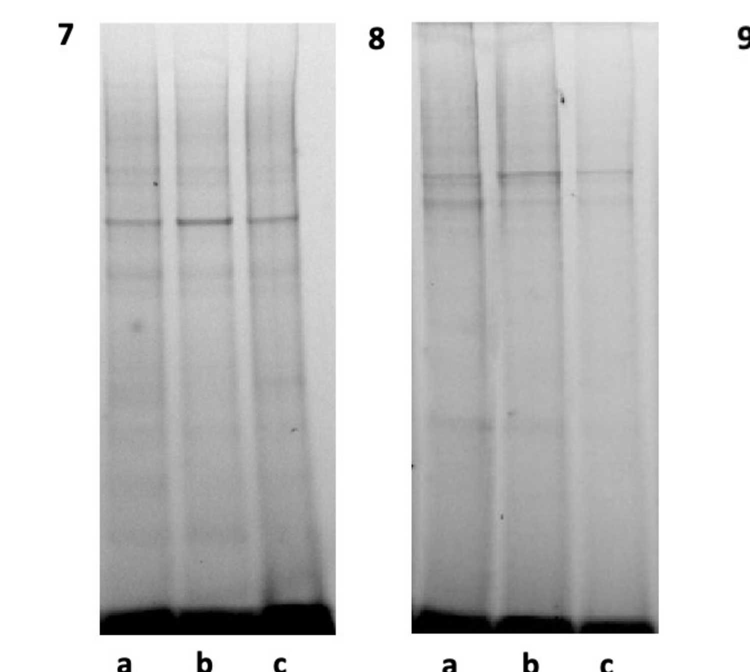

Figure 7abc. Comparison of mitochondrial translation labeling in cells with *MTFMT* defect, cells with *MRPL3* defect, and in fibroblast control cells.

Figure 8abc. Comparison of mitochondrial translation labeling in cells with *MTFMT* defect, cells with *MRPL3* defect, and in fibroblast control cells.

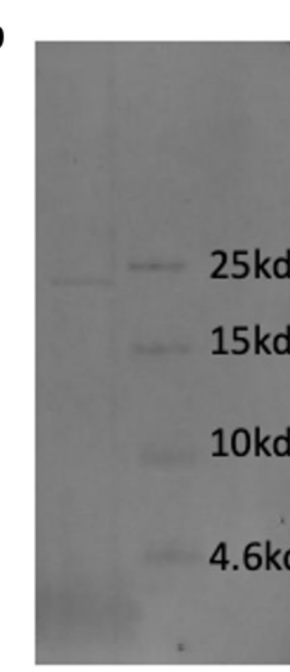

Figure 9. Western blot of fibroblast control cells probed with MT cytochrome oxidase 2. The sample was first run on a gel electrophoresis and then transferred to a PVDF membrane.

DISCUSSION

A global decrease in fluorescence intensity was observed in the bands of subject cell lines, consistent with their known translation defects. However, interestingly, cell lines were distinct from each other: in the YARS2 mutant cell line there was consistently an accessory low molecular weight band, putatively correlating to mitochondrial tyrosyl-tRNA synthetase that was not present in any other cell line (red arrow in figure 5). We hypothesize that this corresponds to premature protein-truncation due to the relative unavailability of tyrosyl-tRNAs. Overall, we conclude that click chemistry can be utilized for a viable and sensitive semi-quantitative mitochondrial translation assay, but further testing is required to explore its sensitivity and ability to discriminate among different translation defects.

One downside to this assay is that decreases in fluorescence intensity can be subtle and challenging to fully quantify. Our future aims include developing a more fully quantitative assay with internal standards for normalization. A first step is to complete western blot analysis with multiple antibodies for other mitochondrial encoded proteins, such as ATP6, ATP8, cytochrome c oxidase subunits, and more. Another future avenue is to focus on a SILAC (stable isotope labeling by amino acids in cell culture) approach. Overall, we conclude that non-radiographic approaches to mitochondrial translation in accessible tissue (fibroblasts) can produce reproducible results and may be a viable direction for clinical assay development.

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