Charcot-Marie Tooth Neuropathy Causing Mutations in Histidine tRNA Synthetase Increase Stress Granule Number

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INTRODUCTION

Charcot-Marie-Tooth neuropathy (CMT) is a heritable disorder that manifests in the development of progressive distal neuropathy. While CMT is one of the most common inherited neurological diseases, no cure currently exists and treatment is limited to physical therapy and the use of orthopedic devices. Further, while several deleterious mutations have been associated with the development of CMT, the pathogenic mechanism for many of these variants remains unknown.

Mutations in aminoacyl tRNA-synthetases are associated with CMT

The primary function of aminoacyl tRNA-synthetase enzymes (AARSs) is the charging of tRNA molecules with their cognate amino acid for use in translation. Mutations in many of these proteins are associated with the development of CMT.

Some CMT associated mutations do not cause loss of aminoacylation activity

- Yeast complementation studies have demonstrated the ability of mutated AARSs to complement strains lacking functional AARS protein
- This suggests an alternative pathogenic mechanism for this subset of CMT variants

The role of tRNA synthetase localization to stress granules remains unknown

- Stress granules are mRNA-protein granules that form under a variety of stress conditions, it has recently been found that AARS proteins localize to these granules
- Stress granule like assemblies of protein and RNA are observed in many neurodegenerative diseases, such as ALS
- This suggests that the localization of tRNA synthetase to stress granules is of important consideration when investigating the pathology of CMT

Mutations altering mRNA binding may cause misregulation of stress granules

- Proteins play an important role in the regulation of gene expression via modulation of transcriptional and translational activity
- mRNA binding assays in yeast and mammalian cells have demonstrated mRNA binding activity in several tRNA-synthetases, including histidyl tRNA-synthetase (HTS1) and tyrosyl tRNA-synthetase (Tys1)
- Stress granule formation sequesters protein and mRNA away from the cytoplasm during stress conditions
- If mutations in HTS1 alter mRNA binding, they may affect the relocalization of mRNA into stress granules, causing misregulation of translation
- This misregulation of mRNA-protein assemblies is commonly associated with neurological diseases

OBJECTIVES

1. Identify changes in stress granule formation caused by CMT associated mutants of Hts1 and Tys1
2. Investigate the stress granule localization of other tRNA synthetases in yeast

PREPARATION OF YEAST FOR FLUORESCENT MICROSCOPY

CMT Causing Mutations of Hts1

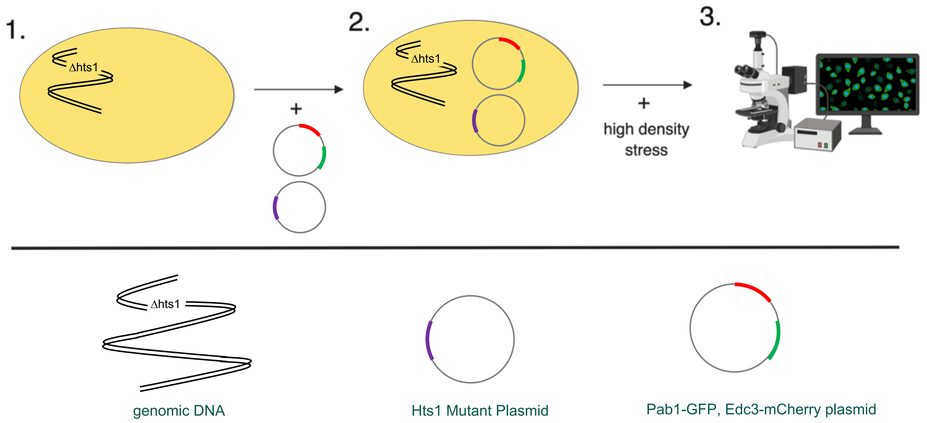

Figure 2: Preparation for fluorescent microscopy by transforming mutant Hts1 plasmid and plasmid with stress granule and P-body markers and applying high-density stress conditions.

tRNA-Synthetase Localization to Stress Granules

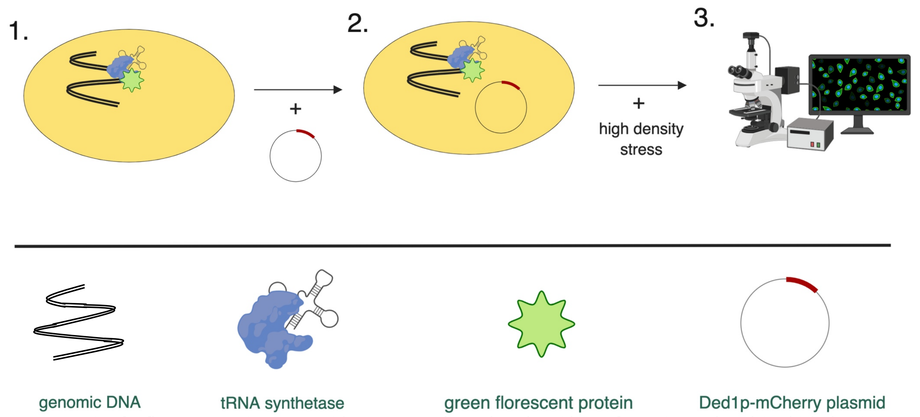

Figure 3: Preparation for fluorescent microscopy with stress granule marker plasmid transformation and applying high-density stress conditions.

EXPLANATION OF METHODS

Transformation with mutant HTS1 plasmids

- Leu2 marked plasmids with CMT associated variants of Hts1 were obtained from the Antonellis Lab at University of Michigan Medical School, transformed into *E. coli* and cultured to increase concentration
- The HTS1 mutant plasmids were then purified (Mini Prep) and transformed into a heterozygous null strain of Hts1
- Similar strains are currently being created for CMT associated variants of Tys1

Primary selection of tRNA synthetases for GFP-fusion

- 11 yeast strains were plated, each with a different tRNA synthetase-GFP (Ths1, Tys1, Frs1, Hts1, Vas1, Ses1, Ala1, Wrs1, Mes1, Gus1, Ils1)

Microscopy following high density growth stress

- Mutant HTS1 yeast were transformed with a plasmid containing fluorescently labeled stress granule (Pab1-GFP) and p-body (Edc3-mCherry) markers.
- The proteins Pab1 and Edc3 are markers of stress granules and p-bodies respectively, allowing for visualization of these intracellular assemblies via the fluorescent tags GFP and mCherry
- Cells were cultured in for approximately 48 hours. High OD served as a stressor to the cells.
- Wet mounts were prepared and imaged using a Zeiss Axioimager Epifluorescence Microscope (Imager Z1)
- Images were analyzed using Image J. Stress granules were counted by a researcher unaware of sample identity.

PRELIMINARY RESULTS AND MICROSCOPY

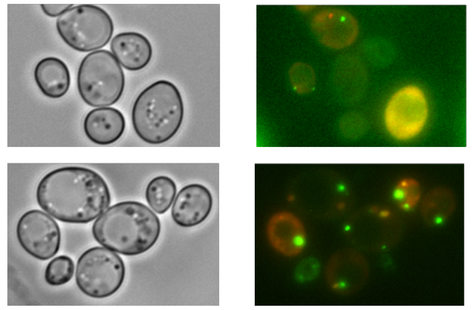

Figure 3: Preliminary Microscopy for mutation D175E. Top Left: WT White Light; Top Right: WT Overlay of Edc3-m-Cherry (red) with Pab1-GFP (green); Bottom Left: D175E White Light; Bottom Right: DI175E Overlay of Edc3-m-Cherry (red) with Pab1-GFP (green).

Summary of Preliminary Results

- The desired strains were successfully synthesized
- Of the five mutant strains tested, T132I, P134H, D175E, AND D364Y showed a significant increase in stress granule formation when compared to wild type cells.

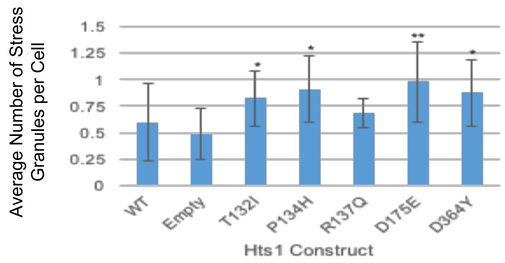

Figure 4: Bar graph displaying average stress granules observed per cell under high density growth conditions. Error bars are standard deviations.

DISCUSSION: IMPLICATIONS OF FINDINGS

- Mutations manifesting in delayed recovery of stress granules after alleviation of stress have also described as pathogenic in other neurological diseases
- Increased stress granule formation caused by the Hts1 mutations could alter mRNA metabolism and gene expression
- IF AARS binding plays an important role in the relocalization of mRNAs to stress granules, mutations in the binding domain could alter mRNA fate and cause shifts in decay or translation

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