The Ribosome Assembly Disorder Bowen-Conradi Syndrome Includes Changes in Protein Translation

Courtney Geer, Derek Harris, Joel Hardy, and J. Michael Charette

Department of Chemistry, Brandon University, Brandon, Manitoba, Canada

INTRODUCTION

Bowen-Conradi Syndrome (BCS)
- Ribosome assembly disorder (ribosomopathy)
- Lethal genetic disorder in the Hutterite population
- 1 out of every 355 lives births (1)
- Autosomal recessive disorder (1)
- Due to a missense D86G amino acid change in the Emg1 protein (2)

Emg1 Protein
- Member of the SSU processome in ribosome assembly
- Methylates the 18S rRNA at residue 1191 in *S. cerevisiae*, 1248 in humans (3)
- Ntd 1191 essential to translational fidelity during decoding
- Disease variant may cause issues in translation, leading to changes in the proteome

AIM

- To comparatively analyze the translation of normal and BCS ribosomes
- Through this analysis we can identify translational differences between normal and BCS ribosomes

BACKGROUND

Figure 1. Presentation of Bowen-Conradi Syndrome
- Failure to thrive/grow
- Small size (5.3 kg at 6 years)
- Developmental delay
- Microcephaly
- Micrognathia
- Flexed limbs and digits

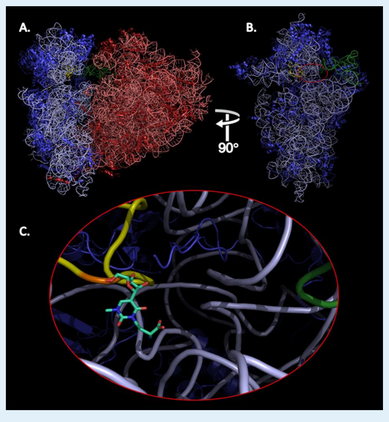
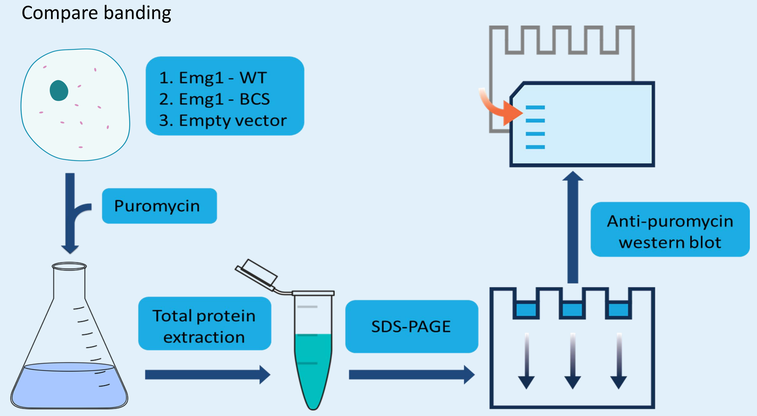


Figure 2. Modification site of Emg1
- Residue 1191 on 18S rRNA
- Isomerized from U to Ψ by snR35 (4)
- Methylated by Emg1 (4)
- Acetylated by Tsr3 (4)
- Close proximity to P-site

in decoding region
- Critical for translation

METHODS

RiboPuromycylation Assay
- Grow yeast WT and BCS cells in dextrose
- Treatment with puromycin for incorporation into nascent proteins (5)
- Extract total protein
- SDS-PAGE
- Anti-puromycin western blot
- Compare banding



RESULTS

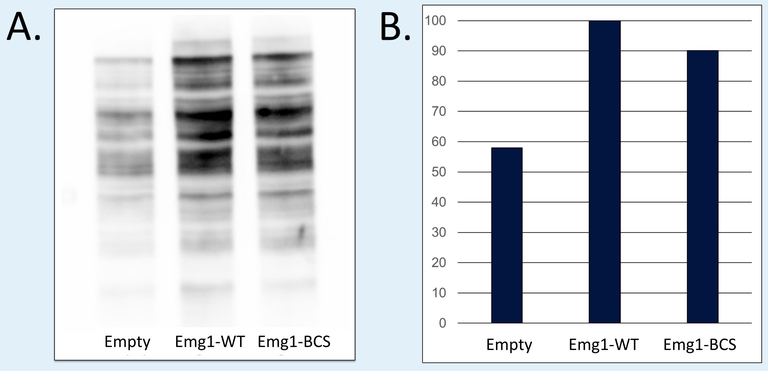


Figure 3. (**A**) Anti-puromycin western blot showing nascent peptides labeled with puromycin. All treatments show similar banding patterns, but different band intensities. Empty shows the lightest banding, Emg1-WT banding is the darkest, and Emg1-BCS shows an intermediate banding intensity. (**B**) Densitometry analysis of the anti-puromycin western blot quantifies the western blot results presented above. BCS cells demonstrate a decreased translational capacity compared to WT cells.

CONCLUSIONS

- RiboPuromycylation assay compared translational capacity of ribosomes assembled with either WT or BCS Emg1 protein
- Emg1-WT cells show the most intense banding, giving a baseline for normal translational function
- Empty cells show the lightest banding, demonstrating the negative effect of complete depletion of the Emg1 protein
- Emg1-BCS shows an intermediate banding intensity, indicating sub-optimal translation in BCS cells
- Suggests that decreased translational capacity is part of the molecular pathogenesis of BCS
- Proteomic analysis confirms translational changes in BCS

FUTURE DIRECTIONS

Characterize the mechanism of this decrease in translational capacity in BCS:
- Stop-codon read-through?
- Frameshifting?
- Amino acid mis-incorporation?
- Decreased translation rates?
- Decreased initiation?

Further studies using translation reporter plasmids will elucidate the molecular pathology of BCS, which may lead to treatment strategies.

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