SAM Supplementation Partially Rescues a Yeast Model of the Bowen-Conradi Syndrome Ribosome Assembly Disorder

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INTRODUCTION

Bowen-Conradi Syndrome (BCS)
- Ribosome assembly disorder (ribosomopathy)
- Lethal autosomal recessive disorder (1)
- Exclusive to the Hutterite population (1/355 live births) (1)
- Due to a missense D86G amino acid change in the EMG1 protein (2)

EMG1 Protein
- Pseudouridine methyltransferase protein (homodimer) & a member of the SSU processome during ribosome assembly
- Methylates the 18S rRNA at residue 1191 (yeast) & 1248 (human) as step 2 of a 3 step "hypermodification" process (3)
- Hypermodified residue is essential to protein translational capacity & fidelity
- Methyl group donated by the small molecule *S*-adenosylmethionine (SAM), which is bound by EMG1 in a pocket between dimer subunits
- SAM is synthesized by the *S*-adenosylmethionine synthetase SAM2 in yeast

Structural Rigidification
- Rigidification/stabilization of protein structure has been seen following small molecule binding in other studies (4)
- Does binding SAM stabilize the structure of BCS EMG1?

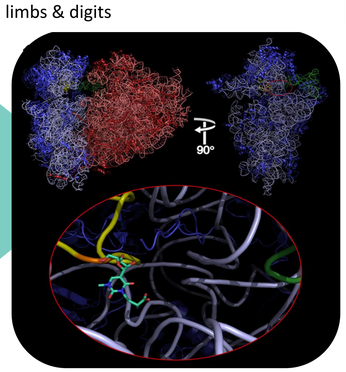
OBJECTIVES

- To investigate the growth of SAM-supplemented & un-supplemented wild type (WT) & BCS cells
- To identify any translational differences between SAM-supplemented & un-supplemented wild type (WT) & BCS cells

BACKGROUND

Figure 1. A 3-year-old female child presents with Bowen-Conradi Syndrome (right) next to her 4-month-old sibling (left); BCS is characterized by:
- Failure to thrive/grow
- Developmental delays
- Craniofacial malformations
- Flexed limbs & digits

Figure 2. Modification site of Emg1:
- Residue y1191/h1248 in 18S rRNA
- Hypermodified U:
1. Isomerized from U to Ψ by snR35/ACA13 (5)
2. Methylated by EMG1 (5)
3. Acetylated by TSR3 (5)
- Close to the P-site in the decoding region
- Critical for translation



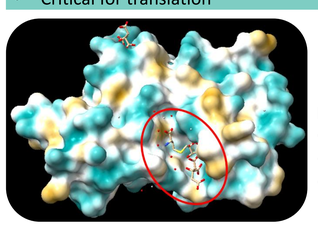


Figure 3. EMG1 SAM-binding pocket:
- Required for catalytic function (methylation)
- Located between dimer subunits
- Dimer interface also critical to 18S rRNA binding

METHODS

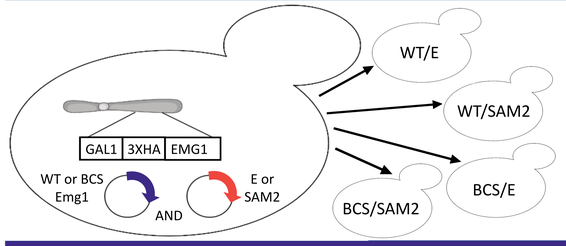
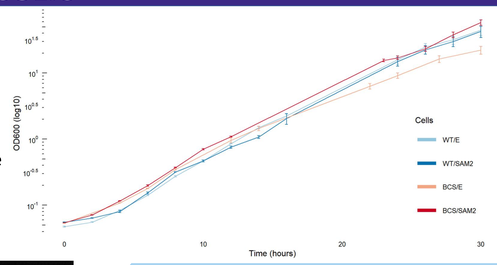


Figure 4. WT/BCS yeast model system with & without the ability to overexpress SAM2. The cells are an Emg1 depletion strain where the endogenous Emg1 is under the control of a GAL promoter. Each cells type contains two plasmids encoding either WT or BCS Emg1 **and** SAM2 or an empty vector (E). The SAM2 gene is under the control of a GPD promoter, which is the strongest promoter in yeast.

RESULTS

Figure 5. Growth curve for SAM-supplemented and un-supplemented WT & BCS cells. Cells were grown in liquid media & optical density (OD) was measured intermittently over a 30-hour period, post-Emg1 depletion. There is a 2.6-fold increase in growth for SAM-supplemented BCS cells (BCS/SAM2) compared to their un-supplemented counterparts (BCS/E), but no difference in the growth of SAM-supplemented (WT/SAM2) & un-supplemented WT cells (WT/E).



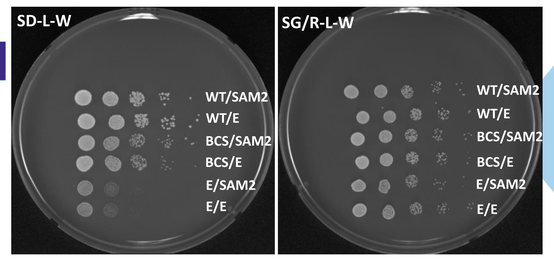
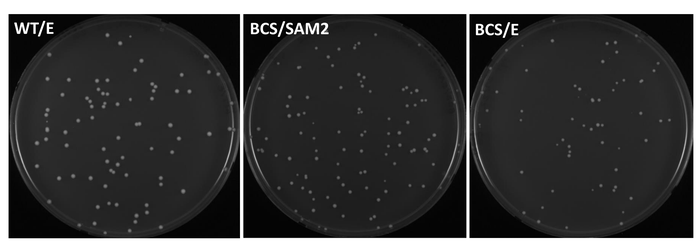


Figure 6. Dot plates showing differences in growth between cell types. Cells were serially diluted (10^{-2} to 10^{-6}) & spotted on experimental (left) & control (right) plates. There is an increase in growth of BCS/SAM2 cells compared to BCS/E cells, but the opposite is seen in WT cells. BCS/SAM2 cells also show larger colony size compared to BCS/E cells. Empty (E) cells were also plated to show the lack of growth when Emg1 is absent.

Figure 7. Colony size assay showing differences in colony size between cell types. Cells were diluted (1:5000) & plated, then grown & imaged/measured for 5 days. There is an increase in the colony area of BCS/SAM2 cells compared to BCS/E cells. WT/SAM2 cells were not assayed as there was no difference in growth compared to WT/E cells in the growth curve & dot plate assays.



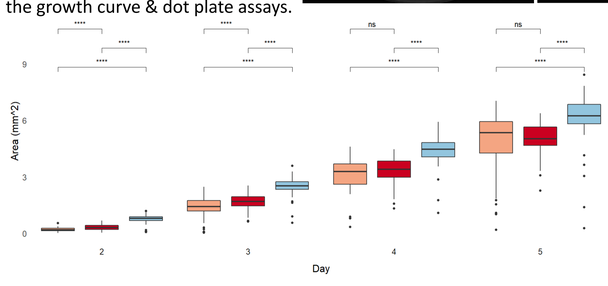


Figure 8. Boxplots showing the average colony area of each cell type for the first 5 days of growth. For days 2 & 3, average colony area is significantly different ($P < 0.001$) between each cell type. WT/E cells are consistently the largest, followed by BCS/SAM2 cells, while the BCS/E cells are the smallest. On days 4 & 5, there is no significant difference between the supplemented & un-supplemented BCS cells.

CONCLUSIONS

- Methylation of the pseudouridine residue is possible since Emg1 binds the small molecule SAM in a pocket between its dimer subunits
- Small molecule binding has been found to rigidify/stabilize protein structure in other studies, perhaps suggesting that Emg1's binding of SAM rigidifies or stabilizes its structure
- Structural stabilization of the Emg1 variant protein associated with Bowen-Conradi Syndrome may improve its function
- SAM-supplementation (through overexpression of the SAM2 gene responsible for its synthesis) resulted in a slight improvement of the BCS growth defect in the growth curve, dot plate, and colony area assays
- This improvement was observed as:
  1. An increase in the growth of BCS/SAM2 cells to near-WT levels, and
  2. An increase in colony size/area of the BCS/SAM2 cells, particularly during the 1st few days of growth

FUTURE DIRECTIONS

Supplement WT & BCS cells with a range of concentrations of exogenous SAM to determine:
1. Whether a similar effect on growth is seen using exogenous supplementation
2. The concentration of exogenous SAM that leads to optimal growth

Perform a cycloheximide chase assay to assess BCS Emg1 protein stability compared to the WT protein with & without endogenous SAM:
- Cycloheximide blocks the elongation step during translation, halting protein synthesis
- Measuring protein decay over time provides information about stability

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