The Bowen-Conradi Syndrome Protein EMG1 Contains an N-Terminal Intrinsically Disorder Region

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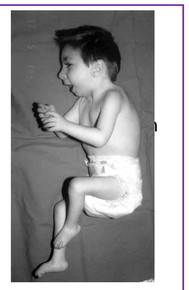
INTRODUCTION



Bowen-Conradi Syndrome (BCS)

-rare, genetic disorder present in the prairie Hutterite population at 1 in 355 live births.

-A congenital ribosome assembly disorder (ribosomopathy) that presents severe developmental delays and death in infancy.

Figure 1[1]. Physical characteristics presented by a child with BCS. Small size (5.3 kg), microcephaly, micrognathia, rocker bottom feet, developmental delay.

-Caused by a D86G missense genetic variant in the essential ribosome assembly protein, EMG1.

Background Information

EMG1 Protein:

-A methyltransferase in the small subunit (SSU) processome, a large ribonucleoprotein complex involved in the biogenesis, assembly, and maturation of the ribosomal SSU.

Intrinsically disordered region of EMG1

-Intrinsically disordered regions (IDR) are functional areas of proteins that do not have a defined 3D structure.

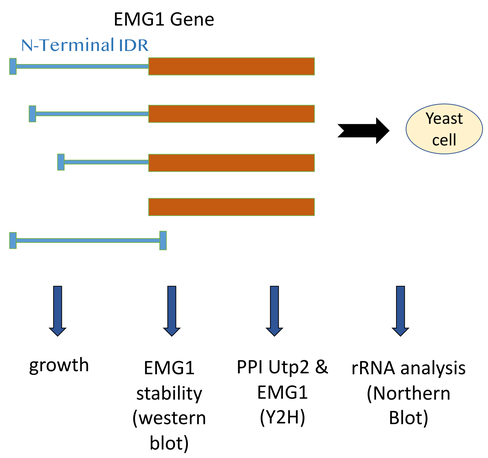
-IDR's play a variety of different roles such as mediating protein-protein interactions.

Objectives

Having predicted an N-terminal IDR in EMG1:

1. **To validate the presence of the N-terminal IDR in EMG1 protein**
2. **Determine the function of the N-terminal IDR in EMG1 in ribosome assembly**

Experimental Design



Objective 1: To validate the presence of an N-terminal IDR

- IDR predictors used to determine the presence of the N-terminal IDR in EMG1.
- Validated by amino acid compositional bias and protein structure.
- **Objective 2: To determine the function of EMG1's N-terminal IDR in ribosome assembly**

-Cloning the full length Emg1 gene into yeast over expression plasmid.

-Create a series of truncation mutations on the EMG1 IDR, removing 5 amino acids with each subsequent truncation.

-Assay by various methods such as dot plates, western blots, Yeast-2-hybrid to determine the IDR function.

RESULTS

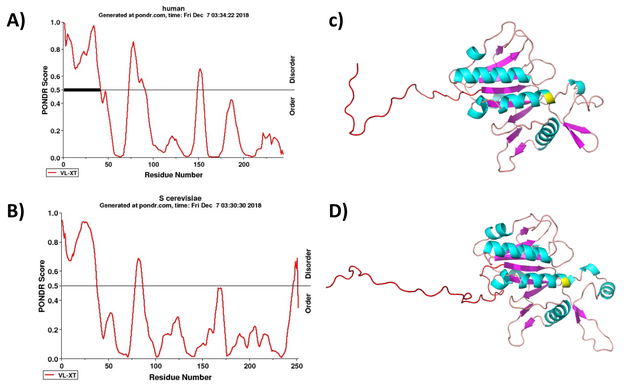


Figure 2: Validating the presence of an N-terminal IDR. **A,B**) Graphs from PONDR program which predicted disordered regions in the human (A) and yeast (B) EMG1 protein. PONDR score of above 0.5 predicts disorder in both humans and yeast at the N-terminal of EMG1. **C,D)** Structure of human (C) and yeast (D) EMG1. EMG1 IDR is presented in red. The BCS mutation, D86G in humans and D90G in yeast is highlighted in yellow.

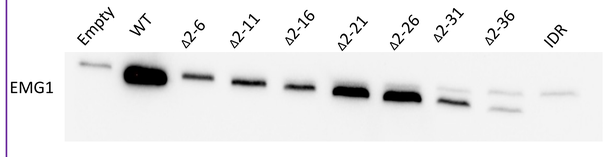


Figure 3: Determining contribution of the N-terminal IDR in EMG1's protein stability. Western blot analysis shows general trend in which protein stability decreases as the IDR truncations get larger.

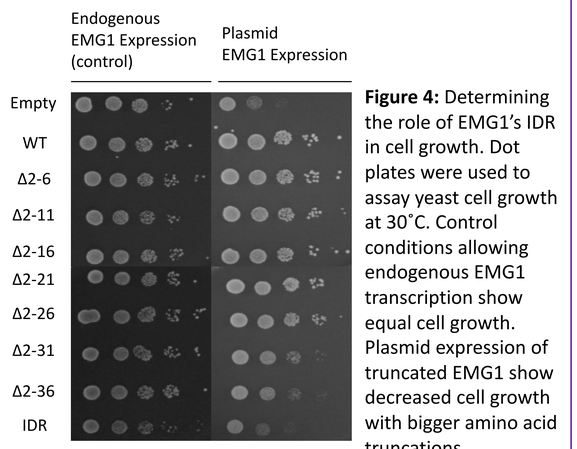


Figure 4: Determining the role of EMG1's IDR in cell growth. Dot plates were used to assay yeast cell growth at 30˚C. Control conditions allowing endogenous EMG1 transcription show equal cell growth. Plasmid expression of truncated EMG1 show decreased cell growth with bigger amino acid truncations.

CONCLUSION

-Using PONDR, a disorder predictor, as well as performing a protein structural analysis, we identified the presence of a novel N-terminal intrinsically disordered region in the EMG1 protein

-We identified that the N-terminal of EMG1, including the IDR is heavily post translationally modified.

-Series of increasing IDR truncations were created and tested in various ways to determine the importance of this disordered region in EMG1. Results show that the IDR is important for both the stability of EMG1 as well as cell growth.

-Experimental analysis of the function of the IDR are ongoing.

References:

(1) Lowry, R., Innes, A., et al (2003). Bowen-Conradi Syndrome: A Clinical and Genetic Study. *American Journal of Medical Genetics,* 120A, 423-428

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