The Bowen-Conradi Syndrome Protein EMG1 Contains an N-terminal Intrinsically Disordered Region

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INTRODUCTION

Bowen-Conradi Syndrome (BCS)

-rare, genetic disorder present in the Hutterite populations of the North prairies.

-Occurs in 1/355 live births

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

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

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

Background Information

EMG1 Protein: A Key Methyltransferase Protein

-A member of the small subunit (SSU) processome, which is a large ribonucleoprotein complex involved in the biogenesis, assembly, and maturation of the ribosomal small subunit. This complex also processes the 18S rRNA that is incorporated into the small subunit of the ribosome.

Intrinsically disordered region of EMG1

-Intrinsically disordered regions (IDR) are functional areas of proteins that do not have the defined 3D structure. Specifically, IDRs lack secondary and tertiary protein structure.

-IDRs play a variety of different roles such as mediating protein-protein interactions and being sites of post-translational modifications.

Aims

Having predicted an N-terminal IDR in EMG1:

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

ACKNOWLEDGEMENTS

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CHRD Child Health Research Days

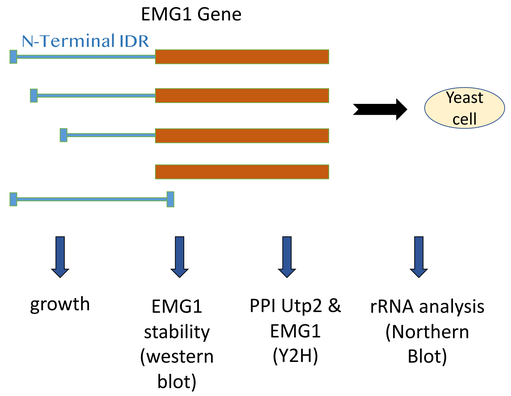
Brandon University Founded 1899

NSERC CRSNG

INNOVATION.CA Canada Foundation for Innovation | Fondation canadienne pour l'innovation

The Charette Ribosome Assembly and Ribosomopathy Lab

Methods



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

- IDR predictors such as Predictor of Natural Disordered Regions (PONDR) were used to determine the presence of the N-terminal IDR in EMG1.
- IDR was validated by amino acid compositional bias and protein structure analysis.
- Analysis for the presence of post-translational modifications in the region were used to validate this IDR.

Objective 2: To determine the function of EMG1's N-terminal IDR in ribosome assembly

- Cloned the full length Emg1 gene into yeast over expression plasmid.
- Created a series of 8 Emg1 IDR truncations by removing 5 amino acids with each subsequent truncation.
- Function was analysed by various methods, such as dot plates, western blots, Yeast-2-Hybrid, rRNA analysis and Co-IPs

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

RESULTS

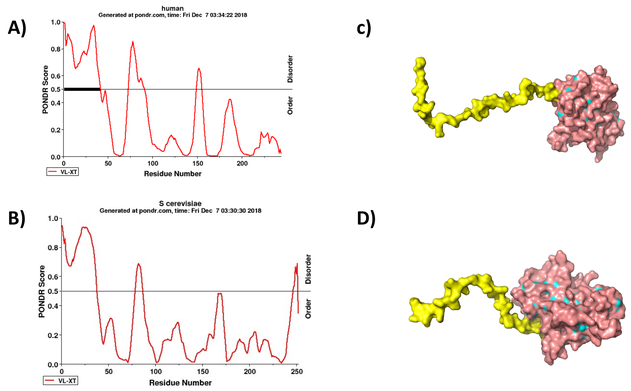


Figure 2: Validation of the presence of an N-terminal IDR. **A,B**) Graphs from PONDR program which predicted disordered regions in the human (A) and yeast (B) EMG1 protein. PONDR score of above 0.5 predicts disorder in both humans and yeast at the N-terminal of EMG1. **C,D)** Structural analysis of human (C) and yeast (D) EMG1 revealed lack of 3D structure in the N-terminal region (yellow). Structured region of EMG1 is pink.

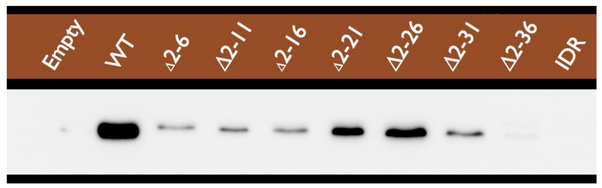


Figure 3: Determining the contribution of the N-terminal IDR in EMG1's protein stability. Western blot analysis shows general trend in which protein stability decreases as the IDR truncations get larger.

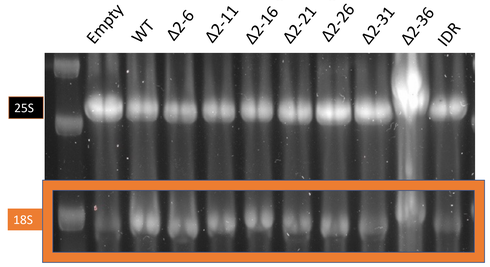


Figure 4: Determining the importance of the N-terminal IDR of EMG1 in SSU processome functioning and ribosome assembly. General trend of rRNA gel analysis shows that as IDR truncations get larger, abundance of 18s rRNA decreases.

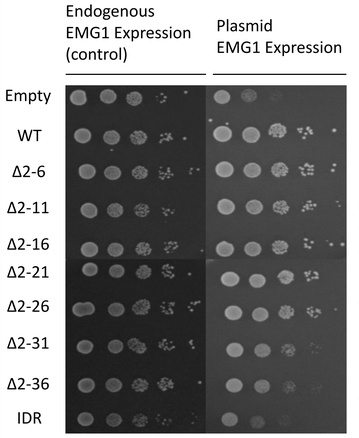


Figure 5: Determining the role of EMG1's IDR in cell growth. Dot plate methods were used to assay yeast cell growth at 30˚C. Control conditions that allowed endogenous EMG1 transcription show equal cell growth. Plasmid expression of truncated EMG1 show decreased cell growth with bigger IDR truncations.

CONCLUSION

Validating the presence of the N-terminal IDR

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

We identified that this N-terminal region of EMG1 lacks secondary and tertiary structure and is heavily post translationally modified.

Determining the Function of the N-terminal IDR

To determine the function of this novel IDR, a series of truncations were created in which the IDR was increasingly shortened from the N-terminal side. These IDR truncations were then tested in various ways to determine the importance and contribution of this disordered region in EMG1 to protein stability, cell growth, and SSU processome functioning.

Results indicate that the IDR contributes to protein stability of EMG1 as seen by the general decreasing abundance of EMG1 in the western blot as the IDR is increasingly truncated. The dot plate assay and RNA gel analysis revealed that the EMG1 IDR is necessary for cell growth, and assembly and processing of ribosomes. Investigations into the functions of this IDR in mediating protein-protein interactions by Yeast-2-Hybrid methods are ongoing.