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Abstract Title

Exploration of novel EMG1 variants using a *Saccharomyces cerevisiae* model offers preliminary evidence for the essential role of multiple residues in ribosome assembly

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Background

EMG1 is an essential component of the small-subunit processome (SSUP) involved in 18S rRNA processing and ribosome assembly. Bowen-Conradi syndrome (BCS), an autosomal recessive developmental disorder, is caused by a homozygous c.257A>G (p.D88G) EMG1 variant. No other EMG1 variants have ever been described. We hypothesized that other EMG1 variants have severe consequences for ribosome assembly and are incompatible with life.

Objective

This project aimed to evaluate the functional impact of other EMG1 variants using a yeast model.

Methods

Site-directed mutagenesis was used to introduce ten novel Emg1 variants (R88K, R88L, R88Q, P89G, P89A, D90E, D90P, D90N, D90L, R132Q) and the equivalent BCS variant (D90G) into a yeast over-expression plasmid. These were functionally assessed using growth complementation assays, protein

stability by western blot, rRNA by gel electrophoresis, and structural consequences using AlphaFold.

Results

Cells expressing the BCS variant display a slight decrease in growth while other mutants showing significantly reduced growth. Western blotting revealed comparable Emg1 levels in wild-type and BCS strains, and reduced levels to no detectable Emg1 in novel mutant strains. The BCS strain exhibits 18S rRNA levels comparable to wild-type, while other mutants exhibit lower levels. Mutant strains demonstrated reduced total protein production. Structural modeling predicted altered interactions with the RNA ligand, disruption of the R88-D90 salt bridge, and loss of hydrogen-bonding with the S49 residue in the various mutant models.

Conclusion

Emg1 variants showed reduced growth, 18S rRNA levels, total protein levels, and reduced or no detectable Emg1 compared to the wild-type and D90G strains. This impact on RNA and protein levels may be due to reduced interactions with the RNA ligand; furthermore, hydrogen-bonding with the S49 residue may be important for ribosome assembly. These results provide preliminary evidence for the essential roles of other Emg1 residues in ribosome assembly.