Modelling, Cloning, and Expression of the J domain of *C. elegans* Rme-8 Protein

Madison Thornhill, My Tran, Bingbing Xiao, Odutayo Odunuga (Advisor)

Department of Chemistry and Biochemistry
Stephen F. Austin State University, Nacogdoches, Texas

Stephen F. Austin State University
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Department of Chemistry and Biochemistry

Abstract

Rme-8 is a J domain-containing plasma membrane protein that is required for endocytosis in various cells. The J domain is a characteristic structural motif found mainly in heat shock protein 40 (Hsp40 or DnaJ) and other proteins such as Rme-8. Within the J domain is a tripeptide, the HPD motif, that is required by the J-domain protein to interact with and stimulate the ATPase activity of Hsp70, a major cellular chaperone. Rme-8 protein in *C. elegans*, CeRme-8, has not been identified with a particular Hsp70 partner. CeHsp70-1 is the only cytosolic Hsp70 in *C. elegans*, therefore, we hypothesize that it is the binding partner for the J domain of CeRme-8. To test this hypothesis, we first need to express and purify the J domain of CeRme-8. We report herein the successful cloning and expression of the J domain of CeRme-8. Computer modelling revealed that the amino acid sequence of the J domain of CeRme-8 folds into the canonical J domain conformation, containing the HPD tripeptide. Complementary DNA of the J domain of CeRme-8 was cloned into the pGEX-Tev-KG plasmid, in-frame with the gene for glutathione-*S*-transferase (GST), to yield a GST-CeRme-8 fusion protein. IPTG-induced expression of the expected 37-kilodalton fusion protein was confirmed by both SDS-PAGE and western blotting using antibody against GST. Work is ongoing to develop a protocol for purification of both GST-tagged and untagged J domains of CeRme-8. Future work will involve testing the effect of the J domain protein on the ATPase activity of CeHsp70-1.

Sequence Alignment and Modelling of CeRme-8 J Domain

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CeRme8J   REILS-VDLTNEEHRKPAFIRRQYYKLAAKYHPDKNPEGREMFERINAAYELL 52
DnaJ      QEALNVFGLSG--ELTEKDIKAAYRKAALKYHPDRNPLGAELMKAVNAAFDVL 51
          :* *. . *:   . .   *:  * * * *****:** * *::: :***:::*
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Fig 1A: CeRme-8 J domain contains the canonical HPD (yellow highlight) motif and other critical amino acid residues necessary for interaction with heat shock protein 70.

Sequences of the J domain regions of *E. coli* DnaJ (CAD6022412.1) and *C. elegans* Rme-8 (CAA99831.3) proteins were obtained from NCBI and aligned using Clustal Omega.

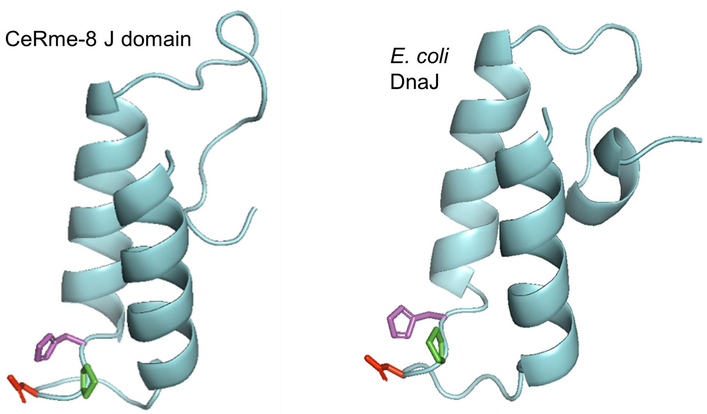

Fig 1B: CeRme-8 J domain structure model (left) resembles the prototype, *E. coli* DnaJ domain (right). The HPD motif sidechains in each structure are represented as sticks.
H (histidine) is magenta, P (proline) is green and D (aspartic acid) is red.

Modelling was performed using Protein Homology/analogY Recognition Engine V 2.0 (Phyre2) software. Coordinates were downloaded and the structures visualized using PyMol.

CeRme-8 J Domain Docks in the J Domain-Binding Site of CeHsp70-1

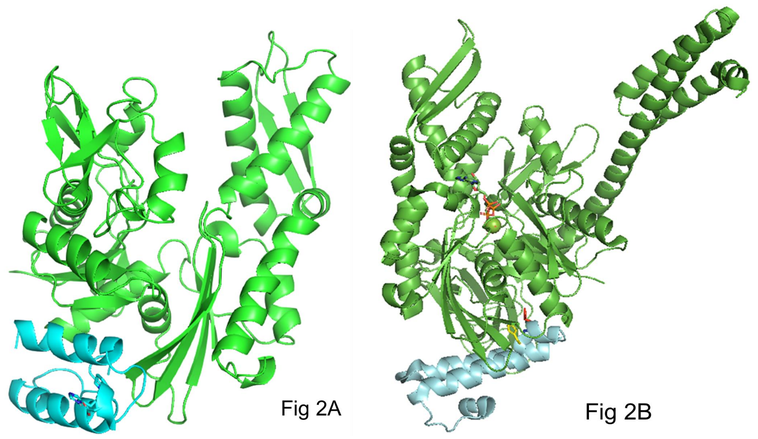

Fig 2A Fig 2B

Fig 2: Modelled CeRme-8 J domain docked in the expected cleft opposite the nucleotide-binding cleft of CeHsp70-1 (2A). This complex is similar to the *E. coli* DnaK and DnaJ interaction complex (2B).

Docking was performed by submitting the PDB files of modelled CeHsp70-1 (protein) and CeRme-8 J domain (ligand) to ClusPro 2.0 protein-protein docking server. DnaK-DnaJ complex was obtained from the Protein Data bank, ID 5NRO. Both complexes were rendered and visualized by PyMol.

CeRme-8 J Domain Gene Cloned into pGEXTevKG Expression Vector

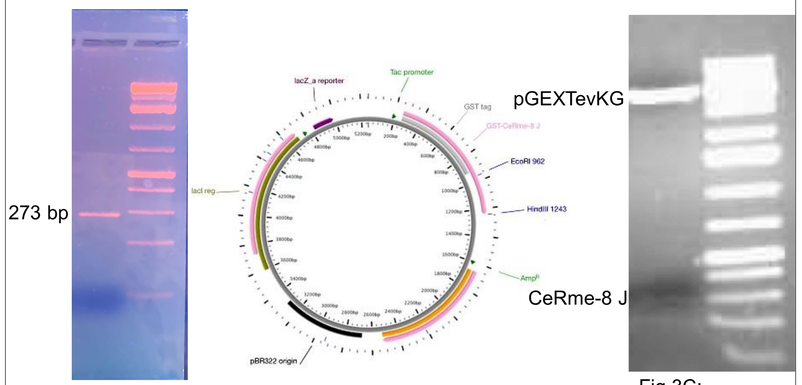

Fig 3A: CeRme-8 J PCR

Fig 3B: Plasmid Construct

Fig 3C: CeRme-8 J Cloning

Fig 3A: The DNA sequence coding for the J domain of CeRme-8 was identified by multi-sequence alignment. The sequence was then amplified from the cDNA of full-length CeRme-8 using forward and reverse primers containing *Eco* RI and *Hind* III restriction enzyme sites respectively. Fig 3B: Amplified CeRme-8 J gene containing appropriate overhangs was ligated into the pGEXTevKG, in-frame with the gene for glutathione-*S*-transferase (GST), to generate a construct that would express a GST-CeRme-8 J fusion protein. Fig 3C: Cloning of the CeRme-8 J gene into the pGEXTevKG plasmid vector was confirmed by restriction enzyme digest using *Eco* RI and *Hind* III, as well as by DNA sequencing.

CeRme-8 J domain Expressed in Fusion with Glutathione-*S*-transferase

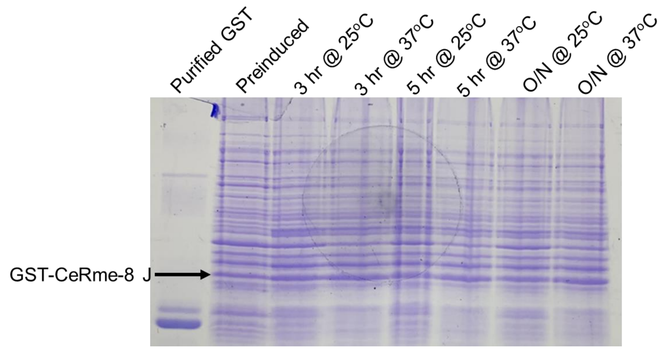

Fig 4A: Expression of GST-CeRme-8 J protein. Actively growing cultures of bacterial cells harboring plasmid construct were chemically induced to express protein at various temperatures by the addition of isopropyl ß-D-1-thiogalactopyranoside (IPTG). Samples of the expression cultures were taken at various times and whole-cell lysates analyzed on SDS-PAGE. The fusion protein was expressed at the expected size of 36 kDa.

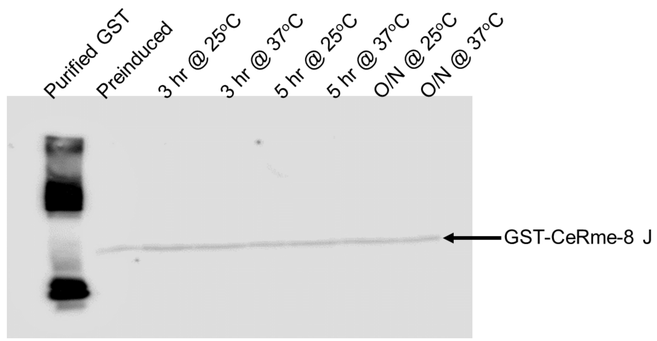

Fig 4B: Confirmation of expression of GST-CeRme-8 J. Whole-cell lysates of bacterial cells expressing GST-CeRme-8 J protein were analyzed on SDS-PAGE. Proteins were transferred onto a nitrocellulose membrane. Expression of the fusion protein was confirmed by immunoblotting using antibody against GST. Multiple bands in the first lane shows the expected oligomerization of GST.

Conclusions and Future Work

1. The J domain of CeRme-8 protein folds into expected conformation and contains the HPD motif.
2. The J domain of CeRme-8 binds to CeHsp70-1 at the predicted site.
3. The J domain of CeRme-8 was successfully cloned and expressed in fusion with GST.
4. Future work will include purification of the protein and testing its effect on the ATPase activity of CeHsp70-.

Acknowledgements

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References

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2. Odunuga et al. Protein Expr Purif 82 (2012)132-137