Life Adapted to the Cold: Isolating Kinesin Sequences from Antarctic Foraminifera

Joseph [redacted][1] and Michael Koonce[2]
[1]Washington & Jefferson College, Washington, PA 15301
[2]Translational Medicine, Wadsworth Center, Albany, NY 12201

Introduction

Macroscopic motion begins at the cellular level with the work of various motor proteins. Kinesin motor proteins move along microtubule filaments (MT) in order to perform tasks from transporting of organelles, to facilitating mitosis. Due to their essential role in cellular processes, disruptions in normal kinesin functioning have been revealed to be involved in certain diseases. These motor proteins have been well documented in organisms at warm temperatures yet there exists little investigation into these proteins with regards to how they function in organisms adapted to the extreme cold. One such group organisms are the Foraminifera, carnivorous protists which inhabit many of Earth's aquatic ecosystems. Though largely recognized for their tests (shells), which play a role in the fossil record and global carbon levels, there exists limited molecular data on forams. Forams collected from sediment of Explorers Cove, Antarctica have been observed to exhibit robust organelle transport activity despite ambient temperatures reaching as low as -1°C. Under similar conditions MT in mammalian cells would depolymerize and the cell would eventually die. To investigate whether there are structural modifications in the kinesins of these Forams which would confer the cold functionality, we attempted to isolate kinesin DNA from the Forams by targeting the motor domain regions of the kinesin heads which are highly conserved across species as well as different kinesin families.

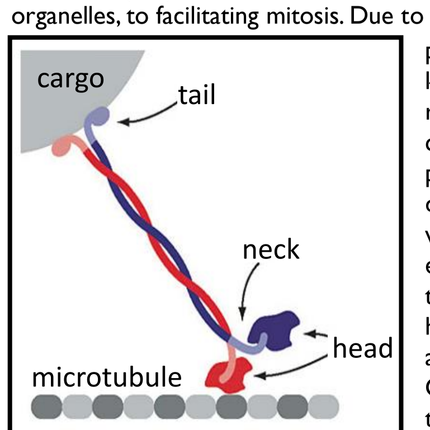
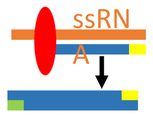


Figure 1. Transport Kinesin Components. Tails bind to cargo. Heads take alternating steps along MT by hydrolyzing ATP, generating force. Adapted from Schwartz 2003.

Methods

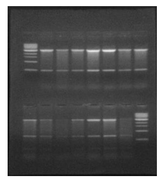
RNA isolated from foram cells using shredder column & 50µL RNase free H_2O

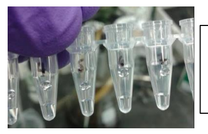
Isolated RNA used as template for reverse transcription-PCR to generate DNA



DNA cloned into competent *E. coli* cells to amplify target sequences

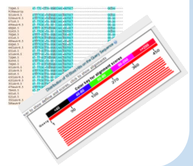
DNA collected from cultures & digested with *EcoR1* to check for vector insertion





DNA with insert isolated & prepped for sequencing

DNA sequences run through Blast and MUSCLE alignment



Materials

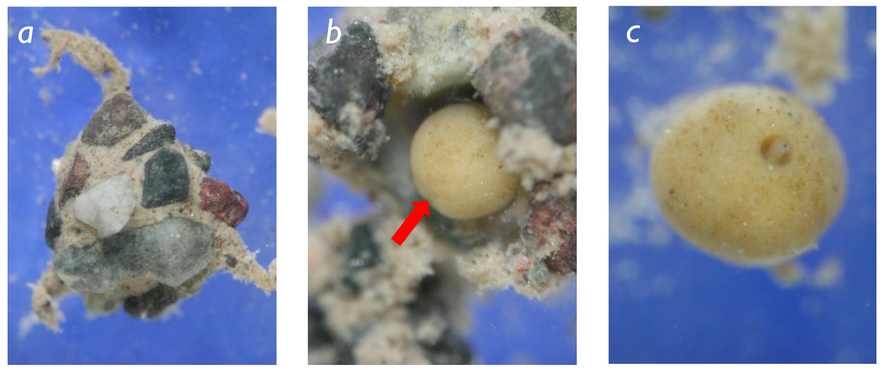


Figure 2. Astrammina rara a. View of intact test. ***b.*** Test opened, revealing foram inside (indicated by arrow). ***c.*** *Astrammina rara* with test removed. Samples used in study had tests removed. Forams provided by Bowser lab. Photo courtesy foramBarcoding, foram database.

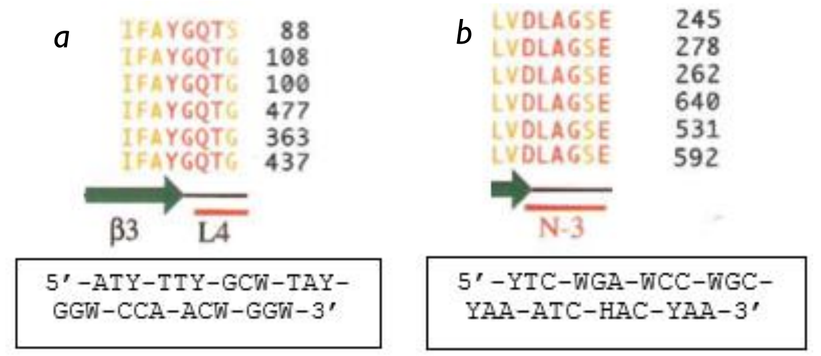


Figure 3. Custom Primers a. Amino acid and DNA sequence of upstream primer ***b.*** Amino acid and DNA sequence of downstream primer. Both regions are conserved motifs involved in nucleotide binding. Primer sequences surround a region of approx. 150amino acids. Adapted from Sablin et al., 1996.

Results

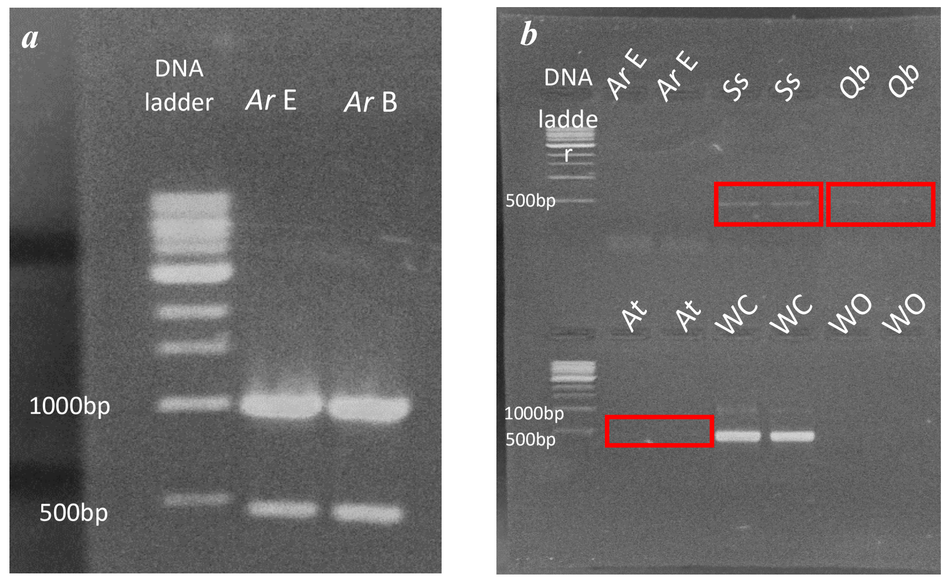


Figure 4. Agarose gel (1.2%) images from RT-PCR product. ***a*** Initial samples of *Astrammina rara* (*Ar*) from ethanol (E) and DNA buffer (B) run with annealing temperature of 52°C. Bands appeared at 500 and 1000bp. ***b*** RT-PCR products from four (4) foram species: *Ar, Silver Saccammina* (*Ss*), *Notodendrodes hyalinosphaira* (*Qb*), and *Astrammina triangularis* (*At*), with two water controls (WC), RT-PCR run with 80µL of RNase free water supplied in RNA extraction kit & WO, RT-PCR run with 10µL RNase free water included in RT-PCR kit for master mix. Bands around 500bp appeared in *Ss*, and faintly in *Qb* and *At* (boxed in red). Bright bands appeared at 500 and 1000bp in the WC. Profile run with 47°C annealing temperature. Gel run with 1kb DNA ladder. Bands were excised, and DNA purified for further analysis.

Table 1: Distribution of Blast Identities

DNA Band Size (base pairs)	Organism	% Identity	No. Queries	% of samples
500	*Sordaria macrospora* Kinesin Protein	80	34	75.6
500	*Fusarium pseudograminearum* Kinesin Heavy Chain	79	2	4.4
500	Other	79-99	3	6.7
500	No Match		4	8.9
1000	*Delftia acidovorans* genome	99	2	4.4

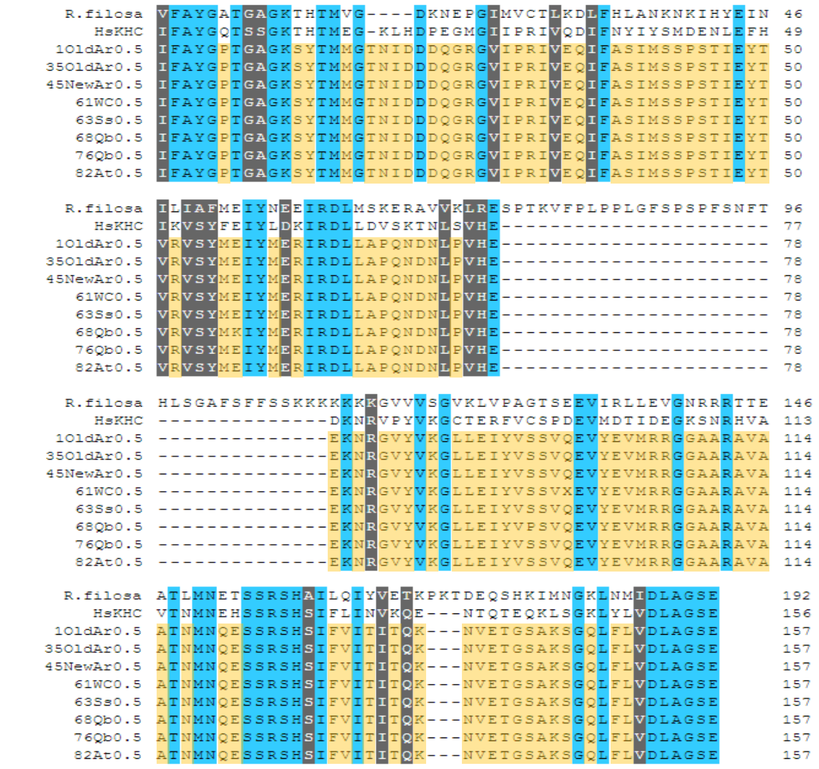


Figure 5. Amino acid alignment of representative samples. Sequences of human and *R. filosa* kinesin were included for comparison (first two lines). Though a freshwater, moderate temperature foram, *R. filose* is the only known sequenced foram. Samples included represent each foram species used. Residues highlighted in blue are completely conserved across all samples; in grey are similar residues. Alignment of just forams, in orange, reveals amino sequences across species are nearly identical. Generated through MUSCLE alignment tool (http://www.ebi.ac.uk/Tools/msa/muscle/)

A total of 45 samples were sent out for sequencing, 43 from 500bp samples and 2 from 1000bp. Sequences were run through a Blast nucleotide query, the results are presented here. A majority (80.0%) of the samples were identified as kinesins but of fungal origins (*s. macrospora* & *f. pseudoaraminearum*). "Other" refers to query outputs which were of prokaryotes or predicted/uncultured samples at 500bp.
*It should be noted that identified species are completely foreign to lab environment.

Discussion

- DNA bands of 500bp were more closely focused upon based on custom primer parameters
- Blast query identified samples as kinesin in origin, but from species of fungi
- The presence of DNA bands in the WC lanes (figure 4b) indicates the presence of contaminants in water used for RNA extraction
- The presence of contaminant bands, however, is inconsistent, not only between multiple trials but also within the same runs (it would be expected to see contaminant bands in all lanes which included the water)
- Nearly identical amino acid alignments support that contaminants were present/introduced to the study
- An alternate explanation for fungal hits is divergence of foram proteins from other Eukaryotes
- There exists little molecular data on forams, which leaves the possibility that generated Blast identifications are false positives, or a closest possible, in the absence of exact matches
- From the few proteins that have been studied in forams, unique structural modifications, such as highly repetitive units have been discovered warranting investigation into longer bands in future studies
- Using *in vitro* motility assays at varying temperatures may provide a more definitive identification of the origin organisms (based on cold stability properties)

References

Atherton J, Farabella I, I-Mei Y, Rosenfeld SS, Houdusse A, Topf M, Moores CA. Conserved Mechanisms of Microtubule-Stimulated ADP Release, ATP Binding, and Force Generation in Transport Kinesins. eLife. 2014. September 10; 3

Bowser SS, DeLaca TE. Rapid Intracellular Motility and Dynamic Membrane Events in an Antarctic Foraminifer. Cell Biology International Reports. 1985. October; 9 (10): 901-910.

Bowser SS, DeLaca TE, Rieder CL. Novel Extracellular Matrix and Microtubule Cables Associated with Pseudopodia of *Astrammina rara*, a Carnivorous Antarctic Foraminifer. J. Ultrastruct Mol Struct Res. 1986. May 27; 94 (2): 149-160.

Glöckner G, et al. The Genome of the Foraminiferan *Reticulomyxa filosa.* Current Biology. 2014. January 6; 24 (1): 11-18.

Habura A, Hou Y, Reilly AA, Bowser SS. High-Throughput Sequencing of *Astrammina rara*: Sampling the Giant Genome of a Giant Foraminiferan Protist. BMC Genomics. 2011. 12 (169).

Hirokawa N, Noda Y. Intracellular Transport and Kinesin Superfamily Proteins, KIFs: Structure, Function, and Dynamics. Physiol Rev. 2008 July. 88. 1089-1118.

Kirokawa N, Tanaka Y. Kinesin Superfamily Proteins (KIFs): Various Function and their Relevance for Important Phenomena in Life and Diseases. Experimental Cell Res. 2015. 334. 16-25.

Sablin EP, Kull FJ, Cooke R, Vale RD, Fletterick RJ. Crystal Structure of the Motor Domain of the Kinesin-Related Motor ncd. Nature. 1996. April 11; 380 (6574): 555-559.

Shwartz M. Limping all the way, protein carries stuff of life across cell. Stanford Report. 2003. December 10.

Stothard, P. 2000. The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. BioTechniques 28: 1102-1104.

Wang W, Cao L, Wang C, Gigant B, Knossow M. Kinesin, 30 Years Later: Recent Insights from Structural Studies. Protein Science. 2015. 24: 1047-1056.

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Further Information

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