Exploration of Possible Alternative Splicing in the *Caenorhabditis elegans* Gene *PMK-3* through Treatment with an Anti-Inflammatory

Faith Rumpp, Jenny Kinard, Alex Romer, Rebecca Seipelt-Thiemann

Middle Tennessee State University Biology Department, Murfreesboro, TN

Introduction

- In the soil nematode, *Caenorhabditis elegans*, important functions such as axon regeneration within the worm are partially attributed to the functions of gene *PMK-3* pathways (Nix et al. 2011)
- *PMK-3* is often referred to as p38 MAPK (mitogen-activated protein kinase) due to sequence similarities with p38 (Berman et al. 2001).
- Signals from this specific gene have been observed to regulate cilium length (van der Vaart et al. 2015) and the formation of muscle connections (D'Souza et al. 2016)
- Microtubules appear to be heavily linked to *PMK-3*, particularly due to the microtubule core which makes up cilia (van der Vaart et al. 2015)
- *PMK-3* is responsive to extracellular environmental stresses and inflammatory devices such as sodium chloride (Berman 2001)
- The stressor sodium chloride was shown to activate "much greater kinase activity" (Berman et al. 2001)
- Changed gene function by treatment of an anti-inflammatory such as sodium chloride should in turn relate to a lack or decrease of kinase function and alternative gene expression when *C. elegans* is placed in the presence of an anti-inflammatory.
- Due to *PMK-3*'s tendency to be activated by inflammatory stresses, the administration of an anti-inflammatory will alternatively splice the gene, therefore reducing gene expression and protein levels in *C. elegans*.

Methods

Selection of *PMK-3* as gene of interest in *C. elegans* and treatment of ibuprofen

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Mapping of gene's exon's & introns/region of possible alternative splicing

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Growth of nematodes & PCR primer design

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Harvesting of nematodes & treatment with 10μM ibuprofen with a control group kept

Methods

Use of forward & reverse PCR primers on gradient PCR to determine best annealing temp with gel electrophoresis

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Use of forward & reverse PCR primers on experimental and control cDNA in best annealing temp (53.9ºC)

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Gel electrophoresis run on PCR cDNA to determine any present alternative splicing based on band sizes

Results

Fig. 1: Gene structure of *PMK-3*. This shows the normal splicing patterns of the gene by the green branches. This structure was derived from WormBase as a basis for targeting alternative splicing (2019)..

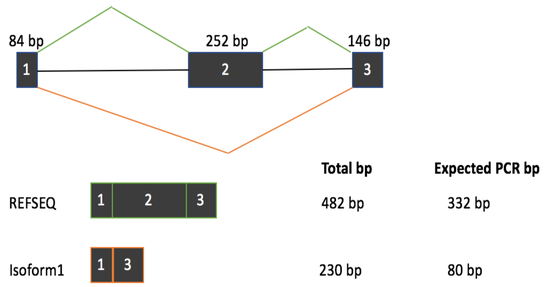

Fig. 2: Gene region of interest with expected PCR sizes. Expected alternative splicing patterns shown in orange along with expected base pair length after PCR with and without splicing (Primer3Plus 2019).

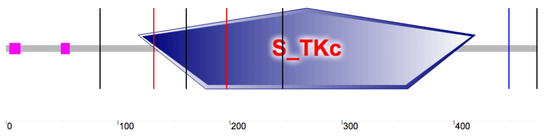

Fig. 3: Domain of *PMK-3* in *C. elegans*. The S_TKc protein's alpha and beta folds create an involvement in ATP binding. Catalytic enzyme activity is driven by its center which holds aspartic acid (UnitProt SMART 2019),

Results

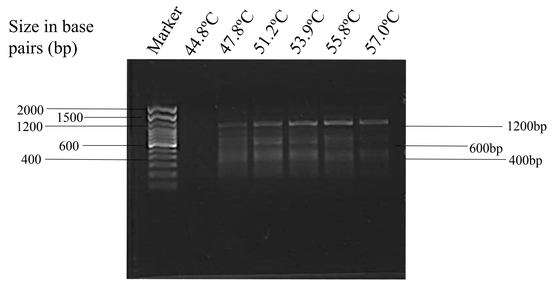

Fig. 4: Gradient PCR. Used to determine best annealing temp, which was determined to be 53.9ºC (Invitrogen™ TrackIt™ 100 bp DNA Ladder from ThermoFisher Scientific used).

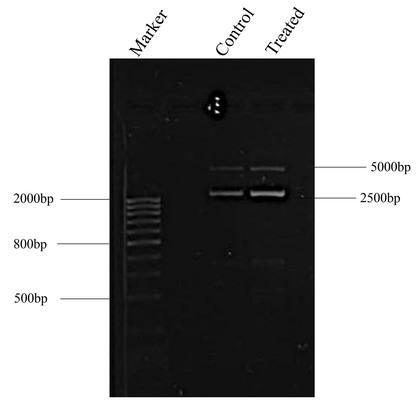

Fig. 5: Control and experimental (treated) RT-PCR. In this gel run, although the annealing temperature was ideal, unexpected band sizes appeared. Such large bands as 5000bp and 2500bp would indicate that the primers did not anneal at the intended locations, but rather very far apart.

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REF_SEQ_pmk-3     1 MASVPSSSSLPVSHVRRHEDVSTPSAPPTKRSNNQSQPPESYEPNTWLQQQREQQQKKL
Isoform_1         1 ------------------------------------------------------------

REF_SEQ_pmk-3    61 AAENIKKQSIEATGNNEMVGEEEEDILSKPCGPHKRRFQFVMIRNITFAIPEGYDVEPNS
Isoform_1         1 ------------------------------------------MIRNITFAIPEGYDVEPNS

REF_SEQ_pmk-3   121 IEYLGGGSFGNVIKTSAVCRDGLRRYVAIKKMREPFFDPHHARRIFRETKLLQLMRHDNI
Isoform_1        20 IEYLGGGSFGNVIKTSAVCRDGLRRYVAIKKMREPFFDPHHARRIFRETKLLQLMRHDNI

REF_SEQ_pmk-3   181 ICALDIYTPDEENDFRDVYVVTEFAGRSLYQILKQQRDYGRRVLTDEHIKFIIYQIIRAL
Isoform_1        80 ICALDIYTPDEENDFRDVYVVTEFAGRSLYQILKQQRDYGRRVLTDEHIKFIIYQIIRAL

REF_SEQ_pmk-3   241 KYIHSANIIHRDLKPGNLALTDDSDLMILDFGLARSLEKKDTSLTQYVQTRWYRSPEVIY
Isoform_1       140 KYIHSANIIHRDLKPGNLALTDDSDLMILDFGLARSLEKKDTSLTQYVQTRWYRSPEVIY

REF_SEQ_pmk-3   301 WKIDSYTNLADMWSLGCIAAELLTGEPLFPGDEPNAQYQRITQLCGSPDEELLTKIENDN
Isoform_1       200 WKIDSYTNLADMWSLGCIAAELLTGEPLFPGDEPNAQYQRITQLCGSPDEELLTKIENDN

REF_SEQ_pmk-3   361 SSAIKAVIQSYTTHKRRNFRDVFSAHNPSEDFIDLLEKLLVLDPEKRITVEEAIQHPYLA
Isoform_1       260 SSAIKAVIQSYTTHKRRNFRDVFSAHNPSEDFIDLLEKLLVLDPEKRITVEEAIQHPYLA

REF_SEQ_pmk-3   421 EFSLPEDEPRADHIFDLDDSQARTRFEWRDAVWKEIMNYKRLSSSPLIPGEADR
Isoform_1       320 EFSLPEDEPRADHIFDLDDSQARTRFEWRDAVWKEIMNYKRLSSSPLIPGEADR
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Fig. 6: Protein translation comparison. Predicted Isoform1 is shortened at the amino terminus of the protein (ExPasy 2019).

Conclusions

- The best observed annealing temperatures were 51.2C and 53.9C, with 53.9C being chosen for final PCR of the treated and control cDNA with primers.
- There are multiple bands in both of the samples, however, both samples, treated and control, contain the same number of and sized fragments. They also are much different than expected in size. The fragments are over 2000 base pairs in length, which would indicate issues in annealing of the selected and diluted primers. It is possible that they annealed at locations far from the anticipated locations, therefore creating exceedingly large bands.
- The resulting fragments of cDNA would indicate that if the RNA had been translated into amino acids, the final proteins would be very different from the anticipated protein which promotes kinase function within the organism. This would be due to the excess base pairs present and the many unintended amino acids in the final product.
- Because the resulting proteins would contain so much extra material, they would most likely not be very functional, or take on another function entirely based on the large sequence of amino acids.
- The proteins which result from the *PMK-3* gene, which is in part responsible for cilium length and function of muscle connections, heavily impact ATP binding and kinase activity. When lacking this function, *C. elegans* mobility would most likely be notably negatively affected and lacking.
- In a similar experiment with *PMK-3,* Berman et al. reported no alternative splicing of the gene (2001). However, other results concluded a decrease in protein levels which would be in direct correlation to splicing (Bounoutas et al. 2011).
- Alternative splicing was not observed in this case due to possible improper primer annealing; however, the prospect of an anti-inflammatory having a substantial effect on *PMK-3* still persists.

Future Directions

More testing is needed

- To test different primers to ensure that annealing at unintended locations does not occur
- To determine if, with proper primer annealing, the concentration/use of the anti-inflammatory, ibuprofen, does not cause alternative splicing and if a stronger/different anti-inflammatory is better suited to induce alternative splicing
- In the case of the same issues occurring with annealing, the smaller bands could be excised and sequenced to see if any alternative splicing occurred.

Literature Cited

Berman K, Mckay J, Avery L, Cobb M. 2001. Isolation and characterization of pmk-(1-3), three p38 homologs in C. elegans. Mol Cell Biol Res Commun [Internet]. 4(6): 337-344. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4460246/pdf/nihms691820.pdf doi: 10.1006/mcbr.2001.0300.

Bounoutas A, Kratz J, Emtage L, Ma C, Nguyen K, Chalfie M. 2011. Microtubule depolymerization in Caenorhabditis elegans touch receptor neurons reduces gene expression through a p38 MAPK pathway. PNAS [Internet]. 108(10): 3982-3987. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3054000/pdf/pnas.201101360.pdf

D'Souza SA, Rajendran L, Bagg R, Barbier L, van Pel DM, Moshiri H, Roy P. 2016. The MADD-3 LAMMER kinase interacts with a p38 MAP kinase pathway to regulate the display of the EVA-1 guidance receptor in Caenorhabditis elegans. PLOS Genet [Internet]. 12(4): 1-24. Available from: https://journals.plos.org/plosgenetics/article/file?id=10.1371/journal.pgen.1006010&type=printable

Nix P, Hisamoto N, Matsumoto K, Bastiani M. 2011. Axon regeneration requires coordinate activation of p38 and JNK MAPK pathways. PNAS [Internet]. 108(26):10738-10743. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3127873/pdf/pnas.201104830.pdf

van der Vaart A, Rademakers S, Jansen G. 2015. DLK-1/p38 MAP kinase signaling controls cilium length by regulating RAB-5 mediated endocytosis in Caenorhabditis elegans. PLOS Genet [Internet]. 11(12): 1-24. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4686109/pdf/pgen.1005733.pdf doi: 10.1371/journal.pgen.1005733