September 2009

Studentship Report 2009: Biochemical Society

Investigation into the conservation of motor activity between myosin V proteins

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1. Aims of the Project:

Schizosacchromyces pombe has two myosinV genes, myo51 and myo52. These motor proteins are involved in a number of cellular processes, and are responsible for the transport of molecular cargoes throughout the cell. My project aimed to examine the motor function conservation between motor domains of class V myosin's. We aimed to insert the myo52 tail domain of the myosin motor from S.pombe into a pREP S.pombe expression vector and fuse the tail with motor domains from different organisms, including Drosophila melanogaster myosin V and myosin VI. Once myo52 chimeras had been acquired these would then be expressed into S.pombe and the cells ability to localise correctly and maintain the correct phenotype would be assessed using live cell imaging.

2. Description of work:

The DNA for the head domains, DmmyoV, DmmyoVI and DmmyoVI –inverter region, successfully inserted into pGEM vectors. This was achieved by firstly using PCR to amplify the DNA. We used high fidelity enzyme to do this and the product was then cleaned using a Qiagen PCR purification kit. The fragments were then ligated together with the pGEM cut vector. This was then transformed into *Escherichia coli* DH5α cells overnight. Once the DNA was extracted from the bacteria, we used the restriction enzyme *EcoRI* as a diagnostic tool to identify the correct inserts. The pGEM DmmyoV, DmmyoVI and DmmyoVI - inverter region are shown in ***Figure 1*** below on a 1% agarose gel. With the head domain chimeras obtained they were then ready to fuse to the myo52-GFP tail.

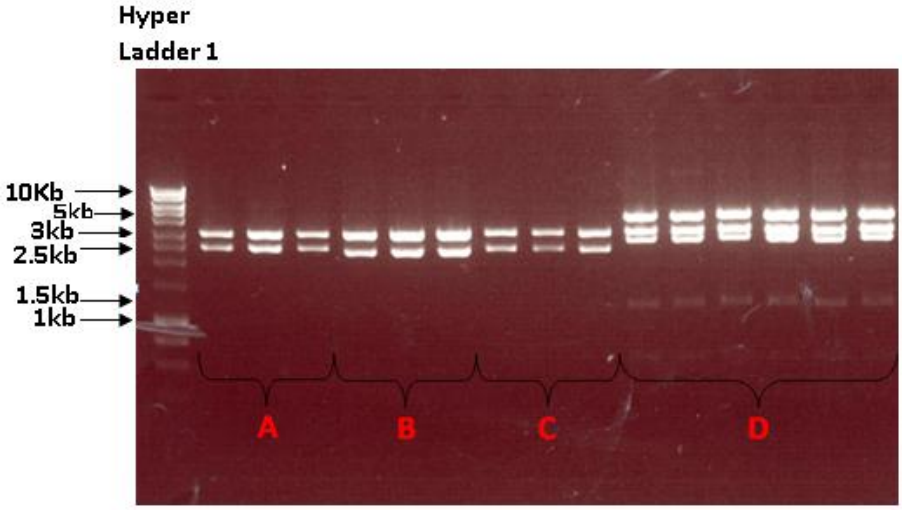

Figure 1. Shows the constructs obtained throughout the project: All DNA here has been cut with restriction enzyme, *EcoRI*.

A:pGEM + Dmmyo V
B:pGEM + Dmmyo VI
C:pGEM + Dmmyo VI – inverter
D:pREP41 + myo52-GFP

The myo52-GFP tail chimera was obtained by inserting the 2.5Kb myosin52 tail domain into a 10Kb pREP, GFP tagged, S.pombe expression vector. This was achieved by firstly linearising the pREP vector and the pGEMmyosin52 tail vector with the endonuclease enzyme, *SmaI*. After an overnight digest, the enzyme was then extracted using the phenol/chloroform technique and left overnight to digest with *SalI*. The *SmaI-SalI* digested DNA was then run on a 1% agarose gel and the pREP vector and the 2.5Kb myo52 tail were cut away and gel purified. The recovered DNA from the gel purification was then left overnight in a ligation and then transformed into *E.coli* DH5α cells the following day.

Once the transformed bacteria were left to grow overnight the DNA from single colonies were extracted until the myo52-GFP chimera was recovered. Unfortunately the myo52 - GFP tail chimera was not obtained until much later in the project so we were unable to progress any further than obtaining the chimeras shown above in ***Figure 1***.

3. Assessment of the results and outcomes of studentship:

We were unable to assess the results any further than having the chimeras sequenced. It was disappointing that we did not manage to move onto the live cell imaging but the chimeras that were produced throughout the project will hopefully aid the labs further research.

4. Future Directions in which the project could be taken:

This project still holds many possibilities; the myo52 head domain from S.pombe can be fused to the pREP myo52-GFP tail domain and expressed in myo52 deletion cells as a control experiment. Once it is certain that the pREP myo52-GFP tail domain is functioning correctly, the poem chimeras with the myosin head domains can then be fused to the tail domain and expressed in yeast. After that, the project can move on by inserting head domains from other myosin's, such as Myo1, Myo2, other S.pombe myosin V's and myosin's from other fungi.

5. Departures from the original proposal:

None, other than we were unable to achieve all of the original aims as the clones were obtained later than we expected and so were unable to express any chimeras into S.pombe. Therefore the ability of these different organisms' motors to function effectively in yeast was not determined.

6. Value of studentship to the student and to the lab:

This studentship has increased my confidence in the lab and greatly improved my basic lab skills, as well as teaching me new skills and reinforcing the teaching from my undergraduate classes. I had a wonderful time in the lab and I think the most important outcome from this experience is that it has confirmed my desire to pursue a PhD after graduation. I hope that the results I have produced will aid the lab in their future research.