RNA Interference Approach to Knockdown Rian IncRNA in Skeletal Muscle In Vivo

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Introduction
Muscle dystrophies result in severe weakness and loss of muscle, which have debilitating effects on patients. By establishing the molecular mechanisms involved in muscle regeneration, better therapies to help boost tissue repair in patients suffering from muscle diseases can be developed.

This project examines the role of RNA Imprinted and Accumulated in Nucleus (Rian), a lncRNA (long non-coding RNA) found in the largest known mammalian miRNA (microRNA) cluster, known as the Dlk1-Dio3 locus. This locus, along with the evolutionarily conserved transcription factor MEF2 responsible for its expression in mouse skeletal muscle, is known for its important role in skeletal muscle regeneration. The locus codes for hundreds of miRNAs and several lncRNAs. MrRNAs are non-coding RNAs which epigenetically regulate gene expression by targeting corresponding RNA transcripts for degradation. LncRNAs often regulate gene expression epigenetically by controlling how tightly DNA is coiled, thereby restricting or allowing access to specific genes. Rian, the lncRNA investigated in this project, is currently known to interact with several chromatin modifying complexes, such as PRC2, in mouse embryonic stem cells. Overall, the main pattern observed is that Rian interacts with repressors of gene expression.

Through this experiment, we hope to determine the role of Rian in muscle gene regulation and muscle regeneration by observing the effects of a Rian knockdown on the regeneration of injured mouse muscle.

Results
• The bacterial transformation of AA V9 U6 gRNA shRian plasmid into DH5α cells was successful. After incubating the bacteria at 37°C overnight, dozens of colonies grew on the agar plates.

• The mini-prep DNA was transformed into DH5α cells along with a helper plasmid and a rep/cap plasmid for virus packaging.

• Packaged virus will be extracted, purified, and injected into injured mouse muscle samples.

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Discussion/Conclusions
The growth of the DH5α bacteria colonies on the ampicillin plates signifies that the bacterial transformation worked. Without the AA V9 U6 gRNA plasmid containing the amplicin-resistant gene, the bacteria would not have survived, because the ampicillin would have killed them.

The last bands of the four mini-prep bands in Figure 4 suggest that the bacteria replicated the correct plasmid and that the mini-preps contain the recombinant plasmid. The surviving colonies and the gels in Figures 3 and 4 suggest that the plasmid in the mini-preps is the correct plasmid and that the mini-preps contain the recombinant plasmid.

Future Research Objectives
Future work on this project will include the packaging of the AA V9 with the shRian gene and RNA interference.

Virus Packaging:
• Mini-prep plasmid will be purified, then transfected into mammalian 293T cells along with a helper plasmid and a rep/cap plasmid for virus packaging.
• Packaged virus will be extracted, purified, and injected into injured mouse muscle samples.

Molecular Cloning of Recombinant Plasmid:
• Ligated plasmid is transformed into a culture of DH5α bacteria. Bacteria recover in 37°C shaking incubator for one hour.
• Bacteria are plated on agar and ampicillin and incubated overnight at 37°C.
• The surviving bacteria express the plasmid and replicate to form stable colonies.
• 4 colonies are inoculated and the bacteria are grown overnight.
• Mini-prep separates and extracts the recombinant plasmid from the bacteria. The mini-prep DNA is sequenced.

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Methods/Materials
Recombinant DNA and molecular cloning are the two main methods and materials that have been used so far in this project.

Creating the Recombinant DNA:
• Digest genome AA-U6gRNA1-U6gRNA2-TntCre at the Aar1 and Sap1 restriction sites using the corresponding Aar1 and Sap1 enzymes.
• Gel electrophoresis of the digested genome to check that it is linear.
• Gel extraction and isolation of digested genome.
• Double-stranded shRian gene with complementary “sticky ends” to those of the digested AA V9 genome annealing an engineered shRian top strand with a shRian bottom strand. The shRian gene uses complementary base pairs to target Rian.
• Ligation reaction using the enzyme ligase inserts the double-stranded shRian gene into the plasmid.

Discussion/Conclusions
The growth of the DH5α bacteria colonies on the ampicillin plates signifies that the bacterial transformation worked. Without the AA V9 U6 gRNA plasmid containing the amplicin-resistant gene, the bacteria would not have survived, because the ampicillin would have killed them.

The last bands of the four mini-prep bands in Figure 4 suggest that the bacteria replicated the correct plasmid and that the mini-preps contain the recombinant plasmid. While the cut AA V9 genome registered at 6 kbp, the original uncle AA V9 genome and the last bands of the mini-prep DNA registered at 4 kbp. This suggests that the plasmid in the mini-preps is the correct plasmid and is not linear, because the uncut and the final plasmids appear to be the same length.

Compact (circular) plasmids can also travel faster in the gel than the cut plasmids, so they appear shorter, just like the last bands of the mini-preps in Figure 4.

The surviving colonies and the gels in Figures 3 and 4 suggest that the shRian plasmid was successfully transformed into and replicated by the DH5α cells. Therefore, the recombinant plasmid was likely successfully created and cloned, and is now ready to be transfected into mammalian 293T cells along with a helper plasmid and a Rep/Cap plasmid for virus packaging.

References

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