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September 30, 1997

Philip Migliore, M.D.
Research Director
Moran Foundation

Dear Phil,

Enclosed please find my progress report for Moran Foundation Project number 96-0083. I would like to express my appreciation for the support provided by the Moran Foundation. Thanks to preliminary results supported by the Moran Foundation, we have received a pilot project grant to continue this investigation. This is the type of project that was perfect for Moran support since it is novel and risky.

If you have any questions, please feel free to contact me.

Sincerely,

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The ultimate goal of this project is to develop novel strategies to regulate exogenous gene expression at the level of pre-mRNA splicing. In the future, this approach could be used to regulate expression of genes in vectors used for human gene therapy. This project is still active

We have recently received funding of a pilot project (listed below) due in part to preliminary results supported by the Moran Foundation Award.

5 PO1 HL51754-05 (Arthur L. Beaudet) 09/1/97-08/31/98 10%
NIH/NHLBI $25,000 (Pilot project)
Somatic gene therapy for cystic fibrosis
Pilot project title: Post-transcriptional regulation of exogenous genes

We have made significant progress characterizing intron elements that promote muscle specific exon inclusion. Four intron elements were shown to be required for muscle specific splicing of the cardiac troponin T alternative exon. In a effort to develop elements that can be used to regulate exogenous genes, we have shown that one of these elements present in multiple copies gives very strong muscle specific regulation of a heterologous alternative exon. These results demonstrate the feasibility of the proposal.

We have also improved the approach originally proposed. Rather than using beta-galactosidase (ß-gal) as the marker gene, we have started using green fluorescent protein (GFP). GFP is an autofluorescent protein. The expression plasmid is transfected into cells then expression of the protein is visualized using standard FITC optics. In contract, visualization of ß-gal activity in transfected cultures requires a precise fixing scheme: enough to break open the cells but not harsh enough to denature the enzyme an destroy activity. We have recently expressed GFP in our primary skeletal muscle cultures and fibroblast cultures and demonstrated a strong signal.

We will continue this project using the remaining Moran funds and funding from the pilot project noted above. We will then include the preliminary data generated in the next year to submit an independent proposal to an outside agency.