

Basic Science Project: Ribozyme-mediated repair of Sickle β -globin RNA and DNA

The overall goal of this proposal is to explore the ability of group I and group II introns to repair mutant β -globin genes and transcripts and assess the potential utility of these molecules in human cells. These have been of great scientific interest because they are able to perform catalysis and because a subclass these RNA enzymes can act as mobile genetic elements. Moreover, their ability to modify RNA and DNA sequences through forward and reverse-splicing reactions makes these introns of particular interest to translational researchers. Previously, we demonstrated that trans-splicing group I ribozymes can convert sickle β -globin encoding mRNAs into γ -globin encoding transcripts following transient transfection of the ribozyme into erythrocyte precursors derived from patients with sickle cell disease. In addition, we have demonstrated that such RNA repair can proceed with low to moderate efficiency (up to 50% repair) in cells cotransfected with ribozyme and sickle β -globin expression cassettes. More recently, we have demonstrated that the *Lactococcus lactis* group II intron can reverse-splice and site specifically insert into desired DNA target sequences in transfected human cells. These proof of concept studies suggest such catalytic RNAs may represent molecules that can be employed to modify genetic instructions for therapeutic ends to treat sickle cell disease and other genetic disorders. These studies also underscore necessity for further evaluation and optimization of these catalytic RNAs if they are to become therapeutically useful. Here we propose to perform more detailed analyses of group I and group II intron activity in human cells focusing upon repair of mutant β -globin transcripts and genes.

Specific Aims are:

- 1.) To evaluate the trans-splicing activity of group I intron variants and expression cassettes and identify those with enhanced ability to repair sickle β -globin transcripts in cell culture.
- 2.) To generate derivatives of the *Lactococcus lactis* group II intron RNP that can insert wild type β -globin exons into mutant β -globin genes in *E. coli*.
- 3.) To evaluate the ability of group II intron RNPs to repair mutant β -globin genes in mammalian cells by exon insertion following direct transfection of the reconstituted RNPs and following expression of the intron and the intron-encoded protein in human cells.

The completion of these studies will establish the needed experimental foundation from which the logical development of therapeutic group group II ribozymes for the treatment of sickle cell disease and other genetic disorders can proceed.