Editorial

RNA repair as a novel approach to genetic therapy

Gene therapy-based treatments of genetic disorders have conventionally been attempted through the transfer of a wild-type version of a gene to the cells of a patient harboring defective copies of a disease-associated gene. Despite significant advances using this paradigm, several major technical hurdles must still be overcome before this 'gene replacement' approach to gene therapy will become useful in the treatment of a variety of human maladies. Chief among these obstacles is the inability to introduce therapeutic genes efficiently into patients that faithfully recapitulate the expression pattern of endogenous genes following gene transfer. For this reason, alternative strategies to genetic therapy have started to attract more attention from the research community. Repair of mutant genetic instructions represents a fundamentally different approach to genetic therapy that may have significant advantages over gene replacement. Herein, I will discuss recent advances with using ribozymes for repair of mutant RNAs as a novel means to correct genetic deficiencies.

RNA molecules are the sole carriers of genetic instructions from the genome to the ribosome. Moreover information embedded in RNA appears to be very malleable and genetic instructions are often revised by splicing and editing of RNA transcripts. Thus RNA molecules appear to be attractive substrates for genetic repair and a few approaches have recently been described that exploit normal RNA processing reactions to re-write genetic instructions for therapeutic ends.¹

Perhaps the most thoroughly understood reaction that revises RNA is the one mediated by the group I self-splicing intron from *Tetrahymena thermophila*.² This intron catalyzes its own excision without the aid of proteins.³ Moreover, this RNA enzyme (or ribozyme) can perform a similar splicing reaction on a substrate RNA that is not covalently attached to the ribozyme. In this trans-splicing reaction, the ribozyme recognizes and binds a substrate RNA by forming base pairs with the targeted transcript. Once bound, the ribozyme cleaves the targeted RNA, releases the downstream cleavage product and splices a 3' exon sequence that is initially attached to the end of the ribozyme on to the upstream cleavage product. This means of shuffling around RNA sequences can in principle be adapted to revise RNA transcripts for a wide variety of applications because very few sequence requirements exist for the target RNA and the 3' exon in this trans-splicing reaction.^{2,4} The main sequence requirement for the substrate is to have a uridine residue at the

cleavage site and the main requirement for the 3' exon is that it must allow the ribozyme to fold into a catalytically competent conformation.

Trans-splicing ribozymes can be employed to repair defective RNA messages. In the first example of this application, the group I ribozyme from *Tetrahymena thermophila* was re-engineered to repair truncated *lacZ* transcripts via targeted trans-splicing in *E. coli*⁵ and in mammalian cells.⁶ In both settings, the ribozyme was shown to be able to splice restorative sequences on to mutant *lacZ* target RNAs with high fidelity and thus maintain the open reading frame for translation of the repaired transcripts. In a subsequent study, the efficiency of RNA repair was monitored and the ribozyme was shown to be able to revise up to 50% of the truncated *lacZ* transcripts when ribozyme and *lacZ* substrate encoding plasmids were cotransfected into mammalian fibroblasts.⁷

More recently, two groups have demonstrated that group I ribozymes can be employed to amend faulty transcripts that are associated with common genetic diseases. Phylactou *et al*⁸ demonstrated that a trans-splicing ribozyme could be employed to amend transcripts associated with myotonic dystrophy, while Lan *et al*⁹ employed this RNA repair approach to correct mRNAs associated with sickle cell disease. In the myotonic dystrophy case, a trans-splicing ribozyme was employed to shorten the trinucleotide repeat expansion found in the 3' untranslated region of the human myotonic dystrophy protein kinase transcript in cell culture studies.⁸ In the sickle cell experiments, trans-splicing was employed to convert sickle β -globin transcripts into γ -globin encoding mRNAs in erythrocyte precursors isolated from patients with sickle cell disease.⁹ In both studies, sequence analysis of the amended RNAs demonstrated that the ribozyme had accomplished such repair with high fidelity forming the proper splice junctions between the targeted transcript and the corrective sequences.

These results demonstrate that a trans-splicing group I ribozyme can be employed to repair pathogenic transcripts in clinically relevant, cellular settings. However, as with the development of almost every novel therapeutic approach, several technical issues must be addressed before ribozyme-mediated repair of mutant RNAs can become useful in the clinic. First, it remains to be determined if repair of any pathogenic transcript can proceed efficiently enough in primary human cells to be therapeutically beneficial. In the case of sickle cell disease, conversion of as little as 5–10% of the sickle β -globin transcripts into mRNAs encoding γ -globin is expected greatly to reduce cell sickling and thus the severity of the disease. Whether this relatively modest level of repair can

be achieved in erythrocyte precursors from individuals with sickle cell disease is unclear but results demonstrating that 50% of the mutant *lacZ* transcripts expressed in mammalian cells can be revised by ribozymes⁷ is at least encouraging in this regard. Second, the specificity of trans-splicing may have to be increased because in mammalian cell experiments the *Tetrahymena* group I ribozyme was shown to react not only with the intended *lacZ* target RNAs but also with other cellular transcripts.⁶ Such limited reaction specificity is fully anticipated from knowledge about the energetics of substrate binding by this ribozyme. This biochemical knowledge is now being utilized to redesign the ribozyme to enhance its specificity.¹⁰ Finally, even if trans-splicing ribozymes can be generated that are efficient and specific, advances in gene transfer technology will probably be required for ribozymes to become useful in the clinic.

In summary, the ability to employ trans-splicing ribozymes to revise genetic instructions embedded in targeted RNAs represents a broad new approach to genetic therapy. Because defective RNAs can only be repaired in the cells in which they are present and only when they are expressed, RNA repair may be a more effective means of recapitulating the natural expression pattern of genes than traditional gene replacement therapies. Moreover, RNA repair may be especially useful in the treatment of genetic disorders that are associated with the expression of dominant or deleterious mutant RNAs and proteins. In these cases, RNA repair should simultaneously engender wild-type protein production and eliminate production of the deleterious gene product. For these reasons, the

concept of RNA repair is likely to continue to attract increased interest from gene therapists.

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