

### SERIES INTRODUCTION

# Targeted genetic repair: an emerging approach to genetic therapy

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The last two decades have heralded a remarkable increase in our understanding of the genetic basis of disease and the endogenous mechanisms responsible for the repair of genomic DNA and the processing of RNA. Targeted gene repair is a powerful yet controversial technique developed to direct base changes in chromosomal genes, while RNA repair is an emerging strategy to alter the coding content of messenger RNAs. This Perspective series examines the inspired techniques for facilitating the simple correction of genetic defects in what would represent a major shift in the paradigm of clinical science, and the hurdles that need to be overcome in order to make clinical use of molecular therapeutics.

Human genetic information is encoded in the sequences of nucleic acids found inside our cells. During the past two decades, it has become increasingly apparent that such instructions are not fixed, but rather that molecular processes exist that can revise them. Recently, a number of investigators have been exploring whether the ability to revise RNA or DNA sequences can be used to repair mutant genetic instructions, to treat inherited disorders such as cystic fibrosis and sickle cell disease, as well as to revise pathogenic genes associated with cancers and infectious diseases. This repair approach has received increasing attention because of the safety and efficacy issues encountered with more traditional gene therapy strategies where additional copies of therapeutic genes are delivered to and expressed in transduced cells. Most notably, the recent observation that retroviral gene transfer apparently induced leukemia in two children treated for X-linked SCIDs has raised significant safety concerns for traditional gene add-back strategies (1). In contrast to the traditional approach to gene therapy, genetic repair strategies attempt to directly correct endogenous genetic mistakes rather than deliver extra copies of genes to cells.

Thus by analogy, genetic repair methods are similar to word processors that correct misspelled words within their intended written context, whereas gene add-back approaches are similar to editors who prepare corrected versions of defective sentences and then randomly insert them into the text without amending the original written mistake.

Genetic repair strategies may have significant therapeutic and safety advantages over traditional gene therapy approaches for the treatment of many genetic disorders. Firstly, because the mutant genetic instructions are directly repaired, the corrected RNAs and/or DNAs will be maintained in their native sequence context and be regulated by their endogenous regulatory machinery. Secondly, in the instance where the mutant gene encodes a deleterious or dominant-negative mutant protein, repair of the mutant should simultaneously engender the regulated production of the wild-type protein while eliminating or reducing expression of the deleterious gene product. Finally, genetic repair strategies attempt to repair defective instructions in a site-specific manner. Therefore, once adequately developed, these strategies will result in less random mutagenesis of the genome and lead to fewer mutagenic side effects than do methods that randomly insert genes into the genome (1).

In this Perspective series, six articles will update the reader on the progress toward and the hurdles that remain for developing such genetic repair strategies. The first half of the series will focus on approaches to RNA repair, while the latter will describe methods for DNA repair.

As therapeutic modalities, RNA and DNA repair have different advantages and weaknesses. For example, RNA repair may represent a safer approach to genetic correction than DNA repair because the revision of unintended target RNA will not result in permanent genetic change within a cell since RNAs undergo continual turnover in vivo. However, the limited half-life of the amended instructions also necessitates that RNA repair strategies have to continually repair the mutant RNAs emerging from mutant DNA. By contrast, DNA repair will amend the cell's genetic blueprint, and such repair need occur only

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once to permanently correct the products expressed from the repaired gene in the treated cell and its progeny. However, since any revised DNA will be stably maintained and propagated, the specificity of DNA repair is a major safety consideration because genes that are unintentionally revised will also be maintained and propagated.

### Strategies for RNA repair

Most protein-encoding RNAs have to be processed by RNA splicing to generate fully functional messenger RNAs (2). This discovery engendered the concept of RNA repair for therapeutic applications. The basic idea is that since RNAs are continuously being revised in human cells, perhaps the RNA revisionist machinery could be redirected to repair mutant RNAs associated with disease. In this Perspective series, three articles will describe the RNA repair approaches that have received the most attention. To begin, two related approaches to RNA repair that both use *trans*-splicing to amend mutant transcripts are discussed (3, 4). The first article, by Meredith Long and colleagues (3), describes efforts to use *trans*-splicing ribozymes to repair a variety of clinically relevant transcripts (5–8), whereas the second article, by Mariano Garcia-Blanco (4), describes a more recent and very promising approach to therapeutic RNA repair that uses the endogenous splicing machinery to perform *trans*-splicing to amend mutant target RNAs (9–11). Following this, Peter Sazani and Ryszard Kole (12) will describe a third and most promising approach to RNA repair based on the use of antisense oligonucleotides to modulate alternative splicing and engender the production of therapeutic gene products (12, 13).

### Strategies for DNA repair

Processes such as homologous recombination and DNA mismatch repair are now being exploited by a number of groups to develop methods to repair mutant DNAs in a site-specific manner. In the latter half of this series, the state of this emerging technology and the challenges that must be overcome before DNA repair approaches can become useful in the clinic are discussed. Michael Seidman and Peter Glazer (14) describe the potential utility of DNA triplexes for DNA repair (14–16). Eric Kmiec (17) and Dieter Gruenert and colleagues (18) conclude the series with their discussions regarding how short DNA oligonucleotides and small DNA fragments can be used to repair mutant genes (17–22).

Collectively these six approaches to genetic repair hold great promise for the treatment of a vast array of human diseases that have a genetic basis. As with the development of any new therapeutic modality, significant developmental issues must be overcome, but progress in this young field has been very

encouraging. Most of these genetic repair approaches have already shown activity in either primary human cells, such as erythrocyte precursors taken from patients with sickle cell disease or  $\beta$ -thalassemia, or in animal models of human disease, such as those for evaluating treatments for cystic fibrosis or hemophilia (6, 11, 13, 16, 20, 22). Current efforts are now focused largely upon making these repair approaches efficient enough for therapeutic benefit in man. Rapid progress on this front may mean that genetic repair will make its debut in the clinic in the not-too-distant future.

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