CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Correcting Mutations by RNA Repair

Robert Reenan, Ph.D.

Imagine a young patient with a debilitating, perhaps lethal, inherited disease showing up for her regular treatment. You inject a viral vector that delivers a personalized nanomachine to affected tissues, which repairs a specific mistake in her protein-making machinery (part of which a d
leagues

[1] represents a step toward making this a reality. They demonstrate an RNA-repairing nanomachine within the limits of current technology.

Therapeutic approaches to mendelian disorders use tactics from gene replacement to small molecules designed to correct functional defects, from drugs that force the synthesis of full-length proteins that would otherwise be truncated to oligonucleotides that alter RNA splicing.[2] The repair machine described by Montiel-Gonzalez et al. is different. It depends on a byzantine contradiction of the central dogma of molecular biology (which states that DNA generates RNA, which in turn generates protein). This contradiction is a process called adenosine-to-inosine (A→I) RNA editing. In this process, enzymes called ADARs (adenosine deaminases acting on RNA) chemically modify adenosine residues to inosine residues in RNA transcripts.[3] Because cellular machines "see" the inosine residue as a guanosine residue (G, one of the other four standard bases in RNA), the final effect is the conversion — mutation, if you will — of adenosine to guanosine (A→G). In nature, mRNAs that encode proteins are reworked by ADARs so that they incorporate A→G changes. More than half the amino acids of the genetic code can be altered in this fashion. Curiously, recoding by A→I editing seems to occur almost exclusively in the nervous systems of animals. Important to the story, ADARs require that target adenosine residues reside in a region of double-stranded RNA (dsRNA) (Fig. 1A), which is usually formed between a coding sequence (an RNA sequence that encodes protein) and a downstream noncoding sequence.

The authors posit that the deleterious consequences of any mutation caused by the substitution of a guanosine nucleotide by an adenosine nucleotide (G→A) could be reversed at the RNA level by an appropriately directed ADAR. But how would one precisely target an ADAR to reverse a patient-specific mutant adenosine in a particular mRNA in double-stranded form?

Montiel-Gonzalez et al. used an RNA oligonucleotide with homology to the mRNA region of interest, thus generating a region of duplex RNA (Fig. 1B). Next, they wanted to direct the enzymatic activity of an ADAR to this duplex region and avoid off-target (nonspecific) effects of rampant ADAR expression on other dsRNAs expressed in a cell. The authors solved this issue by replacing the dsRNA-binding domains of human ADAR2, which will generically bind any dsRNA, with an exquisitely specific and small bacterial virus protein called lambda N (λN). Normally, λN binds exclusively to a small viral

Figure 1. ADAR in Nature and as Part of an RNA-Repairing Machine.

In Panel A, ADAR (adenosine deaminase acting on RNA) is depicted as binding to a newly synthesized messenger RNA (mRNA) encoding a protein in a region of double-stranded RNA (dsRNA) formed by base pairing between exonic coding sequences and intronic noncoding sequences. Normally, ADAR binds to RNA through its dsRNA-binding domains. Here a glutamine codon is recoded to an arginine codon by conversion to inosine. As shown in Panel B, the lambda N–ADAR (λN-DD) enzyme and the boxB–guide RNA are encoded as part of a viral-vector delivery system (hexagon). Once expressed, these components assemble into a highly specific RNA-repairing machine targeted to, in this case, a premature termination codon (UAG = stop) that is a disease-causing mutation. Repair of the UAG to UIG results in read-through by the ribosome: UIG is "interpreted" as a tryptophan codon (UGG = W), and the full-length protein is synthesized.