CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Correcting Mutations by RNA Repair

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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 is messenger RNA [mRNA]), effectively curing her. Your healthy patient heads off to soccer practice. Sounds like science fiction? That's because it is. But a recent study by Montiel-Gonzalez and colleagues[1] represents a step toward making this a reality. They demonstrate an RNA-repairing nanomachine within the limits of current technology.

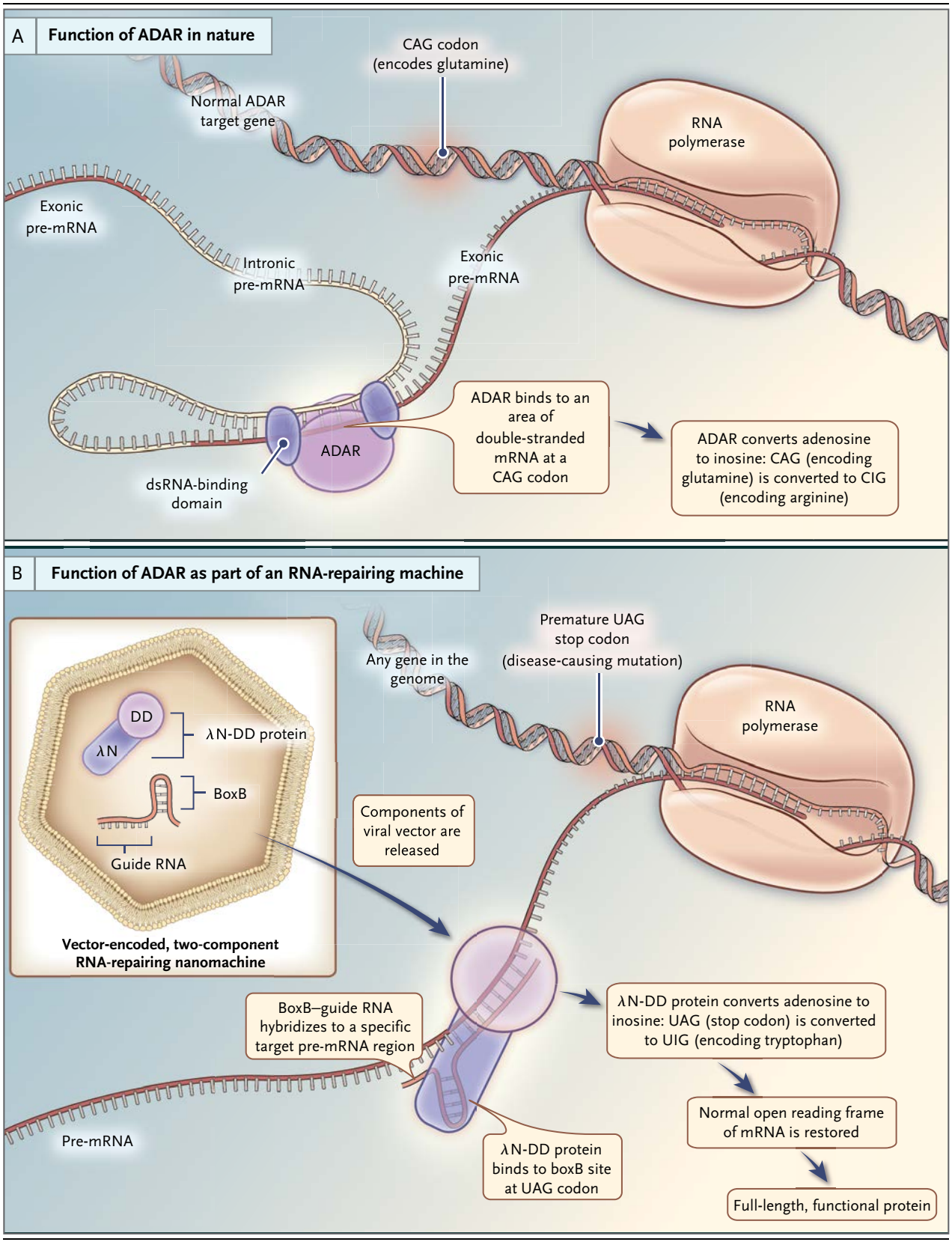
Therapeutic approaches to mendelian disorders use tactics from gene replacement to small molecules designed to correct functional defects, from drugs that force the synthesis of full-length proteins that would otherwise be truncated to oligonucleotides that alter RNA splicing.[2] The repair machine described 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.



RNA sequence called boxB. The authors found that the chimeric protein, λN–ADAR, binds to the boxB sites when they are placed near any dsRNA region of the experimenter's choice and converts A→I in the dsRNA region.

The authors tested their approach on a mutation that causes cystic fibrosis, the W496X mutation in *CFTR*. W represents the amino acid tryptophan, encoded in RNA by the triplet UGG, and X represents a stop codon, encoded in RNA by the triplet UAG. Thus, the investigators sought to edit the UAG to UIG, which would then be interpreted as UGG (encoding tryptophan); this process would circumvent the stop codon and allow continued translation of the CFTR mRNA and thus produce a full-length, normal CFTR protein. At this stage, the authors found that they could "tune" the length and sequence of the guiding oligonucleotide to modify precisely one, and only one, adenosine — the one that they targeted for repair. The authors attached the boxB sequence directly to the guiding RNA nucleotide (Fig. 1B). On injecting the modified ADAR and its "guide" into frog oocytes, Montiel-Gonzalez et al. achieved efficient repair of the *CFTR* W496X truncating mutation as well as the production of a full-length protein. Even more exciting, they found that the "corrected" CFTR protein functions as it does in human cells: as an ATP-dependent chloride channel. The authors also found that this approach corrects mutations in a nonhuman gene in a human embryonic kidney cell line.

There is no reason to believe that this system would not work well in any cell type in a eukaryotic organism. The tools are simple, and the approach is particularly effective for premature truncating mutations, because all three stop codons (UAG, UAA, and UGA) can be recoded by ADAR activity to "read-through" UGG codons. Furthermore, any G→A mutation, including many amino acid missense mutations and splicing mutations, could be repaired with the use of this approach. Although the authors did not obtain perfect efficiency in correcting mutant mRNA, a partial restoration of function may be therapeutically effective in many disorders (e.g., cystic fibrosis). Still, the challenges in delivering molecules such as ADARs to affected, critical cells in a complex multicellular organism are formidable, and given the transience of mRNA, the "therapeutic" molecules would have to establish some degree of residence in the cell to have a durable effect. Were these challenges to be overcome, a different class of enzymes, which performs C→U RNA editing, could potentially be harnessed in a similar strategy to that used by Montiel-Gonzalez et al. Perhaps other chemistries could make complete a toolkit for ribonucleoprotein nanomachines to deliver tailored RNA-repairing chemistries to patients.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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