PERSPECTIVES: DEVELOPMENT

Survival Is Impossible Without an Editor

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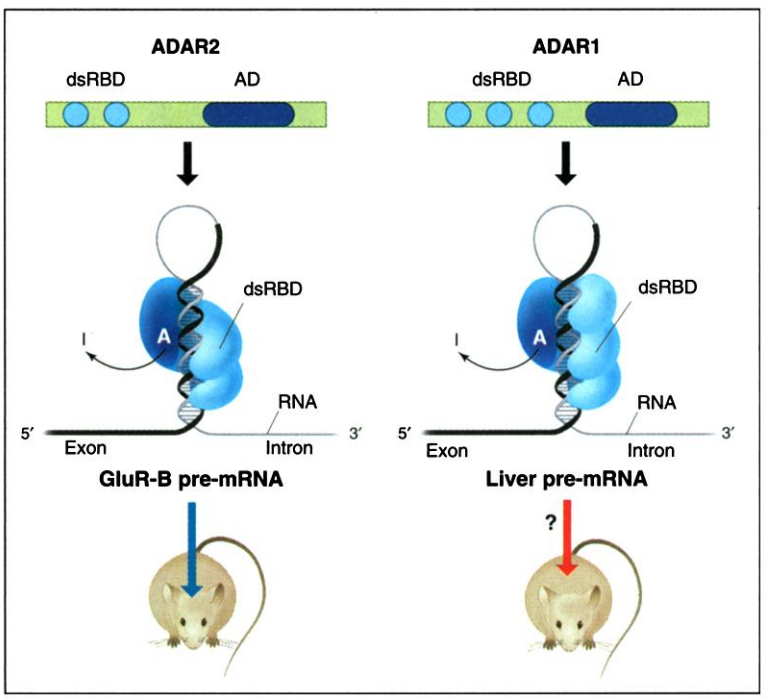
It is almost 20 years since the publication of the DNA sequence of a plant mitochondrial gene and the dawning realization that the genetic code might not be universal (*1*). From this DNA sequence it became clear that some codons in mitochondrial genes were unexpected when compared with the known amino acid sequences of the proteins encoded by these genes. Later, the strange, but true, explanation was discovered: Codons in RNA transcripts of mitochondrial (and chloroplast) genes are altered after transcription by editing of the RNA (*2*, *3*). But RNA editing is not just a phenomenon of mitochondrial genes; it operates equally well on the transcripts of nuclear genes in animal cells (*4*). Three papers published this year, including one by Wang *et al.* on page 1765 of this issue (*5*), describe mutations in nuclear RNA-editing enzymes called ADARs (adenosine deaminases acting on RNA) and the abnormal features (phenotype) that defective versions of these enzymes cause (*5*–*7*). This trio of papers clearly demonstrate that editing of transcripts is necessary for normal development of mouse and fruit fly embryos.

The ADARs convert certain adenosine bases in an RNA transcript into inosines by removing an amino group involved in Watson-Crick base pairing (*8*). Inosine has the base-pairing properties of guanosine (*9*), and so the RNA codon containing a converted adenosine will now encode a different amino acid, resulting in an altered protein. The ADARs recognize a double-stranded RNA (dsRNA) structure in the transcript that is formed between the editing site complementary sequence (ECS)—usually located in the downstream 3′ intron of the transcript—and the sequence to be edited (*10*). It is not known why the ADARs convert some adenosines in dsRNA and not others. The best-characterized target transcripts that undergo RNA editing by ADARs encode glutamate-sensitive ion channels that are expressed by neurons of the central nervous system (CNS) (*11*).

The ADARs have two or three dsRNA binding domains, as well as an adenosine deaminase domain that catalyzes the conversion of adenosine to inosine (see the figure). They probably evolved from similar enzymes that convert adenosine to inosine in transfer RNAs (*12*). There are three known *ADAR* genes in vertebrates. *ADAR1*, the first to be discovered, is widely expressed together with *ADAR2* in different tissues. *ADAR3* is expressed only in the brain, and it is not yet known whether it has any enzymatic activity (*13*).



Editing makes a difference. The ADAR editing enzymes contain dsRNA binding domains (dsRBD) and a catalytic adenosine deaminase domain (AD). They bind to and deaminate specific adenosine bases in the dsRNA region of the transcript, formed between the editing site and the ECS. The ECS is usually located in a downstream intron. It is the duplex structure of the dsRNA that is recognized by ADARs and not a particular sequence in the transcript. The most important transcript that is edited by ADAR2 is the GluR-B transcript at the Q/R site; the critical transcripts that are edited by ADAR1 are unknown. [Figure adapted from (*15*)]

The ADAR2 enzyme edits the pre–messenger RNA (mRNA) that encodes the glutamate-sensitive ion-channel receptor subunit B (GluR-B). By deaminating adenosine, ADAR2 converts a codon encoding the amino acid glutamine (Q) to one encoding the amino acid arginine (R) (*10*). This is the only site that is edited with >99% efficiency. The amino acid change generated by editing is critical for producing an assembled glutamate receptor that is less permeable to calcium ions. Higuchi *et al.* (*6*) have generated a heterozygous mouse that lacks one copy of the *ADAR2* gene. These animals appear normal and show no decrease in the amount of editing at the Q/R site of the GluR-B transcript. Mice with two defective copies of *ADAR2* appear to develop normally but die during or soon after weaning. These homozygous mice are prone to epileptic seizures, and their tissues contain the unedited form of the GluR-B transcript. Higuchi *et al.* genetically engineered their homozygous mice to express a form of GluR-B (GluR-B^R) that had its arginine residue genomically encoded instead of introduced by editing. Bypass of the editing requirement at this position rescued the phenotype of the ADAR2-deficient mice and also showed that the GluR-B transcript is the principal target of ADAR2.

Inosines have been found in mRNAs from many tissues (*14*). The Higuchi *et al.* study demonstrates that the main target of ADAR2 editing is expressed in the CNS. Thus, the question arises whether a wider range of phenotypes would be generated if *ADAR1* rather than *ADAR2* was eliminated. This step has now been taken by Wang *et al.* (*5*). They generated heterozygous embryonic stem (ES) cells that carried a mutation in one copy of the *ADAR1* gene. The mutation resulted in the deletion of two exons that encode part of the adenosine deaminase domain of ADAR1; the exons were replaced with a marker gene. Heterozygous mice with mutations in one copy of the *ADAR1* gene could not be generated because chimeric animals (made by injecting ES cells into donor blastocysts) that would normally be mated to produce the heterozygous mice died at about embryonic day 12 (E12) of development. The chimeric mice suffered

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from defects in the production of progenitor red blood cells, which are made after hematopoiesis shifts from the yolk sac to the liver at stage E12 of embryonic development. Expression of ADAR1 increases in the liver at this stage, suggesting that there is a critical transcript in liver tissue that requires efficient editing at this time. Apparently, the activities of ADAR1 and ADAR2 do not overlap sufficiently to enable one enzyme to take over the responsibilities of the other (defective) enzyme.

There are probably other transcripts that are edited by ADAR1, but chimeric mouse embryos do not survive long enough to allow other phenotypes to emerge. In their next set of experiments, Wang *et al.* induced teratomas (embryonic tumors) in nude mice by injecting them with either wild-type or ADAR1 heterozygous ES cells. The teratomas were composed principally of neural tissues in which the amount of editing of known transcripts could be measured. In stark contrast to heterozygous ADAR2 newborn mice that had no decrease in RNA editing (*6*), the teratomas formed in nude mice from heterozygous ADAR1 ES cells showed decreased editing of glutamate receptor (GluR-B R/G and GluR5 Q/R) and serotonin receptor transcripts (but no decrease in editing of the GluR-B Q/R site). Thus, ADAR1 editing is very dependent on whether two copies or only one copy of the gene are expressed. This sensitivity to gene dosage could arise if, for example, lower levels of ADAR1 result in splicing of the transcript (that is, removal of noncoding introns) before editing, which would remove the essential ECS element.

In the heterozygous ES cells, a truncated protein (that has only the dsRNA binding domains) derived from the mutated copy of the *ADAR1* gene cannot be detected by Western blot analysis. Even if this truncated protein interfered with editing, this would not negate the possibility that ADAR1 is sensitive to gene dosage. However, clarification of the possible interference in the editing process by the truncated protein will have to await generation of ADAR1-deficient mice in which the entire gene has been deleted.

Fruit flies that completely lack any ADAR activity have been generated—this is much simpler to achieve in flies than in mice because flies have only one *ADAR* gene that is expressed exclusively in the CNS (*7*). The phenotype of the mutant flies shows parallels with that of the ADAR2 heterozygous mice. The fruit flies are viable and have a normal life-span, but they walk poorly, are unable to fly, and suffer progressive brain neurodegeneration. Editing is completely eliminated at known target sites in RNA transcripts that encode ion channels. The fly phenotype is consistent with the notion that RNA editing is important primarily in the fly CNS.

The generation of mice deficient in either of the two RNA-editing genes establishes beyond a doubt the importance of RNA editing. But what is the purpose of this nuclear editing process? Does it "correct mistakes" in the genome, as appears to be the case for the Q/R site in GluR-B transcripts, or does it produce a diversity of protein products that do slightly different jobs? Only with the discovery of additional edited transcripts and testing of the functions of the edited and unedited forms will this question be answered. The evidence that editing is important and more widespread than previously thought seems particularly appropriate this year as we busily interpret the human genome sequence. The lesson that was learned from the mitochondrial and chloroplast genomes almost 20 years ago—that there is more to predicting the coding sequences of genes than simply identifying exons—should be remembered as we behold the final genome sequence of human nuclear DNA.

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An Absorbing Study of Cholesterol

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Sterols are essential constituents of the membranes of animal and plant cells. Although structurally very similar, the sterols synthesized by animals and plants differ in the nature of their side chains; for example, the plant sterol sitosterol has the same ring structure as cholesterol (an important animal sterol) but differs in the side chain by an additional ethyl group (see the figure). Plant sterols taken in by animals in their food cannot be used by mammalian cells and are not normally absorbed. The cellular machinery that allows selective absorption of animal sterols but not those of plants is defective in a rare, recessive disorder called sitosterolemia. Patients with this disease accumulate large amounts of plant sterols in most tissues, have elevated plasma cholesterol, and develop coronary heart disease at an early age (*1*, *2*).

On page 1771 of this issue, Berge *et al.* (*3*) report the identification of mutations in two genes in sitosterolemia patients. The genes are new members of the ATP-binding cassette (ABC) family of transporters. Last year, another member of the gene family, *ABCA1*, was found to be mutated in Tangier disease. This disorder is characterized by defective efflux of cholesterol from cells, which results in an inability to make high density lipoproteins (HDLs) (*4*). That finding proved to be a treasure trove for the field of lipid metabolism because it identified the transporter responsible for removing excess cholesterol from cells. The Berge *et al.* study seems likely to yield similar riches. In addition to clarifying how plant sterols are excluded from animal cells, their results address a longstanding mystery in the lipid metabolism field: How is absorption of cholesterol regulated given that cholesterol appears to be passively taken up by intestinal cells?

The intestine is a major barrier to the uptake of plant sterols: Less than 5% of dietary plant sterols are normally absorbed compared to 40% of the available cholesterol. Plant sterols also appear to be preferentially removed from the body by excretion into bile. Berge *et al.* provide strong evidence that ABC transporter proteins pump plant sterols out of intestinal cells into the gut lumen, and out of liver cells into the bile duct. Although the transporters preferentially pump out plant

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