PERSPECTIVES: TRANSCRIPTION

Dirty Transcripts from Clean DNA

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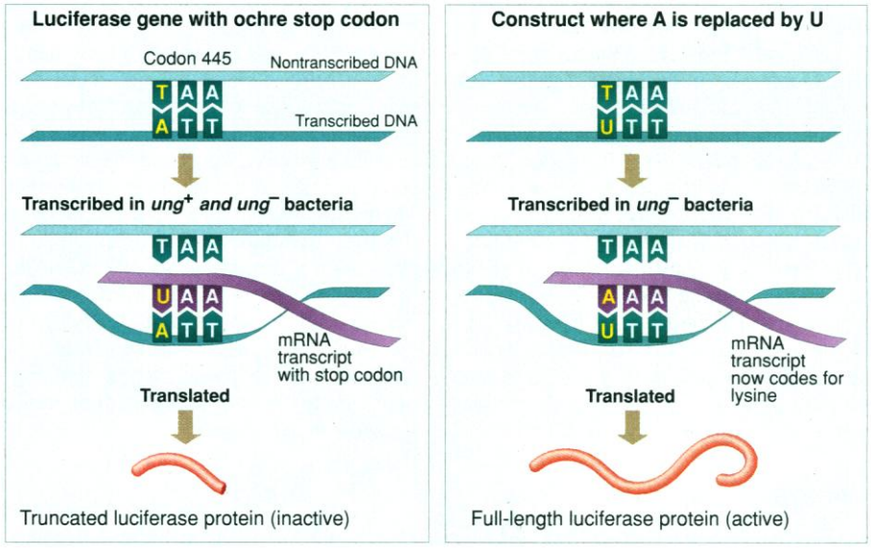
Sometimes aberrant bases appear in the DNA template in place of one of the four correct bases—adenine, cytosine, thymine, or guanine. The presence of an abnormal or damaged base usually halts RNA transcription until the base has been excised and the DNA repaired by special enzymes. However, in the case of some abnormal bases, for example uracil, transcription still proceeds even if excision and repair does not occur. This results in an mRNA transcript in which an incorrect base is inserted in the position opposite to the abnormal base in the DNA template, and synthesis of an altered protein ensues.

Although it is known from in vitro work that mutant mRNA transcripts are made with high efficiency from DNA templates that contain certain altered bases, there has been little evidence that this happens in living cells. Now, on page 159 of this issue, Viswanathan *et al.* present evidence for this process in nondividing *Escherichia coli* bacteria (*1*). They demonstrate that a miscoding base in DNA can give rise to an altered mRNA transcript and a mutant protein in vivo. Such a mechanism has been proposed as an explanation for "adaptive" mutation in starved bacterial cultures in which mutations arise at a rapid rate and are almost exclusively confined to those that restore growth (*2, 3*).

Viswanathan *et al.* designed an elegant assay to analyze transcription in *E. coli* that carry a mutation in the *ung* gene. This mutation renders the bacteria unable to excise and repair DNA containing uracil, which frequently arises by spontaneous or chemically induced deamination of cytosine. They introduced a stop codon—either with or without a deliberate thymine-to-uracil mismatch—into the firefly luciferase gene and transfected this into nondividing ung^+ and ung^- cells. (The bacteria were prevented from dividing so that transcription could be measured in the absence of DNA replication.) When DNA containing uracil is transcribed in ung^- bacteria, an adenine is inserted in the mRNA strand, resulting in the conversion of the stop codon to the wild-type codon. With the wild-type amino acid coding sequence restored, a full-length, active luciferase protein is synthesized that can be readily detected by its fluorescence (see the figure). In contrast, ung^+ bacteria repair the uracil mismatch mutation, resulting in translation of the stop codon and synthesis of truncated, nonfluorescent luciferase. In this way, the investigators demonstrated that nondividing *E. coli* could transcribe DNA containing uracil at high efficiency and could generate an altered protein resulting in changes in the characteristics (phenotype) of the cell.



Bypassing DNA mutations. Sometimes bases in the DNA become damaged; for example, deamination of cytosine produces uracil. In ung^- bacteria that are unable to excise uracil and repair the DNA, the mRNA strand that is transcribed from this DNA template has an adenine inserted opposite the uracil, which may result in synthesis of a "mutant" protein. Viswanathan *et al.* mimicked this situation in vivo by inserting a stop codon, with either adenine or an aberrant uracil, in the firefly luciferase gene. When the DNA template (transcribed) strand contained adenine, both ung^+ and ung^- bacteria produced a truncated, inactive luciferase protein (**left**). When the DNA template strand contained uracil, ung^+ bacteria repaired the uracil, resulting in a regenerated stop codon and synthesis of an inactive luciferase protein. However, ung^- bacteria are unable to repair the uracil defect and transcribe the DNA containing uracil into an mRNA transcript that eliminates the stop codon and instead codes for the correct amino acid of the wild-type, fluorescent luciferase protein (**right**).

But does this occur in normal cells? After all, Viswanathan *et al.* used cells that were unable to repair the DNA mutation. But in fact, in cell populations subjected to starvation, DNA repair capacity may decline over time creating just such a DNA repair-deficient situation. Although transcriptional misreading of DNA damage might also occur in repair-proficient cells, damage that actually blocks RNA polymerase is known to be subject to a specific and rapid repair process.

A day may seem a long time in the life of bacteria, but some mammalian cells, neurons and oocytes for example, remain functional for many years without dividing. Even here relatively stable changes have been observed in vivo. Perhaps the best characterized of these changes is in postmitotic neurons of the homozygous (*di/di*) Brattleboro rat. This animal suffers from severe diabetes insipidus due to a single base-pair deletion in the second exon of the vasopressin gene. Yet immunostaining studies show that cells expressing wild-type vasopressin begin to appear early in life in the supraoptic nucleus and the paraventricular nucleus of the rat brain hypothalamus, and that they increase in number as the animals age. The sequencing of mRNA transcripts from these cells revealed that the reading frame in the mRNA had been corrected by a deletion of two base pairs or an insertion of one base pair, and that these changes tended to occur in particular regions (hotspots) (*4–6*). Mutant vasopressin proteins have also been observed in neurons of the human hypothalamus (*7*). Initially, these phenotypic changes were interpret-

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ed to be the result of somatic mutations in the DNA, but it now seems more likely that they have arisen through mistranscribing of the vasopressin gene.

On the basis of their work with the Brattleboro rat, this group looked for two base-pair deletions in mRNA transcripts from other genes. They discovered such deletions in aberrant amyloid precursor protein and ubiquitin B protein in the brains of Alzheimer's disease patients. These aberrant proteins were found in the dystrophic neurites that contribute to the characteristic pathology (neuritic plaques, neuropil threads, and neurofibrillary tangles) of Alzheimer's disease (*8*) but not in the brains of individuals without dementia. Sequencing confirmed that the mRNA transcripts had two base-pair deletions and were confined to a subpopulation of cells in brain tissue samples from Alzheimer's disease patients. Examination of the genomic DNA, however, failed to reveal any evidence for the two base-pair deletions, so they are presumed to have arisen as errors of transcription.

How do these mutant RNA transcripts come about? The bacterial work of Viswanathan *et al.* suggests that aberrant bases accumulate in the DNA template, resulting in errors of transcription. Transcriptional mutations in neurons are only found in specific cell subpopulations that have a high metabolic activity (*7, 9*); active metabolism is known to be associated with elevated mutation rates. Of course, there may be other mechanisms for the generation of erroneous mRNA transcripts in neurons. There may exist age-related epigenetic or genetic changes that alter the fidelity of transcription or of mRNA editing (*10*). These mechanisms would explain the observation that mutant transcripts are produced from different genes within the same cell (*11*).

Thus, erroneous mRNA transcripts clearly arise in nondividing bacteria and in nondividing mammalian cells in vivo. They can alter the phenotype of the cell and are associated with clinical disease in humans. Yet we know very little about the rules governing erroneous transcription, the nature of any mechanisms that might correct such errors, or the consequences of mutagenic transcription for the cell. The clinical implications of transcriptional bypass of DNA damage and of errors introduced during transcription itself should ensure that this field comes under increasing scrutiny in the future.

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PERSPECTIVES: EVOLUTION

A Deadly Double Life

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Two years ago, the groups of Eduard Hurt at the University of Heidelberg and Eric First at Louisiana State University made the remarkable discovery that the carboxyl-terminal domain of human tyrosyl-transfer RNA synthetase—the enzyme that catalyzes covalent attachment of the amino acid tyrosine to the corresponding tRNA molecule in preparation for protein synthesis—has extensive amino acid sequence homology (49% identity) with a cytokine (*1*). The cytokine in question, endothelial monocyte-activating polypeptide II (EMAPII), activates endothelial cells to express tissue factor and surface adhesion molecules, and stimulates phagocytic cells to express tissue factor and tumor necrosis factor–α (TNF-α), and to migrate to sites of inflammation (*2*). Does human tyrosyl-tRNA synthetase lead a double life as the cytokine EMAPII? Apparently so, as Wakasugi and Schimmel report on page 147 of this issue (*3*). They show that human tyrosyl-tRNA synthetase is secreted as cells undergo programmed cell death (apoptosis) and is cleaved into not one but two cytokines.

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These investigators demonstrate that tyrosyl-tRNA synthetase, which normally resides in the cell cytoplasm, is secreted by a transformed human hematopoietic cell line that was forced to undergo apoptosis by serum deprivation. Secretion of tyrosyl-tRNA synthetase is specific; other tRNA synthetases cannot be detected in supernatants derived from these apoptotic cells. The secreted tyrosyl-tRNA synthetase is full length and inactive but, like many other cytokines, it becomes activated after cleavage into two fragments by extracellular proteases.

When tested in a panel of bioassays, the EMAPII-like, carboxyl-terminal fragment of tyrosyl-tRNA synthetase (just like EMAPII itself) is capable of stimulating leukocyte and monocyte chemotaxis, and inducing myeloperoxidase, tissue factor, and TNF-α synthesis. More surprising still, the amino-terminal fragment of tyrosyl-tRNA synthetase also appears to have cytokine activity. This fragment binds the interleukin-8 (IL-8) type A receptor (that until now has lacked an identifiable ligand) and behaves like the cytokine IL-8, yet still retains complete tyrosyl-tRNA synthetase activity.

Apoptosis is a physiologically normal process by which multicellular organisms—from flies and worms to humans—get rid of injured, infected, or developmentally unnecessary cells. It begins when a death signal causes a cell to activate a variety of intracellular proteases (death enzymes called caspases) and at least one nuclease (*4*), which together destroy the "guts" of the cell and prepare its corpse for phagocytosis. It is possible that the EMAPII-like and IL-8–like cytokines derived from tyrosyl-tRNA synthetase attract phagocytic cells to sites of apoptosis. It is even conceivable, as Wakasugi and Schimmel suggest, that secretion of tyrosyl-tRNA synthetase may help to shut down residual protein synthesis in the dying cell. However, this may be an effect, not a cause, as secretion of tyrosyl-tRNA synthetase coincides with destruction of the protein synthesis machinery by other means, including cleavage of the essential translation initiation factor, eIF4G (*5*).

Programmed cell death can be initiated from without, by binding of extracellular ligands such as TNF-α to dedicated cell surface receptors; or from within, by signals induced in response to DNA damage, viral infection, or other injury. One intracellular death messenger is cytochrome c, an essential component of the oxidative phosphorylation apparatus that is normally sequestered in the space between the inner and outer mitochondrial membranes. After induction of apoptosis, mitochondria release cytochrome c into the cytoplasm, where it works together with adapter molecules to activate caspases (*6*).