Self-Correcting Messages

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MOLECULAR BIOLOGY

Self-Correcting Messages

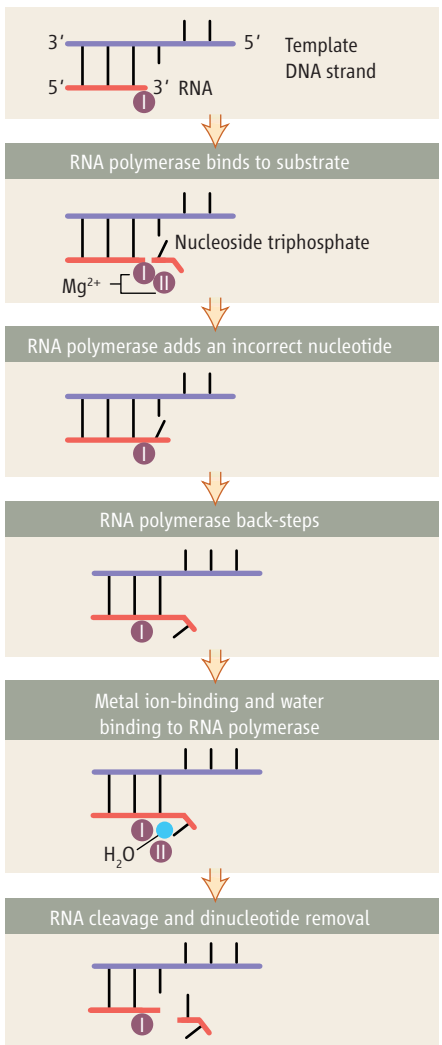
Patrick Cramer

Mistakes can occur as RNA polymerase copies DNA into transcripts. A proofreading mechanism that removes the incorrect RNA is triggered by the erroneous RNA itself.

Precision can be vital. Living cells transcribe their DNA genomes into messenger RNA (mRNA), which then directs protein synthesis. These processes are not without mistakes, but cells have evolved processes for proofreading and correction to shut down the propagation of errors. On page 518 of this issue, Zenkin *et al*. report that mRNA itself helps correct errors that occur during its own synthesis (*1*). This finding helps to explain the fidelity of gene transcription and suggests that self-correcting RNA was the genetic material during early evolution.

During gene transcription, the enzyme RNA polymerase moves along the DNA template and synthesizes a complementary chain of ribonucleotides, the mRNA. Errors arise when the growing mRNA incorporates a nucleotide that is not complementary to the DNA template. Nucleotides could, in principle, be removed by an RNA cleavage activity of the polymerase (*2*), but this intrinsic activity is very weak. Transcript cleavage factors enhance the polymerase's cleavage activity, and render error correction efficient in vitro (*3*, *4*). These cleavage factors are, however, not essential in vivo. These observations have led to the widespread belief that transcriptional error correction may not be critical for cellular function. However, erroneous mRNA could produce nonfunctional or harmful proteins, arguing for the existence of a mechanism that increases transcriptional fidelity.

Zenkin *et al*. now describe a simple mechanism for efficient, factor-independent error correction during transcription (see the figure). The authors assembled complexes of bacterial RNA polymerase with synthetic DNA and RNA. The RNA chains contained at their growing end either a nucleotide complementary to the DNA template, or a noncomplementary nucleotide that mimicked the result of misincorporation. In a key experiment, addition of magnesium ions triggered efficient cleavage from a polymerase-DNA-RNA complex of an RNA dinucleotide containing an erroneous nucleotide, but not from error-free complexes. Further biochemical experiments showed that RNA polymerase within an erroneous complex slides backwards or "backsteps" along DNA and RNA, and that the terminal, noncomplementary nucleotide participates in catalyzing removal of itself, together with the penultimate nucleotide. When the experiments were repeated in the presence of nucleoside triphosphates, the substrates for RNA synthesis, most of the RNA in erroneous complexes was still cleaved, although a fraction of the RNA was extended past the misincorporation site. Thus, RNA-stimulated RNA cleavage after misincorporation may suffice for transcriptional proofreading.



RNA-assisted transcriptional proofreading. Correction of misincorporation errors at the growing end of the transcribed RNA is stimulated by the misincorporated nucleotide. Mg^{2+} ions are bound to the catalytic region of RNA polymerase.

What is the chemical basis for such observed transcriptional proofreading? Both RNA synthesis and RNA cleavage occur at a single, highly conserved active site (*5*–*8*), and require two catalytic magnesium ions (*5*, *9*–*12*). The first metal ion is persistently bound in the active site, whereas the second is exchangeable. Binding of the second metal ion is stabilized by a nucleoside triphosphate during RNA synthesis, or by a transcript cleavage factor during RNA cleavage. Zenkin *et al*. show that the base of the back-stepped misincorporated nucleotide can also stabilize binding of the second metal ion (*1*). In addition, the misincorporated nucleotide and transcript cleavage factors may both activate a water molecule that acts as a nucleophile in the RNA cleavage reaction. Thus, the terminal RNA nucleotide plays an active role in RNA cleavage.

These results strengthen and extend the model of a multifunctional, "tunable" active site in RNA polymerases. Nucleoside triphosphates, cleavage factors, and back-stepped RNA can occupy similar locations in the active site, and position the second catalytic metal ion for RNA synthesis or cleavage. Because RNA dinucleotides are generally obtained in the presence of cleavage factors, the terminal RNA nucleotide and a cleavage factor likely cooperate during RNA cleavage from a back-stepped state. If the RNA is further backtracked, cleavage factors become essential for RNA cleavage, because the terminal nucleotide is no longer in a position to stimulate cleavage. In both scenarios, RNA cleavage provides a new, reactive RNA end and a free adjacent substrate site, allowing transcription to resume.

The discovery of self-correcting RNA transcripts suggests a previously missing link in molecular evolution (*13*). One prerequisite of an early RNA world (devoid of DNA) is that RNA-based genomes were stable. Genome stability required a mechanism for RNA replication and error correction during replication, which could have been similar to the newly described RNA proofreading mechanism described by Zenkin *et al*. If self-correcting replicating RNAs coexisted with an RNA-based protein synthesis activity, then an early RNA-based replicase could have been replaced by a protein-based RNA replicase. This ancient protein-based RNA replicase could have evolved to accept DNA as a template, instead of RNA, allowing the transition from RNA to DNA genomes. In this scenario, the resulting DNA-dependent RNA polymerase retained the ancient RNA-based RNA proofreading mechanism.

Whereas an understanding of RNA proof-

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reading is only now emerging, DNA proofreading had long been characterized. DNA polymerases cleave misincorporated nucleotides from the growing DNA chain, but the cleavage activity resides in a protein domain distinct from the domain for synthesis (*14*). The spatial separation of the two activities probably allowed optimization of two dedicated active sites during evolution, whereas RNA polymerase retained a single tunable active site. This could explain how some DNA polymerases achieve very high fidelity, which is required for efficient error correction during replication of large DNA genomes.

In the future, structural studies will unravel the stereochemical basis for RNA proofreading. Further biochemical and single-molecule studies should clarify how back-stepping and other rearrangements at the tunable polymerase active site are triggered. Techniques must also be developed to probe the in vivo significance of different aspects of the transcription mechanism discovered in vitro.

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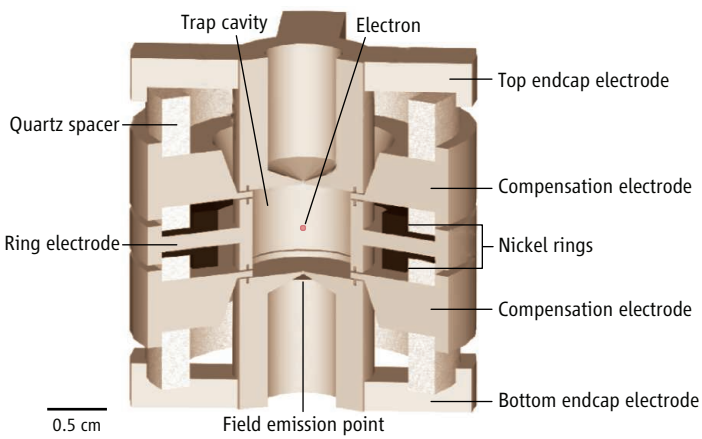
PHYSICS

A More Precise Fine Structure Constant

The fine structure constant, a vital quantity in quantum theory, sets the scale for the physical world. Recent measurements have improved its precision by a factor of 10.

Daniel Kleppner

Relativistic quantum electrodynamics (QED)—the theory that describes electromagnetic interactions between all electrically charged particles—is the most precisely tested theory in physics. In studies of the magnetic moment of the electron (a measure of its intrinsic magnetic strength), theory and experiment have been shown to agree within an uncertainty of only 4 parts per trillion. This astounding precision has just been improved. A new measurement by Odom *et al*. (*1*) has increased the experimental precision by a factor close to 6. In a parallel theoretical effort, Gabrielse *et al.* (*2*) have extended the QED calculations of the magnetic moment to a new level of precision. By combining these advances, the precision with which we know the value of the fine structure constant is now 10 times as high as that obtained by any other method. The fine structure constant is a dimensionless number, ~1/137, which involves the charge of the electron, the speed of light, and Planck's constant. It is usually designated α, and it plays a ubiquitous role in quantum theory, setting the scale for much of the physical world. Thus, α occupies an honored position among the fundamental constants of physics.



One-electron cyclotron. A magnetic field along the axis confines the electron radially; an oscillating electric field applied to the endcap electrodes confines it longitudinally. Nickel rings slightly perturb the magnetic field so as to couple the radial and longitudinal motions. The electron is trapped in a cavity that inhibits spontaneous emission. Other electrodes are used to control the electric field so as to reduce QED effects of the vacuum.

The quantity that has been measured by these researchers is the ratio of the magnetic moment of the electron to the fundamental atomic unit of magnetism known as the Bohr magneton. This dimensionless ratio is called the *g*-factor of the electron. Because the *g*-factor is a basic property of the simplest of the elementary particles, it has played a prominent role both in motivating and testing QED. According to Dirac's theory of the electron (*3*, *4*), for which he received the Nobel Prize in 1933, the *g*-factor should be exactly 2. In the period immediately following World War II, new data on the spectrum of hydrogen led to the creation of QED by Schwinger, Feynman, Tomonaga, and Dyson (*5*). According to QED, the electron *g*-factor would differ slightly from 2. Kusch and Foley discovered experimentally that the *g*-factor differed from 2 by about 1 part in a thousand (*6*). For this work Kusch received the Nobel Prize in 1955, followed by Schwinger, Feynman, and Tomonaga, who received the Nobel Prize in 1965. In 1987 Dehmelt published the measurement referred to above, accurate to 4 parts per trillion, for which he received the Nobel Prize in 1989 (*7*). The major experimental innovation in Dehmelt's measurement was a technique that allowed him to observe a single electron. The experiment of Gabrielse and colleagues builds on Dehmelt's work but incorporates major innovations that make the isolated electron into a quantum system whose energy levels can be probed.

The experiment compares the two types of motion of an electron in a magnetic field. The first is circular motion around the direction of the field at a frequency known as the cyclotron frequency f_c because the motion is described by the same equation as that for charged particles in a cyclotron accelerator. The second type of motion is spin precession. An electron possesses intrinsic spin, somewhat in analogy to the spin of a flywheel in a gyroscope. If a gyroscope is suspended by one end of its axle, it

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