How cell's master transcribing machine achieves near perfection

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One of the most critical processes in biology is the transcription of genetic information from DNA to messenger RNA (mRNA), which provides the blueprint for the proteins that form the machinery of life. Now, researchers have discovered new details of how the cell's major transcriptional machinery, RNA polymerase II (Pol II), functions with such exquisite precision. With almost unerring accuracy, Pol II can select the correct molecular puzzle piece, called a nucleosidetriphosphate (NTP), to add to the growing mRNA chain, although these puzzle pieces can be highly similar molecules.

Two papers in the June 6, 2008, issue of the journal *Molecular Cell*, published by Cell Press, describe advances in understanding Pol II copying fidelity. The papers are by Craig Kaplan of Stanford University and his colleagues; and Mikhail Kashlev of the National Cancer Institute Center for Cancer Research and his colleagues.

The researchers said their findings not only offer unprecedented details about the fidelity mechanism of Pol II, but likely about fidelity in all cellular genetic copying machines. They said their discoveries also offer understanding of how defective Pol II can generate errors in transcribing mRNA—errors that can promote cancer formation.

Both groups concentrated on the function of the Pol II "active site" region, where the enzyme captures an RNA component, called a nucleosidetriphosphate (NTP), and chemically attaches it to the RNA chain. Although Pol II uses the DNA genetic sequence as a template to

specify the RNA sequence, another largely unknown fidelity mechanism exists by which Pol II discriminates against incorrect NTPs. This fidelity mechanism is extremely precise; it can distinguish the NTPs that make up RNA from the deoxyNTPs used in DNA—although the two molecules differ only in one small chemical group.

In their paper, Kaplan and colleagues explored a key component of the active site known as the "trigger loop." This small bit of protein is highly mobile, and although researchers have believed that it plays a critical function in discriminating the correct NTP, that function was poorly understood.

In studies with yeast, Kaplan and his colleagues produced a mutant form of Pol II with a subtly crippled trigger loop. This mutation substituted one amino acid with another in what was believed to be a key position in the trigger loop, His 1085, for interacting with incoming NTPs to discriminate the correct one. The researchers compared the detailed molecular function of normal and His 1085 mutant Pol II enzymes during the encounter with both correct and incorrect NTPs. They also compared the behavior of the mutant with the action of the mushroom toxin alpha-amanitin, which is theorized to block Pol II by interfering with the trigger loop. The researchers' studies of the mutant and alpha-amanitin revealed crucial details showing how the trigger loop determines fidelity, said Kaplan.

"We found that the amanitin-treated wild-type enzyme behaved very similar to our mutant enzyme," said Kaplan. In fact, he said, the experiments, as well as structural information on the active site, indicated that alpha-amanitin targets the same His 1085 position in the trigger loop as does their mutation. Kaplan concluded that the findings reveal a specific and critical role for the trigger loop.

"These findings reveal what is called a 'kinetic selection' mechanism for

Pol II, which is like many polymerases," he said. "That is, the active site in one condition has a similar affinity for both correct and incorrect NTPs. However, because of motion within the active site—in this case the action of the trigger loop—catalytic activity in the active site proceeds much faster with the correct NTP than with the incorrect NTP. The trigger loop is mobile, and only when it is positioned properly in response to a correct substrate can it really function.

"We think this mode of substrate recognition is a general theme for systems that have to select the right molecule out of a giant pool of the wrong molecules," said Kaplan. An example, he said, is when the protein-making ribosomal machinery must select the correct transfer RNA from among similar-but-incorrect transfer RNAs.

Besides Kaplan, other co-authors on the paper were Karl-Magnus Larsson and Roger Kornberg.

In the other Molecular Cell paper, Kashlev and colleagues used a different yeast mutant to explore the function of the Pol II active site. In their screen for Pol II mutants, they identified one, E1103G, that shows a several-fold increase in error rate over the normal, wild-type Pol II.

Importantly, said Kashlev, the researchers could precisely measure the transcription error rate using a new assay, called a retrotransposition assay, developed by co-author Jeffrey Strathern.

The researchers' analysis of the effects of E1103G yielded significant insights into the function of the trigger loop, said Kashlev.

"Normally, when an NTP diffuses into the active site of the polymerase, the trigger loop closes behind it like a door, long enough for the polymerase to perform the chemistry to add the NTP to the end of the RNA chain," he said. "If the NTP is incorrect, there is a tendency for

this door to stay open for a longer time, which means that the NTP has a chance to diffuse out of the active site before the polymerase can proceed to chemistry.

"Our mutation occupies a strategic position important for keeping the loop open, like a latch," said Kashlev. "So, in the mutant, the door wants to stay in the closed state for a longer time, which means if an incorrect NTP migrates into the active site, there is time for the polymerase to add this incorrect NTP to the RNA chain."

Kashlev said the motivation for their studies of Pol II transcription fidelity is to understand the effects of Pol II errors on genome stability. Specifically, error-prone Pol II could generate mRNA that produces aberrant versions of the critical enzyme DNA polymerase. As DNA polymerase is responsible for gene replication, the result of its malfunction could be a burst of gene mutation causing an "error catastrophe" that could lead to genome instability and cancer formation.

Source: Cell Press