Nobel Laureate finds 'elegant' explanation for DNA transcribing enzyme's high fidelity

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Last month, Roger Kornberg of Stanford University won the Nobel Prize in Chemistry for his efforts to unravel the molecular basis of eukaryotic transcription, in which enzymes give “voice” to DNA by copying it into the RNA molecules that serve as templates for protein in organisms from yeast to humans. Now, Kornberg and his colleagues report in the December 1, 2006 issue of the journal Cell, published by Cell Press, new structures that reveal another critical piece of the puzzle: how the so-called polymerase II enzyme discriminates among potential RNA building blocks to ensure the characteristic accuracy of the process.

The researchers found that a portion of the enzyme known as the trigger loop acts like a “trap door,” swinging beneath a matching nucleoside triphosphate (NTP) building block, to close off the active center and form an extensive network of interactions with the NTP and other parts of the enzyme. Those interactions leave another side chain in the trigger loop precisely positioned, such that it may literally “trigger” the formation of the chemical bonds that link components of the growing RNA chain together. If the NTP is even slightly misaligned, Kornberg said, those critical interactions fail.

The trigger loop mechanism therefore couples NTP recognition and catalysis, ensuring the fidelity of transcription, they reported.

“Of all revelations from the structure [of the transcription machinery] since it was first solved, this is perhaps the most fundamental since it gets at the underlying mechanisms,” Kornberg said. “It’s long known that

the enzyme operates with high fidelity—selecting the correct base and sugar—but it's been a mystery how that is accomplished."

These findings offer "an unexpected and elegant explanation that's both beautiful and simple, as nature invariably proves to be."

The fundamental mechanism of transcription is conserved among cellular RNA polymerases, the researchers explained. Common features include an unwound region of about 15 base pairs of the DNA with some eight residues of the RNA transcript hybridized with the DNA in the center of the "transcription bubble." The enzyme polymerases involved are capable of moving both forward and backward on the DNA. Forward movement is favored by the binding of NTPs, while backtracking occurs especially when the enzyme encounters an impediment, such as damaged DNA.

Kornberg's group captured the first picture of the polymerase II transcribing complex by X-ray crystallography in 2001. Those structures revealed the complex with a nucleotide still in the enzyme's addition site, just after it had been added to the RNA transcript.

Later X-ray structures revealed the transcribing complex with the addition site available for entry of a matched NTP. Those crystals uncovered a second NTP-binding site on the transcribing enzyme, dubbed the entry site. While all NTPs can bind the entry site, only an NTP matched for base-pairing with the DNA template binds the addition site for attachment to the growing RNA strand, Kornberg said.

Yet the question of how the enzyme achieves such a high degree of discrimination between matched and mismatched NTPs remained unanswered.

The chemical attraction alone between RNA bases—adenine, cytosine,

guanine, and uracil—and their complementary bases on the DNA template strand is far from sufficient to account for the incredible selectivity of polymerase II, Kornberg said. And the scientists didn't know either how the polymerase avoids substituting the NTPs that constitute DNA for the correct RNA building blocks, molecules that differ by only one oxygen atom.

In search of an explanation in the current study, the researchers screened hundreds of crystals to achieve higher data quality and resolution than ever before.

"In the course of the work, we saw something that had never been noticed before— additional protein density beneath the matching nucleotide," Kornberg said.

The team traced that protein density back to a portion of the polymerase II enzyme: the trigger loop.

"Of the 14 crystal structures now reported in which the trigger loop was observed, only in two is it seen in that location, directly beneath the NTP," Kornberg said. Those were the only two crystals in which the NTP was correctly matched to the DNA template, evidence of the trigger loop's "clear relationship to NTP selection."

Further study revealed that, when a matching NTP reaches the addition site, the trigger loop swings from its usual position some distance away until it rests parallel to the NTP. It then forms a network of interactions—some 20 to 30 in all—with components of the NTP, a process that serves to "recognize all features of the NTP in the addition site and detect its precise location," the researchers reported.

"The specificity is a result of the alignment with the NTP that is critically dependent upon the base, sugar, phosphate and location when

the trigger loop swings into position," Kornberg said. "If it is misaligned even slightly, that set of contacts cannot occur."

As a consequence of that alignment, to angstrom (a unit of length equal to one hundred millionth of a centimeter) precision, a histidine side chain of the trigger loop rests on the ß phosphate, the chemical constituent that must have its bond broken in order for the NTP to join the RNA chain through the formation of a phosphodiester bond, Kornberg said. The finding suggested the side chain acts as a trigger for bond formation.

The whole decision-making process occurs extremely rapidly, he added, on the order of picoseconds. A picosecond is one trillionth of a second.

"The basis for the extraordinary specificity with which RNA polymerases transcribe DNA lies in a structural element termed the trigger loop, which makes both direct and indirect contact with all features of the nucleotide in the polymerase active center," the researchers concluded.

Source: Cell Press