A mutational timer is built into the chemistry of DNA

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If you had to copy billions of letters from one sheet of paper to another, you'd probably make a few mistakes. So it might not come as a surprise that when DNA makes a copy of its three-billion-base genetic code, it can slip up too.

The human's excuse could be fatigue or boredom, but scientists have

long wondered how DNA's nearly infallible replication machinery makes the mistakes that it does. Now, they think they may know a big part of the answer.

Scientists have discovered that the helical structure of DNA contains a kind of built-in timer that determines the frequency at which specific mutations spontaneously occur. They show that certain DNA bases can shape-shift for a thousandth of a second, transiently morphing into alternative states that can allow the replication machinery to incorporate the wrong base pairs into its double helix. Such mismatches, though rare, could serve as the basis of genetic changes that drive evolution and diseases, including cancer.

"Increasing or decreasing the rates of spontaneous mutations could significantly alter the ability of an organism to evolve or alter its susceptibility to disease," said Hashim M. Al-Hashimi, Ph.D., senior author of the study and James B. Duke Professor of Biochemistry and Chemistry at the Duke University School of Medicine."An interesting question is: What determines the mutation rate in a living organism," Al-Hashimi said. "From there, we can begin to understand the specific conditions or environmental stressors that can elevate errors."

The findings are published Feb. 1 in the journal *Nature*.

Every time our cells divide, the DNA within them must replicate so that each new cell receives the same set of instructions. Molecular machines known as polymerases make these copies of DNA by recognizing the shape of the right base pair combinations—G with C and A with T—and adding them into each new double helix, while discarding those that don't fit together correctly. Though they are good at their job, polymerases are known to slip up from time to time, generating a mistake roughly one out of every 10,000 bases. If not fixed these become immortalized in the genome as a mutation.

In their landmark 1953 paper describing the iconic structure of the DNA double helix, Watson and Crick hypothesized that DNA bases might be able to change their shape so that mispairs could pass as the real thing. A few years ago, Al-Hashimi and his colleagues used a sophisticated technique called NMR relaxation dispersion to capture these tiny movements or "quantum jitters," which only last for the blink of an eye.

The study, published in a 2015 issue of *Nature*, showed the bases G and T nudging aside the atoms on their surface so they could connect like puzzle pieces. The researchers found that these rearrangements came in different varieties, called "tautomeric" and "anionic" forms, though it wasn't clear which ones were responsible for replication errors.

In this study, Duke graduate students Isaac Kimsey and Eric Szymanski used an enhanced version of their previous technique to examine the relationship between these shape-shifting bases and the errors made by the DNA-copying polymerase. Again, they caught the G and T bases in the act, and showed that their shape-shifting occurred at about the same rate that polymerases incorporate G-T mismatches.

Together with their collaborators at The Ohio State University, they fed their NMR data into a "kinetic model" that traced the nearly invisible movements taken by the atoms in the mismatches that result in replication errors. They found that, though the different alternative states each contributed to errors, the tautomeric forms dominated under normal conditions and the anionic forms dominated in the presence of mutagens and environmental stress.

"In the past, we knew DNA polymerases make mistakes during DNA replication but did not know how they do it," said Zucai Suo, Ph.D., Ohio State professor of chemistry and biochemistry. "Now, our study provides a mechanistic sense for how the mistakes arise."

The results provide "convincing validation for the chemical origins of mutations proposed by Watson and Crick in 1953," said Myron Goodman, Ph.D., a professor of molecular biology and chemistry at the University of Southern California, who was not involved in the study. "It is significant scientifically, and even though it took about 65 years to prove, it also demonstrates the folly of ever betting against Watson and Crick."

The textbook depiction of the iconic double helix shows a static double-stranded structure, but it turns out that on rare occasions it can morph into other shapes that exist for exceptionally small periods of time," Al-Hashimi said. "Though some might question the importance of such states, there are a growing number of studies showing they can be major drivers of biology and disease. Given the difficulty in observing these phenomena, it makes you wonder how many more states are out there dictating the outcomes of biology that we don't even know about."

One of the surprising discoveries made by the team was that the frequency at which bases shifted their shapes varied with DNA sequence. In one of their experiments, Ohio State biochemists Zucai Suo and Walter Zahurancik essentially counted the number of times that polymerases incorporated the wrong base into the DNA. They found that mistakes were indeed not uniform: they appeared more frequently in some sequences than others. For example, a region with more Gs and Cs might form more quantum jitters, and subsequently more mutations, than an area that was rich in As and Ts.

The quantum jitters may be responsible not only for errors in replication, but also in other molecular processes such as transcription, translation, and DNA repair. Therefore, the researchers plan to continue to investigate how these alternative states might disrupt the seamless flow of information contained within our DNA.

More information: "Dynamic Basis of dG-dT misincorporation via tautomerization and ionization," Isaac J. Kimsey, Eric S. Szymanski, Walter J. Zahurancik, Anisha Shakya, Yi Xue, Chia-Chieh Chu, Bharathwaj Sathyamoorthy, Zucai Suo, and Hashim M. Al-Hashimi. *Nature*, Feb. 1, 2018. DOI: 10.1038/nature25487