Watching a polymerase be unfaithful in real time

By Robin Arnette

To make illustrations appear to come to life, animators project a sequence of drawings at high speed to create the illusion of movement. At any time, the artists may slow the process down or even stop the action to study an individual frame in detail.

In much the same way, scientists may now take snapshots of an enzymatic reaction in real time, then compile the images to view the key steps during the reaction. Others have utilized this new technique called time-resolved crystallography, but NIEHS postdoctoral fellow Bret Freudenthal, Ph.D., and colleagues William Beard, Ph.D., and David Shock, Ph.D., were the first to use the method to examine how a model human DNA polymerase beta (pol beta) chooses a nucleotide during DNA synthesis.

All three scientists are members of the NIEHS DNA Repair and Nucleic Acid Enzymology Group led by Samuel Wilson, M.D. They said since pol beta fills gaps in DNA following the excision of mutagenic damaged nucleotides, the work will help researchers better understand DNA repair and the processes that protect genomic DNA from mutations that could lead to cancer and other diseases. Their results *(http://www.ncbi.nlm.nih.gov/pubmed/23827680)* appeared online July 3 in the journal Cell.

Freudenthal, who won a 2014 NIH Fellows Award for Research Excellence for work leading to this study, is first author on the paper. "By utilizing the technique, we were able to look at steps that have effects on the downstream signals following DNA repair," he said. "We can now get a feel for how things are handled in the cell." (Photo courtesy of Steve McCaw)

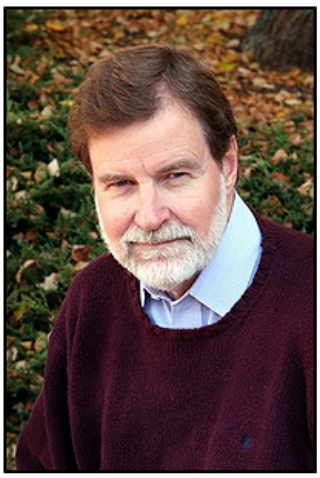
Uncovering pol beta's secrets

Wilson said the new X-ray crystallography technique confirmed features of the computational results his team and collaborators generated earlier, but revealed important surprises. They found that pol beta changes its shape depending on whether it incorporates a complementary base pair or correct nucleotide, which happens during faithful replication, or a noncomplementary base pair or incorrect nucleotide, which results in a mistake, or mutation.

The study also found that pol beta forms a third metal ion-binding site during the reaction, and likes to hang on to its reaction product pyrophosphate. Prior to this report, and other recent work, scientists believed that all polymerases used only two metal ion binding sites in their mechanism of action and released pyrophosphate instantly.

Using the new technique, Freudenthal and other colleagues performed the polymerase reaction in the crystal using pol beta and natural substrates, and froze the reaction at various time points for time-resolved crystallography. These quick peeks, in essence, slowed down the reaction about 100-fold, and allowed them to observe pol beta making critical decisions in molecular detail.

"Typically, with an enzyme like this, you'd have to look really fast because the entire reaction would be over in one second," Wilson said. "This method allows you to observe the reaction in whole seconds, rather than a nanosecond timescale."



"In general, the results are consistent with previous observations, but we had not actually seen the incorrect incorporation occur until now," Wilson said. (Photo courtesy of Steve McCaw)

Differences between right and wrong

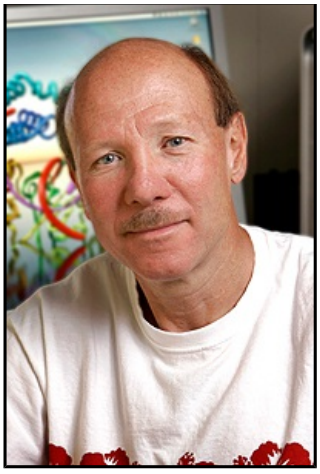
Freudenthal found that if pol beta incorporated the wrong nucleotide, the polymerase switched to an open configuration, which released the two products normally generated by the reaction, newly synthesized DNA and pyrophosphate. If it incorporated the right nucleotide, the pyrophosphate remained for an extended time and prevented pol beta from changing to the open structure and releasing pyrophosphate. This pyrophosphate hang-up or delay following correct incorporation could facilitate downstream channeling of DNA substrates during DNA repair. In contrast, the rapid release following incorrect incorporation may facilitate passage to alternative

DNA repair pathways or release of pol beta from DNA.

Freudenthal explained it this way. "Time-resolved crystallography revealed that the speed of the reaction and the shape of pol beta vary if the incorrect base pair is used. Are these differences important to figuring out how abnormal DNA repair leads to cancer? Right now, we don't know, but we're a bit closer to teasing out the processes that may be crucial."

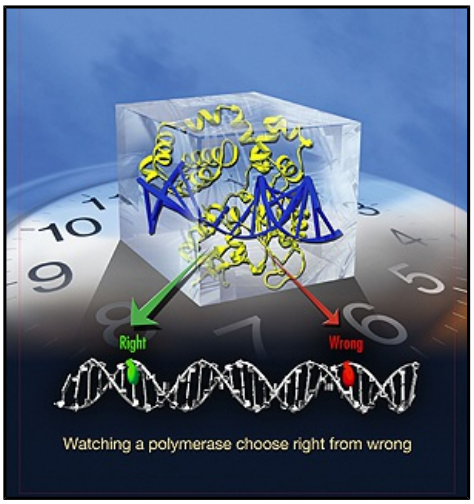
Although the technique used in this work is called time-resolved crystallography, Freudenthal also likes to call it time-lapse crystallography because the procedure reminds him of the time-lapse photography used in some animated features. Regardless of the name, he's sure other researchers will use the approach to make their own enzymatic movies and, in the process, learn more about the potential causes of disease.

Citation: Freudenthal BD, Beard WA, Shock DD, Wilson SA. *(http://www.ncbi.nlm.nih.gov/pubmed/23827680)*
2013. Observing a DNA polymerase choose right from wrong. Cell 154(3):157-168.



As a staff scientist in Wilson's group, Beard has participated in several breakthroughs involving DNA repair. (Photo courtesy of Steve McCaw)

Shock used his expertise as a research chemist to contribute to the work. (Photo courtesy of Steve McCaw)



DNA polymerases must choose a nucleotide from a pool of structurally similar molecules to preserve Watson-Crick base pairing during DNA replication and repair. This diagram illustrates a crystallized DNA polymerase making a temporal decision to incorporate a right or wrong nucleotide. (Design by Bret Freudenthal and artwork by Donna Corcoran)

The Environmental Factor is produced monthly by the National Institute of Environmental Health Sciences (NIEHS) *(http://www.niehs.nih.gov/)*
, Office of Communications and Public Liaison. We welcome your comments and suggestions. *(bruskec@niehs.nih.gov)*