Researchers watch antibiotics, bacteria meet at atomic level

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A new understanding of an enzyme important for the transfer of genetic information in bacteria may help scientists improve current antibiotics and also create antibiotics that are less vulnerable to resistance.

Researchers used extremely powerful imaging techniques to see, for the first time, exactly what happens between bacteria and antibiotics at the atomic level. They report their findings in two studies in the journal *Nature*.

The work provides the most detailed view yet of an enzyme structure that is key to turning on the genes that make bacteria work, said Irina Artsimovitch, a co-author on both studies and an associate professor of microbiology at Ohio State University.

Artsimovitch worked with Dmitry Vassylyev, the lead author of both studies and a professor of biochemistry and molecular biology at the University of Alabama at Birmingham. The two conducted the study with researchers from the University of Alabama at Birmingham , the University of Wisconsin-Madison and the University of Nebraska Medical Center.

In the first study, the team found that they could create a detailed image of the elongation complex, a structure formed by RNA polymerase. RNA polymerase is the enzyme responsible for setting gene expression in motion, a process called transcription. Without a properly functioning RNA polymerase, a cell will die.

“RNA polymerase spends most of its working hours as the elongation complex,” Artsimovitch said. “The complex makes RNA's messages one step at a time, many thousands of times, until its completion.

“This structure is important from a physiological point of view, not only for antibiotic design, but also because faults in the complex have been implicated in many diseases such as hereditary cancers.”

Artsimovitch and her colleagues used the bacterium Thermus thermophilus to run their experiments. While T. thermophilus won't make a human sick, the bacterium is widely used to gather structural information at the molecular level.

The researchers first isolated the RNA polymerase from T. thermophilus. They then created an active elongation complex by mixing the enzyme with small molecules of DNA and RNA. This solution hardened into a crystal, which the researchers could then examine using an imaging technique called X-ray crystallography.

X-ray crystallography let them create a computerized image showing the minute details of the elongation complex.

In the second study, the team learned how the antibiotic streptolydigin blocks transcription. Streptolydigin has been around for several decades and the researchers already knew that this antibiotic stops RNA polymerase activity inside a cell. But they didn't know what controlled this mechanism.

“We have to know what we're looking at – and working with – before it's possible to make a useful antibiotic,” Artsimovitch said. “Now we can. Now we can see where the enzyme and antibiotic make contact at the atomic level.”

Upon examining the X-ray images, the researchers found that the antibiotic prevented the normal operation of the elongation complex by freezing it in the inactive state.

They saw a loop-shaped element that must close every time the elongation complex adds a nucleotide (a building block of DNA or RNA) to the growing RNA chain. This loop must open again to allow the next cycle to happen. If something such as an antibiotic keeps the loop from closing, RNA polymerase can't properly function and stalls.

“This loop is a target for antibiotics, including streptolydigin,” Artsimovitch said. “If we can design new drugs that will prevent its movements, then we will immediately stop the action of RNA polymerase, and bacteria will die soon thereafter.”

The team's findings may be applicable to realms outside microbiology and drug discovery. Such a clear picture of the RNA polymerase elongation complex may be useful to a number of research areas, including manipulating the complex to increase the efficiency of bacteria that can harvest biofuels, Artsimovitch said.

“We think that this mobile loop is a hot spot for regulating transcription in all living organisms, not only by using antibiotics but also by manipulating cellular factors,” she said.

Source: Ohio State University