Detour leads to antibiotic resistance

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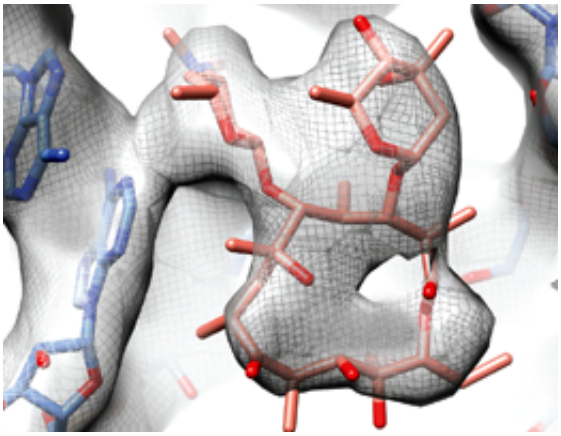

Ludwig Maximilian University researchers have used cryo-electron microscopic imaging to characterize the structural alterations in the bacterial ribosome that are required for induction of resistance to the antibiotic erythromycin.

Bacteria that have become resistant to multiple types of antibiotics are a growing problem in clinical medicine. Many bacterial species carry genes that cause them to be intrinsically resistant to specific antibiotics. Resistance can arise as a result of random mutations or be picked up via genetic exchange with other bacteria. In order to impede the development of resistance to existing antibiotics and guide the design of more effective anti-bacterial agents, it is crucial to understand the mechanisms that underlie known resistances. LMU biochemist Daniel

Wilson and his colleagues have just taken a significant step toward this goal in the case of erythromycin, a member of the so-called macrolide class of antibiotics. The LMU team has elucidated the mechanism which activates production of a protein that makes the bacterial ribosome insensitive to inhibition by the drug.

Antibiotic-dependent induction of resistance

Most antibiotics, including erythromycin, bind to ribosomes, the cellular organelles that are responsible for protein synthesis. As a consequence, the bacterial cell is unable to produce the proteins it requires for its growth and proliferation. Resistance to erythromycin is known to occur via methylation of a specific position in the so-called ribosomal RNA, which is an integral part of the ribosome. Moreover, binding of the antibiotic to the ribosome is a prerequisite for the production of ErmB, a protein enzyme that carries out the modification by attaching one or two methyl (CH3-) groups to the target site in the ribosomal RNA. "These modifications inhibit binding of erythromycin to the ribosome, rendering the antibiotic ineffective," says Wilson.

"The ErmB enzyme required for resistance is only produced when needed, and the short signal peptide ErmBL plays a key role in controlling synthesis of the ErmB protein", Wilson explains. The genetic information for both ErmBL and ErmB is encoded on the same messenger RNA molecules, which are transcribed from the bacterial DNA and program the synthesis of the proteins by the ribosome. However, the genes encoding ErmB are usually not accessible for translation. In the presence of the antibiotic, synthesis of ErmBL comes to a premature halt before the peptide is complete. This stalling of the ribosome enables the mRNA to undergo a structural rearrangement, which exposes the ribosome binding site of the downstream sequence that encodes ErmB, allowing it to be synthesized and thus inducing erythromycin resistance.

"Until now, the structural basis for the premature halt in ErmBL synthesis was completely unknown," says Wilson. "With the aid of cryo-electron microscopy we have, for the first time, imaged ribosomes stalled by through the cooperation of the ErmBL signal peptide, the inducing antibiotic and the ribosome. This structure provides insights into the mechanisms that underlie the induction of resistance," Wilson explains.

Diversion of the signal peptide

To everyone's surprise, the data revealed that ErmBL does not interact directly with the antibiotic. Instead, in the presence of erythromycin, the path of the growing peptide is apparently diverted within the tunnel of the ribosome, leading to specific interactions with the ribosome that inhibit its active site, bringing the ribosome to a premature halt.

"Ultimately, these findings should facilitate the development of better macrolide antibiotics," Wilson says. "But first we must gain a better understanding of how these mechanisms operate on the ribosome itself." To do this, Wilson and his coworkers now want to increase the resolution of their cryo-electron micrographs, and also examine the structures of other drug stalled ribosomes that have been brought to a halt by other agents.

More information: "Molecular basis for erythromycin-dependent ribosome stalling during translation of the ErmBL leader peptide." Stefan Arenz, et al. *Nature Communications* 5, Article number: 3501 DOI: 10.1038/ncomms4501 . Received 28 January 2014 Accepted 24 February 2014 Published 24 March 2014

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