Genetic switch potential key to new class of antibiotics

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Researchers have determined the structure of a key genetic mechanism at work in bacteria, including some that are deadly to humans, in an important step toward the design of a new class of antibiotics, according to an accelerated publication that appeared online today as a "paper of the week" in the *Journal of Biological Chemistry*.

Information stored in genes is translated or expressed into proteins, the workhorse molecules that make up the body's structures and carry its messages. In the classical view of gene expression, instructions stored in deoxyribonucleic acid (DNA) chains are copied into messenger ribonucleic acids (mRNAs). The mRNAs are then transported to ribosomes that pair them with transfer RNAs that contribute amino acids into a protein chain, thereby decoding the gene. In recent years, groundbreaking work has revealed that RNA is much more than a passive middleman, and instead exerts decisive control over expression.

Researchers in the Breaker lab at Yale and Nudler lab at NYU reported in 2002 that regulatory mechanisms arising from riboswitches regulate gene expression at the level of the mRNA by changing shape in ways that govern the genetic decoding process. Clarifying the principles of how riboswitches change their spatial organization, which entails binding to a small signaling partner, promises to inform the design of a new class of antibiotics. The current study clarified for the first time the exact structure of nature's smallest known riboswitch, and detailed how its structures control life processes in bacteria.

"The work has gained attention because interfering with riboswitches in bacteria known to cause major human infections may provide a new generation of antibiotics at a time when bacteria have become frighteningly capable of resisting current drugs," said Joseph E. Wedekind, Ph.D., associate professor with the Department of. Biochemistry & Biophysics at the University of Rochester Medical Center, and the study's senior author. "Among the bacteria now known to contain riboswitches are E. coli and streptococcus, as well as the bacteria behind forms of anthrax, gonorrhea, meningitis and dysentery. Riboswitches have not yet been found in human cells, and the hope is future riboswitch drugs will kill bacteria without side effects."

Riboswitches typically work by turning off the ability of an mRNA to decode its genetic message. Specifically, the current study looked at the preQ1 riboswitch that controls the ability of bacteria to produce a molecule called queuosine. In many organisms, queuosine or "Q" enables accurate gene expression by overcoming a built-in defect (tRNA wobble) in the mRNA-ribosome-tRNA system charged with translating genes into proteins. Without Q many important bacteria lose their ability to produce gene products necessary for survival or to confer human disease.

Landmark work by Yale's Ron Breaker first revealed the mechanism by which some bacteria ensure that they possess the right amount of cellular Q - i.e., a riboswitch "senses" whether there is enough of a key Q precursor called preQ1, which is the namesake for the riboswitch in the study. When too much preQ1 is present, bacterial genes responsible for producing preQ1, or its uptake from the environment, are shut down. The preQ1 precursor known as preQ0 also has the same effect.

The theory is that when preQ1 or preQ0 binds to the preQ1 riboswitch, the shape of the mRNA changes to mask signals necessary for a productive "handshake" with the ribosome, i.e. the cellular machine that

brings together mRNA and tRNA for protein building. The mRNA's ribosome binding site is instead locked up by the preQ1 riboswitch, like putting a hand in a pocket. In some cases, preQ1 riboswitches regulate the expression of a gene that codes for the enzyme queF, which converts preQ0 into preQ1, a key step required for Q production. They can do so because the riboswitches are located on the same mRNA that encodes the queF protein. Hence the system regulates how much Q is around by restricting the quantities of its precursors. When too much preQ1 is present, the enzyme that makes preQ1 is shut off by the riboswitch.

In the current study, Wedekind and colleagues solved the crystal structure of a preQ1 riboswitch in complex with its precursor metabolite preQ0. They chose to study the riboswitch from Thermoanaerobacter tengcongensis, a tough bacterium that lives at extreme temperatures and was first found in a Chinese hot spring. The team suspected that the hot spring preQ1 riboswitch must be stable enough to crystallize due to its need to function at high temperatures. The more stable the riboswitch, the easier it is to capture and map its molecular structure. The team also applied for and was granted access to a high-energy synchrotron radiation source located at the Stanford Synchrotron Radiation Laboratory (SSRL, CA) for experiments. Synchrotrons are particle accelerator facilities that direct high energy X-rays at crystals to produce detailed electron density maps from which extremely accurate 3-D pictures can be constructed.

The new snapshots revealed fascinating insights about the riboswitch structure and preQ0 interactions, the researchers said. They show for instance that preQ0 binds into a buried pocket of the riboswitch, and achieves a structure usually associated with the stability of DNA or RNA chains. When the riboswitch twists into the well-known double helix structure, the RNA composing part of its structure aligns the bases in its chain such that they stack on top of each other, adding to their stability. Because preQ1 is a modified form of an RNA base, it stacks on the

RNA bases in the riboswitch binding pocket with high affinity, adding stability to the switch that tightly locks up the mRNA's ribosome binding site.

The study further detailed how the first base of the mRNA's ribosome binding site binds to a loop of the riboswitch, the "lever" by which the ribosome-binding site is sequestered. Every riboswitch is made of an aptamer, the domain that binds to the signaling molecule, and a neighboring expression platform. The binding of the aptamer to the signaling molecule changes the platform's shape. Oftentimes, the expression platform is located next the code specified in a given mRNA, and the change in the platform takes away the ability of that mRNA to decode genetic information.

The order of bases in a typical aptamer domain of the riboswitch causes the chain to fold back and bind to itself to form a double helix that ends in a loop like a lollipop. The current study revealed some surprising insights into how the loop end of the preQ1 riboswitch aptamer domain binds to preQ0, and about how that binding empowers the switch to lock up, or sequester, the mRNA's ribosome binding site.

The true surprise came when the study revealed that the mechanism which enables the preQ1 aptamer loop to bind to the first base in the ribosome binding site is a standard G to C base pairing similar to that first described by Watson and Crick when they modeled the structure of DNA in 1953. The riboswitch, made possible by dazzling structural diversity within its loop structure, owes its function to one of the most straightforward of base pairs.

The study also confirmed Breaker's results that the preQ1 riboswitch is uncommonly small, meaning its economical and elegant design accomplishes more function than expected for an RNA of this size. The mRNA exhibits not only genetic coding ability but also contains its own

switch to shut off the decoding process. The current structural study found that in contrast to other riboswitches, the preQ1 aptamer is unusually small (34 nucleotides), which is about 2.5-fold shorter than functionally related riboswitches that recognize similar metabolites. This could imply that it is widespread in nature because evolution tends to pass on efficient mechanisms.

The next step for Wedekind's team is to see how other bacterial species sequester their ribosome binding sites using divergent preQ1 riboswitch aptamers. The design of a new class of antibiotics will be based on the specific base pairs that occur in each riboswitch loop, and on a clear understanding of the rules by which the preQ1 riboswitch aptamer buries neighboring mRNA signals that control protein translation.

Any drug would have to bind in place of the natural signaling molecule (e.g. preQ0), and lock the mRNA into a stable conformation to counter that mRNAs role in a bacterium's disease-causing ability. Previous experiments showed that bacteria like Escherichia coli (a main cause of food poisoning) and Shigella flexneri (a main cause of dysentery) are limited in the ability to grow or cause disease when genetically engineered to lack the genes for Q production.

Along with Wedekind, the work was performed in the Department of Biochemistry & Biophysics at the University of Rochester Medical Center by doctoral students Robert C. Spitale and Andrew T. Torelli who played key roles in the structure determination and refinement. Jolanta Krucinska is a senior technical associate in Wedekind's lab who grew the RNA crystals. Assistant professor Vahe Bandarian is an expert on the enzymes that produce preQ0, and led a partnering effort in the Department of Biochemistry at the University of Arizona in Tucson. The work was funded in part by a research grants to Dr. Wedekind from the National Institutes of Health and the National Institute of General Medical Sciences. SSRL is supported by the National Center for

Research Resources and the U.S. Department of Energy. The JBC paper of the week designation is given to the top one percent of papers published each year, and made special mention of the student contributions by Torelli and Spitale. The paper was also selected for the cover illustration.

"At least one riboswitch, the glmS, has been found to control gene expression as an RNA enzyme," said Spitale, who received his Ph.D. in Chemistry on April 16th, 2009. "The RNA World Hypothesis holds that RNA performed these tasks in ancient life before DNA and protein enzymes emerged. Our preQ1 aptamer structure tells us just how small and efficient a gene regulation element can be, and suggests that metabolite-binding to riboswitches may have been a preface to the emergence of more complex, catalysts that have yet to be discovered. The relative complexity and economy of the preQ1 riboswitch fold also forces us to reevaluate the numerous, small non-protein-coding RNAs present in the human genome. In the future, we hope to unravel the biology of these molecules, which promises to be of enormous benefit in our understanding of gene regulation, as well as of human disease."

Source: University of Rochester Medical Center (news : web)