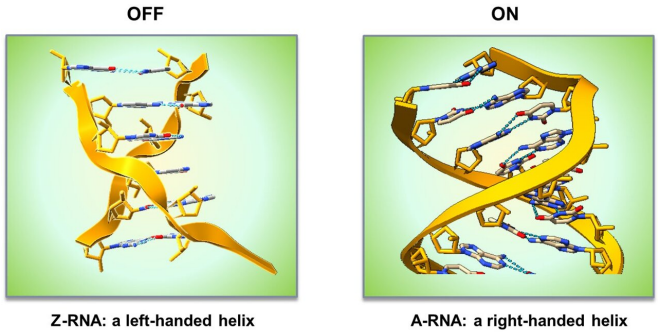
A Z-RNA nanoswitch encoded by 'junk DNA' turns-off immune responses against self

13 May 2021



The front ribbon of the Z-RNA Double-Helix points upwards to the left. In A-RNA and in Watson-Crick B-DNA the foremost ribbon points upwards to the right. Left handed Z-RNA can form from right-handed A-RNA by flipping the steps of the double helix upside down. The steps can be flipped by holding, then twisting each end of the helix Credit: Alan Herbert

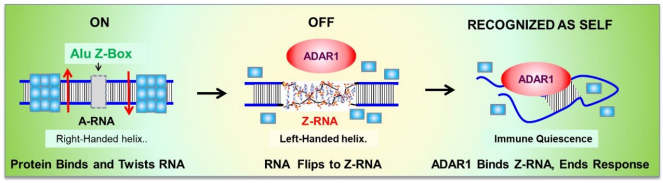
In a paper published in the May 13th, 2021 issue of *PLOS Genetics*, a Z-RNA nanoswitch that regulates interferon immune responses is described. The switch, less than 5 nanometer in length, is based on sequences, called flipons, that change outcomes by altering their three dimensional conformation. The Z-RNA nanoswitch flips from the shorter right-handed A-RNA helix ("on") to the longer left-handed Z-RNA helix ("off"). The flip ends immune responses against self RNAs, but not against viruses. Surprisingly, the Z-RNA nanoswitch sequence is encoded by "junk DNA." The Z-RNA nanoswitch is used by some cancers to silence anti-tumor immune responses. In other cases, a malfunction of the Z-RNA nanoswitch causes inflammatory disease.

In the paper, Dr. Alan Herbert of InsideOutBio describes how nature uses a nanoscale-Z-RNA switch to turn-off immune responses against self. The nanoswitch utilizes sequence elements called flipons that are capable of changing their three dimensional conformation under physiological conditions. The "on" state of the switch is represented by the right-handed A-form double-stranded RNA helix (dsRNA), while formation of the left-handed Z-RNA double helix turns the switch to the "off" position. The switch is used to defend against viruses that produce dsRNA when they infect cells. When the switch is "on," it starts an immune response against the virus. However, in normal cells, the nanoswitch is set to "off" as only self dsRNA is present. When the switch malfunctions and fails to turn off, Inflammatory diseases like Aicardi-Goutieres disease result. These diseases are characterized by the over-production of type I interferons. Although rare, these diseases are considered by some to provide insight into more common inflammatory diseases like systemic lupus erythematosus. Others believe that the Z-RNA "off" nanoswitch is used by cancer cells to silence immune responses against them.

The finding of the Z-RNA nanoswitch is also remarkable because it is based on the unusual left-handed double-stranded RNA conformation. Normally, double-stranded RNA is right-handed like the right-handed Watson-Crick B-DNA double helix. Since the discovery of the left-handed double helix, scientists have wondered if this structure has a function in biology. Many had given up hope that it did anything useful. The current paper, published in the prestigious open access journal *PLOS Genetics*, is not only important because it confirms that the left-handed Z-RNA has a key role in regulating immune responses, but the article also details the mechanism by how that happens. The question whether flipon switches regulate other genetic programs is currently under active investigation.

The paper highlights the way self-made RNAs are tagged with sequences encoded by so-called "junk" DNA. "Junk" DNA received this name because it does not code for protein. Instead, all "junk DNA" does is "copy and paste" itself throughout a host

genome. By doing so, the RNA sequences encoded by "junk DNA" become a way to distinguish self-made RNAs from foreign RNAs which lack these sequences. By forming Z-RNA, the "junk" sequence acts as flipons that turn "off" immune responses against self These "junk" elements, once an existential threat to the human genome now serve to protect normal cells from damage by preventing inappropriate activation of the immune system. Z-RNA formation is important in directing attacks to pathogens and other evolving viral threats.



Double-stranded A-RNA (dsRNA) formed by "junk" RNA elements is twisted and shortened as immune proteins binds to it. If the right-handed dsRNA (helical length =2.46 nm) contains a Z-Box, then the tension generated is relieved by flipping the sequence to the longer left-handed Z-RNA conformation (helical length = 4.56 nm). The flip causes the immune proteins to fall off the RNA. The Z-RNA engages an enzyme called ADAR1. ADAR1 modifies dsRNA, producing single-stranded RNA that doesn't cause immune activation. The Z-Box sequences are called flipons. They are often found in RNAs made from "junk" DNA named "Alu Repeats". Credit: Alan Herbert

How does the switch turn "off"? The answer illustrates how nature works at the nanoscale. RNA is short for ribonucleic acid. It is normally made from the DNA (deoxyribonucleic acid) that makes up the human genome. Double-stranded RNA (dsRNA) is formed by pairing of two RNA strands that have matched sequences. Some dsRNA can adopt different three-dimensional conformations under physiological conditions. The sequences are called flipons. The Z-RNA nanoswitch is made from dsRNAs that adopt either a right-handed double-helix (called A-RNA) or a left-handed double helix (called Z-RNA). The two flipon conformations represent the "on" and "off" setting for the switch. The switch is "on" when it is A-RNA. In this state, the switch allows the assembly of proteins that drive the inflammatory response. The switch is "off" when it flips to Z-RNA. The flip to Z-RNA promotes the release of pro-inflammatory proteins from self dsRNA. It also localizes a protein, called ADAR1, that binds the Z-RNA flipon conformation. After modification by ADAR1, the self dsRNA becomes single-stranded and is no longer capable of activating an immune response.

What causes the nanoswitch to change to the Z-RNA conformation? The pro-inflammatory proteins twist the dsRNA as they assemble on it. As a result of twisting, the bound dsRNA shortens in length, stretching the adjacent dsRNA segment. One way to relieve the tension is to flip a portion of the unbound dsRNA segment into the left-handed Z-RNA conformation. The flip relieves tension because the Z-RNA helix is 4.56 nanometers long whereas the A-RNA helix is only 2.46 nanometers in length. By being left-handed, the longer Z-RNA helix also offsets the right-handed twist caused by the pro-inflammatory proteins. The extra-slack produced by the Z-RNA flip causes these proteins to fall off the dsRNA, ending the immune response. The Z-RNA nanoswitch works similarly to an electrical "pull-switch" used to operate a light or a fan. In the case of a "pull-switch," tugging on the cord with sufficient force twists the internal components to the "off" position. With the Z-RNA nanoswitch, the action of ADAR1 prevents the switch from being turned back "on" as the changes made by the enzyme are irreversible.

"How does the Z-RNA nanoswitch protect normal cells?" The switch is based on flipon sequences present in the human genome, but absent from viruses and other disease-causing organisms. What are these sequences? Rather surprisingly, the flipons are encoded by what was formerly called "junk DNA." They arise from "junk DNA" elements called Alu repeats that make up about 11% of the human genome. Many Alu repeats are in reverse orientation to each other. They are called inverted repeats. When copied into RNA, the inverted repeats fold back on themselves to form A-RNA. The Alu inverted repeat dsRNA contain a Z-RNA forming element called a Z-Box that Is highly conserved. The Z-box enables the switch to turn "off" an immune response against self RNA. These

repeats are absent in viruses and serve to protect the host against attack.

The reported findings synthesize the discoveries of many scientists from different disciplines over the last 40 years. The Z-RNA nanoswitch is the first example of a flipon where an alternative nucleic conformations where there is direct genetic evidence for biological function. It is an example of a genetically encoded binary switch. Other types of flipons such as G-quartets and triplets are more varied in type than Z-flipons. Their different roles in cellular pathways are also being actively investigated. The new appreciation of the important role played by flipons in biology represents quite a paradigm shift. Until recently, these alternative conformations were considered oddities of interest to physical chemists only. The recent reversal in the status of Z-DNA and Z-RNA shows that science sometimes moves slowly, but eventual finds the correct answer. As Dr. Herbert comments "I'm very happy that we finally made it across the finishing line—along the way, there were many good reasons to give up."

InsideOutBio is an early-stage biotech company developing therapeutics for the treatment of cancer based on the role of complement proteins in self-recognition. By correctly identifying cancer cells as abnormal, the therapeutics initiate responses against them, leading to their rejection. Proof of principle has been accomplished in pre-clinical models. The company is operating remotely and has taken advantage of the new biotech ecosystem to discover and prototype the new therapeutics at low cost. The access to the enormous databases created by collaborative international efforts has helped the InsideOutBio scientists make fundamental discoveries such as those reported by Dr. Herbert in the *PLOS Genetics* publication. Previously Dr. Herbert discovered a family of proteins that bind the left-handed Z-DNA conformation and performed pioneering human genetic studies in the Framingham Heart Study. InsideOutBio is privately funded.

More information: *PLOS Genetics* (2021). DOI: 10.1371/journal.pgen.1009513

Alan Herbert. A Genetic Instruction Code Based on DNA Conformation, *Trends in Genetics* (2019). DOI: 10.1016/j.tig.2019.09.007

Provided by InsideOutBio