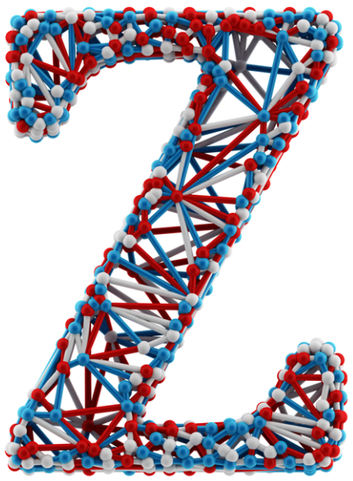
Z-DNA the new biology:

The third dimension of cancer therapeutics

DNA comes in many different shapes and sizes. Z-DNA, also known as left-handed DNA, is different from the more familiar right-handed B-DNA. Until recently, the role of Z-DNA in humans was a mystery. In a scientific breakthrough, Dr Alan Herbert of InsideOutBio Inc., Charlestown, Massachusetts, has identified the purpose of unusual DNA sequences called "flipons". Flipons get their name from their ability to "flip" their conformation, from right-handed to left-handed DNA. Flipons change the way that genes are read out, altering the programming of cells and their response to the environment. Flipons can turn-off the immune response, an ability sometimes hijacked by cancer cells to avoid rejection by the body's immune system. These discoveries hold the promise of new treatments for diseases like cancer in the future.



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The unique structure of the DNA double helix is one of the most recognisable images in biology. Most of the DNA in our cells takes the form of B-DNA. The familiar B-DNA is also known as right-handed DNA because the DNA strands wind to the right. The B-DNA double helix structure was discovered by Watson and Crick in 1953. Less well-known, however, is a different type of DNA: the left-handed Z-DNA. Z-DNA was discovered by chance and, until recently, its role – or even whether it had any purpose at all – remained a mystery. Z-DNA is not a mirror image of B-DNA, but has its own unique shape, a feature nature makes use of.

UNRAVELLING THE MYSTERY OF Z-DNA

An important step towards deciphering the role of this unusual form of DNA came from an RNA-editing enzyme called ADAR. Enzymes are vital proteins that speed up the chemical reactions in the cell through a part of their structure called the catalytic domain. Without enzymes, a single reaction would take hours to complete.

Flipons change how RNA messages are made, codons change how proteins are made.

When a cell makes proteins, RNA (ribonucleic acid) is made from a gene – a piece of DNA. The RNA carries the code for the particular protein that is to be made. Basically, the ADAR enzyme has the ability to re-code the RNA messages that are made by genes before the RNA information is translated into protein sequence. The re-coding performed by ADAR is known as RNA editing. In this process, ADAR changes one of the RNA building blocks to something else – meaning that the RNA now carries different information. The altered RNA then directs the production of a protein that functions differently from the unedited version. RNA editing thus allows a single gene to produce a number of different proteins, each with unique properties.

ADAR AND CANCER

Significantly, cancer cells can use ADAR and RNA editing to make proteins that give them a survival advantage. Other times cancer cells can use ADAR and RNA editing to avoid being terminated, especially when they make excessive amounts of RNA from genes. Built-in sensors within the cell signal when such a problem occurs. The alarm sounded leads to a rapid counter-response that can cause a cancer cell to die, either because it closes down the cell growth machinery or because it stimulates the immune system to kill the cell. The immune response generated by the abnormal cancer cells is similar to that produced by viruses, which also produce large amounts of their RNA in order to replicate.

To escape death, cancer cells must find a way to stop the excess RNA they create from triggering an immune response. Cancer cells do this by recruiting ADAR for their own purposes. The RNA-editing capability of ADAR removes much of the RNA that alerts the cell's alarm sensors. Indeed, many tumour cells can only survive by over-producing ADAR themselves. If ADAR can no longer function, they die; their dysfunction catches up with them.

Often, the excess RNA in tumour cells arises from parts of the genome that do not normally make RNA. Many of these RNA strands do not code for protein and frequently arise from regions of DNA where there are certain sequences that are repeated many times over. One common class of these repetitive sequences is known as ALU repeats. Altogether, ALU repeats make up about 11% of the human genome. They undergo extensive editing by ADAR.

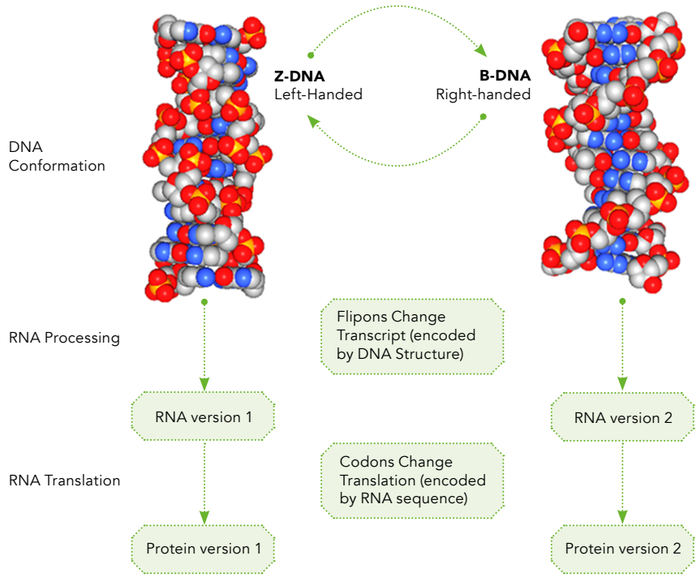
Normally, RNA is single-stranded. However, when two ALU repeats lie close to one another, but in opposite orientations, they naturally form double-stranded RNAs. When this happens, two such ALU copies are described as an inverted repeat. When an inverted repeat is copied into RNA, the two ALU copies fold back on each other to make double-stranded RNA. The ADAR enzyme only edits RNA that is double-stranded.

Cancer cells make more ADAR than normal cells to stop the inverted repeat RNA from triggering the interferon response. This allows cancer cells to evade the immune response and survive. Inhibiting ADAR is, therefore, a very effective way to prevent the unchecked growth of cancer cells. Specifically, preventing Z-DNA binding stops cancer cells from using ADAR to shut down the interferon response. Solving the mystery of Z-DNA formation and function has proven to have immense potential practical value in the search for new strategies to fight cancer.

THE SECRET UNLOCKED BY Z-DNA: FLIPONS

At InsideOutBio Inc., Dr Alan Herbert has made huge strides in unlocking the potential of Z-DNA in cancer treatment. Dr Herbert first identified ADAR as a Z-DNA binding protein. He was then able to show how Z-DNA binding by ADAR turns off the immune response. He analysed families who have alterations to the Z-DNA binding domain that prevent binding to Z-DNA. Affected individuals can no longer turn-off interferon production, an important step in regulating immune responses. Their immune systems are always on, leading to inflammatory diseases.

Dr Herbert has proposed the term "flipons" to describe sequences in the human genome that can adopt either the right- or left-handed DNA conformations under normal conditions.



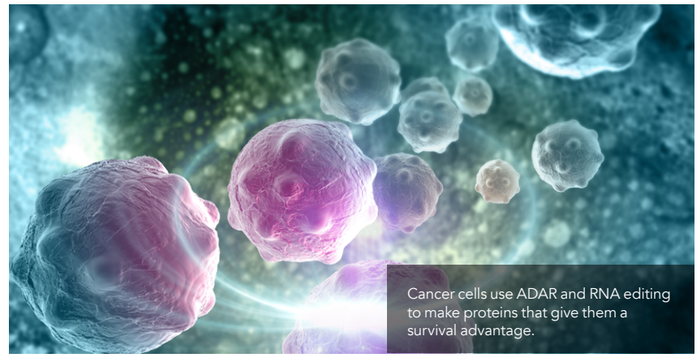
Flipons change how RNA messages are made, codons change how proteins are made.

Significantly, flipons have the ability to change the RNA output from a gene. In some cases, flipons may even act as an "on/off" switch for a gene – "on" with one conformation, "off" with the other. In other situations, the flip may lead to different edits in the RNA message, causing the production of different protein variants. Crucially, all this occurs without any change to the DNA sequence. The flip allows a rapid response by cells to different environmental challenges.

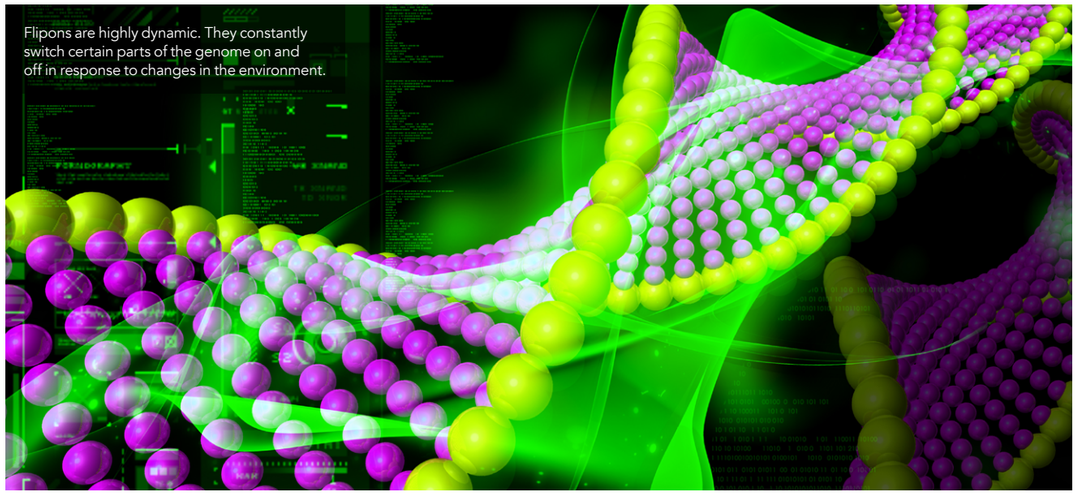
These capabilities naturally raise the question: what controls whether flipons are right- or left-handed? The flip from right- to left-handed DNA requires energy. One possible source of this energy is that generated by enzymes that copy DNA into RNA. When a gene is actively making RNA, flipons are more likely to be pushed into the left-handed conformation. The flip is favoured in gene regions (known as promoters) where RNA transcription starts. Indeed, flipon sequences are more frequent in promoters, meaning that they are often located in regions where they can regulate gene activity.

WHAT ELSE CAN FLIPONS DO?

Sometimes, DNA is damaged (for example, through exposure to radiation or substances that cause mutations). In these circumstances, due to the way the DNA bases are modified, less energy is needed to push flipons to Z-DNA. This allows flipons to act as DNA damage-sensors. The "flip" enables the cell to respond to the challenge and repair the damage. The flipon does this by



Cancer cells use ADAR and RNA editing to make proteins that give them a survival advantage.



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changing which RNA is made by a cell, effectively causing a different set of genetic programmes to run.

Flipons are highly dynamic. They constantly switch certain parts of the genome on and off in response to changes in the environment. By using flipons to change the RNA readout, DNA can store different sets of information using the same genomic base sequence.

As with any other genetic variation, flipons are subject to evolutionary change. They may be inserted, deleted or lose their ability to adopt the Z-DNA conformation. As a result, new genetic programs arise and others run differently. Those that provide an evolutionary advantage are transmitted to the next generation. ALU repeats contain flipon sequences that form Z-DNA. They flip into the Z-DNA conformation whenever RNA is made from the ALU repeat element. The Z-DNA allows localisation of ADAR to the repeats through a particular part of the enzyme, called the Zα domain. When ALU flipons are copied to other sites in the genome, they alter the RNAs made there. This process creates new genetic programs for natural selection to act on.

In some cases, flipons may even act as an on/off switch for a gene.

ADAR MUTATIONS CAUSE INHERITED GENETIC DISEASES

The primary challenge in unlocking the mystery of Z-DNA was to show that it had a biological function. To do so proved difficult, as formation of Z-DNA is highly dynamic – it can happen, for example, due to the conditions used in a particular experiment. This does not necessarily mean that Z-DNA forms that way in nature.

One way to tackle this problem is to observe human genetics. In these studies, there is no experimental intervention, so any Z-DNA can be assumed to have formed naturally. The challenge, however, is to find a phenotype (or observable characteristic) that provides the necessary information. Dr Herbert found a solution in the form of DNA mutations that inactivate the Zα domain of ADAR. Dr Herbert's analysis showed that mutations that prevented ADAR from binding Z-DNA are responsible for two genetically-inherited human diseases: Aicardi–Goutières syndrome (AGS) and bilateral striatal necrosis/dystonia (BSD). These conditions are closely related: both cause deficiencies in the regulation of interferons. AGS affects the brain and skin, causing significant intellectual and physical problems, while BSD affects movement and vision. These debilitating genetic conditions provide unambiguous proof of a biological role for Z-DNA.

TARGETING ADAR IN CANCER

The overexpression of ADAR has been found to occur in many types of cancer, including breast and lung cancer. In cell culture, over 40% of cancers are dependent on ADAR for survival. In mice, there is some experimental evidence that knocking out ADAR improves the response to immunotherapy, especially when combined with checkpoint inhibitors (drugs that help the immune system to fight cancer). Targeting pathways regulated by flipons, rather than just genetic mutations in DNA, is an entirely new way to treat cancer.

Other therapies are in development that use RNA editing to treat genetic disease. Here, ADAR recodes RNAs that have mutations that lead to disease. Efforts are underway to improve editing efficiency by targeting the Zα domains of ADAR. The approach avoids the permanent DNA changes made using enzymes like CRISPR; with these enzymes, an unintended alteration could produce negative outcomes.

AN EXCITING FUTURE

The recent discovery of flipons shows that our DNA holds many secrets that can take a lot of work to discover. Dr Herbert's insightful research into Z-DNA – a form of DNA that was, until recently barely understood – shows us just how much we still have to learn about our DNA. The flip from B- to Z-DNA that creates flipons can clearly have a great impact on the way our cells respond to their environment and to diseases like cancer. This new understanding offers hope for novel, targeted, individual therapies in modern medicine, taking us one step closer to true personalised medicine.

Behind the Research

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Research Objectives

InsideOutBio Inc. are developing a novel class of therapeutics that "light up" tumours for the immune system to kill by reprogramming ancient self/nonself pathways that cancer cells hijack.

Detail

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Bio

Dr Alan Herbert is originally from New Zealand where he earned his MBChB and PhD form the University of Auckland. He initiated his research on Z-DNA at MIT. Following this, his genetics research led to the publication of the first genome-wide association study on a human population in the journal *Science*. Following a period at Merck translating human genetics into therapies, Dr Herbert founded the start-up InsideOutBio where he leads research to improve cancer therapy using approaches validated by human genetics.

Collaborators

The ADAR community has provided huge insights into the way ADAR regulates the interferon system by editing gene messages. The work on Z-DNA by Dr Herbert and its recognition by ADAR was originally started at MIT in collaboration with Dr Alexander Rich. The Biophysics community has provided other structural and kinetic insight into the factors that influence Z-DNA and Z-RNA formation. Many labs and companies worldwide now focus on ADAR due to the cancer connection and the therapeutic potential to use this enzyme in the recoding of RNA.

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Personal Response

What direction should future research into flipons take?

"With the advances in microscopy, we will be able to see flipons change conformation in real time. These studies will enable us to understand what causes them to flip and the consequences that follow. The development of specific, small molecules that inhibit ADAR from binding to the Z-conformation will also increase understanding of the biological processes regulated by flipons. In combination, the microscopic and small molecule approaches will open a new window into the biology of cells and provide novel insights on how cells develop, on pathways that lead to disease and on how life evolves order out of chaos."