The flipside of DNA

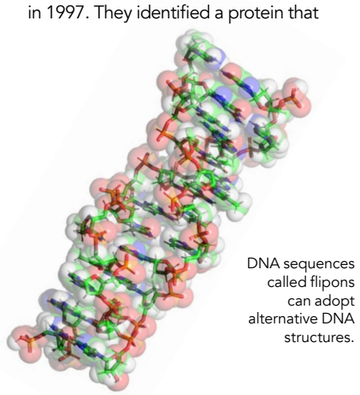
Flipons and alternative nucleic acid structures

DNA sequences called flipons can adopt alternative DNA structures. New research suggests that they have important biological roles. In a quest to further unravel the mystery of these dynamic DNA elements, Dr Alan Herbert, InsideOutBio Inc, USA, and colleagues have spent years conducting experiments at the cutting edge of genomic and molecular science. Now, they demonstrate that by targeting flipons, small pieces of highly conserved RNAs are able to kickstart gene expression during early development. The findings raise questions about how these insights can be applied therapeutically.

Our DNA holds a wealth of information coding for an almost endless number of end-product possibilities. Seventy years on from the discovery of DNA, we're still learning about the intricacies of our genome – and have much more to learn. An area of focus for Dr Alan Herbert, InsideOutBio Inc, USA, is alternative nucleic acid structures. These differ in structure from the original B-DNA first described by Watson and Crick in 1953. However, the genome contains many repeat sequences capable of forming alternative DNA structures under physiological conditions, like left-handed Z-DNA and four-stranded quadruplexes. These genetically encoded elements are called flipons.

FLIPONS

The existence of proteins binding to an alternative DNA conformation was first shown by Herbert and colleagues in 1997. They identified a protein that specifically recognised Z-DNA, a double helix that twists to the left rather than the right as found in B-DNA. For many years scientists believed Z-DNA did not have a biological function, but recent research by many groups – some in collaboration with Herbert – have shown the contrary to be true. In fact, Herbert has demonstrated that Z-DNA plays a critical role in regulating our immune responses.



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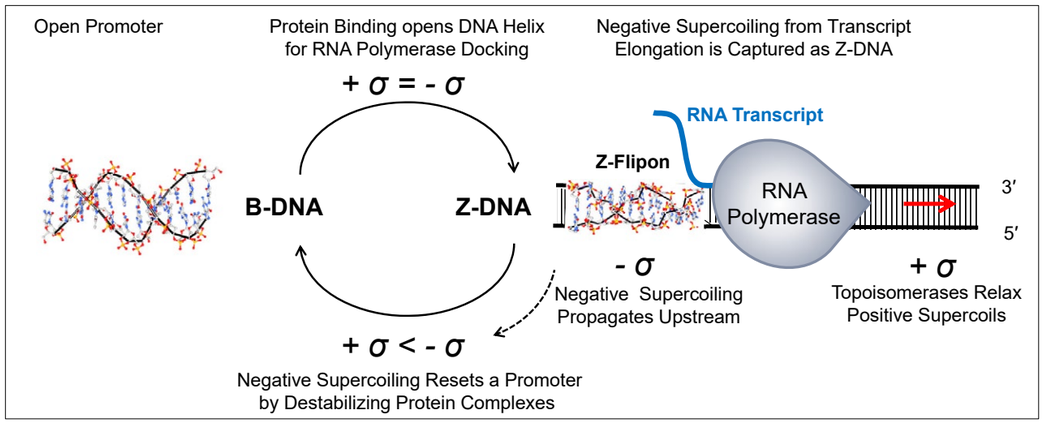
DNA ACROBATICS AND ENERGETICS

Building on this pioneering work, Herbert and team now describe how the formation of Z-DNA is initiated in cells. Flipons can flip from right- to left-handed, forming alternative DNA structures such as Z-DNA (called Z-flipons). In addition to Z-DNA, other classes of flipons can initiate the formation of different types of non-B-DNA conformations (NoBs). Recently published research from the Herbert laboratory sheds light on the energy requirements, sources, and dynamics for forming these NoBs in a cell.

Herbert notes that the energy needed to power these flips to a NoB comes from different sources, including energy liberated by enzymes (polymerases and helicases) during transcription. He also proposes that nucleosomes act as batteries storing the required energy to flip flipons on demand when environmental conditions dictate. This enables rapid response and almost immediate change to chromatin. Z-flipons can rapidly forward and reverse flip – and in doing so, acquire and release energy, acting to power changes in gene expression.

Inherently, these alternative DNA structures possess more energy than B-DNA. This excess energy can be released and used for other cellular needs, such as building cellular machines. The formation of different flipon classes described above have distinct energy requirements. For example, Z-flipons are quite dynamic and rapidly change conformation. They can act as a catalyst to accelerate the exchange of protein complexes on DNA. One example is with promoters, where the capture of energy from an RNA polymerase is used to dislodge proteins used to assemble the transcription complex. The reset enables the recruitment of a fresh set of factors to start the cycle over again. G-flipons are quite stable and can act as memory elements. They require reset by a class of proteins that are involved in many biological functions, including the enhancement or repression of gene expression and in the repair of DNA.

Other factors can also impact flipon conformation. Herbert's most recent study provides novel insights into how small conserved RNAs (called microRNAs, or c-miR) are implicated in the process. By binding to the flipon DNA in a sequence-specific manner, they can promote or block formation of the alternative DNA structure. This process appears important during embryonic development. By coding flipon conformation, c-miR can kickstart the process, much as a starter program is necessary to bootstrap loading of an operating system in a computer. In both cases, a microcode is involved that initiates the readout of key instructions necessary for programs to run.



Z-flipons catalyse the reset of promoters. To unwind B-DNA, proteins binding to the promoter must torque the DNA in the opposite direction. To reset the promoter, these proteins must be removed. Flipons help catalyze this process by capturing the energy released by the RNA polymerase as it reads through a gene to make a transcript. The energy powers the flip to the Z-DNA conformation. As flipons revert back to B-DNA, they can twist the DNA in a way that destabilises the proteins bound to the promoter, causing the promoter to reset for the next round of transcription. The σ symbols capture these changes in DNA winding in either the '+' or '–' direction.

ADAR BINDS TO Z-DNA

The key to unlocking the biological role of Z-DNA was the study of ADAR, an enzyme that binds to Z-DNA. Herbert previously showed that people with mutations affecting the ability of ADAR to bind to Z-DNA may experience inflammatory disease. ADAR serves to suppress interferon responses. Another protein in the human genome called ZBP1 also recognises Z-DNA. In contrast to ADAR, ZBP1 induces necroptosis, a form of inflammatory cell death. The regulation of ZBP1 by ADAR is important in regulating immune responses to foreign pathogens. Thanks to this work performed in collaboration with Balachandran and Poptsova, we now know of many diseases where sensing of Z-DNA protects the host against viral infections.

ADAR is also implicated in cancer. Tumours use ADAR to suppresses immune responses against them. In recent work also performed in collaboration with Balachandran and Poptsova, Herbert demonstrated how small molecules that induce Z-DNA in cells could bypass ADAR to activate ZBP1. In this case, targeting flipons with drugs showed the potential of this new class of therapeutics for improving cancer survival.

FROZEN FLIPONS

Flipons are frequent in the genome. Many sequences that can adopt alternative conformations are composed of nucleotide repeats that can form Z-DNA or other non-B-DNA conformations. The repeats vary in size and their length can change due to errors in replication. In some cases, long flipons can 'freeze' in the NoB conformation within cells. The junctions between B-DNA and the alternative conformation are prone to damage as they are often single-stranded. Thus, the frozen flipons can lead to DNA breakage and the development of Mendelian diseases, especially when enzymes critical to the repair process are faulty. These rare outcomes have led some researchers to believe that flipons destabilise DNA and do not have a fundamental biological role because of the damage they cause. However, the embryo locks down flipons prone to freeze during development as B-DNA. The other, more dynamic flipons act to increase the range of phenotypes possible. By allowing the rapid switch from one genetic program to another, flipons offer a survival advantage that can evolve through natural selection.

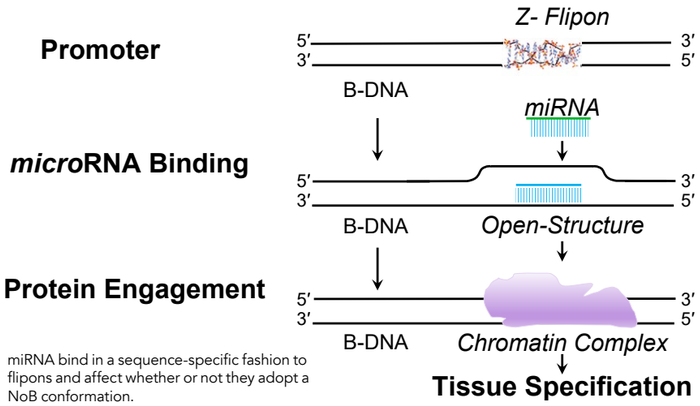
c-miR have binding sites on flipons, and these microRNAs likely help 'jumpstart' embryonic development.

THE BIOLOGICAL ROLE OF FLIPONS

The beauty of flipons is that different RNA messages and proteins can be formed from the same DNA sequence, leading to increased phenotypic diversity. In this way, flipons encode genetic information. The information content of flipons is different from that found in codons. In contrast to flipons that provide information through the structure they adopt, codons store information in their sequence. Codons consist of three DNA bases and play a key role during translation by specifying the amino acids incorporated into a protein. Codons lie in the body of a gene. Flipons are usually found in the promoter region of genes, positioning them perfectly to regulate gene expression through a change in their structure. Flipons of different types allow promoters to adopt a variety of different shapes associated with different outcomes.

The particular conformation adopted by a flipon can be programed using the miRs Herbert and colleagues described in their recent paper. The sequence-specific binding of the microRNA allows them to set the conformation of a flipon. The recognition of each flipon conformation by structure-specific proteins, each with different effects on gene expression, is key to understanding how this system of gene regulation works.

Over time, gene regulation can evolve by using different microRNA sequences to target flipons and to switch their conformation. The system then develops by changing the set of miR expressed in



miRNA bind in a sequence-specific fashion to flipons and affect whether or not they adopt a NoB conformation.

a cell. Such alterations can occur faster than it is possible to generate sequence-specific proteins that produce the same sequence-specific outcome.

FLIPONS AND CONSERVED microRNAs: EMBRYONIC DEVELOPMENT

Historically, microRNAs were identified by their effects in the cytoplasm of a cell. They can destabilise RNA transcript and impair translation of RNA into protein. These miR exert their influence during development by controlling when and for how long proteins are expressed in tissues and cells. Herbert and colleagues recently focused their attention on the role of microRNAs in the nucleus and their effects on RNA transcription, rather on the cytoplasm and translation. They found that highly conserved c-miR from placental animals bound flipons in promoters of genes that play a very important role in development, one distinct from the effects that microRNAs have in the cytoplasm. The flipons in the proximal promoter regions are bound by the c-miR in genes essential for multicellular development and neural transmission. The genes also code for proteins involved in several cellular mechanisms important in cell-to-cell interactions that decide the fate of each cell type. The findings offer an explanation of how these microRNAs likely help 'jumpstart' embryonic development by setting the scene for the sequence-specific proteins that characterise an organism's general operating system.

We need to better understand the RNA microcode that controls gene expression.

The scientists found that Z and G flipons were particularly abundant in the candidate cis-regulatory elements (cCRE) of proximal promoters. Not only are Z and G flipons the most enriched in promoter regions, but c-miR favours interactions with these flipons. Their study showed that c-miR have the potential to set the timing and location of when and where flipons form NoBs. They thus control flipon conformation to direct gene readouts and regulate gene expression. This is a non-standard way of regulating gene expression. But how are these interactions powered? As previously explained, polymerases and helicases liberate energy during transcription, enabling flipons to form NoBs. During early embryo development, the significant amount of transcription ensures sufficient energy is available to power flipons. Thus, energy can also be stored in nucleosome to facilitate readout of genetic information at a later stage in development to help assemble complexes containing sequence-specific transcription factors.

The miRNA bind in a sequence-specific fashion to flipons and affect whether or not they adopt a NoB conformation. Proteins specific for each flipon structure can then act as a bridge – localising protein complexes to modify chromatin deposition in different ways. This design cleverly allows the repurposing of protein complexes that have worked well in the past to regulate gene expression in new ways. It's important to note that no change to the DNA promoter sequence or to the protein binding preference is required. The miRs that determine the conformation of specific flipons in a promoter are the only element that changes. The team proposes that the miR (and other non-protein coding RNAs) ultimately control the chromatin landscape and direct the genetic output of a cell, a model supported by data from other studies on miR and gene expression. In this manner, flipon-miRNA interactions can influence developmental outcomes. Ultimately, this leads to increased phenotypic diversity of the offspring, which aids in the survival of a species.

THERAPEUTICS AND THE FUTURE

The evidence presented by Herbert and colleagues strongly suggests that c-miR interact with flipons to regulate their structure. In doing so, c-miR control gene expression in early embryonic development, providing the code to kickstart this process. However, the study focuses on only a portion of flipons – it is likely that far more interactions exist between flipons, microRNAs, and other noncoding RNAs than have been so far discovered. Flipons may also play a role in more biological processes. The current findings may be just the tip of the iceberg. The researchers encourage more studies to be done to confirm and fully elucidate these newly discovered interactions and roles.

Our understanding of the physiological and pathological role of flipons is growing in this fast-evolving field. As evidence mounts and points towards a key role for flipons in different cellular pathways, the question turns to if and how flipons could be targeted in therapeutics. Possibilities range from genomic editing to rectify frozen flipons, or even to synthesising flipons to modify gene expression for therapeutic benefit. Currently, these are only designs awaiting implementation. More research is needed on how flipons function both in health and diseases.

Behind the Research

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Research Objectives

Alan Herbert investigates how conserved microRNAs and flipons shape gene expression during development by altering promoter conformations.

Detail

Bio

Dr Alan Herbert trained in New Zealand as a physician. His work at MIT led to the discovery of the Z-DNA specific Zα domain. His subsequent Mendelian genetic studies of Zα variants confirmed a biological role for Z-DNA and helped to identify a new class of cancer immunotherapeutic targeted to Z-DNA flipons.

Collaborators

- Professor Maria Poptsova (HSE University, Moscow)
- Professor Sid Balachandran (Fox Chase Cancer Center, US)

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Personal Response

How could your findings with regards to flipons be used to develop new targeted therapeutics?

" The genome is much more dynamic than was once imagined. It can change shape to modify the read-out of information from it, enabling the creation of many different versions of a cell and many different body plans. Our ultimate goals are to understand the microcode used by RNAs that allows retrieval of the different genetic programs from DNA and the role flipons play in this process. The hope is to use this microcode to therapeutically reset diseased cells and tissues to a functional state. "