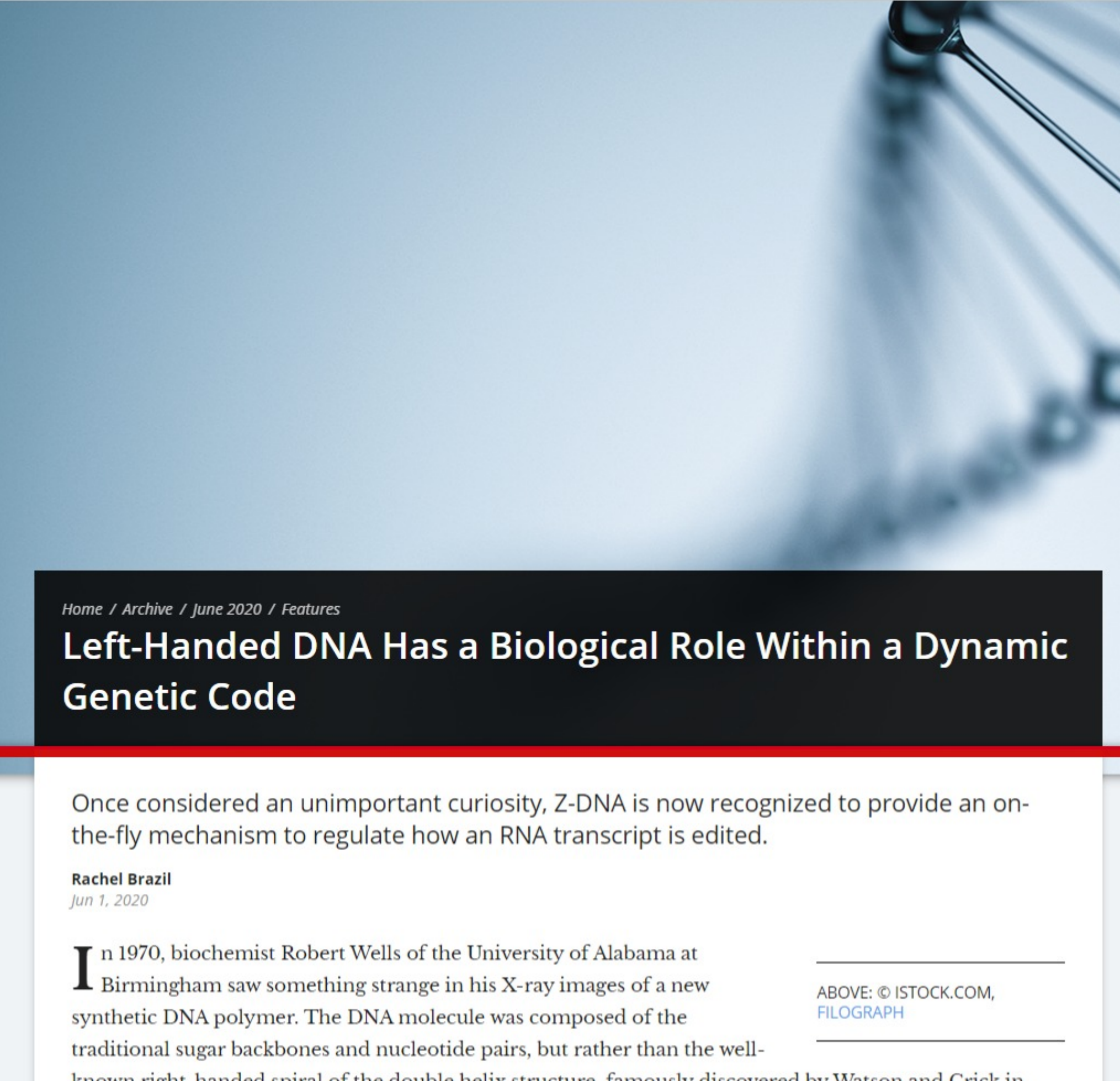

Left-Handed DNA Has a Biological Role Within a Dynamic Genetic Code

Once considered an unimportant curiosity, Z-DNA is now recognized to provide an on-the-fly mechanism to regulate how an RNA transcript is edited.

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In 1970, biochemist Robert Wells of the University of Alabama at Birmingham saw something strange in his X-ray images of a new synthetic DNA polymer. The DNA molecule was composed of the traditional sugar backbones and nucleotide pairs, but rather than the well-known right-handed spiral of the double helix structure, famously discovered by Watson and Crick in 1953, Wells's polymer spiraled in the opposite direction, giving it a zigzag appearance.

Whether this bizarre form of DNA existed in cells and had any function, and what that might be, was hotly debated for nearly half a century. But research has recently confirmed its biological relevance. So-called Z-DNA is now thought to play roles in cancer and autoimmune diseases, and last year scientists confirmed its link to three inherited neurological disorders. Today, molecular biologists are beginning to understand that certain stretches of DNA can flip from the right- to the left-handed conformation as part of a dynamic code that controls how some RNA transcripts are edited. The hunt is now on to discover drugs that could target Z-DNA and the proteins that bind to it, in order to manipulate the expression of local genes.

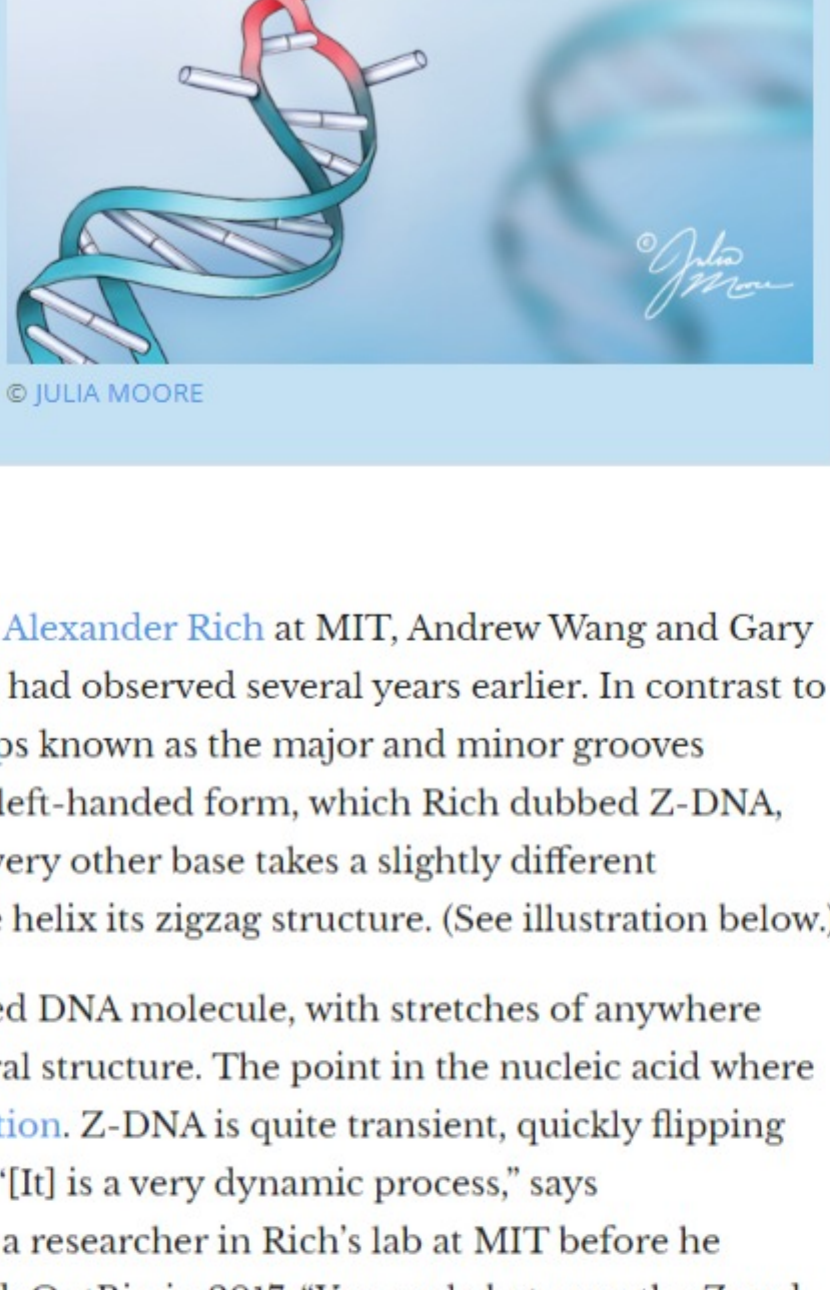
The story of Z-DNA is an unusual one, says Alekos Athanasiadis, an expert on protein–nucleic acid interactions at the Gulbenkian Science Institute in Portugal. "Usually you have a biological function and then structural work is used to identify the mechanism behind it," he says. "In this case, the research community was starting from structural information and the biology followed."

> It's clear this has some important function in nature.
> —Burghardt Wittig, Free University of Berlin

B- Versus Z-DNA

The left-handed Z-DNA double helix is held together by traditional Watson-Crick base pairs, but unlike righthanded B-DNA, which has major and minor grooves between the twists of its sugar-phosphate backbones, Z-DNA's grooves show little difference in width. In addition, every other base in a stretch of Z-DNA takes on a different orientation relative to the sugar backbone than the arrangement in B-DNA, giving this alternative form of DNA the zig-zag shape for which it was named.

Z-DNA exists transiently in short stretches of up to 100 base pairs within some right-handed DNA molecules. The site where the DNA molecule switches chirality is called a B-Z junction. At this point in the polymer, one A-T base pair projects to the outside of the double helix.



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Looking for a biological function

In 1979, working as postdocs in the laboratory of the late Alexander Rich at MIT, Andrew Wang and Gary Quigley solved the left-handed DNA structure that Wells had observed several years earlier. In contrast to right-handed B-DNA, which has two differently sized gaps known as the major and minor grooves between the twists of its sugar-phosphate backbone, the left-handed form, which Rich dubbed Z-DNA, has grooves that are much more uniform. In addition, every other base takes a slightly different orientation compared to how it sits in B-DNA, giving the helix its zigzag structure. (See illustration below.)

B-DNA and Z-DNA typically coexist on a double-stranded DNA molecule, with stretches of anywhere from a dozen to 100 base pairs taking on the reverse spiral structure. The point in the nucleic acid where the direction of its spiral changes is known as a B-Z junction. Z-DNA is quite transient, quickly flipping back to the B conformation, sometimes within seconds. "[It] is a very dynamic process," says immunobiologist Alan Herbert, who worked for years as a researcher in Rich's lab at MIT before he founded a DNA-based therapeutics company called InsideOutBio in 2017. "You cycle between the Z and B." This makes it exceptionally challenging to study.

To try to understand whether Z-DNA has a biological function, Colorado State University structural biologist P. Shing Ho, another former member of the Rich group, and colleagues looked for potential Z-forming sequences in the human genome. Using Z-DNA specific antibodies to identify Z-DNA stretches in a variety of plasmids, the research team discovered that Z-DNA formed most readily in repeated sequences of alternating purines and pyrimidines, particularly the purine guanine (G) and the pyrimidine cytosine (C). Using an algorithm to identify more than 300 similar sequences across the genome, the team found that "the Z-DNA regions tend to cluster right around the transcription start sites of most eukaryotic genes," says Ho. "They are widely distributed across different types of genes, but they are not found within genes themselves."

Researchers have also found that Z-DNA formation is linked to the "supercoiling" of DNA molecules, which are twisted around themselves into tangled configurations. During transcription, as an RNA polymerase moves along the DNA strand, it causes over- and underwinding of the DNA, compressing or relaxing the double helix. "These changes produce strain on the molecular configuration in the vicinity of the enzyme," explains Burghardt Wittig, yet another former Rich lab member, now at the Free University of Berlin. The strain behind the polymerase changes the thermodynamic stability of the DNA molecule, making it more likely to flip to the Z-DNA conformation. In 1990, Wittig was able to detect Z-DNA in cells during active transcription, and showed that inhibiting RNA transcription decreased the amount of Z-DNA in the genome.

This link to transcription led some researchers to speculate that Z-DNA provided some sort of epigenetic switch, turning gene transcription on and off. "But the data didn't really support that simplistic switch model," notes Herbert, and many scientists and funders gave up on the field altogether. But Herbert remained convinced that Z-DNA had a biological function, and was determined to prove it.

Z-DNA's relevance revealed

Herbert took a big step toward convincing the field of Z-DNA's importance in 1995 when he and others at MIT discovered a Z-DNA binding protein. The researchers found that the RNA-editing enzyme ADAR1 contained a domain, which they named Zα, that bound to Z-DNA. The Zα part of the protein binds to Z-DNA's backbone, rather than to any of the bases, and so is not specific to a DNA sequence, but to the left-handed conformation.

The Zα domain is present in a small number of other proteins in organisms from viruses to humans, but initially "it was not clear why these proteins bind to Z-DNA," says Wittig. The Zα domain seems to stabilize otherwise transient Z-DNA regions, so it's possible that the domain itself may induce B-DNA to flip to the Z formation, rather than the Z-DNA attracting proteins with a Zα domain. "This is a chicken and egg question," says Chi-Hua Lee, a postdoc in the lab of Wang, Rich's former mentee, who is now at the Academia Sinica in Taiwan.

ADAR1 also binds to double-stranded RNA, which forms when an RNA transcript folds back and base pairs with itself. Normal cells produce a large number of double-stranded RNAs during routine transcription of genes. The ADAR1 enzyme is known to edit double-stranded RNAs—specifically, it helps change adenosine bases into inosine, a base which is read by ribosomes as guanosine when present in codons being translated. This base change hampers RNA's ability to take on a double-stranded conformation. Double-stranded RNA molecules initiate cell signaling pathways involving type 1 interferons, proteins that trigger immune responses that can damage cells. By editing double-stranded RNAs, ADAR1 limits the interferon immune response, ultimately stopping more-widespread cell damage.

One source of double-stranded RNA targeted by ADAR1 is Alu elements within the genome. These are transposable DNA stretches, also known as jumping genes, named for their identification using a restriction endonuclease extracted from *Arthrobacter luteus* (ALU) bacteria. Alu elements make up about 10 percent of the human genome and are known to produce "junk" double-stranded RNA in cells. Alu elements include many alternating purine-pyrimidine base sequences—which Herbert realized were the exact sequences that are known to form Z-DNA. So when the ADAR1 protein binds to these Z-DNA–forming regions, it is often close to an Alu element that yields double-stranded RNA that ADAR1 also acts on. Herbert suspected this was no coincidence.

Sure enough, Herbert's team last year published evidence of a causal link between mutations in *ADAR1* that prevent the encoded protein from binding to Z-DNA and a number of inherited inflammatory diseases that involve the over-production of type 1 interferons. By looking at multiple mutations in the human ADAR1 gene, Herbert showed that only those that led to changes in the Zα domain of the protein—not in the binding domains that recognize double-stranded RNA—were associated with the diseases, suggesting that it was the loss of the enzyme's ability to bind to Z-DNA that was causing the inflammatory symptoms.

> It's a different way of thinking about the biology.
> —Alan Herbert, InsideOutBio

Herbert concluded that Z-DNA regions provide sites for ADAR1 to bind, allowing the protein to orchestrate the editing of double-stranded RNAs produced by transcription near the Z-DNA forming region. (See illustration below.) "The Z-DNA domain is actually localizing the ADAR1 editing to those Alu elements within the genome, and this allows the RNA they produce to be edited, which in turn will then inhibit any interferon response," says Herbert. But patients with the rare diseases he studied have genetic mutations that prevent the enzyme's Zα domain from binding to these Z-DNA regions in the genome; "because [they're] not able to localize the enzyme where it needs to be, you are unable to take care of business and stop the interferon response from being amplified."

Wittig says that Z-DNA's link to these diseases was "the nail in the coffin" showing that the left-handed conformation of nucleic acids is biologically relevant. "It's clear this has some important function in nature," he says. "This is definitely the final proof." And these are likely not the only diseases in which Z-DNA plays some kind of role, Herbert adds. The involvement of immune response pathways also suggests that Z-DNA could be involved in other ailments, including cancer, and recent work points to Z-DNA or proteins that bind it as potential therapeutic targets.

Drugging the Z-conformation

Z-DNA's ability to regulate the interferon cell signaling pathway provides a route to fight a range of conditions, from viral diseases to cancers. For example, some viruses use their own Z-binding proteins to downregulate host immune responses triggered by viral RNA. This is seen in the variola virus, a member of the pox virus family that expresses the protein E3L, which mimics ADAR1 in binding to Z-DNA and in turn prevents the interferon response from ramping up against the virus (*RNA*, 20:214–27, 2014).

"In principle, blocking the viral protein, which contains the Zα domain [that binds to Z-DNA], will allow an immune response to control the virus," says Alekos Athanasiadis, who studies protein–nucleic acid interactions at the Gulbenkian Science Institute in Portugal.

Alan Herbert, founder of the DNA-based therapeutics company InsideOutBio, sees Z-DNA as a potential target in cancer immunotherapy. Cancer cells make a lot of double-stranded RNA, which stimulates an interferon response and tends to lead to the death of the malfunctioning cells. But some 40 percent of tumors rely on the enzyme ADAR1 to protect themselves from this response by removing those RNAs. For these tumors, inhibiting ADAR1 could prevent RNA removal and in doing so, facilitate cancer cell death, Herbert and his colleagues proposed last year. Indeed, the deletion of ADAR1 in certain cancer cell lines causes cell death in vitro.

The therapeutic potential of molecules that inhibit ADAR1 could turn out to be extremely broad, says Robert Copeland, cofounder and chief scientific officer at Massachusetts-based Accent Therapeutics. The company's most immediate focus is on treating solid tumors, says Copeland, but he foresees applications in several inflammatory diseases, including autoimmune conditions such as lupus and Crohn's disease. After developing a proprietary assay for detecting ADAR1 inhibition and screening diverse libraries of molecules, Accent researchers now have confirmed hits. "Obviously, you never know what's around the next corner, but we anticipate being able to bring an ADAR1 inhibitor into the clinic sometime in 2022—that's our ambitious objective," says Copeland.

Accent Therapeutics isn't alone in its optimism. "I know that in the Boston area, there are at least three companies really seriously looking at the role of Z-DNA in these processes and its druggability," says Herbert, who expects new drug candidates to enter preclinical testing within the next few years. He says he suspects that treatments for cancer will be the first out of the gate, but like Copeland, he emphasizes that that would be the tip of the iceberg. There is even some evidence for an alteration from the usual B-DNA to Z-DNA conformation in the hippocampus of Alzheimer's disease patients, though he cautions that much more work needs to be carried out to fully establish what this might mean.

In the meantime, researchers continue to look for ways to target Z-DNA or induce or inhibit flipping between the right- and left-handed conformations. Last year, Kyeong Kyu Kim, a structural biologist at Sungkyunkwan University in South Korea, discovered that the antibiotic aklavin can induce Z-DNA formation. Kim also suggests that the bases extruded from DNA where the B and Z conformations meet could be another avenue for modulating Z-DNA formation.

Research into Z-DNA has had "a lot of fits and starts," says P. Shing Ho, a structural biologist at Colorado State University. "I think interest is going to [increase] again because of the potential link to disease states."

A dynamic code

For Herbert, the biological relevance of Z-DNA is massive, as he suspects that flips in DNA chirality influence how RNA molecules are processed across the genome. He suggests that the formation of Z-DNA and the localization of Z-binding proteins during transcription could quickly turn on and off the editing of RNA products at many active genes.

Because Z-DNA is so unstable, Herbert named DNA sequences that can flip into the left-handed conformation "flipons." He hypothesizes that the final readout of genetic information from the genome depends on the activity of these flipons at the time of transcription. "It's not an on-and-off switch for the gene, but it does play a role in regulating how the initial transcript is compiled into different RNAs," he explains.

Herbert suggests flipons take on the Z conformation only once transcription is underway, because the DNA supercoiling that accompanies active transcription is thought to promote the conformational change. But a 2012 study provided some evidence that Z-DNA may help open up the DNA that is normally tightly wound around histone proteins in nucleosomes, in preparation for transcription to begin. Keji Zhao of the National Heart, Lung, and Blood Institute found that a protein complex called SWI/SNF (SWItch/Sucrose Non-Fermentable), which is involved in loosening DNA-histone interactions, caused DNA near the promoter region of a gene to flip into the Z conformation.

Zhao speculates that Z-DNA modulates the placement of nucleosomes on the genome. "Formation of Z-DNA by the activity of SWI/SNF complexes may first generate an unstable nucleosome, which can slide to a nearby B-DNA region or eject the core histones to form a nucleosome-free region," thus allowing transcription to start, he explains. The idea that Z-DNA could be present on DNA molecules wound around histones is somewhat surprising, notes Ho. "Most of the data that we've seen from other laboratories have shown that Z-DNA doesn't actually sit on nucleosomes, primarily because [Z-DNA] is a very stiff structure," he says. "It's very rod-like, whereas nucleosomes require a very large amount of flexibility in the DNA in order to make essentially 200 base pairs wrap around the small complex."

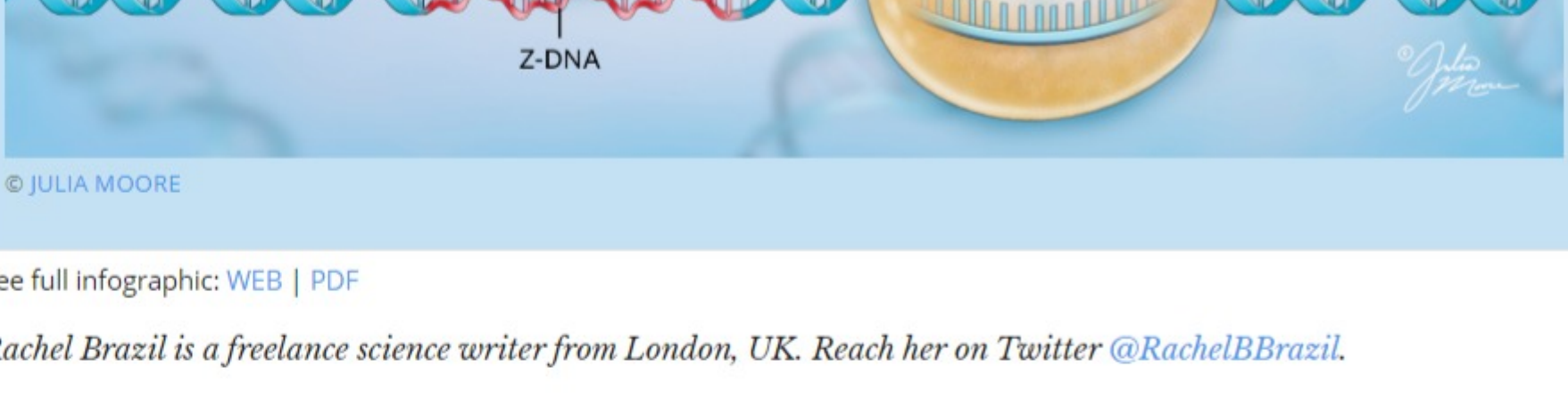
Zhao's work also supports the idea that Z-DNA formation may be influenced by DNA methylation. He and his colleagues created DNA templates assembled into nucleosomes that contained known Z-forming regions, including DNA with methylated and non-methylated guanine bases. The researchers could detect Z-DNA using a restriction enzyme modified with two copies of the Zα binding domain that would cleave the DNA if the Z configuration was present. The team found that Z-DNA was only present when the DNA fragments were made with methylated guanines. An older study had similarly found that DNA tends to switch conformations in the presence of methylated cytosines. Herbert adds that there are other types of DNA modifications, such as the hydroxymethylation of cytosine, that make the formation of Z-DNA more difficult and favor the B-DNA conformation.

The link to epigenetics will need more investigation, but it has led Herbert to the idea that flipons ultimately have a quick and spontaneous role in controlling how cells react to their environments. Herbert speculates that there could be a link between Z-DNA formation and oxidative stress in cells. During oxidative stress DNA bases themselves get oxidized, which could favor the Z-DNA conformation, with its formation acting as a sensor to activate protective pathways. "It's just a really great way of signaling that something needs to be responded to quickly, so [the cell] can then quickly assemble a complex in the right place to either repair DNA damage, or transcribe a damage response gene," he suggests. "You can actually change the genomic programming on the fly."

It has taken decades to understand that Z-DNA has significance in biology. Although there is still much to discover, it's becoming apparent that Z-DNA provides another mechanism to influence the decoding of genomic information, says Herbert. "It's pretty exciting. . . . It's a different way of thinking about the biology."

Z-DNA in Action

Z-DNA is linked to control of the interferon immune response through the RNA-editing enzyme ADAR1, which contains a Z-DNA binding domain called Zα. By editing double-stranded RNAs (dsRNAs), produced by stretches of repetitive DNA known as Alu elements, ADAR1 limits the interferon immune response normally caused by the dsRNA molecules. Recent work has shown that Z-DNA regions provide ADAR1 binding sites, allowing dsRNA editing to be localized to areas where dsRNA is produced following transcription. Genetic mutations of the Zα domain have been linked to serious neurodevelopmental disorders that are caused by the overproduction of type 1 interferons.



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