

It's one of science's sacred cows. Yet what if the DNA in your body isn't always double helical after all? Robin Orwant explores some unexpected twists

IMAGINE DNA. Chances are, the double helix leaps to mind. Ever since James Watson and Francis Crick proposed this elegant structure in 1953, the image of two gracefully intertwined ribbons framing the rungs of a spiralling ladder has become the very symbol of DNA. A model of stability and order, the double helix is so firmly established it seems heretical to envision DNA any other way.

But growing numbers of researchers are saying there may be much more to DNA than the double helix. It seems DNA can perform all sorts of gymnastic feats, twisting and folding into many different structures and even forming triple helices. Scientists have known about these structures for decades but mostly dismissed them as oddities created in the lab, with no function in living cells. Now, however, more and more evidence suggests these forms not only exist in living cells, but may have crucial roles in an ever-expanding list of **human** diseases, including the neurodegenerative **disease** Huntington's and perhaps even autism, schizophrenia and cancer. They may play an important part in our **health** too, policing the behavior of our genes and protecting them from damage. DNA may be a lot more dynamic and versatile than once thought and we are just beginning to understand what that means.

Much of the DNA in your cells does take the form of the conventional double helix, dubbed B-DNA which resembles a ladder twisted into a spiral. The "legs" of the ladder are a long chain of sugar and phosphate units, while each rung is formed by a pair of chemical units called bases. These bases form the letters of the genetic code: adenine (A), thymine (T), cytosine (C) and guanine (G). Each base on one side of the ladder pairs up with a specific partner on the other side. T normally pairs with A and C normally pairs with G. A form of chemical attraction called hydrogen bonding glues these two halves of the rung together. Importantly, this gluing is reversible, allowing the helix to be "unzipped" to read or copy the DNA, and zipped back up afterwards.

Base pairing plays a key role in DNA's dazzling gymnastic talent. Bases can twist and tilt in different ways when they pair up and this affects the three-dimensional structure of the molecule. In B-DNA, the helix winds to the right, with 10 rungs per turn. In Z-DNA, the winding is leftwards and the legs of the ladder form an unusual, zigzag pattern (see Diagram, page 40). "Hairpin" structures form when one strand of a double helix separates from the other and folds back on itself. The bases pair with others on the same strand. And a cruciform structure occurs when a hairpin forms directly opposite one on the other strand.

DNA can even form a triple helix, or triplex. This can happen in several ways. For example, when part of a double helix unravels, one strand can fold back and pair up with the part of the double helix that didn't unravel, via an unusual form of base-pairing (see Diagram). Two triple helices can even glue together to form "nodule DNA" and a tetra-helix can arise when two hairpins glue together. All of these are thought to form in the cell at times when the DNA molecule unzips. They are but a few examples: the tally of alternative structures currently stands at about 9, and the list just keeps growing. So what makes DNA adopt one kind of structure or another? It turns out the sequence of bases along a DNA strand is the key.

Forty years ago, when Robert Wells of Texas A&M University began his career, most scientists had closed the book on DNA structure. At the time, Wells was studying long stretches of DNA containing defined repeating sequences of bases to work out how these coded for proteins. While doing so, he noticed something odd. The DNA molecules behaved differently in several biochemical tests depending on which repeating sequences appeared. This led him to a startling conclusion. "I contended that the sequence of bases dictated the properties of the DNA," he remembers. At the time, this was pure heresy. Later, Wells showed that different repeating sequences were responsible for the formation of various alternative DNA structures, and suggested that these structures accounted for the distinct properties of the molecule.

Of course, he was working with synthetic DNA and many argued that the unusual DNA structures he had observed were just artefacts that would never form outside a test tube. But the advent of more advanced molecular **biology** techniques allowed Wells and his team to test this. They inserted various repeating DNA sequences into plasmids — small circles of bacterial DNA that are able to copy themselves — and watched what happened when they grew them inside live bacteria. Time and again, they found the same unusual structures forming in the repeating sequences.

Still, the sceptics remained unconvinced. Even if these novel DNA structures could be coaxed into forming in bacteria, the experiment could not show whether they existed in any other organisms or that they had a function. Interest in them waned. But some DNA researchers persevered, and found a handful of examples to suggest that they did indeed have a role in living cells. They showed that, in certain plasmids, cruciform structures form at the point where the bacterium's machinery starts reading the DNA as a first step toward making a protein. Some viruses do the same, and even use the cruciform structures to start copying DNA.

A few researchers suggested that these structures could play a role in **human** cells, too. They proposed that triplexes or four-stranded tetra-helices fold up the ends of chromosomes, known as telomeres, to help protect them. And triplexes have been implicated in control of gene activity. Certain sequences that form triplexes in the test tube have been found in the regions of DNA that switch genes on or off in living cells. What's more, if researchers alter or remove these sequences in cells, the activity of the neighbouring gene is affected. One idea is that a triplex attracts or repels key proteins, which in turn control gene expression.

No one has seen these structures at work in **human** cells. But in 1991, scientists announced a discovery that breathed new **life** into the alternative-structure field. An international team of researchers found the cause of a neurological **disease** called fragile X syndrome, which affects about 1 in 4000 people. They discovered that the **disease** was caused by a long stretch of DNA with the same CGG (and complementary GCC) triplet of bases repeated over and over again, lying next to a gene called FMR1. The repeats block the expression of the gene. Healthy people also have these repeats, but usually less than 40 and never more than 200. People with fragile X have between 230 and 1000. The fact repeating base sequences can lead to the formation of alternative DNA structures immediately suggested that they may have a role in the **disease**.

More than 30 disorders, including Huntington's and another neurodegenerative **disease** called Friedrich's ataxia, have been found to be associated with similar repeats. The researchers suggested the alternative DNA structures might help explain a puzzling aspect of some of these diseases. Within families affected by fragile X, for example, symptoms such as learning difficulties grow increasingly severe and show up earlier in **life** with each successive generation. This phenomenon is called genetic anticipation. It occurs because the repeating base sequences are unstable and tend to expand in length from one generation to the next, but the exact mechanism is unclear. Could alternative DNA structures be responsible for this process?

It's a question that may have implications for understanding other, more common diseases. Some studies suggest that conditions such as autism, bipolar disorder and schizophrenia also show genetic anticipation. This raises the possibility that expanding repeats play a role in these disorders too, although this has yet to be proved.

No one really knows what makes repeated sequences become unstable. But Sergei Mirkin, a researcher at the University of Illinois in Chicago recently suggested how unusual DNA structures might cause already unstable repeats to keep expanding. In work published in March, Mirkin suggests a mechanism for how a DNA triplex might lead to the expansion of the repeats involved in Friedrich's ataxia (*Molecular Cell Biology*, vol 24, p 2286).

The **disease** is characterised by GAA repeats in a gene called X25. It was already known that these repeats formed triplex structures that prevented DNA from being copied in the test tube. When Mirkin inserted these

repeating sequences into plasmids in bacteria, copying was also blocked showing that the process could take place in living cells. However, it was only blocked when the number of repeats exceeded 40. Mirkin suggested that when the number of repeats exceeds this number, a triplex forms which "stalls" DNA replication.

So how does stalled copying cause repeats? DNA gets copied by an enzyme called DNA polymerase. This normally chugs along the double helix, unzipping it with the help of other proteins, and using the unzipped strand as a template for copying the DNA sequence. However, blockages on the strands can result in DNA polymerase "stalling" in one place. Mirkin suggests that this stalling may cause the repeat expansions in humans. In his model, when DNA polymerase hits an obstruction it behaves like a car stuck in the sand, spinning its wheels and throwing up piles of sand behind. As it tries to break free of the obstruction — a triplex — and restart, DNA polymerase keeps churning out new DNA, and these extra repeats are incorporated into the copied DNA.

[Slipping up](#)

Richard Sinden of Texas A&M University and colleague Chris Pearson, now at the University of Toronto, have suggested how this incorporation happens. They reckon that jammed replication in regions of repeated sequence can itself cause a weird structure called "slipped strand DNA" to form. As the jammed DNA polymerase churns out extra copies of the repeats, the copied strand can fold back on itself to form a hairpin. When this strand glues back together with the original template, it will be out of register thanks to the hairpin, and will form a slipped strand structure (see Diagram). When this area is unzipped and copied again, the extra repeats in the hairpins are copied too, leading to repeat expansions. Sinden and Pearson discovered slipped strand DNA in 1996, using fragments of DNA containing the repeats found in fragile X and the muscular wasting **disease** myotonic dystrophy. Pearson found that the longer the repeat sequences are, the greater the likelihood that slipped strand structures will form. This correlates well with their propensity to expand and cause **disease** in people.

Sinden thinks that yet another unusual structure called "unwound DNA" could help explain what causes the repeats to expand in a neurological **disease** called spinocerebellar ataxia type 10 (see Diagram). He found that the base pairs in the long repeating AATTCT sequence involved in the disorder are prone to falling apart. Unwound DNA was originally identified in 1984 in areas of DNA known to trigger replication. Sinden suggests the repeat sequence causes multiple rounds of aberrant DNA replication, causing expansions.

These are just a couple of models for how DNA structures might cause expansion and there is no real consensus. But that's to be expected, Wells says. "The field is very young, and studies on mechanisms are in their infancy." Besides, there is no reason to pick any one model — they could all be right in one way or another. It's unlikely that any one mechanism will explain expansions in all diseases and in every situation, says Sinden.

Sceptics point out that no one has proved that alternative DNA structures actually form in the chromosomes in **human** cells to cause these diseases. But in March, Michael Lieber of the University of Southern California found evidence that an unusual DNA structure forms in the chromosomes of living **human** cells and can help cause cancer.

Abnormal chromosomes are often associated with cancer. Sometimes a piece of one chromosome can break off and join on to the end of another. These "translocations" often activate genes that predispose cells to becoming cancerous. Lieber was studying a translocation that commonly occurs between chromosomes 18 and 14. He noticed that the break on chromosome 18 always happened at the same place (Nature, vol 428, p 88).

Lieber suspected this break might be due to an unusual structure within that area of the chromosome. He analysed the chromosomes in the test tube, and discovered that there were several regions of single-stranded DNA in the key area. Crucially, he was able to test for these regions on chromosomes in living cells to confirm that the single-stranded regions existed in a more natural context. Unpaired bases at the sites implied that unusual DNA structures were present, but Lieber says it will take many more experiments to determine exactly which structures are forming.

Mirkin hails the work as an important contribution, but Sinden is more sceptical. He points out that probing cells with harsh chemicals can alter DNA structure and result in misleading artefacts. But Lieber stands by his methods. He believes his study provides strong evidence that unusual DNA structures are implicated in cancer. He suspects they may also be involved in other translocations associated with cancer, and his lab is working on some candidate chromosome sites.

As for the roles of various other structures in repeat expansion disorders, Lieber is waiting to see proof that they actually form at the expansion sites on **human** chromosomes in living cells before jumping on the alternative DNA structure bandwagon. But he may have a long wait, according to Pearson. Some structures may be so rare or transient that they are nearly impossible to find on the chromosome in normal situations. "It's like detecting a grain of sand on the biggest beach in the world," he says.

But for Wells, there's no question that all these structures are important. "I don't think they were put on this earth for the amusement of molecular biologists," he says. "I think they're here for a reason. Our challenge is to discover what that reason is."

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DIAGRAM: DNA'S MANY GUISES: DNA takes many forms — some may contribute to **disease**

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