

# Research in Progress

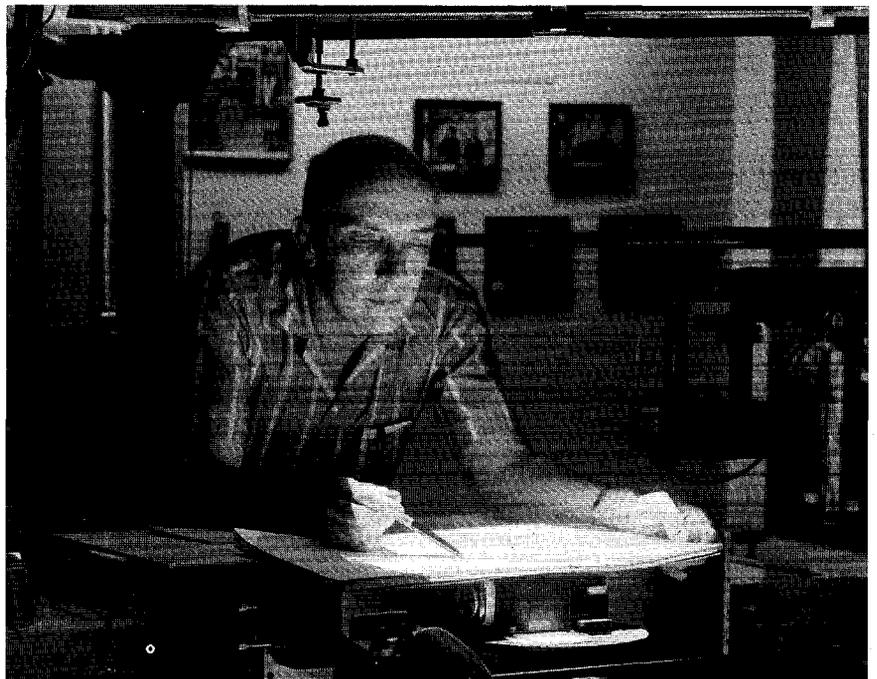
## An Eleven-Year Twitch

Sunspots were first observed by Galileo, and their 11-year cycle was noted in the mid-19th century, but the reason for this repeating period of solar activity has remained one of the sun's biggest mysteries. Recent evidence uncovered by Robert Howard and Barry J. LaBonte links this cyclic activity to solar-mass movements, offering a solution to the mystery. Howard is a staff member and LaBonte a research fellow of the Hale Observatories, which are operated jointly by Caltech and the Carnegie Institution of Washington.

What is known about sunspots is that

they contain highly magnetized material and are associated with violent storms, the largest of which appear as solar flares. At the beginning of the cycle, sunspots appear at the intermediate latitudes (about 35°) in both hemispheres and increase in frequency and size as they drift toward the equator over an 11-year period. As this activity then vanishes at the equator, small new sunspots show up closer to the poles as the start of the next cycle. The polarity of the magnetic field of this new group of sunspots is opposite to that of the previous ones, creating a cycle of 22 years.

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Robert Howard checks a solar image at Mount Wilson's 150-foot Tower Telescope.

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Howard and LaBonte analyzed velocities (measured from the wavelength shifts of an absorption line of iron) representing horizontal east-west motions of solar material in 12 years of data from the magnetograph of Mount Wilson's 150-foot Tower Telescope. A series of calculations from these data, including subtracting other motions, and a sensitive method of constructing maps of the east-west flows led to a contour plot of the velocity differentials. An obvious organized pattern could be seen — a traveling torsional wave with alternating horizontal zones of slow and fast rotation originating at the poles and drifting over 22 years toward the equator, where they disappear.

Four zones (two fast and two slow) start from each pole in that interval. LaBonte and Howard theorize that, as the torsional wave reaches the intermediate latitudes, the differential velocity of the fast and slow zones increasingly "stretches" the subsurface magnetic fields, causing them to erupt to the surface as sunspots. The sunspot regions form in a latitudinal strip centered on the poleward shear boundary of the fast zone.

When solar activity is at a minimum (when sunspots have disappeared at the equator and are just beginning to appear closer to the poles), a new fast zone originates at each pole. About 11 years later this zone reaches sunspot latitudes, and sunspots begin to form along its poleward boundary with the slower zone. After another 11 years it merges at the equator with the corresponding zone from the other hemisphere and disappears. So two full cycles of the torsional oscillation are always present in each hemisphere.

This is the first evidence that the sunspot cycle is not a random process, but a large-scale, deep-seated phenomenon arising from a fundamental property of the sun — the subsurface wave oscillation. The two astronomers conclude that the solar magnetic cycle is not generated by the drift of fields across the surface; it is driven from underneath by the torsional oscillations. Investigations into the phenomenon will continue, but this evidence provides an important clue to a mystery that has endured for nearly 400 years.

## A Second Left-Handed DNA Helix: One Good Turn Deserves Another

Since it was first proposed by Watson and Crick in 1953, the right-handed DNA double helix has been almost an article of faith among molecular biologists. That faith was put to the test last fall when Wang, Rich, and coworkers at MIT discovered that the double-helical DNA hexamer d(CpGpCpGpCpG), a six-base-pair sequence of alternating cytosine and guanine or CGCGCG, is in fact a *left-handed* helix, and has a zigzag backbone such that the repeating unit of the helix is two base pairs rather than a single step. (A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel, and A. Rich (1979). *Nature* 282:680-686.)

Caltech graduate student Horace Drew and Professor of Chemistry Richard Dickerson have found a similar but not identical left-handed helix in crystals of the DNA tetramer d(CpGpCpG), a four-base-pair sequence or CGCG, prepared under high-salt conditions. They have found a possible mechanism for the transition between low-salt (MIT) and high-salt (Caltech) helices involving the binding of ions in the groove of the helix. If valid, this represents the first detailed single-crystal study of a conformation change in DNA induced by the binding of small ions or molecules.

The MIT group has christened its structure the Z helix after its zigzag backbone. The Caltech helix also has a zigzag backbone — the differences are more subtle than that — but it has been named the  $A_L$  helix to differentiate it from the low-salt version and to call attention to its formal resemblance to the right-handed A helix. The  $A_L$  double helix, formed by making Watson-Crick base pairs between two strands of CGCG running in opposite directions, is shown in Figures 1 and 2. The zigzag backbone that is common to both Z and  $A_L$  helices arises because of an

alternating geometry of the bonds between bases and deoxyribose sugar rings: *syn* (rings turned toward the Watson-Crick base pairing) at guanines and *anti* (rings turned away from the base pairing) at cytosines. This feature alone probably limits left-handed helices of this type to alternating sequences of purines (adenine and guanine) and pyrimidines (thymidine and cytosine), since the *syn* conformation, although tight but acceptable for purines, leads to energetically unfavorable close contacts between atoms in pyrimidines.

The difference between the  $A_L$  and Z helices lies in the geometry of the backbone chain and the puckering of the deoxyribose sugar rings. Classical right-handed A helices have C3'-*endo* sugar puckering, meaning that carbon atoms (numbered from the atom connecting to the base) C1', C2', C4', and the oxygen (O) of the sugar ring are essentially planar, and atom C3' projects out of the ring on the same side of the plane as the attachment of atom C5' to C4' (Figure 3). The classical B helix has C2'-*endo* puckering, meaning that only the C2' atom is out of the ring plane in the same direction. The low-salt hexamer structure from MIT has an alternation of C2'-*endo* at cytosines and C3'-*endo* at guanines. In the high-salt tetramer, the sugar puckering at cytosines is C2'-*endo*, but that at guanines is C1'-*exo* (that is, projecting on the opposite side from the C5'-C4' bond) instead. Figure 3 shows that this is only a slight deformation of C2'-*endo*, so to a first rough approximation the  $A_L$  helix has uniform sugar puckering along the chain rather than alternating conformation. An ideal left-handed A helix would be expected to have C2'-*endo* puckering at every position, which is why the Caltech helix has been called the  $A_L$  helix.

Not only can a CG polymer of DNA adopt two different helical conformations;

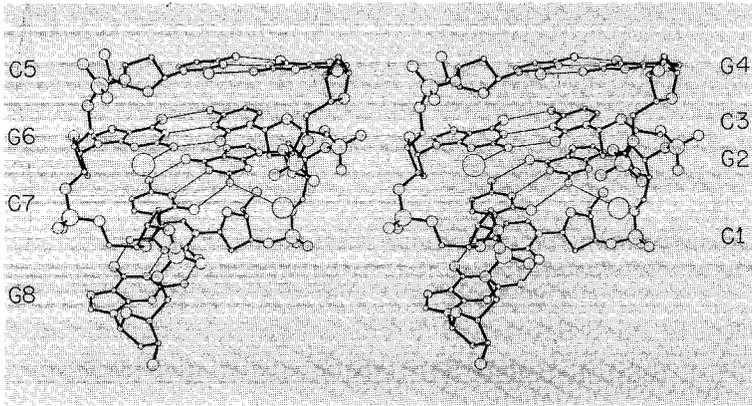


Figure 1. Stereo drawing of the double-helical CGCG molecule, looking into the groove of the helix. One of the two tetramer molecules has its cytosine (C) and guanine (G) bases numbered 1-4 to the right of the right-eye image, and the other has its bases numbered 5-8 to the left of the left-eye image. This view corresponds to a view into the narrow groove of right-handed helical DNA. Spheres in order of decreasing size represent  $\text{Cl}^-$ , P, O, N and C atoms.

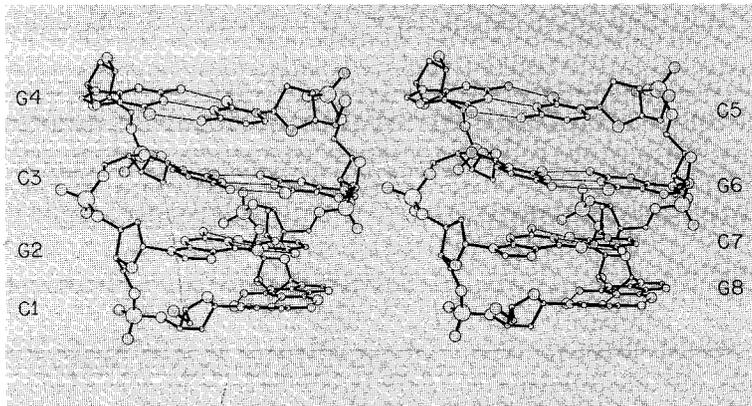


Figure 2. Stereo drawing of CGCG viewed from the outside of the helix, in what formally corresponds to the wide groove of a right-handed helix. Each guanine has the oxygen atom of a neighboring deoxyribose pointed toward its six-membered ring, and the base pairs are puckered in a manner suggesting steric repulsion between sugar oxygen and the guanine ring.

it can go from one to the other in the crystalline state. Drew established in 1978 that two different crystal forms of CGCG were obtained by crystallizing it from low- and high-salt conditions (H. R. Drew, R. E. Dickerson, and K. Itakura (1978). *Journal of Molecular Biology* 125:535-543), and that crystals could be induced to change from one form (and one x-ray diffraction pattern) to the other by changing the surrounding crystal medium, without leading to destruction of the crystals. The low-salt form of CGCG has not yet been solved here, but it appears likely that it will turn out to be a Z helix like the MIT hexamer.

The difference between the two structures can be accounted for in terms of binding of chloride ions. Drew found several  $\text{Cl}^-$  ions in his crystal structure, two especially significant ones being 3.4 Å away from the N2 amino groups on guanine rings G2 and G6. (These are the

largest two circles in Figure 1.) The phosphate groups linking G2 and C3 on one chain, and G6 and C7 on the other, are turned out, away from the chloride ions as if they were being repelled by their mutual negative charges. (Figure 4, right.) The  $\text{C3}'\text{-C4}'$  torsion angle is  $122^\circ$  and the sugar has  $\text{C1}'\text{-exo}$  puckering. In the MIT low-salt hexamer, these phosphate groups are rotated inward and connected to the guanine amino groups by hydrogen-bonding water molecules. (Figure 4, left.) The torsion angle decreases to  $82^\circ$ , and the sugar conformation becomes  $\text{C3}'\text{-endo}$ . Drew and Dickerson propose that the origin of the salt-induced structure change in CG tetramers and hexamers lies in the displacement of bridging waters by anions under high-salt conditions, resulting in repulsion of the phosphate backbone.

Left-handed DNA helices are not new: They have been proposed in the past by

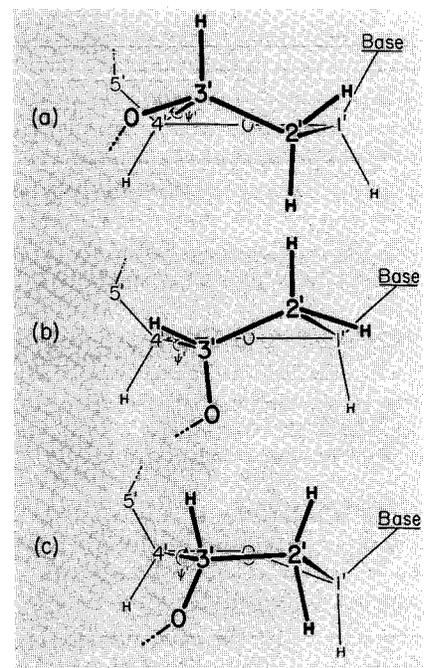


Figure 3. Deoxyribose sugar pucker and torsion angle  $\psi'$ . (a)  $\text{C3}'\text{-endo}$ , with  $\psi' = 82^\circ$ . (b)  $\text{C2}'\text{-endo}$ , with  $\psi' = 144^\circ$ . (c)  $\text{C1}'\text{-exo}$ , with  $\psi' = 120^\circ$ . Conformations (b) and (c) are closely related, and less like (a).

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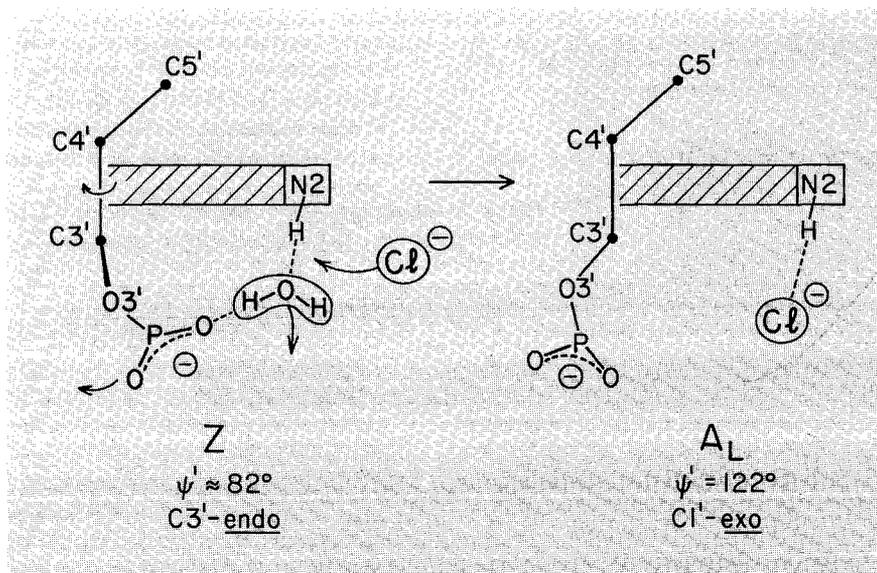


Figure 4. Proposed mechanism for salt-induced conformation change in a left-handed poly (CG) helix. C3' and C4' are atoms in the sugar ring and C5' and O3' are continuations of the helix backbone. The shaded slab represents an edge view of a guanine double ring with a -NH<sub>2</sub> amino group at position 2 on the rings (N2). In the Z helix this group and a backbone phosphate are bridged by a water molecule, but in the A<sub>L</sub> helix a chloride ion bound to the amino group at N2 repels the phosphate and changes the helix backbone geometry.

people who worried about the topological difficulty of unwinding a long helix for duplication and readout. (This turned out to be a red herring.) But no one thought of incorporating an alternating *syn/anti* geometry into a zigzag backbone with two base steps per helix repeat, and the structures that were proposed looked like right-handed Watson-Crick structures which some atomic-level weight lifter had wrenched bodily in the wrong direction. As so often happens, Nature turns out to be much more imaginative than its chroniclers are.

What relevance do these left-handed helices have to DNA *in vivo*? The answer is not clear. It is easy to explain why these two zigzag helices can only form with alternating pyrimidine/purine copolymers such as CGCG; it is less easy to see why even such alternating copolymers should prefer the left-handed helix over a conventional right-handed one. It has been suggested that something similar to a zigzag left-handed helix occurs in chromosomal DNA in short regions of alternating CG sequence, as a means of relaxing total

helix coiling. Several carcinogens and other alkylating agents are known to alkylate (attach methyl or other alkyl groups to the ring atoms) the guanine and cytosine rings along their edges that in right-handed helices are buried in the major groove but in left-handed helices are pushed to the helix surface (nearest the viewer in Figure 2). Rich has suggested that a low but measurable susceptibility of chromosomal DNA to such modification might represent a low but finite amount of left-handed helical regions. Such drastic changes in the middle of a helix might function as markers or recognition sites for proteins.

Drew's structure is part of a longer range study in Dickerson's laboratory designed to help answer the question: To what extent is the helical structure of DNA modified by the particular base sequence, and might this be a factor in recognition of specific DNA sequences by repressors and other control proteins? Graduate student Ben Conner is working on the structure of CCGG, which is not expected to be left-handed. Drew and Visiting Professor Richard Wing from the University of Cali-

fornia at Riverside are collecting data on a self-complementary DNA 12-base-pair sequence — CGCGAATTCGCG, which is of biological interest because it contains the recognition site for the *EcoRI* restriction enzyme, one of a family of enzymes that makes sequence-specific cuts in DNA. A left-right-left helix sense change may be possible in this molecule. Research chemist Mary Kopka is attempting to crystallize the 21-base-pair *lac* operator, which controls expression of the lactose gene, and a sequence variant, both in its double-stranded form and in single-strand hairpins. All of these sequences have been synthesized by or under the guidance of Dr. Keiichi Itakura of the City of Hope National Medical Center in Duarte. Postdoctoral fellows Peter Dembek from Poland and Shoji Tanaka have worked at Caltech to help prepare some of the sequences, under a joint Caltech/City of Hope collaborative arrangement. These structures, and others now being planned by Itakura and Dickerson, will help shed light on the sequence-structure-recognition problem with DNA.