

DISSECTING THE DANCE

BY STEFI WEISBURD

The molecular sculpture is unmistakable: that slow sweep of the phosphate and sugar backbone from which interlocking pairs of bases are strung like a grand spiral staircase. The DNA helix is one of the most familiar images in science, the very logo of modern biology.

Since James D. Watson and Francis Crick created their classic model 30 years ago, biologists have uncovered a whole repertoire of DNA architectures. Models of these structures fill journals and textbooks; they have blessed researchers with an understanding of how genetic information is encoded. Inspired by such knowledge and fascinated by the variety of DNA forms, biologists are delving deeper into the atomic arrangements, looking now for clues explaining how this code is transcribed — how enzymes find, unlatch and copy the genetic recipes for making proteins.

But the structural models tell only half of the transcription story, because they are static, inert. And real DNA is rich in movement. It bends and breathes, vibrates and shimmers. It is a living mobile that passes on the message of inheritance. Predicting the function of DNA from its static structure, comments one researcher, is like trying to determine what human beings can do by photographing the shape of their arms and legs and fingers without saying how these appendages bend and move.

It is the movement, the vibrations, of DNA that a community of biophysicists has come to think is central to the macromolecule's interaction with the enzymes that unzip the helix and read the genetic code within. Led by Earl W. Prohofsky at Purdue University in West Lafayette, Ind., these researchers have set out to characterize and hunt for DNA vibrations. Borrowing techniques from solid state physics, they have had remarkable success at predicting DNA's vibrational modes at frequencies spanning the electromagnetic spectrum from radiowaves to the infrared.

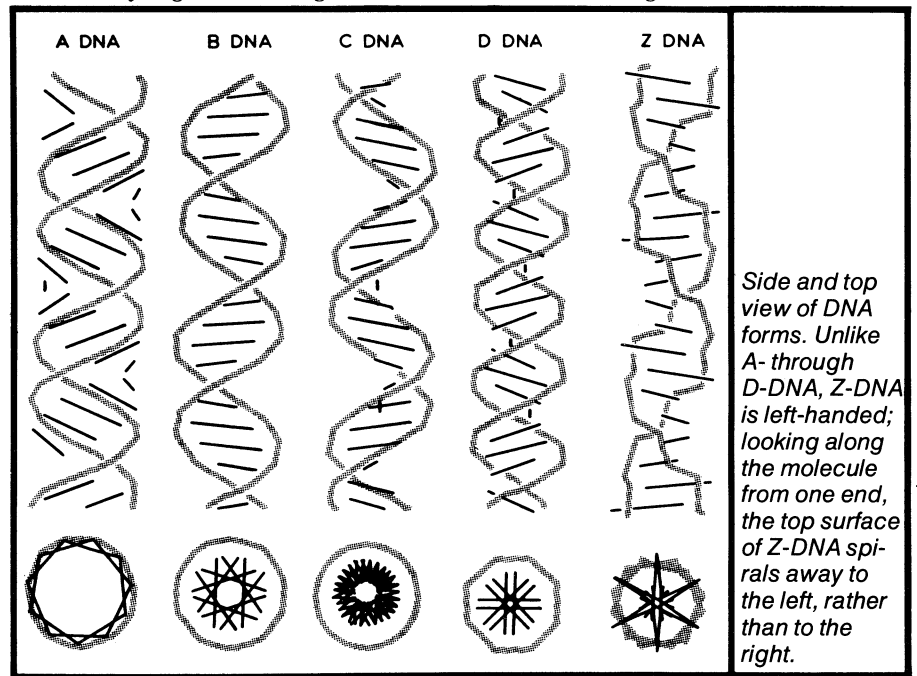
Out of a veritable city of movement inhabiting a DNA molecule, Prohofsky and his colleagues have found a number of distinct vibrational modes, or phonons, that

may be especially important to biological processes. The researchers speculate that enzymes — which themselves vibrate — may capitalize on the DNA vibrations to get at its genetic information. They might trigger or enhance the unwinding mode of the DNA strands, for example. Vibrational modes that are unique to a certain segment of DNA might also serve as a mechanism for matching enzymes with specific binding sites. Vibrational mechanisms, suggests Prohofsky, could be the key that activates and facilitates enzymatic processes.

Prohofsky began cultivating these ideas

atoms together; hydrogen bonds of intermediate strength; and so-called non-bonded forces such as the electrostatic interactions, which are weak but far-reaching. Considering the complexity of the forces and the fact that a small segment of DNA housing a thousand base pairs contains a million atoms, the theorist's job is formidable indeed.

Luckily, from the previous structural studies of DNA Prohofsky knew that there is some order in that jungle of atoms. The periodicity and symmetries of the DNA helix could be exploited to reduce the task to a more manageable form. In his lattice



Side and top view of DNA forms. Unlike A- through D-DNA, Z-DNA is left-handed; looking along the molecule from one end, the top surface of Z-DNA spirals away to the left, rather than to the right.

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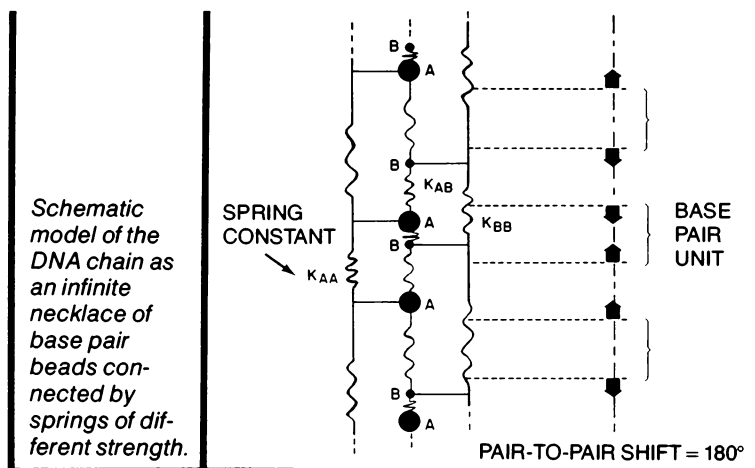
a decade ago when he heard about an experiment in which viruses were killed by illuminating them in the infrared at their resonance frequency. "It struck me that there were vibrational modes that were probably important in DNA too," he says. "This started me thinking that vibrational modes not only existed in DNA, but might be active [biological] agents."

So Prohofsky began doing what physicists do well — cataloging and predicting all the ways that atoms in DNA can oscillate in response to a myriad of forces latching the molecule. These include the strong valence bond forces that weld neighboring

dynamics theory, Prohofsky first assumes that the DNA is an infinitely long homopolymer — made up of one kind of base pair. He then models the DNA as a necklace of base pair "beads" latched together by mechanical springs with stiffnesses determined by the strength of the various forces.

Since this is the same picture used to model crystalline solids, he has been able to adopt mathematical methods long employed in solid state physics to radically simplify the problem of DNA vibrations. Out of the chaotic menu of thermal motions buzzing through the DNA molecule, a

IN DNA



Lindsay, Prohofsky/ADENINE

clean set of allowable vibrational modes triumphantly emerged.

To characterize and keep track of these modes, they are plotted on what is known as a dispersion curve, which relates the frequency of each mode to its allowed wavelengths. (For the DNA problem, Prohofsky translates this information into a graph of frequency versus the phase shift between base pair units.) There are two classes of modes — optical and acoustic. The four acoustic modes in DNA are at the lower frequencies and include a compressional mode, two transverse bending motions and a torsional mode.

In general, experiments done at high frequencies provide information about the strong forces between nearby atoms whereas the acoustic data are used to estimate the weaker, long range forces in the DNA helix. According to Stuart Lindsay, a physicist at Arizona State University in Tempe, most of the information relevant to biology and gene expression is contained in the lower frequency motion involving the cooperative movement of atoms over large sections of DNA. Adds his co-worker Johnny Powell, "these low frequency motions due to long range forces make it so that DNA can essentially talk to itself over a large distance and know what is going on from one end to the other."

Lindsay and Powell are among a few experimentalists testing the theories of Prohofsky's group. The experiments have necessitated the development of very sensitive high-resolution spectroscopy techniques. "The experiments are hard to do," comments Prohofsky, "and they're not all in by any means. But there is enough of a pattern of agreement now to make it look very hopeful."

One of the recent successes of the theory was the prediction of resonant absorption of microwaves by DNA (SN: 4/21/84, p. 248), a finding that could have important ramifications in the study of the bioeffects of radio frequency radiation. As for discoveries linking vibrations to the way in which DNA normally conducts its genetic business, the researchers are most excited about three kinds of DNA motion — softening, melting and local modes.

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Probably the most exciting twist in the overall DNA saga has been the discovery

that the macromolecule comes in a variety of forms. Watson and Crick's so-called B-DNA is the natural structure most commonly found in solutions and cells. But three other right-handed varieties have also been found — A, C and D DNA — each having their base pairs tilted relative to the longitudinal axis. Most intriguing is the finding several years ago of a left-handed version of the helix, the Z-DNA, so named because its backbone zigzags around the molecule rather than spiraling smoothly as in the right-handed structures. Z-DNA also has more base pairs per turn and these pairs are flipped compared to the conventional geometry.

Scientists have noticed that changes in the environment of DNA such as temperature, acidity, salt level and water content can drive transformations between these DNA structures. Adding water to dehydrated A-DNA, for example, creates B-DNA. Similarly, a B-to-Z switch accompanies a reduction in salt concentration.

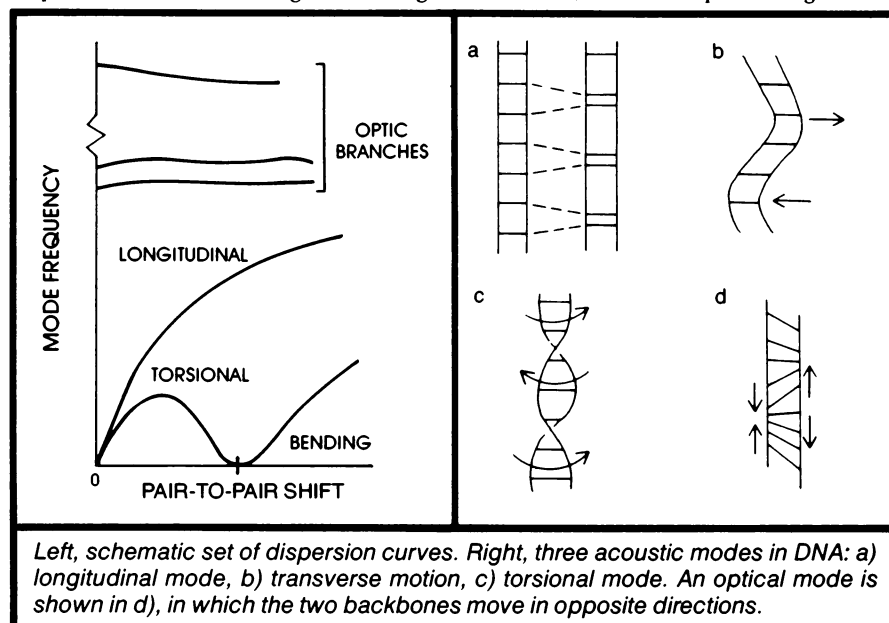
Concomitant with these conformation shifts is a dramatic change in one particular vibrational mode of DNA. As in solid state physics, this mode is called the soft mode because its frequency approaches zero or "softens" as the atomic geometry is rearranged. When the mode softens, the amplitude of the vibration grows so large

that the original structure is destabilized and the molecule is driven into a new geometry.

By relating the change in water or salt content to changes in the dielectric constants that enter into the calculations of forces between atoms, Prohofsky has predicted that in the B-to-A shift, it is the lowest lying optical mode that softens. "The actual mode," he says, "is a conglomerate motion which has the backbones beating against each other (thereby tilting the bases), coupled with breathing or swelling and untwisting of the helix. . . . It looks just like the motion required for moving from one conformation to another."

The theory predicts that an optical mode at 12 inverse centimeters (cm^{-1}), when the pair to pair phase shift is zero, will soften. Experimenters in Japan and at Arizona State using a technique called Raman spectroscopy have succeeded in observing a mode with the same general character, except the phase shift is not zero, soften from 22 cm^{-1} to 16 cm^{-1} . Presumably, say the researchers, the 12 cm^{-1} segment of the mode is softening as well, but it is very hard to detect experimentally.

Prohofsky has also just finished preliminary calculations for the B-to-Z transformation, a more complex change involv-



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ing an intermediate geometry and hence two soft modes.

Researchers think that the first step in both transcription (DNA is used as a template for the production of proteins) and replication (more DNA is created) entails special enzymes that separate or unwind the intertwining DNA strands so that the message held in the sequences of the bases can be read.

To unzip the helix, the hydrogen bonds linking the bases in a pair must be broken. If the enzyme breaks these bonds in the same way that an ice cutter separates ice, says Prohofsky, an unreasonable amount of force would be required. Instead, Prohofsky proposes another scheme in which vibrations from the enzyme set the single DNA strands oscillating against one another, stretching the hydrogen bonds in the process. The amplitude of these oscillating stretches progressively increases as the heat generated by this motion weakens the bonds. Larger amplitudes create more heat and so forth until the bonds break, a process known as melting.

Prohofsky has identified a number of optical phonon modes between 65 and 111 cm^{-1} (corresponding to different mixtures of bases in the DNA polymer) that would be suitable candidates for melting modes. This agrees well with observations of a broad hydrogen bond band centered at 85 cm^{-1} , which, unlike any other vibrational mode, softens and disappears as the helix melting temperature is approached.

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Biophysicists believe that the most tantalizing biological prospects for the lattice dynamics approach lie mainly with local modes — motion unique to specific spots in the helix. After all, explains Lindsay, if DNA was a perfect homopolymer, without variations in the sequences of bases, we

would be uninteresting creatures indeed. "That is not biology," he says. "Biology of course arises for special motions at local sites: special sequences of bases, termini, replicating forks. So the real interest in this work is in pushing it to describe motion at special places on the DNA double helix."

To do this, Prohofsky has calculated the effect of adding a defect, or a structural change, at a particular spot in his infinite homopolymer model by applying a mathematical trick (Green's functions) commonly used in solid state theory to study defects in crystals. In this way, he and his colleagues have calculated the local modes that might occur at a replicating fork, where the DNA strands separate in order to make new DNA helices. Their results show increased stretching of hydrogen bonds and breathing modes in the fork region. Prohofsky believes that these local modes enhance the melting process so that it propagates down the helix. He is currently working on the calculations and planning experiments to test this idea.

For the case of a DNA chain in the area of its terminus, Prohofsky's group has predicted a resonance at about 600 million hertz (MHz), corresponding to the unwinding of the DNA at the end of the strands. This resonance can be "pumped" by other vibration waves with the correct symmetry that run up and down the length of the chain. The result is that motions that involve separation of the bases perpendicular to the chain axis are enhanced by a factor of two relative to the interior of the chain.

Lindsay's group did indeed find a sharp, narrow peak at 600 MHz in the spectra from fibers of the B form of DNA from a calf thymus. "I certainly don't want to tell you that this is a terminous resonance," he

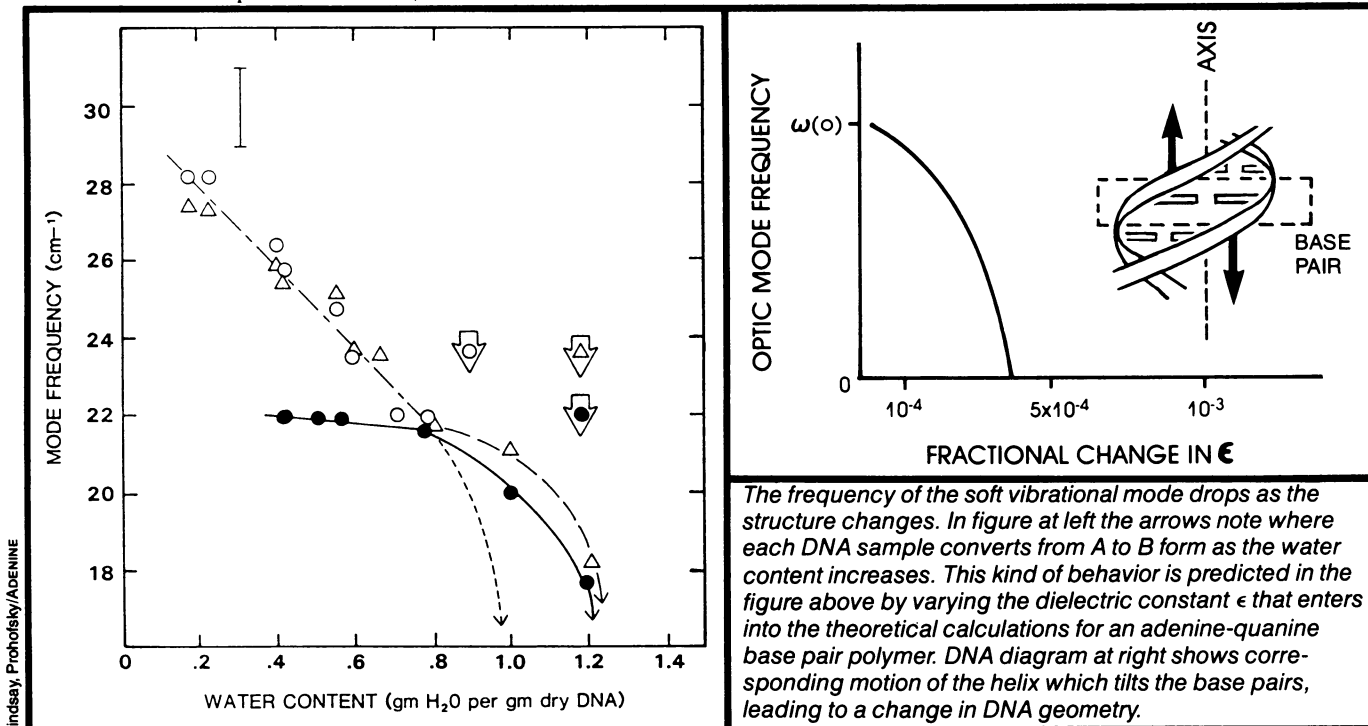
says. "However, it is exciting and if the promise is fulfilled then it appears that we can begin to make some inroads in understanding local motion from first principles."

Prohofsky thinks that local resonances, and all vibrational modes for that matter, may fit into a larger question that pervades biology: How is the undirected energy stored in chemical bonds converted into specific motions of molecules, especially those involved in enzymatic processes?

For DNA, Prohofsky's speculative answer is a scenario that begins when an enzyme harvests the energy released from the splitting of the triphosphate bond in an energy storage ATP (adenosine triphosphate) molecule. This energy, he suggests, excites a vibrational phonon in the enzyme that is transferred to the helix when the enzyme attaches to it. This phonon will wander around the helix, like a vibrational key looking for the matching lock. If its frequency is correct the phonon will resonate with a local mode at some part of the DNA. "This excitation," says Prohofsky, "could create directed motions for doing enzymatic actions — bending the DNA helix so that it allows the enzyme to penetrate, for example."

This vibrational conversion mechanism, he cautions, is just an idea, a theory. But Prohofsky and others think that with the fundamental science being verified, the speculation holds enough promise to continue, as he says, probing and tickling the DNA machinery. □

Stefi Weisburd, a former SCIENCE NEWS intern, will be with BUSINESSWEEK as an American Association for the Advancement of Science Mass Media Fellow this summer.



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