X-RAY CRYSTALLOGRAPHY

Researchers Get Their First Good Look at the Nucleosome

The cell's nucleus is a miracle of packaging. Stretch out human DNA, and it would be 2 meters long. Yet the cell manages to cram it into a space just a few micrometers in diameter. Now, Timothy Richmond and his team at the Swiss Federal Institute of Technology (ETH) in Zurich have provided the first detailed look at a key piece of the molecular machinery responsible for this feat: a fundamental DNA packaging unit called the nucleosome core particle.

An average cell nucleus contains 25 million of these particles. Each consists of a discus-shaped core of eight small proteins, called the histone octamer, encircled by 146 base pairs of DNA that spiral 1.65 turns around the edge of the discus. The nucleosome particles, first described by Roger Kornberg of Stanford University in 1974, are connected, much like beads on a string, by stretches of linker DNA. In this week's *Nature* (18 September), the Richmond team reports that they have determined the x-ray crystallographic structure of the nucleosome core particle to a resolution of 2.8 angstroms—good enough to distinguish about 80% of the atoms in the proteins and all of those in the DNA.

With a molecular weight of 206,000 daltons, about half of it protein and half DNA, the nucleosome is by far the largest DNA-protein complex to be imaged at atomic resolution. But more than that, researchers say, the structure will be very important for understanding such dynamic aspects of nuclear function as gene transcription, which is the first step of protein synthesis, and DNA replication and repair.

In order for these activities to occur, the DNA and its associated proteins, collectively called chromatin, must be at least partially unwrapped, and recent evidence suggests that changes in nucleosome structure play a role in that unwrapping. Already, this new close-up of the core particle is giving researchers fresh insights into how that happens. For example, the tails of the histones project out of the nucleosome, making them good targets for enzymes involved in controlling transcription.

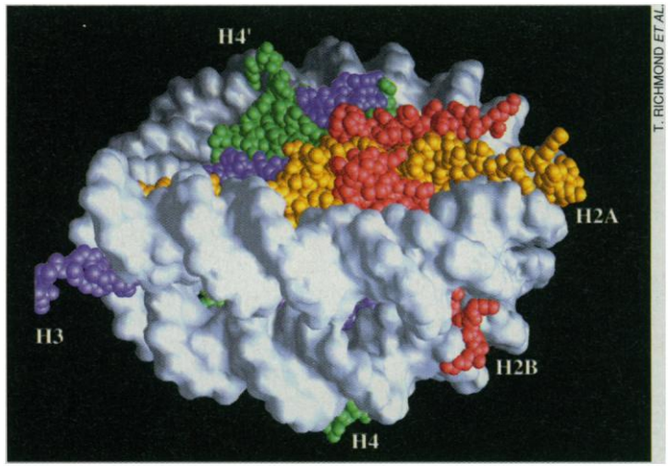
"A few years ago, nucleosomes were thought of simply as rocks in the way of transcription," remarks postdoc Karolin Luger of the ETH team. "Now, it's all active participation." She adds that "it was good timing" to finish the structure just when the nucleosome's dynamic role was being appreciated. Transcription expert David Allis of the University of Rochester in New York, who got his first look at the structure during a presentation Luger made at last month's transcription meeting at Cold Spring Harbor Laboratory in New York, agrees: "I was dazzled by the pictures. It's fantastic to sit back and see the structure we've all been working on."

The ETH team's accomplishment is the culmination of a 20-year quest that began in the lab of Nobel Prize–winning crystallographer Aaron Klug at the Medical Research Council Laboratory for Molecular Biology in Cambridge, United Kingdom. In 1984, the Klug team, including Richmond, a postdoc at the time, produced a crystal structure of the particle at 7 angstroms resolution that allowed researchers to see the overall shape of the histone proteins and how the DNA bends as it twists around the protein mass. Individual atoms couldn't be seen at all, however. The resolution was poor because subtle variations in both the nucleic acid and protein components of natural nucleosomes prevent them from forming the well-ordered crystals needed. "Those [early] crystals [of natural nucleosomes] weren't all that good," Klug recalls.

Richmond, who moved from Cambridge to the ETH in 1985, decided that the only way to be sure of getting the desired crystal quality would be to systematically remove the heterogeneity from the nucleosomes. In effect, he set out to synthesize his own nucleosomes.

He and his colleagues began by making a recombinant DNA with a defined sequence. They also produced all four types of histone—H2A, H2B, H3, and H4—that together form the octamer by expressing the genes in bacteria, which do not perform the chemical modifications that alter the histones in eukaryotic cells. Then the researchers performed site-directed mutagenesis of the histone genes to create sites in the proteins into which they could insert heavy-atom labels to make the eventual diffraction pattern interpretable.

Each of the four proteins and the DNA then had to be purified and enticed to refold into their native conformations in the synthetic nucleosomes. And, finally, the particles had to be crystallized in a form from which good x-ray diffraction data could be collected. Even today all that would be no small feat, but in the early 1980s when the necessary technology was in its infancy, it was daringly ambitious. Indeed, Richmond's team didn't get their first truly high-resolution crystals until nearly 10 years after they started. "It's taken a long time, but understandably so," says Klug. "It's a great achievement."



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New portrait. The exact contacts between the nucleosome histones (colored as indicated) and the nucleic acid can now be seen.

In a fortuitous coincidence, the new European Synchrotron Radiation Facility (ESRF) in Grenoble, France, which produces high-intensity and focused x-ray beams, came online just as the Swiss team got their first crystals. Christian Riekel, who was setting up the high-brilliance x-ray beam at the ESRF, provided the crystallographers with his share of beam time. At a time when no equivalent x-ray source was available in the world, "it really did make the whole thing possible," says Richmond.

The overall structure that resulted from this effort looks like the 1984 structure. And the trace of the central protein structure is almost identical to that Evangelos Moudrianakis and colleagues at Johns Hopkins University found in a 1991 x-ray crystallography analysis of the histone octamer alone. But the new structure shows in molecular detail exactly how the DNA makes contact with the histone proteins as it wraps around them.

The DNA contacts the histone octamer at 14 main points, most of which have quite different structures. This means that the DNA does not follow a regular path around the protein but is more curved at some positions on the nucleosome surface than at others, which may have important functional

consequences. For example, the curvature might distort the DNA so that some of the factors that regulate gene transcription are encouraged to bind, while others are not.

The distribution of the contacts between DNA and protein also allows researchers to envisage how a large enzyme complex like the one that replicates DNA can travel along the DNA strand without completely displacing the nucleosome. "The DNA is like a piece of Velcro on the outside of the histone octamer," explains Richmond. An enzyme could displace 30 or 40 base pairs of DNA from the protein at a time, but when it has passed, that DNA can stick back to the very same nucleosome, so the histone octamer may never be totally removed from the DNA.

Previous biochemical studies had indicated that the histone tails extend beyond the DNA. The new structure provides a more direct view of the position of the tails, which may play an important role in making contact with adjacent nucleosomes as the chromatin folds back on itself to form the higher order structure needed to pack all the chromatin into the nucleus.

That folding would make large stretches of the DNA inaccessible when the genes encoded in them need to be kept inactive. The structure suggests how active genes become accessible. The projecting histone tails contain some of the sites that are modified by histone acetyl transferases—the enzymes that have been hot news in the past couple of years because of their role in regulating transcription. By adding acetyl groups to the tails that poke out, these enzymes would almost certainly disrupt a higher order structure and open up the chromatin to infiltration by the transcriptional machinery.

An atomic-level description of the nucleosome core particle is a tremendous technical achievement in itself but is only the first step toward understanding chromatin structure. To see the structure of two particles connected by the linker DNA would be nice, Richmond says. The structure of three particles, the central one wrapped by uncut DNA, might be even better. But the real goal is "to see what two or three turns of a higher order structure looks like." With luck, this next quest will not take another 2 decades.

–Carol Featherstone

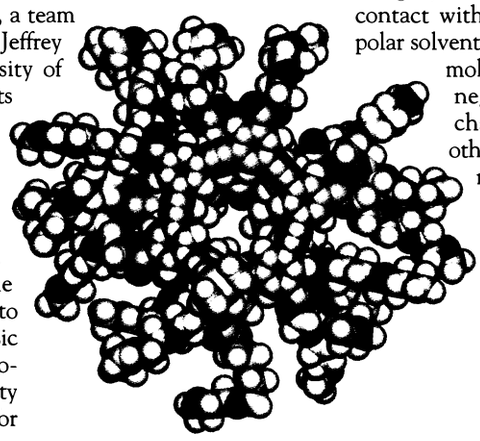
Carol Featherstone is a writer in Cambridge, U.K.

ORGANIC CHEMISTRY

Polymer Folds Just Like a Protein

The exact linear arrangement of amino acids in a protein is not the only thing that determines how it behaves: Also key is the protein's precise three-dimensional shape. For decades, chemists have sought to understand what forces make a string of amino acids bend and curl into a particular configuration, with the hope of one day making their own synthetic polymers that can duplicate the functions of natural proteins. But they have had a hard time getting anything other than a protein to fold in solution.

Now on page 1793, a team led by organic chemist Jeffrey Moore of the University of Illinois, Urbana, reports achieving this goal with a polymer they made from repeating units of a hydrocarbon molecule called phenylacetylene. They found that the polymer readily coils into a helix, one of the basic folding motifs of proteins, and forms a cavity that can be modified for different purposes.



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21st century chemistry. This spontaneously folding polymer may be the wave of the future.

Other organic chemists are enthusiastic, because it is a new addition to the small number of synthetic polymers, sometimes called "foldamers," that they have coaxed into folding. The achievement shows "Mother Nature doesn't have a monopoly on folded structures," says Brent Iverson, a chemist at the University of Texas, Austin. "This is a very important new direction for chemistry."

Researchers hope that the work will point the way to new types of tailor-made complex molecules that have the specificity and self-organizing capabilities of proteins. If they can be made to catalyze chemical reactions as the body's own enzymes do, these tailor-made molecules could be useful as industrial catalysts or as biomedically active substances that would not degrade as easily as proteins themselves.

The new results may also shed light on a long-standing disagreement among protein chemists. Some chemists think that proteins in solution fold to protect those amino acids that are uncharged, or "hydrophobic," from contact with water, a so-called polar solvent because each water molecule carries partial negative and positive charges. In contrast, others have argued that relatively weak links between a hydrogen atom and two adjacent atoms are responsible for a protein's kinks and curls. But the phenylacetylene polymer folded even though it has no such hydrogen bonds, showing that at least in this case "you can drive the ordering and folding just using a hydrophobic effect," says Moore's collaborator Jeffery Saven, now at the University of Pennsylvania, Philadelphia.

To test his ideas about protein folding, Illinois physical chemist Peter Wolynes teamed up with Moore 2 years ago to make a nonprotein polymer that could fold like a protein. For their polymer building block, they chose a molecule, phenylacetylene, that has no nitrogen or oxygen atoms in its backbone and thus lacks the key hydrogen-bond ingredients. Computer modeling by Saven and Wolynes indicated that if a chain had at least eight of these uncharged, ring-shaped phenyl groups, it would be able to twist into a helix and thereby avoid contact with a polar solvent.

The team then synthesized a selection of polymers, ranging in length from two to 18 links, and dissolved them in different polar organic solvents. Using several different spectroscopic techniques, the team found that chains 10 links or longer did form helices as predicted. The Illinois team also showed that the new foldamer, like proteins, can be made to fold, unfold, and then refold.

Changing the solvents also confirmed that hydrophobic-like forces were responsible for the folding. The more polar the solvent, the "stronger the induced organization," Moore says, adding: "This is a step along the way of understanding the rest of protein behavior."

Not everyone is convinced, however, that this polymer reflects what is going on in proteins. "The network of forces [in the polymer] is very clearly different from [the forces] in a protein," comments organic chemist Sam Gellman of the University of Wisconsin, Madison. He points out that proteins are a mosaic of uncharged and charged amino acids, and that the latter could also help drive folding.

But even if foldamers aren't complete protein mimics, researchers hope they will be able to duplicate the sophisticated chemistry that goes on in cells. "This is a field in which almost anything you can imagine, you can try to do," Gellman predicts. "For chemists, it will be the challenge of the 21st century."

–Elizabeth Pennisi