

hot papers

EDITED BY EUGENE RUSSO

CRYSTAL STRUCTURE

K. Luger, A.W. Mader, R.K. Richmond, D.F. Sargent, T.J. Richmond, "Crystal structure of the nucleosome core particle at 2.8 Å resolution," *Nature*, 389:251-60, 1997. (Cited in more than 250 papers since publication)

Comments by **Timothy J. Richmond**, professor for X-ray crystallography of biological macromolecules at the Institute for Molecular Biology and Biophysics, Swiss Federal Institute of Technology, Zurich

In 1996, after 18 years of painstaking work, researchers led by Timothy Richmond were finally able to peer into the microscopic world of genomic complexity and see the tiny object of their search. They had captured an X-ray crystallography snapshot of the 11-nanometer nucleosome, the most fundamental unit of DNA organization and the most basic level of protein-DNA interaction relevant to humans. All eukaryotic cells assemble their DNA into nucleosomes—bundles of DNA wrapped around small proteins called histones. "This structure rivals DNA itself for importance," says Richmond, senior author.

Investigators' challenges were many. Scientists racing to crystallize a structure typically make attempts with the equivalent structures of all available species until they find the one that works best. But with highly conserved structures like nucleosomes, investigators don't have that option. Despite this hang-up, six years of work under **Sir Aaron Klug** at Medical Research Council (MRC) laboratory in Great Britain yielded a seven-angstrom, low-resolution nucleosome structure in 1984.¹ The crystals the team had in hand, however, continued to diffract poorly. "It was clear that they had to be fixed up in some way," explains Richmond.

Aided by recombinant DNA and sequencing technologies even as both evolved from cutting-edge science to standard

techniques, Richmond overcame doubts of many that his goal—to solve a high-resolution structure considered enormous by the X-ray crystallography standards of the time—was even attainable. Says Richmond, "At the time when I went to make the defined-sequence DNA, people said, 'Ah, but you can only synthesize 20 base pairs at a time. How are you going to make 140?'"

Richmond persisted. He started to take advantage of new recombinant DNA technology techniques by piecing together

to a solid 3.5 angstroms. The histones were crucial, says Richmond, because they were heavy-atom derivatives; Richmond and coworkers were the first to successfully use compounds containing multiple heavy atoms to solve a structure, a technique first developed in 1954 by 1962 chemistry Nobelist **Max Perutz**.² Some of the protein mutants bound heavy atoms well, and after further experiments with different DNA sequences, the resolution improved again to the 2.8 angstroms reported in this paper. "I never dreamed it would take quite so long," he admits.

The nucleosome structure has, says Richmond, frequently served as a useful reference for studies related to histones, acetylation, and chromatin remodeling, research areas that have been extremely active in the last few years. Since the paper, his group has improved the resolution to 1.9 angstroms, results that haven't yet been published. They've revealed more than 1,000 water molecules, many ions, and details related to the nucleosome's DNA "tails," pieces thought to be important for forming the higher-order structure of nucleosomes. "How relevant the tails are, I'm not sure yet," remarks Richmond. "The real drive now is to try and crystallize some chunk of the higher-order structure so we can actually see where the tails go in a meaningful system." According to Richmond, to better understand transcriptional regulation and DNA packaging, researchers must elucidate the transition between the higher-order nucleosome structure and the more open structure in which the DNA is essentially dependent on individual mononucleosomes.

Richmond's group is also trying to confirm his contention that all DNA sequences have the same conformation at each binding site on the nucleosome. "I'm hoping it is true," he says, adding that if it's not, discovering what each sequence will do at each of the seven unique positions on the nucleosome will require a massive amount of work. ●



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Pictured is the nucleosome core particle along two axes: Down the DNA superhelix axis (left); perpendicular to the DNA superhelix axis (right). The particle is composed of phosphodiester backbones (brown and turquoise) and eight histone protein main chains (H3 in blue, H4 in green, H2A in yellow, H2B in red).

homogeneous DNA target sequences and producing them in large quantities in bacteria. The end result: an improvement to a resolution of better than four angstroms.

In 1990, **Karolin Luger**, now at Colorado State University in Fort Collins, came to Richmond's lab in Zurich and successfully used recombinant techniques to get the fourth and final histone, H4, helping bump up the resolution

1. T.J. Richmond et al., "Structure of the nucleosome core particle at 7 Å resolution," *Nature*, 311:532-7, 1984.

2. D.W. Green et al., *Proceedings of the Royal Society, London*, 225:287-307, 1954.