Chromatin Structure: The Supercoil Is Superseded

A typical mammalian cell contains DNA that would, if extended, be about 1 meter long. This DNA is covered with proteins and is folded into the cell nucleus, whose diameter is only about 10^{-3} centimeter. Recently, several kinds of experiments have led most investigators to believe that this condensed DNA has a regularly repeating structure that resembles beads on a string. The "beads" are DNA complexed with globular clusters of a special group of proteins, the histones. The "string" consists of short stretches of DNA between the histone clusters. As is well known, DNA structure and function are intimately related. The questions then arise as to how histones interact with DNA to cause this condensation and how the condensed structure of DNA relates to the control of gene expression.

Apparently some aspect of the structure or composition of chromatin—that is, DNA and its associated proteins—affects gene expression in cells of higher organisms. Only a limited portion of the DNA in a cell from a higher organism consists of actively transcribed genes. The remainder of the DNA is thought to consist of inactive genes, regions of DNA used for the control of gene expression, and regions used for chromosome organization.

Gary Felsenfeld, Richard Axel, and Howard Cedar of the National Institute of Arthritis, Metabolism, and Digestive Diseases showed that when DNA is packaged in chromatin, gene expression, as measured by in vitro transcription of DNA sequences, is severely restricted. Globin genes, they reported, are transcribed in vitro from chromatin isolated from duck immature red blood cells, where globin is made, but not from chromatin from duck mature red blood cells or duck liver cells, where globin is not made. The DNA in the three kinds of cells, however, is the same. Moreover, an enzyme that binds to DNA to begin transcription apparently has less access to DNA in chromatin than to naked DNA. Cedar and Felsenfeld find that this enzyme binds to ten times as many sites in naked DNA as in chromatin.

Crucial to the structure and, most likely, to the function of chromatin are a group of five histones, which are positively charged proteins. Four of these five histones, apparently, are necessary for bead formation. Histones are associated with DNA in cells of all higher organisms. Since DNA contains negatively charged phosphate groups, it has long been realized that histones could bind electrostatically to DNA. Other proteins, called acidic proteins, are also associated with DNA, but these form a very heterogeneous group and, unlike the histones, seem not to affect the overall structure of chromatin, although they apparently are important in determining which genes are transcribed.

The first evidence that histones affect the structure of chromatin arose from x-ray diffraction studies. Various investigators noticed that chromatin gives rise to a series of regularly spaced diffraction rings, which is evidence of a repeating structure. When the histones were stripped from the DNA, this structure was destroyed. In 1970, John Pardon and Brian Richards of the Searle Research Laboratories in High Wycombe, England, together with Maurice Wilkins of Kings College in London proposed that the x-ray diffraction patterns of chromatin could be explained if the DNA double helix were coated with histones and twisted on itself to form a larger single helix, which these researchers called a supercoil. The regularly spaced turns of the supercoil could account for the x-ray patterns. Partly because there was no evidence of any other kind of structure of chromatin, the supercoil model had been widely accepted. Recently, however, most researchers have abandoned some elements of the supercoil in favor of the model of beads on a string.

Beads on a String

The most direct evidence that chromatin looks like beads on a string is derived from electron microscopy. In 1974, Ada Olins and Donald Olins of Oak Ridge National Laboratory published electron micrographs of stained chromatin from rat liver cells, rat thymus cells, and chick erythrocytes in which the chromatin appeared to consist of thin fibers connecting globular particles (Fig. 1). Subsequently, similar structures have been observed in electron micrographs of chromatin from other kinds of cells. The possibility remains that these linear arrays of spherical particles arise as artifacts when the cells are prepared for electron microscopy, but the model of beads on a string is consistent with results of other kinds of experiments related to chromatin structure.

R. J. Clark, of the National Institute of Arthritis, Metabolism, and Digestive Diseases, and Felsenfeld obtained information about chromatin structure in the process of investigating the extent to which DNA of chromatin is accessible to particular molecular probes. They reported that only about 50 percent of the DNA of chromatin can be degraded by staphylococcal nuclease, an enzyme that specifically attacks DNA. The remainder of the DNA is inaccessible, presumably because of the way it is packaged. Subsequently, Dean Hewish and Leigh Burgoyne of Flinders University of South Australia, and later Markus Noll of the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, found that discrete regions of DNA within cell nuclei that are about 200 base pairs long are protected from attack by nucleases, that is, enzymes that degrade DNA. The current interpretation of these results is that the protected regions of DNA lie in beads where they are associated with histones.

Further evidence that DNA in the beads is protected from nuclease degradation was obtained by Marilyn Senior of Oak Ridge National Laboratory together with Olins and Olins and, independently, by Kenneth Van Holde and his associates at Oregon State University. These researchers observed that, when chromatin is broken up by nuclease treatment or by high-frequency sound, it can be seen in electron micrographs as individual particles that resemble beads. Both groups of investigators used an analytical ultracentrifuge to estimate the molecular weights of these particles and determined what fraction of that weight corresponds to DNA. Senior, Olins, and Olins report that each particle contains a strand of DNA of molecular weight about 140,000, which is equivalent to about 210 base pairs per molecule.

J. P. Baldwin, P. G. Boseley, and E. M. Bradbury of Portsmouth Polytechnic in England, reconciled the x-ray diffraction patterns of chromatin with the model of beads on a string. By using neutron diffraction, they showed that the regularly repeating units that cause the characteristic diffraction pattern of chromatin are proteins, not DNA. Neutron diffraction differs from x-ray diffraction because it is relatively easy to alter the scattering power of the material in which the chromatin is suspended. Baldwin and his colleagues suspended chromatin in a mixture of deuterium and water that scatters neutrons to the same degree as proteins scatter neutrons. In this solution, any diffraction bands that arise from proteins should be canceled out. These investigators found that some of the regularly spaced diffraction bands

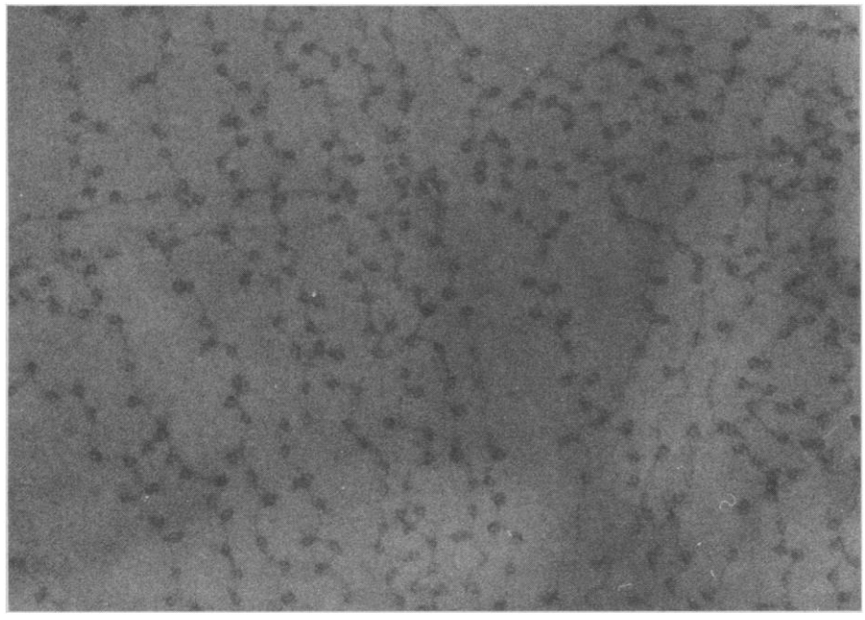

Fig. 1. Chromatin fibers streaming out of a chicken erythrocyte nucleus. The distance between beads is about 140 Å and the diameter of each bead is about 69 Å. [Source: Donald Olins, Oak Ridge National Laboratory]

(which correspond to the same reflections obtained from x-ray diffraction) disappear when the chromatin is in such a solution of deuterium and water. This indicates that these reflections probably come from proteins rather than DNA.

The model of beads on a string is attractive not only because it is consistent with various kinds of results relating to the structure of chromatin, but also because it provides an explanation of what is presumably the first step leading to the condensation of DNA so that it fits into the nucleus of a cell. When a fragment of DNA is tied up in a bead, it is about one-sixth the length it would be if it were extended. Because the DNA in a bead is still partially accessible to enzymes that can modify or degrade it, most investigators believe that DNA wraps around the outside of a globular cluster of histones to form a bead. These beads are on a flexible fiber consisting of DNA and nonspecific proteins that could be folded when the chromatin is inside a cell nucleus.

Now that the model of beads on a string is accepted, many investigators are trying to determine whether histones recognize specific DNA sequences. When histones bind to DNA they affect the physical accessibility of certain DNA sequences and thus restrict gene expression. This could occur if histones recognize specific DNA sequences and bind to them or if specific DNA sequences are shielded from histones (perhaps when acidic proteins bind to them).

Evidence is in fact accumulating in support of the hypothesis that the four histones responsible for generating the beads do not bind to specific DNA sequences. One reason is that chromatin can apparently be reconstituted when DNA from cells of one kind of organism is mixed with histones from cells of another kind of organism. The resulting complex gives rise to the same x-ray diffraction pattern as chromatin and to the same sizes of DNA fragments that are resistant to nuclease degradation. In fact, Felsenfeld and his colleagues find that they can even use DNA from bacteria or bacterial viruses (neither of which are normally associated with histones) to reconstitute a structure that resembles chromatin when it is degraded with nucleases. However, no functional criterion for accurate reconstitution of the overall structure of chromatin exists; thus these experiments do not rule out the possibility that histones recognize specific DNA sequences in living cells.

The most direct way to determine whether histones bind to specific DNA sequences would be to isolate beads, strip them of their histones, and analyze the sequences of the resulting DNA fragments. Such experiments have not as yet been done. One reason is that there is no way to ascertain whether isolated beads represent the same structures that occur in vivo. Some investigators, including Roger Chalkley of Iowa State University and Brian McCarthy of the University of California at San Francisco, find that histones may migrate along the DNA when beads are isolated. Thus the DNA fragments complexed with histones in vitro might not represent the fragments complexed with histones in vivo.

Lacking a direct means to analyze where histones bind to DNA from animal cells, McCarthy and his associate Barry Polisky studied histone binding to the small, well-defined DNA molecule of an animal virus: simian virus 40 (SV40). Others have shown that SV40 could provide a useful model system to study chromatin structure and function. For example, Jack Griffith of Stanford University isolated this viral DNA from the nuclei of infected monkey cells and found that each viral DNA molecule is complexed with an equal weight of histones, as is typical of chromatin from animal cells. He then fixed the complexes of SV40 DNA and histones and observed them by electron microscopy. The structures looked like beads on a string, with each bead containing on the order of 200 base pairs of DNA.

McCarthy and Polisky were able to determine the location of histone molecules on SV40 DNA by using an enzyme—a restriction endonuclease—that cleaves SV40 DNA at six well-defined sites. However, if such a site is complexed with histones, this enzyme will be physically prevented from cutting the DNA. Polisky and McCarthy analyzed the DNA fragments resulting from cleavage of SV40 chromatin by this enzyme. Their result led them to conclude that, since the probability of cleavage is equal at each of the six recognition sites for the restriction enzyme, histones are randomly arranged on SV40 DNA.

Although histones appear not to recognize specific sites on SV40 DNA, they may still recognize specific sites on animal cell DNA. Polisky and McCarthy point out that histones may bind to SV40 DNA only because both SV40 DNA and histones are present in the nucleus of an infected cell. Since histones bind to any sort of DNA, their binding to SV40 DNA could be unrelated to their possible functional role in animal cell chromatin.

An alternate way to study the structure of chromatin is to analyze how histone molecules interact with each other. Since there are five different histones there are, theoretically, a large number of ways that these proteins could combine when beads are assembled. Thus various beads could have distinctive histone compositions or conformations and could have different specific sequences of DNA wrapped around them. Recent results, however, indicate that only a few combinations of histones may be found in chromatin.

Many investigators believe that only four of the five histones (that is, the histones called F2A1, F2A2, F2B, and F3) in-

teract to form the globular clusters associated with beads. The fifth histone, F1, is proposed to be associated with regions of DNA between the beads. This is consistent with the observation by Richards and Pardon that removal of F1 does not affect the x-ray diffraction pattern of chromatin, whereas removal of any of the other histones destroys this pattern.

Roger Kornberg of the Medical Research Council in Cambridge, England, and Jean Thomas of the University of Cambridge in England showed that histones form pairs in solution. Histone F2A1 associates with F3 and F2A2 associates with F2B. Kornberg and Thomas suggest that these same pairs exist when the histones are bound to DNA and that the beads of chromatin are composed of two each of these four histones together with 200 base pairs of DNA. The model of Kornberg and Thomas is the first attempt to coordinate the x-ray data, results from studies of histone stoichiometry and chemistry, and results from studies of the degradation of chromatin by nucleases. Irvin Isenberg of Oregon State University finds that not only do F2A1-F3 and F2A2-F2B pairs form in solution but also the pair F2A1-F2B forms. Thus, in principle, all four of the histones could be in contact with each other. The problem is to show that histone interactions in solution reflect their interactions when they are associated with DNA.

One test of whether histones interact in solution in the same way as when they are associated on DNA is to analyze the sensitivity of these various histone complexes to an enzyme—in this case, trypsin—that degrades histones into their constituent amino acids. Trypsin can only gain access to histones on the outside of beads. Thus trypsin degradation can be used to determine whether the same parts of histone molecules are on the outside of beads when the beads are on DNA as when they are removed from DNA.

Harold Weintraub and Frederick Van Lente of Princeton University showed that, when histones are associated with DNA in chromatin, trypsin degrades only 20 to 30 amino acids from one end—the amino terminus—of each histone molecule in a cluster. The remaining 70 to 100 amino acids that make up a histone molecule are very resistant to trypsin, presumably because they are interacting with each other in a compact globular conformation. The same pattern of histone degradation by trypsin is obtained when the enzyme acts on histones that have been gently removed from the DNA. The histones removed from DNA form clusters, each of which is made up of one each of the four histones.

Further evidence that histones may interact as beads on the chromatin fiber in a manner similar to the way in which they interact in solution was recently reported by Harold Martinson of the University of California at San Francisco together with McCarthy. These investigators used a reagent, tetranitromethane (TNM), to irreversibly join specific regions of interacting histones while they were on chromatin. Then they removed the histones from the chromatin and determined which of them were bound together. In this way, they found that F2A1 and F2B are paired on chromatin.

Martinson and McCarthy also report that histones F2A1 and F2B can be cross-linked by TNM on chromatin that is reconstituted from the individual histones and DNA. However, the F2A1-F2B pairs are only found if the histones are first mixed together and then added to the DNA (the pairs are not formed when the histones are added separately to the DNA) and if histone F2A2 is present during this initial mixing of the histones. These results are consistent with Isenberg's demonstration that, in solution, histones F2A1 and F2B and histones F2B and F2A2 associate.

The work of Martinson and McCarthy and the work of other groups of investigators who are probing the structure of beads by means of enzymes that degrade histones or that degrade DNA are leading many to believe that, if the globular clusters of histones in chromatin are not all alike, they are at least put together according to a set of rules. These rules are still unknown. However, there is evidence that a specification of these rules may be the next advance in understanding chromatin structure.

—GINA BARI KOLATA

Relativity: Experiments Increase Confidence in Einstein

Astronomers and astrophysicists are becoming more and more convinced that Einstein's general theory of relativity is the correct formulation from among a host of competitive theories of gravity. Although no single experimental result can thus far be regarded as definitive, scientists feel that the trend indicated by a succession of increasingly accurate experiments of various types is to rule out theories other than general relativity. One of the most recent and the apparently most accurate experiments is reported by Edward B. Fomalont and Richard A. Sramek of the National Radio Astronomy Observatory (NRAO), Green Bank, West Virginia, in which they measured the deflection of radio waves passing near the sun with an uncertainty of about 1 percent. Other experiments of comparable or greater accuracy are expected to be reported in the near future.

In the years since the publication of Einstein's general theory in 1916, a number of alternatives and modifications to general relativity have arisen. Many have been discarded, and those that remain as viable competitors are all metric theories, as is general relativity itself. Metric refers to a quantity called the metric tensor whose components are determined by the structure of the four-dimensional space-time continuum. Thus the components of the metric tensor are a measure of the curvature of space. In Einstein's theory the metric tensor plays a role akin to a potential in classical mechanics. The competing theories of gravity differ from general relativity in including additional potential-like terms. Depending on whether these additional terms are scalars, vectors, or tensors, the modified theories are called scalar-tensor, vector-tensor, or two-tensor theories.

The best known of these theories is the Brans-Dicke theory (also sometimes referred to as the Dicke-Brans-Jordan theory) which was put forth by Carl Brans and Robert H. Dicke of Princeton University in 1961. (Brans is now at Loyola University, New Orleans.) The Brans-Dicke scalar-tensor theory in particular has stimulated much theoretical and experimental work by researchers interested in astrophysics and cosmology. Moreover, because it makes specific predictions that are different from what general relativity would predict, the Brans-Dicke theory has been the theory most often compared with general relativity when experimental tests of relativity are made.

Until the last few years, the best experimental confirmation of general relativity was provided by the excess advance of the perihelion of Mercury. The apsis of Mercury, or the line connecting the parts of its elliptical planetary orbit that are nearest to