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A model for particulate structure in chromatin

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ABSTRACT

A model is proposed for the structure of nuclease-resistant chromatin particles. The model is novel in that it proposes that the DNA in such a particle is wound about a protein core, made up of the hydrophobic regions of histone molecules.

Recent experiments in our laboratory have been directed to the investigation of that portion of chromatin that is preferentially resistant to staphylococcal nucleases[1,2]. Hydrodynamic studies show that this fraction - which may constitute up to 50% of calf thymus chromatin - consists of reasonably homogeneous, compact particles[2]. These fragments, which we have termed "PS-particles", have a weight average molecular weight of about 180,000, and each contains a single piece of double-strand DNA of 70,000-80,000 daltons. The f/fo ratio is 1.1, indicating these DNA-protein complexes to be as nearly spherical as most globular proteins. Their Stokes' law diameter is 82 Å.

More recently there has appeared confirmation that these "PS-particles" are probably of significance in chromatin structure in vivo. Olins and Olins[3] have presented electron micrographs of swollen, burst eucaryotic nuclei which show the chromatin to have a "string of beads" structure. The beads (which they term "ν-bodies") appear to be quite homogeneous in size. In the case of rat-thymus nuclei, they are found to have an average diameter of 83 Å ± 23 Å (S.D.). Such beaded fibers, and clumps of such beads, appear to form a substantial portion of the chromatin.

We have carried out electron microscope studies[4] on the PS-particles from calf thymus, and find them to be compact, roughly spherical objects, with a number average diameter of 74 Å ± 20 Å (S.D.). Furthermore, we find in these digests a number of particles with 20 Å thick "tails", and a

few examples of "doublet" particles which are connected by such strands. These results lead us to believe that our "PS-particles" are, in fact, the "ν-bodies" of Olins and Olins. We shall refer to them herein as "chromatin particles", or simply as "particles", and the portion of chromatin which they constitute as the "PS-fraction"[1].

We have continued our studies on these chromatin particles, and while details will be published elsewhere, we wish to mention a recent finding which is relevant to the discussion to follow: the calf-thymus chromatin particles contain the normal complement of histones, with the exception of F1, which is progressively reduced as the chromatin is digested toward the PS-particle limit. Particles prepared by long digestion have little or no F1.

The nature and properties of these particles raise fundamental questions about their structure. The DNA fragment, if extended in a B or C conformation, would have a length of about 400 Å. Therefore it must be tightly coiled or folded to fit into a particle with a maximum dimension of about 100 Å. Since we now know that there is little histone F1, and little non-histone protein, we can make a good estimate of the number of proteins in each particle. There are roughly 100,000 daltons of protein per chromatin particle; this corresponds to about eight histone molecules, if F1 is excluded as our analyses indicate. Kornberg[6] has suggested that an eight-histone complex, containing two molecules of each of the histones except F1, may constitute a basic unit of chromatin structure.

Is it possible to make any prediction as to the relative disposition of the DNA and protein in such particles? We believe that it is, based on the following known properties of the DNA and the histones which are involved:

1. To avoid exceedingly sharp bends in the DNA, it must be coiled at a considerable distance from the center of the particle. Note that even a single sharp kink will not do, for this could at best yield a particle of nearly 200 Å in length.
2. All of the histones present have one property in common. The N-terminal region (about 40-50 residues) is strongly basic[7], and it is this region that interacts most strongly with DNA[8,9,10,11].
3. Each of these histones also contains a highly hydrophobic region[7], which extends to, or nearly to, the C-terminus. In a number of cases this portion of the molecule has been shown to interact only weakly with DNA, but to have a strong tendency to self-associate[8,11].

4. There is mounting evidence for specific interactions between pairs of histone molecules. For example, histone F2b and F2a2 are known to associate[12,13,14], as are histones F2b and F2a1[15]. Very recent evidence shows that histones F3 and F2a1 form a tetrameric 1:1 complex[16,17,18]. All of these complexes are formed in salt concentrations comparable to those presumed to exist in the nucleus.

The model which we propose on the basis of these properties has some features in common with those of Bradbury and Rattle[11], Hayashi and Iwai[19], and Kornberg[6]. However, it is much more detailed than any of these. Specifically, we propose:

1. There exist specific complexes of histones F2a1, F3, F2a2 and F2b, involving about eight protein molecules in all. Small amounts of non-histone protein might or might not be included, and there may be more than one type of complex. We suggest that these complexes are formed by interactions of the C-terminal halves of the histones, leaving the N-terminal regions free to interact with DNA. It has been observed[20,11] that the N-terminal portions of the molecules are free in aggregates of histone F2a1.
2. These associated C-terminal regions form a hydrophobic core, about which the DNA is wrapped or twisted. The portion of the DNA touching the core will interact with some of the basic residues in the C-terminal regions.
3. The projecting N-terminal portions of the histone molecules are then wrapped outside the DNA, probably in the major groove as suggested by Richards and Pardon[21]. Thus, these N-terminal histone regions will serve to both hold the DNA to the core, and also to render it partially inaccessible (see below).

The fundamental feature of the model, then, is that histone complexes can act as nuclei upon which DNA can be wound. While it is obviously premature to speculate on details of such a model, it should be noted that the volume of the protein core is such that the DNA could be neatly wrapped around it with an average diameter (center to center) of about 50 Å if we assume an overall particle length on the order of 100 Å. The entire 400 Å DNA piece can be easily accommodated, and will be largely neutralized by the high concentration of positive charges on the N-terminal half extensions of the histone core proteins. D'Anna and Isenberg[18] have pointed out that the total number of lysine and arginine residues in the set of eight

histone molecules is 220, in good agreement with the number of DNA phosphates in the particle.

A schematic drawing of one of a number of possible structures is shown in Figure 1. A particle such as this would result in a "looping out" of the DNA into a knobby protuberance along the chromatin fiber. It is quite possible, of course, that there is not one unique type of particle, but a class of these, corresponding to different assemblies of histones.

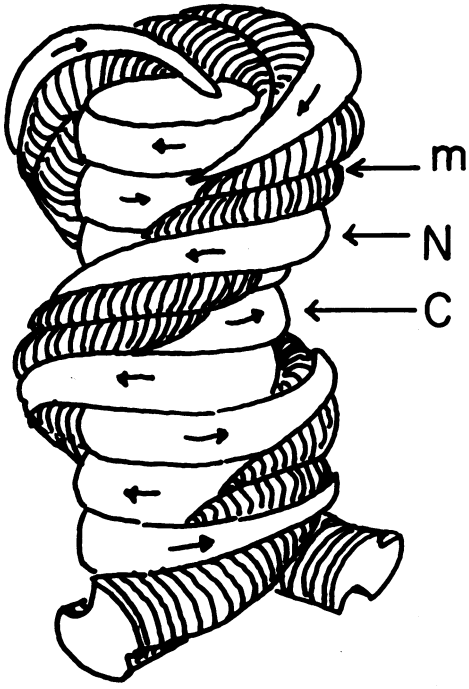

Figure 1. A highly schematic view of one possible kind of chromatin particle. The DNA is shaded. "N" indicates the N-terminal region of a histone molecule, lying in the major DNA groove. The arrows on the histone molecules point toward the N-termini. "C" denotes the C-terminal half of a histone. The minor groove of the DNA is indicated by "m".

There are a number of consequences and predictions that arise directly from the model:

1. The DNA will be partially protected against reagents which interact wholly or partly with the major groove. This may explain the partial resistance to nuclease by these chromatin particles[1,2], as well as the recent observation by Mirzabekov and Melnikova that dimethyl sulfate reacts poorly with residues in chromatin which in free DNA are generally accessible via the major groove[22]. We would predict that any reagent which binds to the major groove will bind hardly at all to these chromatin particles.
2. Since the DNA is on the outside of the particle, and there is not enough protein to occupy both grooves, the minor groove should be quite accessible. It has been observed by Simpson[23] that a reporter molecule, which is believed to interact with the minor groove[24], is bound to the same extent by chromatin as by native DNA. Similarly, Mirzabekov and Melnikova[22] have found that the DNA in chromatin is not protected against dimethyl sulfate methylation of the N3 of adenine, which is accessible only through the minor groove. These results are difficult to explain by any model in which the protein covers the DNA.
3. The chromatin particles should be strongly stabilized by cooperative DNA-protein and protein-protein interactions. We observe that the PS-fraction melts at the upper end of the chromatin melting range[2].
4. Hydrophobic bond-breaking reagents like urea should disrupt the protein-protein interactions, unraveling the chromatin structure, but leaving the histones bound to the DNA through the N-terminal regions. This is observed[25].
5. The reactivity of all lysine residues, including those in the core portion should be reduced over that in free histones (as observed[26]); but some could be partially available and those points should be accessible to limited tryptic hydrolysis. This has been observed by Simpson[27] for whole chromatin, and by ourselves for the chromatin particles[2]. Significantly, in the latter case less hydrolysis occurs. The effect should be, as observed[2,27], an unfolding of the structure of the chromatin particles.
6. Since the DNA in the chromatin particles must be quite tightly coiled, it might be expected to differ in conformation from the remainder of the

DNA in chromatin. We have observed an unusual circular dichroism spectrum in the PS-fraction[1,2], which appears to account for the difference in circular dichroism between free DNA and whole chromatin[28].

Two major problems remain. First, there are numerous electron micrographs that show irregular (often knobby) fibers in chromatin. See, for example, Bram and Ris[29], Slayter, et al.[30]. Two classes are frequently mentioned: fibers about 100 Å in diameter and others about 250 Å. Second, there is the observation of low angle X-ray diffraction rings from chromatin samples[21,29,31,32]. These are most frequently observed at spacings of about 110 Å, 55 Å, 37 Å, 27 Å and 22 Å, and have been generally ascribed to a superhelical structure. Any model of chromatin structure should be compatible with these two kinds of observations. In terms of the model proposed here, these data would be explained in the following ways:

1. Since the chromatin particles are of the order of 100 Å in diameter, a closely spaced series of such particles could give, in electron microscopy, the impression of an irregular 100 Å fiber. Olins and Olins have pointed out that in many regions the particles appear to be tightly packed or clustered[3].
2. In regions where the particles are evenly spaced, it would be very easy for them to array themselves in a helical pattern. Such helices could be of varying widths, but hardly much less than 200 Å in diameter. Further, being made up of 100 Å subunits, they might be expected to display the diffraction pattern characteristic of an axial repeat of about that distance. A final analysis must depend upon detailed calculations of the diffraction pattern to be expected from such helices.

Finally, we should add a word about the possible role of histone F1. It is not an integral constituent of our PS-chromatin particles; it must play some other role in chromatin structure, perhaps acting as a cross-linking agent between particles. This is in accord with a number of observations on the effects of addition or removal of F1 histone to chromatin[33,34].

A fundamental element of our model is the proposal that the DNA lies largely on the surface of the protein. This concept may make it much easier to understand how such processes as transcription and replication can occur. The kind of structure we have proposed (Fig. 1) bears some resemblance to structures suggested by Crick[35]. However, we leave open the question as to

whether any DNA may be unpaired. Further, the idea that specific protein complexes can form loci for the local winding of DNA suggests a reasonable mechanism for the self-assembly of the chromatin structure.

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