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A possible explanation for the nuclease limit digestion pattern of chromatin

(nucleosome structure/re-entrant binding/DNA movement)

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ABSTRACT **The general pattern of DNA fragments in the limit digest of nuclease-treated chromatin could arise from a single, unique nuclease-susceptible site per nucleosome. If DNA binds to the histone core of the nucleosome along a circularly re-entrant path, the location of the DNA entrance and exit can occur at any of a number of distinct sites. This very specific type of heterogeneity together with the natural 10-fold periodicity of DNA B can account for the observed digestion pattern. Such a general picture of the nucleosome structure could also easily explain how nucleosomes might move along the DNA. This type of structure should be easy to distinguish experimentally from more conventional explanations of the origin of the limit digest pattern of chromatin.**

Evidence for a chromatin subunit structure has accumulated during the past few years from techniques as diverse as electron microscopy and nuclease digestion (1, 2). There appears to be general agreement that individual subunits (nucleosomes) isolated by mild nuclease treatment of intact nuclei or chromatin have a structure that is representative of at least some states of native chromatin (3–5). While there are unresolved quantitative discrepancies in various studies, most recent work is consistent with a nucleosome core having two each of the histones H2A, H2B, H3, and H4 and somewhere between 140 and 200 base pairs of DNA (6–10).

Numerous models of nucleosome structure have been constructed (7, 11–13). However none of these easily explains one of the most puzzling findings about the nucleosome. When preparations are subjected to vigorous digestion with *Staphylococcus aureus* or other nucleases, an apparent limit digestion product can be obtained. As first shown by Clark and Felsenfeld (14), this contains, in acid-insoluble form, about half of the original DNA in chromatin. The product is unaffected by increased incubation times or further addition of the nuclease that generated it (15). When DNA of the limit digest product is examined by gel electrophoresis a series of discrete lengths is observed. While the pattern differs, depending on the nuclease used and the source of the chromatin, the essential feature, a series of lengths spaced every 10 nucleotides is seen consistently (3, 5, 15–18,†). The shortest lengths seen are about 20 base pairs, which is close to the minimum length expected to be acid-insoluble. The largest lengths seen are about 140–160 base pairs. The distribution of material from some sources is roughly bimodal, and lengths near 70 base pairs are essentially absent (3). Other sources show a more monotonic mass distribution of DNA lengths (5), while still other experiments suggest that the yields of alternate lengths vary periodically and the digestion pattern has a center of symmetry between lengths 90 and 100†.

Considerable insight into the detailed structure of the nucleosome should be potentially available from the nature of the limit digest. However, at present there is insufficient information about the quantitative yield of the products and the idiosyncracies of individual preparations to allow full exploitation of existing data. The purpose of this short communication is to contrast two general mechanisms by which a limit digest can yield a discrete set of products that seem to form a mutually exclusive set. One mechanism is of particular interest because it should be experimentally distinguishable and because, if it is correct, it offers a natural explanation of the way in which a nucleosome might move along a DNA strand.

To explain a discrete set of nuclease digestion products it seems necessary to argue that only certain positions along the DNA in the nucleosome are potentially susceptible to enzymatic attack. This could arise as a result of a periodic perturbation of the DNA structure. Kinks occurring once per helix turn are certainly a possibility consistent with the structure of the DNA B duplex helix (19). However, the fact that susceptible sites are spaced one per helix turn allows for a simple alternative. Consider a DNA helix constrained to lie in a groove along a histone core. The actual topology of the groove might have to be quite complex to explain the superhelical turns that are coupled to nucleosome formation (20, 21). Regardless of the topology, a B form helix in a groove will have every tenth base pair positioned at a maximum distance away from the nucleosome core. In more general terms, a DNA helix on a surface will automatically have a structure perturbed or exposed with the same periodicity as the helix.

Thus the striking enigma of the limit digest product of nucleosomes is not the periodicity of 10. It is the fact that cleavage occurs at only a small fraction of the potentially susceptible sites. In fact, the simplest way to rationalize the observed digestion pattern is to conclude that cleavage can occur only once per nucleosome. This immediately accounts for the breadth of the product lengths seen, and it can explain bimodal or alternating distributions. We shall accept single hits as a premise and then try to explain it in the discussion that follows. Relaxing this assumption does not really change any of the arguments that follow. It merely adds more complications to each possible hypothesis. The critical fact is that nuclease hits cannot occur many times on all nucleosomes in the population.

A general, but not very attractive, way to rationalize the limit digest is to argue that the intact nucleosome is a structure with nuclease-susceptible sites spaced every 10 residues. Cleavage at any site leads to a structural rearrangement that eliminates all remaining susceptible sites, as shown in Fig. 1a. This mechanism implies, although it does not demand, that the DNA leave and enter the nucleosome at unique points. It predicts that the chain cleavages resulting from vigorous digestion are located at a set of different sites on the nucleosome. Note that the schematic mechanism in Fig. 1a allows for the possibility that some loose ends of DNA may be trimmed away during the nuclease treatment. To explain the discrete lengths found in the

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† D. Pulleyblank and M. Shure, personal communication.

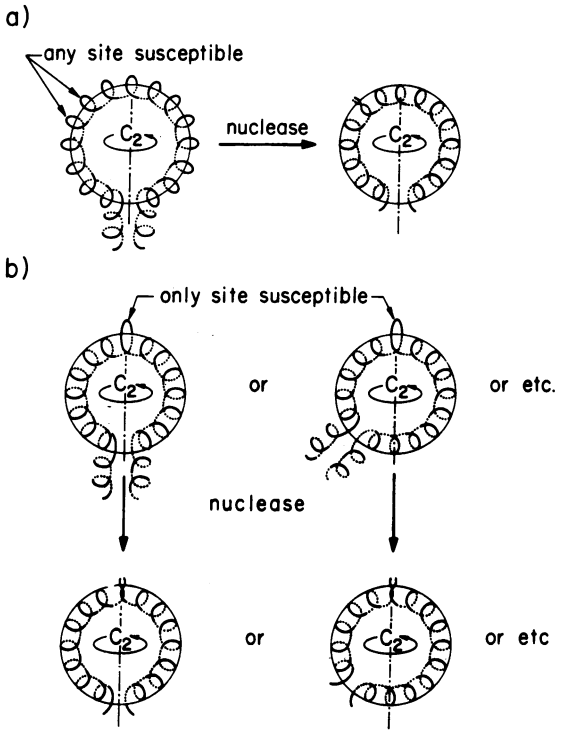

FIG. 1. Two schematic general schemes for the structure of the nucleosome. Both are assumed to have an unique core of eight histones. (a) Any one of the sites spaced one helix turn apart is susceptible to nuclease cleavage. One hit causes a relaxation of the structure, which protects against further digestion. (b) Only one site susceptible to nuclease cleavage exists. For reasons of clarity, only, this is shown as an extended loop. DNA can enter and leave the nucleosome at any pair of adjacent sites spaced one helix turn apart. Only two of the many possible structures are shown. Possible locations of a 2-fold rotational axis in the plane of the page are shown.

final products it is necessary to postulate that these ends can be cleaved down to well-defined points. Thus in actuality three specific cuts are required to generate the observed limit digest.

The mechanism of relaxation after these cleavages can be likened to the relaxation of a superhelix with a high conformational free energy. Once the topological constraint is removed, the free energy is lowered substantially. There is no real flaw in such an explanation. However, except for special cases like superhelices, usually one would expect cleavage of a covalent bond to render a structure less tightly bound and therefore more susceptible to subsequent enzymatic attack.

An alternative general explanation of the limit digest is structural heterogeneity. The context in which this has been suggested usually brings to mind populations of nucleosomes with different histone arrangements (17) or histone conformations (18) or the presence of covalent modifications or nonhistone components. What would be simplest, however, is the very specific and limited structural heterogeneity illustrated schematically in Fig. 1b. Here the histone core is assumed to be identical in every nucleosome. The arrangement of DNA on the histone core is also assumed to be identical in every nucleosome. The only variable is the site at which DNA enters and leaves each nucleosome.

According to the scheme in Fig. 1b, DNA can enter at any one of a class of equivalent or near-equivalent sites. It must fill each in an ordered succession, and then it leaves once the last site is filled. There is a fairly natural explanation for the entry and departure positions. Crick and Klug have shown that the favored direction of bending of DNA will rotate with the period of the double helix (19). Thus, only once in 10 base pairs will it be possible to bend the DNA out directly away from the nucleosome core. While the DNA path is shown topologically as a circle, for simplification, in Fig. 1b, the actual structure could be any re-entrant pathway without altering the arguments that follow. Note that if DNA bends to fill a superhelical re-entrant pathway, the resulting change in supercoils will be a constant independent of the points of entrance and exit.

Suppose that one site along the nucleosome is unique in such a way that it generates a highly susceptible locus for nuclease attack either by distorting the DNA or because of its local environment. On every nucleosome cleavage occurs only at the unique site and at the two loose ends of the DNA. However, the multiplicity of DNA entry points on the nucleosome leads to a set of discrete cleavage points spaced every 10 residues along the DNA. In this scheme there is no need to postulate any nuclease-induced structural changes. It is still necessary to predict favored cleavage positions for the DNA free ends. These would be easy to rationalize if DNA makes a bend at its point of entrance and departure from the histone core. The mechanism in Figure 1b makes two very specific predictions, both of which should be experimentally testable. The DNA termini in an intact or limit digested nucleosome should be located at a set of different sites. However, the new DNA ends generated by the limit digest should be at an unique site on the nucleosome. Note that this prediction is directly opposite to the results expected if the scheme in Fig. 1a is correct or expected for any histone core heterogeneity. Therefore the mechanism in Fig. 1b lends itself to clear experimental discrimination.

The histone composition of the nucleosome strongly suggests that the particles should have a 2-fold rotational symmetry axis. The dyad axis of the DNA B helix is a pseudo-2-fold axis. It would be surprising if these symmetries were not incorporated into the packaging arrangement of DNA on the histone core of the nucleosome. As a result, most sites of DNA binding or potential nuclease susceptibility should occur in symmetry-related pairs. Exceptions occur only at two locations right at or near the symmetry axis itself. If there is one unique nucleosome cleavage site it must be on the dimer axis. Here there might be a distortion in the nucleosome structure. Alternatively the 2-fold axis could be located in a region of the histone core that is devoid of protein. This could easily allow increased accessibility of nucleases to DNA where it crosses the symmetry axis.

The schemes in Fig. 1 could easily be elaborated to rationalize detailed features of the available data. For example, the low yield of DNA lengths of 70 base pairs can be explained by either scheme. In the case shown in Fig. 1a one argues that certain potential cleavage sites are unfavorable. For the scheme in Fig. 1b one argues that certain DNA binding configurations are unfavorable. By making either hypothesis more complex one could explain why the individual DNA limit digest products may be distributions several residues wide rather than homogeneous unique lengths. Similarily it is relatively easy to alter the hypotheses to explain an alternating pattern of yields of successive fragments. However it seems premature to construct such detailed models until some of the available experimental findings have been clarified further. These are fundamental differences implied by the two general schemes in Fig. 1. Since both explain existing data about equally well, new experiments will be needed to see if either is realistic.

One feature of the scheme in Fig. 1b is particularly appealing. It is reasonable to think that under some conditions nucleosomes may be able to move from one region of DNA to

another. The scheme in Fig. 1a or others like it would require either sliding or dissociation to accomplish translational motion. Both processes are likely to be energetically quite unfavorable. In contrast, the scheme in Fig. 1b would readily facilitate movement by rolling. DNA could dissociate transiently from just the initial binding site and be replaced by an equal length of DNA adjacent to the terminal binding site. Thus each distinct structure of the kinds shown in Fig. 1b might actually represent intermediates in a rolling nucleosome mechanism. The activation energy for such movement could be fairly modest.

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