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The helical periodicity of DNA on the nucleosome

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The precise number of base pairs per turn of the DNA double helix in the nucleosome core particle has been the subject of controversy. In this paper the positions of nuclease cutting sites are analysed in three dimensions. Using the midpoint of the DNA on the nucleosome dyad as origin, the cutting site locations measured along a strand of DNA are mapped onto models of the nucleosome core containing DNA of different helical periodicities.

It is found that a helical periodicity of 10.5 base pairs per turn leads to cutting site positions which are sterically inaccessible. In contrast, a periodicity of 10.0 base pairs per turn leads to cutting site positions which are not only sterically sound, but which fall into a pattern such as would be expected when the access of the nuclease to the DNA is restricted by the presence of the histone core on one side and of the adjacent superhelical turn of DNA on the other. As proposed earlier by us (1), a value for the helical periodicity close to 10 base pairs per turn on the nucleosome, taken together with a periodicity close to 10.5 for DNA in solution - a value now established - resolves the so-called linkage number paradox.

THE LINKAGE NUMBER PROBLEM AND THE SCREW OF DNA

When the results of the X-ray crystal analysis on the organization of DNA in the nucleosome core were first obtained and compared with certain physico-chemical data, a paradox emerged (1) which has come to be known as the linking number problem. The X-ray data showed that the double helix of DNA in a nucleosome is wound (about a histone core) into about two turns of a (shallow) superhelix, whereas measurements on closed, circular DNA extracted from SV40 chromatin gave a degree of supercoiling nearer one superhelical turn per nucleosome (2). However, as first realised by Crick (3), what was being measured in the physico-chemical experiments was not the "number of super-coils", but the change in the linkage number of DNA (i.e. the number of times one strand of the DNA double helix is wound round the other strand) when it changes from being free in solution to being wound on the nucleosome.

We pointed out (1) that a change of linkage number can be identified with the number of superhelical turns only if the helical screw of the DNA double helix remains constant (as measured in a local frame of reference) and

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suggested that the X-ray and supercoiling results could be reconciled if the helical periodicity of the DNA was reduced by about 0.5 base pairs per turn (i.e. by about 5%) on passing from solution on to the nucleosome. (This corresponds to an increase of 2° in the angle of screw rotation between base pairs.) Taking the screw of DNA on the nucleosome to be 10 base pairs per turn, the value then given by the first DNAse I digestion studies (4), we therefore predicted that the helical periodicity of DNA in solution was close to 10.5 base pairs per turn (rather than the value of 10 found from X-ray diffraction of fibres). Measurements of the helical repeat of free DNA, made by two quite different approaches (5,6) have now borne out this prediction.

Ironically, however, one of the quantities used in making the prediction, namely the screw of DNA on the nucleosome, was shortly thereafter called into question by more accurate measurements, made by ourselves and our colleagues, of the average distance between DNAse I cutting sites of the DNA in nuclei, which give a value of about 10.4 base pairs per turn, rather than 10 (7,8). It would at first sight now seem that the helical periodicity of DNA in solution and on the nucleosome are nearly the same, thus apparently invalidating our proffered solution to the linking number problem. This belief has induced other authors to look for other solutions: we discuss these below. However, when we reported our new DNAse I digestion measurements, we pointed out that the periodicity of cutting of the DNA, although closely related to the structural periodicity, need not be exactly the same. This is because of the geometry of the nucleosome structure, where all the cutting sites on the DNA do not offer the same environment, i.e. angle of attack, to the enzyme, as they would to an isolated segment of DNA lying on a flat surface. In fact, we concluded (7) that the DNAse I fragments from nucleosome DNA that are approximate multimers of 10.4 bases could have been produced from a helical repeat of 10 base pairs per turn.

In this paper we take up this question again, with an analysis and interpretation of the new data that has become available. The distribution of single-stranded fragment lengths determined in the DNAse I experiments on nuclei (7) does not reflect the actual locations of cleavage sites but only the average distances between them. In a further study by one of us (8), using nucleosome cores radioactively labelled at their 5'-ends, the locations of the DNAse I cleavage sites along the DNA relative to the 5' end have been determined with high precision. This technique has now been applied to study the digestion of nucleosome cores by a second nuclease, DNAse II, of different size and mode of action, and the results are described in the preceding paper

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(9). Although there are differences in frequencies of cutting, the two enzymes attack essentially the same positions in the DNA (within less than half a base on the average), showing that these depend not on the enzyme used but rather on the structure and organization of the DNA.

We shall see below that this nuclease cutting data enables us to calculate the angle of attack by the enzyme at each cutting site for any given postulated structural periodicity of the DNA. We find that the variation of angle of attack deduced on the basis of a 10.5 fold screw in the DNA double helix leads to steric improbabilities, if not impossibilities, whereas all the data are compatible with a periodicity of 10 base pairs per turn of the helix.

A further development which provides support for our interpretation of the action of nucleases on DNA wrapped on the nucleosome core, is that similar nuclease digestion experiments have now been done with DNA bound to various flat surfaces (6; cf. Fig. 3b below). There the geometry of the experiment is simple: the access of the enzyme to one side of the double helix is hindered in a uniform way along its length, so that the only exposed phosphodiester bonds accessible to the enzyme are on the far side and these "sites" recur with the periodicity of the helix. Here, as expected, the accessibility of each turn of the double helix is found to remain essentially constant along the length of the DNA, quite unlike the result for DNA on the nucleosome (10). Moreover, this experiment enables one to deduce the angular range of cutting about any one "site", a useful datum in considering the experiments on the nucleosome. This work, though carried out primarily to determine the periodicity of free DNA, provides a geometrically defined platform of data and experience from which to proceed to the more complicated case of DNA in the nucleosome core considered in this paper.

ANALYSIS OF DNAse I CUTTING RESULTS IN THREE DIMENSIONS

The locations of DNAse I cleavage sites along one strand of the nucleosomal DNA have been determined using high resolution gel electrophoresis to measure the lengths of the single-stranded DNA fragments produced by the action of DNAse I on 5'-terminal radioactively labelled nucleosome cores (8). The results are listed in Table 1, column 2. The values obtained are not integers because they represent averages over a distribution. These lengths represent the distances, measured in numbers of bases, of the cleavage sites along a strand of the DNA measured from its 5' end. By virtue of the two-fold symmetry of the nucleosome core (see e.g. ref. 11), these will be the same for both strands, here denoted C and W respectively.

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Now these distances do not tell one where the cleavage sites are in three dimensions on the nucleosome core. First, one does not know the location in space of a 5' end of DNA relative to the nucleosome, and, secondly, distances measured in bases along a DNA strand have to be translated into real distances measured in space along the DNA double helix. This conversion will depend on the structural periodicity of the helix, which, as we have said before, need not be the same as the periodicity of DNAse I cutting.

There is a way of dealing with the first question. There is one fixed point for placing the DNA double helix on the nucleosome, namely the position of the overall dyad which is common to both the DNA superhelix and the histone core, and which passes through the local dyad at the midpoint of the DNA double helix. This dyad is that which lies half-way along the DNA, between cleavage sites $S7_C$ and $S7_W$ (Fig. 2). Now from Lutter's data (8) we have the information from which to place these sites relative to the dyad axis: they are staggered with respect to each other by 1.4 bases in a known direction, and so are each 0.7 bases away from the dyad, along their respective strands. This gives a fixed point on a strand from which to measure off the distances of the remaining cleavage sites. The observed distances from the 5' terminus can then be converted into distances from S7 (Table 1, column 4).

There still remains the second point. One must convert distances along a DNA strand, measured in numbers of bases, into true spatial terms, in particular into angular settings (azimuths) about the double helix axis. This conversion will depend on the helical screw of the DNA double helix, and this is not known precisely on the nucleosome: indeed it is this which is being sought and what prompted our detailed nuclease digestion study in the first place. Our approach is thus as follows: we postulate a particular helical periodicity for the DNA double helix and see what the consequences are for the spatial and angular disposition of the cutting sites when these are mapped onto nucleosome core models, containing different helical screws for the DNA duplex.

For definiteness we take two cases of special interest: (1) a periodicity of 10.0 base pairs per turn, a value which would, in our view, resolve the linking number paradox, and (2) a periodicity of 10.5 bases per turn, which takes the simple view that the average DNAse I cutting periodicity directly reflects the structural periodicity. In the first case there is a rotation of 36° between successive bases along a strand and in the second a rotation of 34.3^o. The calculation of the angular positions of the cleavage sites is given in Table 1 and Figure 1 and the results plotted in Figs. 2 and 4. In

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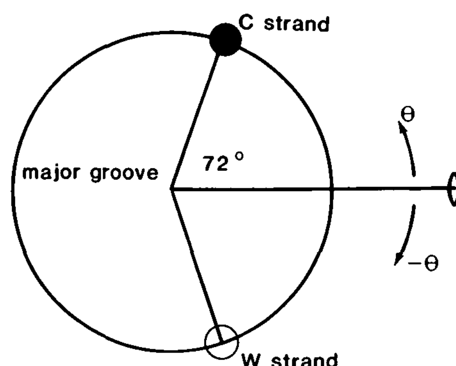
Table 1: Angular Positions of DNAse I cutting sites

Site numbered from 5' end	Distance in bases (a) from 5' end	Site renumbered (b) from nucleosome dyad	Distance in bases from dyad	θ, angular displacement from dyad (Fig. 1)	
				10.0 bp/turn	10.5 bp/turn
S1	12.0	$\bar{6}$	-61.1	111.6°	3.4°
S2	22.5	$\bar{5}$	-50.6	93.6	3.4
S4	42.5	$\bar{3}$	-30.6	93.6	37.7
S5	53.2	$\bar{2}$	-19.9	68.4	30.9
S7	73.8	0	0.7(c)	46.8	44.6
S9	95.0	2	21.9	3.6	37.7
S10	105.9	3	32.8	-28.8	24.0
S12	126.7	5	53.6	-57.6	30.9
S13	136.5	6	63.4	-50.4	54.9

(a) Data from Lutter (1979, Table 1). b) Bar to read 'minus'.
(c) Stagger at S7 from Lutter (1979).

e.g. S10 for 10.0 bp/turns $\theta = 72^{\circ} - 36^{\circ} \times (32.8 - 3 \times 10) = -28.8^{\circ}$
S10 for 10.5 " $\theta = 68.6^{\circ} - 34.3^{\circ} \times (32.8 - 3 \times 10.5) = 24.0^{\circ}$
S5 for 10.0 " $\theta = 72^{\circ} - 36^{\circ} \times (-19.9 - \bar{2} \times 10) = 68.4^{\circ}$
S5 for 10.5 " $\theta = 68.6^{\circ} - 34.3^{\circ} \times (-19.9 - \bar{2} \times 10.5) = 30.9^{\circ}$



Section of double helix through the dyad

Fig. 1: The angular separation of the two helical strands on which the phosphate groups lie is calculated to be about 144° from the coordinates for 10-fold DNA (28); the fact that this is a multiple of 36° is a coincidence. For 10.5-fold DNA, where the helical parameters are unknown, we have reduced this angle to 137°, pari passu with the change of the screw: this is a "best possible" calculation, - had the value been kept unchanged, the cutting sites would have moved even further into the cleft between the superhelical turns (Fig. 4b).

We use a continuous approximation to the phosphate sugar backbone in order to deal with non-integral numbers of bases (arising from an average over a distribution) and there is no other simple way of representing these. Since the enzyme cuts the P-O3' bonds, we might have considered the helices on which these lie rather than the phosphate helices, but the bonds on the two strands are not exactly opposite each other so that the stagger is not zero and a correction would have to be made for these on the continuous approximation. The effect of these approximations does not change the angular disposition of the cleavage sites by more than 5°, and since any error will be in the same direction for both the 10-fold and 10.5-fold double helices, the essential difference between columns 5 and 6 above and hence between Figs. 4a and 4b will remain.

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these calculations we have used the simplest possible assumptions about the organisation of the DNA, i.e. that it follows a smoothly bent superhelical path and that the helical periodicity of the double helix is constant all along this path. This is sufficient to give a general picture of the effect of the helical repeat on the results, but obviously intermediate cases and combinations of these two models could be taken.

The positions of the cleavage sites along the DNA superhelix calculated from the digestion data for the two cases chosen are shown in Figs. 2a and 2b respectively. In these diagrams the DNA double helix is represented on a ribbon which follows the superhelical path, the superhelix itself having been cut in a direction parallel to its axis near sites S3 and S11, opened out and laid flat with its outer face uppermost (i.e. towards the viewer). Pairs of sites across from one another on the two strands lie close together, e.g. $S2_C$ and $S12_W$, or $S1_C$ and $S13_W$, with staggers varying from about 1.4 to about 3.0 bases (8). This variability of staggers means, as discussed earlier (8), that the enzyme does not act at a common "double-stranded site" at all positions (cf. refs. 10, 12). Nevertheless, to get a general picture of the direction of attack by the enzyme at various regions of the nucleosome, we consider such a pair of cleavage sites together and treat the point half-way between as marking the centre of local accessibility in this region of the DNA. The angular positions of these points along the superhelix will then indicate the general direction of attack of the enzyme on the DNA. In Fig. 2a these points are joined together by a line.

We see from Fig. 2a that for the 10 fold double helix there would be a progressive change in the angle of attack of the enzyme as we proceed outwards from the dyad point in either direction along the superhelix towards the ends of the DNA. The sites of attack move away from the adjacent superhelical turn towards the outside of the nucleosome. It is thus the upper surface of the upper turn of the superhelix and the lower surface of the lower turn, both of which face outwards from the nucleosome, that are exposed to enzyme attack. This is sterically sound, and is indeed just what we and our colleagues proposed would happen (7).

In contrast, Fig. 2b for the 10.5 fold helix shows that, rather than move towards the outer faces of the nucleosome, the cleavage sites near the ends of the superhelix move inwards in a direction towards the neighbouring superhelical turn of DNA. This is highly implausible, if not sterically impossible, for the enzyme would have to gain access to sites located in a 5 Å wide crevice between the two turns of DNA. (The pitch of the superhelix is 27 Å (1) and the diameter of DNA is 22 Å.)

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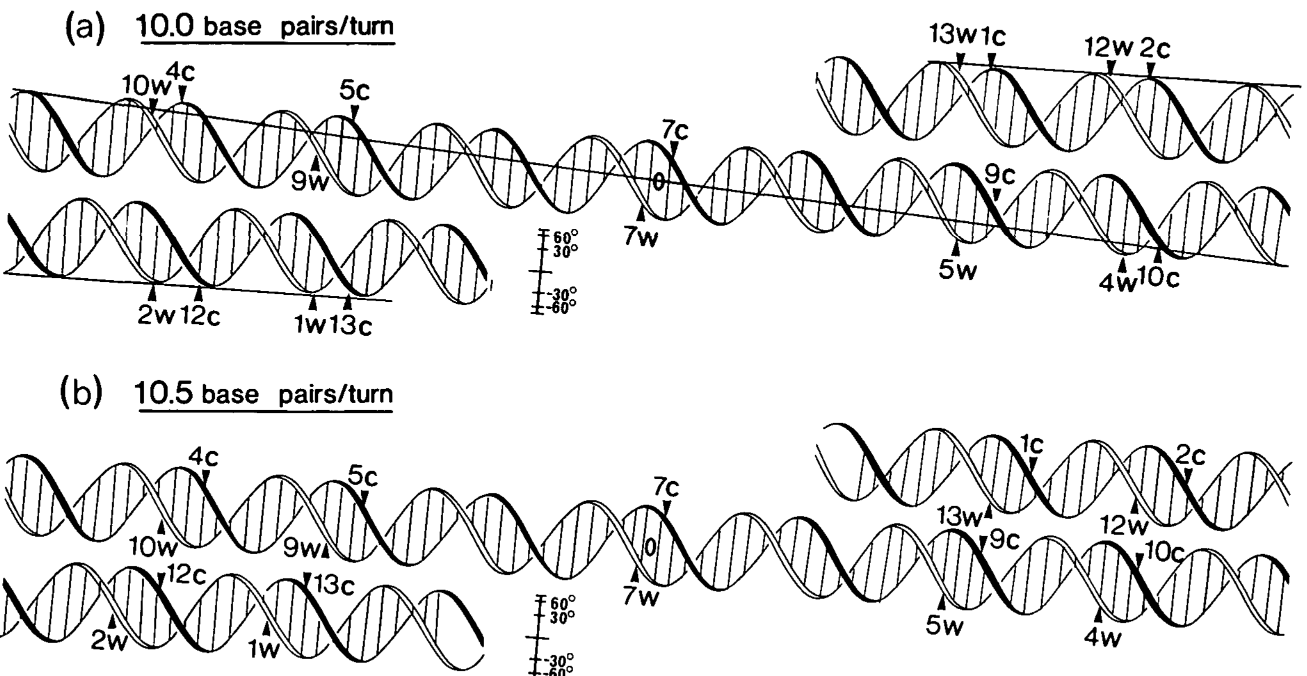

Fig. 2: Location of DNAse I cutting sites on the DNA superhelix plotted for double helical periodicities of 10.0 and 10.5 base pairs per turn respectively (Table 1). The superhelix is represented as a ribbon on a cylindrical surface which has been slit along a line parallel to the axis, then opened out and laid flat. The thin straight line in the top diagram represents the average angle of attack by the enzyme, being drawn through the midpoints of neighbouring pairs of cutting sites on opposite strands. (The dimensions of the double helix are not drawn to scale, but angular positions are maintained; the two strands are denoted C and W respectively and are related by the overall dyad.)

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The reason for this restricted access lies in the large size of the enzyme compared with the dimensions of the DNA superhelix. The limited angular range of attack of an enzyme the size of DNAse I (31,000 daltons) - and DNAse II which gives similar results is even larger (38,000 daltons) - is brought out in Fig. 3a, which shows schematically the accessibility of an enzyme of diameter 30 Å to the DNA of the nucleosome. We do not know the exact shape of the histone core but it is clear that the direction of attack will be towards the upper or lower surfaces of the nucleosome and the crevice between the superhelical turns will not be accessible (unless, of course, the nucleosome were to open up, for which there is no evidence under the conditions of the nuclease digestion experiments).

The theoretical diagram in Fig. 3a can be compared with diagrams of the actual range of accessibility found from the nuclease digestion results for the two cases of a 10 fold and 10.5 fold helical periodicity (Figs. 4a and 4b). In these diagrams the angular positions of the cleavage sites (Table 1) are marked without regard to their location along the superhelix, and so they summarise the angles of attack by the enzyme. It will be seen at once that whereas a 10 fold helix is compatible with the steric limitations, a 10.5 fold helix would require the enzyme to have access to the narrow crevice between the two superhelical turns of DNA, which, as Fig. 3a shows, is highly implausible for an enzyme as large as 30 Å in diameter. Moreover, the range of angles of attack calculated for the model in Fig. 4a compares well with the expectations from the necessarily simplified situation drawn in Fig. 3a.

THE DISTINCTION BETWEEN NUCLEASE CUTTING PERIODICITY AND STRUCTURAL PERIODICITY

It might be helpful to look at the analysis of the last section in a somewhat different way, and to see how the results on the three dimensional mapping of the cutting sites follow from the raw data. If the linear or one-dimensional locations of the DNAse I or DNAse II cleavage sites, i.e. the raw data (Table 1, column 2) are plotted against site number, the distances being expressed as excess in number of bases over 10.0 x n, where n is the site number starting at the 5' end, then a plot like that of Fig. 5 is obtained (ref. 8, Fig. 3 and ref. 9, Fig. 4). The plot has been idealised to bring out the main trends in the periodicity of the DNAse I and DNAse II sites. First, although the average spacing between cutting sites (i.e. between sites 0 and 14) is about 10.4 bases per turn, there are significant deviations from this average, as pointed out earlier (8), corresponding to changes in the

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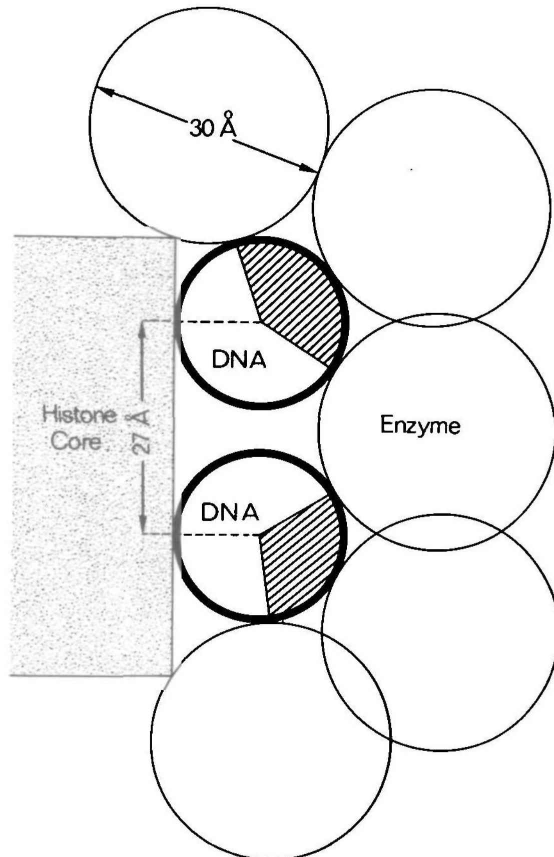
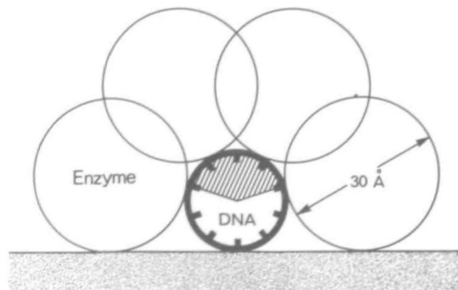
Fig. 3



a)

Schematic drawing (to scale) of the accessibility of an enzyme of diameter 30 Å to two superhelical turns of DNA wound on a histone core. A section through the DNA superhelix of pitch 27 Å is shown. The proximity of the two turns of DNA and the presence of the backing surface of histone restricts the angular access of the enzyme to the regions shown hatched; in particular the cleft between the two superhelical turns is inaccessible.



b)

The case of an isolated segment of DNA bound to a flat surface (from ref. 6).

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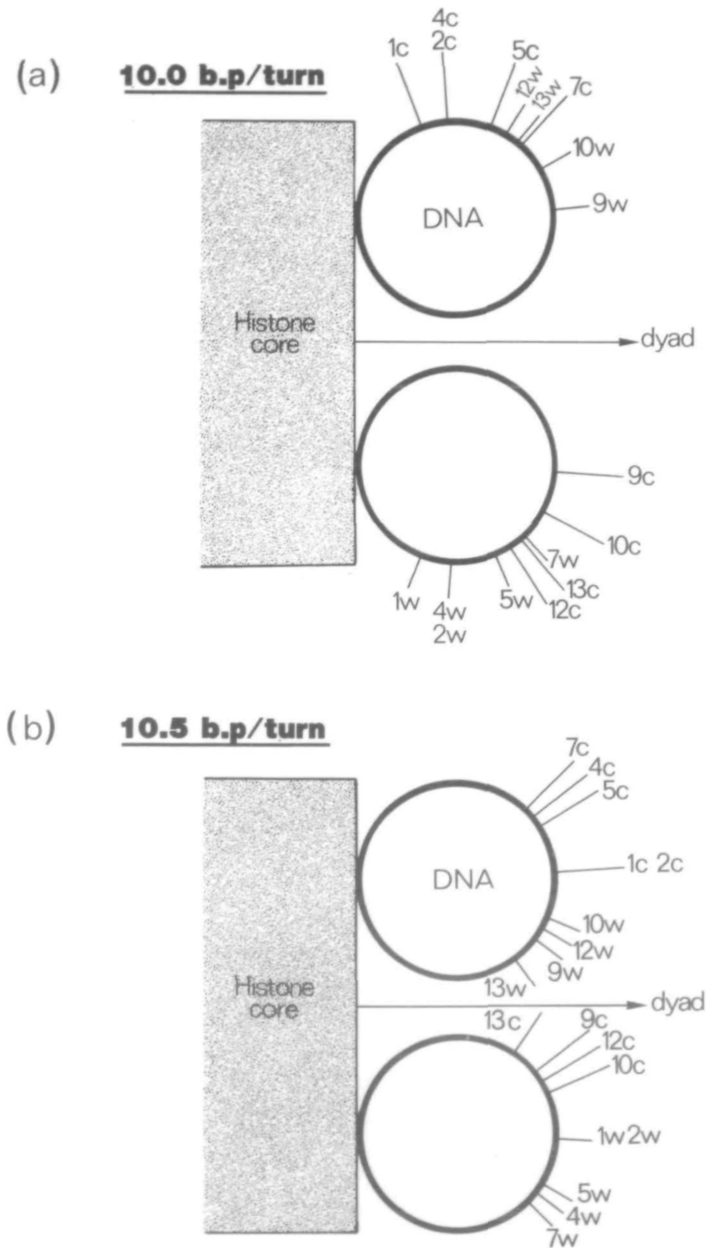

Fig. 4: Angular dispositions of DNAse I cutting sites calculated for DNA double helical periodicities of 10.0 and 10.5 base pairs per turn respectively (Table 1). These diagrams represent a projection of the sites in Fig. 2 along the superhelical direction on to a plane containing the superhelix axis and the dyad of the nucleosome core.

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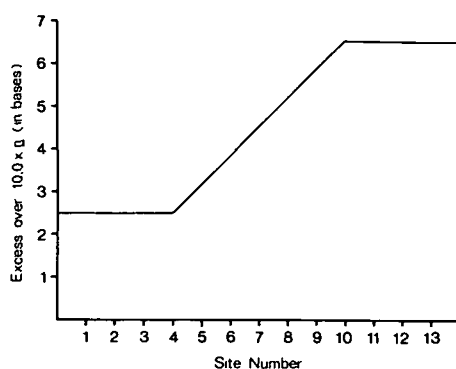

Fig. 5: Idealised plot of cutting site locations (measured from the 5' end of a DNA strand), embodying the results for DNAse I (8) and DNAse II (9).

spacing between sites as one goes from site to site along the DNA. However, the main feature of the plot, and this is what is emphasized in Fig. 5, is that it is relatively flat from S1 to S4 (corresponding to a periodicity of ∿ 10.0), followed by a straight steep section from S4 to S10 (with a periodicity of ∿ 10.6) followed by another flat section between S10 and S14. This behaviour is also found in the case of nucleosome cores reconstituted from poly (dA-dT), for which the spacing of DNAse I cuts is higher (∿ 10.7) near the middle of the DNA than at the ends (∿ 10.2) (ref. 13). The variation of digestion periodicity is therefore not a property of the enzyme, since it is found with both DNAse I and DNAse II, nor of the type of DNA, since it is found with both random sequence DNA and a simple double helical polynucleotide.

The kind of variation of cutting periodicity found in Fig. 5, with a steeper slope in the middle of the plot than at the ends, is what would be expected if the angle of attack of the enzyme changes as it moves, so to speak, from the midpoint of the DNA, towards the two ends of the superhelix. Thus at the midpoint of the DNA where there is an overall dyad and the environment is symmetrical "top" and "bottom", the average angle of attack (i.e. average between $S7_C$ and $S7_W$) must be along the dyad (Fig. 1), i.e. at 90° to the superhelix axis. But from sites S8 and S6 outwards to the two ends of the DNA there is steric hindrance from the neighbouring turn of DNA and there is no reason for the enzyme to maintain this angle of attack. If the actual structural periodicity of the double helix is 10.0 base pairs per turn, and the enzyme cleavage periodicity over the middle of the DNA is 10.7, then the

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cleavage sites move faster than the turning of the double helix, and so move towards either the upper or lower surfaces of the nucleosome, i.e. away from the neighbouring superhelical turn of DNA. But, nearer to the ends of the DNA, where the histone core is recessed (11), the cutting periodicity is shorter and follows the structural periodicity at a rate of about 10.0 base pairs; as represented by the thin line drawn in Fig. 2a. This line with its two sections of different slope is, as it were, an image in three dimensions of the plot in Fig. 5. Conversely, if the structural periodicity of the double helix were 10.5 base pairs per turn, then the cleavage sites in the central stretch from S4 to S10 move at about the same rate, and when this rate slows down, as from S4 to S0 and from S10 to S14, the sites would move in towards the neighbouring superhelical turn of DNA, a most unlikely eventuality as we have seen.

Now, of course, one could take the nuclease cutting periodicity literally, and identify it with the structural periodicity. On this interpretation, if one were to plot the cutting sites in three dimensions, beginning at the midpoint of the DNA, ie. at sites $S7_C$ and $S7_W$, as we have done above, then all sites on the C strand would be attacked at the same angle as $S7_C$, and similarly for the W strand. The average angle of attack would then be exactly perpendicular to the superhelix axis. Now this is what would be expected from a structure containing many superhelical turns of DNA, all of which are equivalent (except for the two turns at the ends), but there is no reason why this should be true for a nucleosome with only two superhelical turns, where, so to speak, there are only end-effects. In this case the lower surface of the lower turn and the upper surface of the upper turn are also exposed to enzyme digestion, and the attack should occur on the average at angles away from the perpendicular, towards the upper and lower surfaces of the nucleosome, indeed just the sort of behaviour we find in the model in Fig. 2a.

As a minor variation of the argument that the nuclease cutting periodicity precisely mirrors the structural periodicity, one could argue that the behaviour represented schematically in Fig. 5, means that the helical periodicity is, say, 10.7 base pairs per turn in the middle region of the DNA, over a stretch of 50 or 60 base pairs, while it is closer to 10.0 over the end regions. One can calculate the consequences of this model on the angular positions of the cutting sites, just as shown in Table 1 and Fig. 2, for the simpler models. While the result is an improvement on Fig. 2b, what one finds is a spread of cutting sites centred on the perpendicular to the superhelix axis, but still reaching into the narrow cleft between the superhelical

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turns. For these two reasons we consider this model implausible.

IS THERE A LINKAGE NUMBER PROBLEM?

The argument we have used to resolve the linkage number problem depends on different lines of evidence, namely (a) the number of superhelical turns on the nucleosome, which is now hardly in doubt, (b) the screw of DNA off and on the nucleosome, which is the subject of this paper, (c) the value of ΔL, the change in linkage number when DNA passes from solution on to the nucleosome, and (d) the path and screw of the linker DNA, which is about 30 bases long, taking the complete two-turn nucleosome as containing 166 base pairs. In this section, we discuss the evidence on these last two points, and related matters.

The largest uncertainty in the data is in the precise value of ΔL which comes from measurements on the SV40 minichromosome (2), but it is clear that its magnitude is not far from -1, the negative sign denoting a left handed DNA superhelix on the nucleosome. This is in the same sense as the helical ramp formed by the histone core (11). But a more overriding question is the relevance of these measurements.

It has recently been argued by Stein (14) that the results on SV40 cannot be carried over to cellular chromatin with which we are concerned. Stein has carried out experiments in which small amounts of histones H1 and H5 are added to SV40 DNA previously assembled to a small degree of supercoiling. He finds that H1 and H5 increase the supercoiling and therefore concludes that no firm value for the latter can be obtained. However, it is questionable whether Stein's experiments pertain to the isolated SV40 minichromosome which contains H1 naturally and which has the full number of supercoils, viz. $\sim$ 24 (since there are $\sim$ 24 nucleosomes, $|\Delta L| \sim 1$): Keller and his colleagues showed that such fully supercoiled SV40 DNA did not have its linking number changed by adding H1 (15).

The question, however, must be faced whether even this data of Keller's is relevant to cellular chromatin, in other words, is the structure of SV40 chromatin the same to that of cellular chromatin? Two lines of evidence point to a strong similarity. First the behaviour of the two kinds of chromatin when digested with micrococcal nuclease is very similar right down to the level of mononucleosome. Thus Varshavsky and his colleagues (16) have found a pause in the digestion by micrococcal nuclease of H1-containing minichromosomes at the "160 base pair" stage, leading to a high proportion of so called MN2 mononucleosome particles, indistinguishable in proportion and behaviour

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from their cellular counterparts. This pause is dependent in cellular chromatin on the presence of H1 (16,17). Secondly, electron micrographs of SV40 chromatin (18,19) show the same kind of zigzag structure which has been found in systematic electron microscope studies on cellular chromatin, and which has been attributed to the presence of H1 on the side of the nucleosome at the entry and exit points of the DNA (20). We therefore have good reason to believe that the path of DNA on the nucleosome and its mode of stabilisation is the same for both SV40 and cellular chromatin. The supercoiling results can therefore, in our opinion, be safely carried over to the common nucleosome structure possessed by both SV40 and cellular chromatin. The only difference which would affect our interpretation is that the ∿ 30 base pair linker between nucleosomes might take different paths in SV40 chromatin and in cellular chromatin (where indeed this path changes with ionic strength (20)). But since this affects only ∿ 30 out of ∿ 200 base pairs, and since the periodicity of the linker DNA is unlikely to be grossly different from that either of free DNA or DNA on the nucleosome, it could only make a small contribution to our calculations on the relationship between ΔL and the periodicity of DNA in solution and on the nucleosome.

An alternative view to ours to explain the apparent conflict of crystallographic results on the nucleosome core (2 superhelical turns of DNA) and the physico-chemical results on chromatin ($|\Delta L| \sim 1$) is that the screw of the DNA is unchanged but that the path of the DNA on the complete nucleosome departs from a simple superhelix. Thus Crick (3) has shown that if the bulk of the DNA is wound in a superhelix on the nucleosome, but crosses over itself at the points of entry and exit, then the linkage number will be reduced by 2. Worcel and his colleagues (21) have produced a model in which all nucleosomes contain 2 superhelical turns, but alternate nucleosomes have their DNA crossing over at the entry and exit points, so reducing the linkage number for this path to zero, while the "normal" nucleosomes have a ΔL of -2. The average ΔL is then -1. This model is topologically sound but assumes that the periodicity of the DNA is not changed in passing from solution on to the nucleosome. If, as we maintain, it does, then the model is structurally invalid.

Another argument that the screw of DNA on the nucleosome is unchanged from (or very close to) its value in solution has been presented by Trifonov and Bettecken (22). This is based on the fact that certain potential enzyme cleavage sites are not cut by DNAse I (10, 23, 24). We have interpreted these as arising from protection by the histones (1, 10) but Trifonov

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and Bettecken ascribe the effect to a type of beating effect caused by the changing orientation of phosphodiester bonds when the periodicity of chromatin DNA has a non-integral value (10.33 - 10.4). This explanation requires that the enzyme be only able to attack the DNA through a narrow window, and hence over a very limited angle of attack.

Moreover, Trifonov and Bettecken do not consider all the nuclease cutting data and its quantitative aspects. Their claim is that the "modulation of nuclease sensitivity can be explained solely on the basis of orientational protection". If this were so, their reasoning should apply not only to the maximally protected sites, but also to the maximally exposed sites, which on their argument should lie towards the middle of their postulated window of accessibility (their Fig. 2). But this is not what the data show. The sites frequently cut e.g. S1 and S5, lie right on the edge of the window, whereas S7, which is cut only infrequently, lies right in the centre of the window.

It might be pertinent, in this connection, to make some remarks on the width of the window of attack by the nuclease at a particular site. The range of cutting at a site on the nucleosome extends over 3 to 4 bases (8), a value close to that found by Rhodes and Klug (6) for DNA bound to a flat surface. In the latter case the geometry of the hindering surface is known - it is a plane, so that the DNA is not buried (Fig. 3b). Moreover, Rhodes and Klug have shown that the effect of a non-integral pitch for the DNA is to produce a small modulation in the distribution of the frequency of cutting at different sites. This orientational effect is quite unlike the results on the nucleosome, where there are very large differences in frequency, some sites being almost totally protected (10, 23, 24). There is no doubt that what is being observed on the nucleosome is a histone protection pattern of the DNA, as originally proposed by us (1, 10). Similar patterns which arise in experiments to locate proteins specifically bound to DNA have later come to be called "footprints" (25), though the technique dates back to experiments on the nucleosome core.

CONCLUSION

We have seen that the general trend of the locations of sites of cleavage by both DNAse I and DNAse II of DNA on the nucleosome can be explained by a model in which the average helical periodicity of DNA on the nucleosome is 10-fold rather than 10.5-fold. The basic feature of the model is that the lower surface of the lower superhelical turn of DNA is more exposed to enzyme

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attack than is its upper surface, where it is shielded by the presence of the neighbouring superhelical turn, and mutatis mutandis for the upper superhelical turn (Figs. 3a and 4a).

This model with a 10-fold periodicity for the DNA accounts for the general pattern of enzyme cutting. For simplicity we have assumed a constant periodicity along the path of the DNA, but it might be expected that there will be local variations brought about by interactions with different patches of histones. One might also expect local deviations from the simple pattern of enzyme accessibility drawn in Fig. 3a, arising from special features of the histone surface. For these reasons and since we do not know the exact shapes of the enzymes nor their manner of binding and cutting, we cannot deduce the average helix periodicity precisely, but we can say it is much more likely to be closer to 10.0 than to 10.5. A value of 10.1 is not ruled out, but 10.2 and 10.3 become unfavourable.

However, while we are not able to deduce the periodicity exactly, there is a good structural reason why it might be close to 10.0. We pointed out earlier (1) that an integral number of base pairs per turn of the double helix would mean that the phosphate groups on the two adjacent superhelical turns keep in phase. Since the distance between the turns is only 27 Å, this is an ideal situation for repeated interactions between the phosphate groups (which come as close as 5-6 Å apart) to be stabilised by counterions. A periodicity of 10 base pairs per turn thus permits the same stabilising interactions between adjacent superhelix turns to occur repeatedly along the chain, just as it does between adjacent molecules in fibres of B DNA (26). Yet no matter how persuasive this argument, the matter will perhaps only be settled when the X-ray analysis of crystals of nucleosome cores now in progress in our laboratory reaches higher resolution. All we can say from the present X-ray data is that the DNA is of the B-form with a spacing between bases of 3.4 Å and an approximately 10-fold periodicity (27).

Finally, it will be realised that the order in our original argument (1) on the linkage number problem can be reversed. Thus if the periodicity of DNA in solution is now taken to be established as 10.4 (5) or 10.6 (6) base pairs per turn, and if the observed change in linkage number ΔL on wrapping DNA into two superhelical turns on the nucleosome is only about -1, then the screw of DNA on the nucleosome can be calculated to be close to 10 base pairs per turn. This is the same as we have concluded from the analysis which considers the locations of the nuclease cutting sites in three dimensions.

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