Chromatin structures: dissecting their mixed patterns in nuclease digests

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Received March 20, 1987; Revised and Accepted August 29, 1987

ABSTRACT

Four separate features could be distinguished in Fe-DNAase-I digestions of human lymphoblast nuclei: a di-nucleosomal (2N) repeat, a mono-nucleosomal (1N) repeat, a component of "random" DNA, and triple splitting of major peaks. The random component is major, is unlikely to be completely artifactual, and is what would be expected from the face to face layering model of Subirana et. al.,(1). The 2N pattern appeared to be associated with compact, metaphase-type chromatin, whereas the 1N pattern was associated with more exposed chromatin. These two modes are explained in terms of orderly back-to-back folding of zig-zag nucleofilaments, and face-to-face folding respectively. Hybridization studies indicated that the centromeric classes of repetitive DNA had the same digestion spectra as the major interspersed classes of repetitive DNA, and DNA enriched in transcriptionally active sequences.

It is suggested that current coil models are all inadequate explanations of higher order chromatin packing.

INTRODUCTION

The problems of condensing DNA into a nucleus are still confused. There is a consensus of opinion that DNA is associated with histone molecules and wound into nucleosomes as the first order of packing (reviewed in 2). It is also reasonably well accepted that nucleosomes are commonly arranged into a fibre with a zig-zag pattern (eg. 3-5). The next level of chromatin condensation is subject to dispute (6,8). A number of models have been proposed: an interdigitised foldback of the nucleosome zig-zag (7,8); a "regular distorted helix" of planar orientated zig-zag monofilaments (1); a solenoid coil (3,9,10); a dis-orderly worm-like coil (11); a double helical crossed linker coil (12); and a coiled ribbon (5,18). A "superbead" structure was proposed by Renz et.al.,(19), although this has been largely discounted (13-17).

Coil models dominate most current concepts of chromatin higher order structures. These models do appear, however, to have serious non-compliances with the experimental evidence that is concerned with how chromatin structures breakdown under nuclease attack.

Helical models with the bridge crossing the axis: The left handed double helical model of Williams et.al.,(12) maintains the disomal substructure and rotates it along a helix. Assymmetric exposure of such a helix, that is one-sided exposure of the structure, would generate disomes, tetrasomes and possibly hexasomes depending on helix details, but after the hexasome further rotational exposure disrupts the series. Coherence of the series is lost by one half revolution of exposure, or earlier depending on the angle of exposure. Maximally, three disomes are obtained after which, further exposure causes the coherence of the disomal series to be destroyed. In contrast to this, the disomal series have been experimentally observed to extend to at least 10N in different experimental material in different laboratories (13,38). This renders the double helical model unlikely without supplementary explanations of some type.

In addition, but less critically as it is negative evidence, assymmetric exposure of such a helix should generate another series of very large fragments that would reflect the turn of the helix. Such a "coil signature" series has not been observed in DNA fragmentation patterns.

The earlier models of coils with bridges that cross the axis, as outlined by Staynov (14), have essentially the same problems. In particular, it is the loss of the disomal series coherence due to one-sided exposure of the coil which quickly fails to give a consistent one sided exposure of the "zig-zag" nucleofilament. The coil rapidly rotates both sides of the nucleofilament to its currently exposed side.

Coiled ribbons: The coiled ribbon model of Woodcock et.al.,(5) as displayed in their figure 8, and that of Worcel et.al.,(15) appear not to generate a disomal exposure even though they are based on a disomal zig-zag. This is due to the chosen topography of the bridges which appears to remove the alternating difference between the exposure of the nucleosomes. It is noteworthy that coiled ribbons with a different topography that alternately exposes one bridge to the exterior and the other to the interior of a coil, such as the form that gives a change of 1.5 in the linkage number per nucleotide (see reference 15, figure 5), would give these coiled ribbons a potential disomal exposure and would also allow the generation of long coherent series of disomes. However, despite this possibility this alternating bridge-exposure model has not been generally acceptable. This is partly because the linkage number implied appears to be incorrect (see Worcel et. al.,(15) for linkage number and its topographical implications), and also because the coiled ribbon models fail to vary their width with

bridge length (12) and they predict that longitudinal spacing would be dependent on bridge length and this has not been observed (14).

The coil of McGee et.al.,(16): This is one of the earliest coil models and it could also generate a disomal exposure, and in a coherent series. However, this model can be faulted on other features. The assembly of its structure appears to require a very large distortion from the nucleosome and bridge conformation described by Klug et. al. (45), and it seems incompatible with shorter linker lengths.

The solenoid: The solenoid of Finch and Klug (9) as supported by Widom and Klug (10) makes no obvious case for a disomal repeat, either short or long, although Butler (25) has suggested that some subtle folding of the bridge DNA may create some disomal character.

The dis-orderly worm-like coil (11): The nucleosome and DNA linker positioning of this coil have yet to be presented, hence the characteristics of its breakdown cannot be determined. It does appear, however, to include a random or dis-ordered component, in a similar way to that of Subirana et.al.,(1).

Perhaps the most persuasive feature of coiling models is that they explain persistent reports of helical character in nucleofilament preparations. However, this is rarely very clear and in at least one case (5), helicity of two senses has been reported, and there has also been a spurious large scale helicity reported as a major artifact of nucleosome preparations (17). It may be that nucleosomes have a tendency to pack in pseudo-helices, whatever their actual connectivity.

A variety of techniques have been employed to analyse chromatin structure. For example nuclease digestions (7,13,38), electron microscopy (3,29), various types of scattering analyses (30), sedimentation (31), electric and linear flow dichroism (18,32,33) and birefringence (34). This paper reports further evidence derived from nuclease digestions of chromatin using DNAase-I attached to a bulky ballast of ferritin. The DNAase-I and Fe-DNAase-I molecules can freely access preparations of permeated whole cells (35-37), which allows chromatin to be digested in situ near to its native form, a feature which is not shared by all other techniques. DNAase-I has shown a capacity to reveal higher order structure in digestions of chromatin (7,38-39). Probing nuclei with DNAase-I based probes generates complex mixtures of fragments, as these mixtures reflect all the features of chromatin structure that determine exposure and susceptibility of the DNA to the probe.

DNAase-I digestions of intact chromatin from chicken erythrocytes (38,39), rat liver cells (7) and HeLa cells have all revealed a strong di-nucleosome feature. That is, a proportion of the heterochromatin in these cells is packed in such a manner as to yield DNA fragments in length multiples of two nucleosomes (about 400 bp). A chromatin packing model to explain this di-nucleosome feature has been proposed by Burgoyne and Skinner (7), and primarily consists of a linear, back-to-back stacking of the basic nucleosomal zig-zag. The resultant asymmetrical self-protection causes every second nucleosome in a nucleofilament to be highly susceptible to attack. The concept of alternating projections was supported by Staynov et. al., (27) who showed that simulated DNAase-I digestions of chromatin favoured a packing structure based on "alternative exposure of consecutive nucleosomes" rather than solenoid or coiled ribbon models.

There is also some evidence that chromatin structure is heterogeneous. In particular, groups of highly repetitive DNA may form specific structures in chromatin. The tandemly repeated human alphoid DNA for example, has been considered to be in distinct nucleosome phase frames (41,42).

This work presents material to outline the development of the di-nucleosomal pattern in Fe-DNAase-I digests of human chromatin and define its relationship to major classes of repetitive DNA, to provide evidence for the existence of other forms of self-protecting features of higher order structure besides the di-nucleosome effect, and to re-examine the models explaining nuclease digestion data.

Experimental Procedures

Preparation of the Fe-DNAase-I probe

The Fe-DNAase-I probe was prepared by glutaraldehyde conjugation as previously described (7). The ferritin attachment increases the effective size of the DNAase-I molecule without obviously altering the active centre of the enzyme. Concentrations of Fe-DNAase-I equivalent to about 5-10 ug/ml free DNAase-I were used in all digestions.

Cells, cell permeabilisation and chromatin digestion

Human lymphoblasts (cell line C5-ABR) were grown to log phase, collected by centrifugation and maintained on ice. The pellet was washed twice with phosphate buffered saline (PBS) with phenyl methyl sulphonyl fluoride (PMSF) and 2-mercaptoethanol (BME). The pellet was suspended in HEPES-A (60 mM KCl, 15 mM NaOH, 15 mM HEPES-acid, 0.15 mM Spermine, 0.5 mM Spermidine) with 0.1 mM EDTA, 0.05 mM EGTA, 0.4 mM MgCl₂, 15 mM BME, 40 ug/ml PMSF, and 0.1% W/V bovine serum albumen (BSA). The cells were centrifuged (1200 rpm), then

agitated on ice for four minutes in the permialising solution (HEPES-A with 0.1mM EDTA, 0.05 mM EGTA, 0.4 mM MgCl_2 , 0.5% W/V BSA and 0.05% Triton x-100). After centrifugation the pellet was washed twice with the first suspension solution minus PMSF.

The pellet was taken up in 1.0 ml of buffered Fe-DNAase-I, 10 ul Ca-Mg (500 mM Mg^{++} , Ca^{++} to 1.25mM and 1.0 ul BME), then incubated at 37°C in a shaking bath for the required time. Preliminary experiments have shown Fe-DNAase-1 digests to plateau out, producing a limit digestion time beyond which there is little release of acid soluble DNA. There are changes in the features of the digestion pattern upto about 120 minutes, and thereafter the pattern remains essentially static. These characteristics of Fe-DNAase-1 digestion are visible in figure (1), where there is little further loss of high molecular weight DNA over the 60 to 150 minute digests. The digestion was halted with HEPES-A containing 0.1mM EDTA, centrifuged and washed once. DNA was prepared by standard procedures.

Time course Fe-DNAase-1 digestion of free cycling lymphoblasts.

Cells were prepared and digested with Fe-DNAase-1 as above. At times 15, 30, 60, 90 and 150 minutes , 0.2ml aliquots were taken, and DNA prepared. The DNA was run on 1.0% agarose gel systems in 1x TAE buffer (10mM tris, 5mM sodium acetate, 0.5mM EDTA, pH 7.8) with ethidium bromide. The gels were photographed under UV light with a 4x5 inch land camera. Mono-nucleosomal standards were produced by micrococcal nuclease (MN) digestion of permialised lymphoblasts (100U MN, 2.0 mM CaCl_2 , 5 minutes digestion at 37°C). Lambda standards (Eco-R1/Hind III cut: Boehringer) were also included in each gel run.

DNA was also end-labelled with 32P-dATP and 32P-dCTP by extension with polymerase-1 (Klenow fragment). Labelled digests were then visualised by either direct autoradiography of dried agarose gels, or by autoradiography of the DNA on nylon membranes after Southern blotting of the gels. Autoradiographs and photographic negatives were scanned with an LKB laser densitometer.

Fe-DNAase-I digestion of lymphoblasts blocked in mitosis

Lymphoblast cells were arrested during metaphase by two methods: (a). Cells were synchronized into S-phase using a double thymidine block, allowed to continue growth for a short period, then blocked in mitosis with colchicine. The cells were harvested and prepared for digestion as above, then digested with Fe-DNAase-1 to plateau (120 minutes). Approximately 95% of the cells were in mitosis. (b). Cells were blocked in metaphase with

Figure 1. Time course Fe-DNAase-I digests of free cycling human lymphoblasts run on agarose gels, ethidium bromide stained, photographed, and the negatives scanned with a laser densitometer. lane 1.- 15 minute digest: lane 2.- 30 minute digest: lane 3.- 60 minute digest: lane 4.- 90 minute digest: lane 5.- 150 minute digest: lane 6.- lambda DNA cut with Eco RI and Hind III: lane 7.- micrococcal nuclease digest of human genomic DNA producing a mono-nucleosomal (1N) series.

colchicine without any prior synchronization. This produced a cell culture with a mitotic index of about 20%. Free cycling cell cultures had a mitotic index of around 7%.

Hybridization of Fe-DNAase-I digests with repetitive DNA.

The Fe-DNAase-I and micrococcal nuclease digests of free cycling or mitotically blocked lymphoblasts were run on agarose gels, photographed, then Southern blotted to nylon membranes (Zetaprobe, Biorad).

A sample of human DNA enriched in repetitive sequences was cloned into M13-mp8, and the individual clones sequenced and identified against sequence data banks. Sequences of the interspersed repeat ALU, and the tandem, centromeric repeat satellite 111 were labelled with 32P-ATP by reverse hybridization primer extension on the M13 template, and hybridized to the nuclease digests of lymphoblast DNA on the nylon membranes. A probe was also made from DNA enriched in transcriptionally active DNA using the method of Kumar and Leffak (44), which was nick translated with 32P and hybridized to the nuclease cut lymphoblast DNA. The ALU repeats present in this "active" DNA fraction were blocked from hybridizing by the addition of excess ALU sequences to the hybridization buffer. The membranes were washed to high stringency after hybridization, then autoradiographed.

RESULTS

Resolution of nuclease digestion patterns.

In contrast with earlier studies using closely similar techniques, the chromatin fragments from the cultured human lymphoblast cells were always much more polydisperse than, for example, avian nuclei (13,38). The elements of order were always visible, but were less abundant than the disorderly element. The amount of "background", non-N multiple length DNA was seen to vary consistently with the degree of chromatin condensation, thus the element of disorder seems to be a characteristic of the chromatin rather than a technical problem.

Ethidium stained 1% agarose gels gave the best definition and resolution of the digests over a range of experimental conditions, with in vitro 32P end-labelling providing good band resolution under other digestion conditions.

Time course Fe-DNAase-I digestion of free cycling lymphoblasts

The most prominent features of free cycling lymphoblast nuclei digested

Figure 2. Densitometry scans of Fe-DNAase-I digests of human lymphoblasts which were either free cycling or blocked in mitosis to varying degrees. lane 1.- micrococcal nuclease digest of human genomic DNA producing a standard mono-nucleosomal (1N) series: lane 2.- free cycling cells (7% mitotic): lane 3.- colchicine blocked cells (20% mitotic): lanes 4 and 5.- thymidine synchronised and colchicine blocked cells (>95% mitotic).

in situ with Fe-DNAase-I at time intervals from 15 minutes to 2.5 hours were: (a) the development of a dominant di-nucleosome (2N) banding pattern in nuclei extensively exposed to Fe-DNAase-I (Fig.1-lane 5: 150 minute digest; peaks at 1N,2N,4N,6N, and 8N). (b) contrasting with the presence of a mono-nucleosome (1N) pattern at short digestion times (Fig. 1-lane 1: 15 minute digest; peaks at 1N,5N,6N,7N,8N, and 9N). (c) and the presence of a

"background" of random length (non-N multiple) DNA. (d) and the splitting, or multiple peak feature at each major band (for examples see Fig.3)

The structure of the digestion patterns at 30, 60 and 90 minutes (Fig. 1-lanes 2,3 and 4) represents the transition between the 1N pattern of the most exposed heterochromatin, and the 2N pattern of the most protected heterochromatin in the 150 minute complete digest. This transition period is characterised by a complex structural "signature" which cannot be adequately analysed at the current resolutions of agarose gels.

In this Fe-DNAase-I digestion series of free cycling lymphoblasts, the progression through a 1N to a dominant 2N series suggests a progressive attack on different structural forms, with each structure type offering a different degree of exposure of the DNA to the nuclease. The most resistant higher order structure produced a dinucleosome DNA length pattern.

Fe-DNAase-I digestion of lymphoblasts blocked at mitosis

Free cycling lymphoblast cells were treated with either colchicine or thymidine plus colchicine to produce cell cultures with varying quantities of mitotic cells. Free cycling lymphoblasts contained 7% mitotic cells, colchicine blocked had 20% and cells in the thymidine plus colchicine cultures were greater than 95% mitotic. These three cultures were digested with Fe-DNAase-I, run on a 1.2% agarose gel, and photographed. The photographic negatives were scanned (Fig. 2).

This series of digests were characterised by the development of a strong 2N series when the mitotic index of the cell cultures was very high. The Fe-DNAase-I digest of free cycling lymphoblast chromatin produced an almost featureless pattern in this particular experiment (Fig. 2-lane 2). Under the same digestion conditions, the highly mitotic cell cultures gave a di-nucleosome pattern in which 1N, 2N, 4N, 6N, and 8N multiples were dominant (Fig. 2-lanes 4 and 5). However, when synchrony was poor, colchicine blocking produced a mono-nucleosomal pattern with peaks at 1N, 2N, 3N, 4N, 5N, 6N, 7N, 8N, and 9N (Fig. 2-lane 1) even though the mitotic index was normally higher than in ordinary cycling cells. In all digests a strong background of random length (or non-N length) DNA was always present.

Thus the strong di-nucleosome feature in human lymphoblasts is due to the presence of high levels of matured, condensed, metaphase-type chromatin.

Peak splitting of major bands.

The 1N and 2N peaks seen in the Fe-DNAase-I digests do not seem to be single entities. The most common observation is a series of two or three sub-peaks combining to make any N-multiple peak. Typical examples of sub-

Figure 3. Densitometry scans of Fe-DNAase-I digests of human lymphoblasts in which the triple-peak nature of the digest can be seen in some of the major N-multiple bands which have been highlighted by arrows. (A). autoradiogram of ^{32}P end labelled digest. (B) and (C). photographic negatives of ethidium bromide stained gels.

peaks interpreted as triples are the 2N peak in Fig. 3(a), the 1N peaks in Fig. 3(b), and the 2N and 4N peaks of Fig. 3(c). In numerous cases, gel resolution allowed isolation of only two major peaks at each multiple, with this definition falling even further away at high molecular weight multiples.

Figure 4. Densitometry scans of Fe-DNAase-1 digested lymphoblast DNA run on agarose gels, Southern blotted, and hybridized to either satellite-III, Alu-I or "active" DNA probes, and autoradiographed. (A). The ethidium bromide stained DNA of synchronised and mitotically blocked lymphoblast cells is compared to the same DNA track which has been probed, stripped, and reprobed sequentially with either satellite-III, Alu-I, or "active" DNA. (B). As for (A) using lymphoblast cells that have been blocked in mitosis but not synchronised. The ethidium bromide stained scan is labelled (B).

Lack of strong differential effects using DNA probes for major classes of repetitive DNA, and transcriptionally active DNA.

The hybridization patterns of all sequences used as probes on Fe-DNAase-1 digests of lymphoblast chromatin were essentially identical to the ethidium bromide stained patterns of the DNA in agarose gels (see figure 4(a) and (b)). There was some loss of resolution in the patterns from the gel to membrane DNA transfer, particularly in the lower molecular weight region, but there was no major alteration in the occurrence or position of major peaks. Thus the tandemly repeated satellite III sequences, the interspersed ALU repeats, and the human genomic DNA enriched in

transcriptionally active sequences appear to be loosely packaged in the same way as the bulk of the genome.

DISCUSSION

In this and other work, it has been demonstrated that DNAase-I either free or conjugated, produces a dinucleosome (2N) repeat series in classic condensed chromatin (7,38,39). The work presented here shows that with progressive Fe-DNAase-I digestion, the chromatin of free cycling human lymphoblasts transits through a 1N pattern in the most exposed chromatin, to a predominant 2N pattern with extensive digestion. This work is supported by the Fe-DNAase-I digestion of the mitotically blocked lymphoblasts, in which the emergence of the strong 2N pattern can be subjectively related to the proportion of cells in normal metaphase at the time of nuclease digestion. Thus, these results suggest that a basic feature of condensed chromatin is the structure identified by a 2N pattern with Fe-DNAase-I digestion, and that this structure is a component of the major folding unit during the 250-fold additional chromatin condensation that occurs during cell mitosis. However, it must also be recognised that a high proportion of the digests are always non-integral DNA lengths, and rather than these being dismissed as chromatin in many different structural arrangements, this "random" component may be due to a distinct structural feature, such as the face-to-face layering model of Subirana et. al.,(1). If the major classes of repetitive DNA, particularly those that are centromeric and tandemly repeated, do form distinctive structural types in chromatin, then these are

Figure 5. Two nucleofilaments back-to-back giving assymetric protection for nucleosomal DNA (signature = 2N). This is a schematic drawing without a direct scale.

Figure 6. Single nucleofilaments, and three filaments stacked face-to-face, in which all nucleosomal DNA is equally susceptible (signature = 1N). This is a schematic drawing without a direct scale.

not highlighted by Fe-DNAase-I digestion and bear no special relationship to the 2N feature.

The significance of the 2N feature.

2N DNAase-I susceptibility patterns have been observed in a variety of nuclei: for example, in chicken erythrocyte nuclei (38,39), in resting nuclei from rat liver (7), in colchicine blocked HeLa nuclei, in synchronised, colchicine blocked human lymphoblast nuclei (this work), and

Figure 7. Nucleosomes stacked both face-to-face and back-to-back (signature = 2N). This is a schematic drawing without a direct scale.

in extensively digested free cycling human lymphoblast nuclei (this work). This 2N pattern has already been attributed to be the "signature" of the linear, folded back, zig-zag mode of packing oligonucleosomes (7,8), and this still seems to be an adequate explanation. In this model one side of the zig-zag is constantly protected from DNAase-I attachment, which would produce a 2N digestion pattern (see Fig. 5). Various arrangements of this basic back-to-back fibre can be envisaged to account for higher levels of chromatin packing. The basic back-to-back fibre would have a predicted width of 30nm, the fibre size observed frequently by electron microscopy (eg ref. 3), and considered to be the first level of folding above the nucleosomal zig-zag filament. This back-to-back arrangement of the nucleosomal zig-zag is in agreement with electron microscopy studies which have described a double stranded 25nm chromatin fibre consisting of two fibres lying side by side (46-48).

The significance of the 1N feature.

Chromatin that is relatively resistant to the probe and still gives a mono-nucleosomal pattern, appears to have structures within it that limit the attack of the probe without causing asymmetry in the mode of attack on the nucleofilament. It seems likely that this reflects zig-zag nucleofilaments arranged either singly, or packed in long distance face-to-face layers allowing access of DNAase-I to both sides of the filament (Fig. 6). In principle, this is a structure closely related to the short distanced layered model of Subirana et. al.,(1), but differing in that the individual lengths of the layers are probably much greater. This type of structure was found in the most exposed regions of the bulk condensed chromatin, and may also exist in transcriptionally active chromatin.

A combination of the 1N and 2N packing structures could be envisaged (Fig. 7). Such arrangements would allow high degrees of chromatin condensation.

The significance of non-integral length DNA.

The occurrence of non-N length DNA in Fe-DNAase-I digestions of chromatin is satisfactorily explained by proposing the ubiquitous presence of the disorderly layered structure proposed by Subirana et. al.,(1). Trivial explanations of this disorder do not seem to be adequate because: (a). The 2N pattern is a reproducible feature that is correlated with the presence of a type of condensation. Therefore, the Fe-DNAase-I probe is capable of detecting structural order in chromatin when it exists, but the predominant, often entire state of interphase chromatin appears to display little such

order. (b). Combinations of 2N and 1N would not produce disorder. (c). Diverse localities with their own local structures are not a sufficient source of disorder, as DNA probes made from various repetitive DNA clones that might be expected to be "heterochromatic" have not resolved the disorder. (d). Variations in nucleosomal spacing during the cell cycle are insufficient to account for the disorder, as control digests with micrococcal nuclease give no indication of large variations in the spacing. Thus, the disorderly smears probably reflect structural reality, rather than having a trivial explanation.

The significance of peak splitting.

Splitting of the 1N and 2N multiple peaks is a feature generated by the DNAase-1 type attacks on DNA wound onto a semi-protected surface, which in chromatin, is almost exclusively the histone proteins of the nucleosome core. The availability of exposed DNA is governed in a way described by Lutter (43) which, together with the protection of linker DNA and "inner" nucleosome bound DNA by the zig-zag conformation of the nucleofilament, combine to allow a Fe-DNAase-1 "hit" frequency producing three major lengths. These peaks partially combine to produce one major band at each N-multiple. Staynov et al, 1983 (27) predicted a similar split peak pattern using simulated DNAase-1 cutting on a chromatin model of "alternating consecutive nucleosomes".

Coiling and solenoid theories of chromatin structure.

Inadequacies of the various coiled models of higher order chromatin structure have been criticised (Burgoyne, (8), and reviewed in the introduction of this paper. In our view a major source of irregularity in most coil models is the inability of the coils to be resolved into extended di-nucleosomeal patterns. Other recent work has also doubted regular coil model theories. For example, Subirana et. al.,(1) using electron microscopy found disorderly zig-zag layering rather than coiling in classical 30nm chromatin fibres, and Sen et. al.,(26) considered that the need to vary the nucleosomal tilt angle to accomodate linker DNA of varying lengths would rule out chromatin packing models using coils or solenoids.

The search for the coiled nucleofilament has led to a variety of models, none of which seem satisfactory. Moreover, these models are mainly aimed at explaining a hypothetical "ideal" case found in some sperm and other highly condensed nuclei. Even if these ideal cases exist in some highly condensed chromatins, one coil seems not to be satisfactory, and in the case of normal somatic chromatin, coils may be quite an inappropriate

way to view the presumptive mixture of structures in a nucleus.

Any model of basic chromatin structure should show a means by which extended di-nucleosomal ladders can arise from DNAase-I digestion. Models containing linear arrangements of the zig-zag nucleofilament currently satisfy this criterion; the solenoid or coiled models in their present state still do not appear to be able to do this.

ACKNOWLEDGEMENTS

This work was supported by the Australian Research Grants Scheme.

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