The EMBO Journal Vol. 2 No.8 pp.1367 – 1372, 1983

Higher-order structure of nucleosome oligomers from short-repeat chromatin

Edwin C. Pearson, P. Jonathan G. Butler[1] and Jean O. Thomas*

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, [1]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Communicated by J.O. Thomas
Received on 2 May 1983; revised on 6 June 1983

Sedimentation measurements and electron microscopy at a series of ionic strengths suggest that chromatin from neurons of the cerebral cortex is able to form condensed structures *in vitro* that are probably several turns of a solenoid with about six nucleosomes per turn. Since neuronal chromatin has a short nucleosomal repeat (~165 bp) allowing virtually no linker DNA between nucleosomes, and yet forms apparently 'normal' elements of solenoid, the packing of nucleosomes in the solenoid must be highly constrained. This permits only a limited number of possible models, and enables tentative suggestions to be made about the location of the linker DNA in the typical solenoid.

Key words: short-repeat chromatin/cortical neurons/chromatin folding

Introduction

The nucleosome filament condenses *in vitro* in response to increasing concentrations of mono- or divalent cations. Previous biochemical and biophysical studies of the folding process have been consistent with the formation of a relatively regular, helical structure 30 nm in diameter in the most compact state (Finch and Klug, 1976; Thoma *et al.*, 1979; Suau *et al.*, 1979; Butler and Thomas, 1980; Thomas and Butler, 1980; McGhee *et al.*, 1980; Bates *et al.*, 1981; Lee *et al.*, 1981). There has been relatively little support for discontinuous arrangements of nucleosomes in superbeads (Renz *et al.*, 1977; Strätling *et al.*, 1978). Although various suggestions have been made (Worcel and Benyajati, 1977; Thoma *et al.*, 1979; Suau *et al.*, 1979; McGhee *et al.*, 1980; Lee *et al.*, 1981; Yabuki *et al.*, 1982) the precise details of the solenoidal structure, in particular the path followed by linker DNA between nucleosomes and the location of histone H1, are not yet clear.

Both our previous hydrodynamic studies (Butler and Thomas, 1980; Thomas and Butler, 1980; Bates *et al.*, 1981) and the physical studies of others (Suau *et al.*, 1979; McGhee *et al.*, 1980; Lee *et al.*, 1981; Yabuki *et al.*, 1982) have been carried out on chromatins with repeat lengths of 200–215 bp. We have now investigated chromatin obtained from neurons of ox cerebral cortex; this has a short nucleosome repeat length of ~165 bp, as found earlier for rabbit neurons (Thomas and Thompson, 1977) and neurons from the mouse, guinea pig and rat (Brown, 1978, 1982; Ermini and Kuenzle, 1978; Jaeger and Kuenzle, 1982; Whatley *et al.*, 1981). The aim was to determine whether chromatin with a short nucleosomal DNA repeat length ('short repeat chromatin') can form stable higher-order structures similar to those of rat liver and chicken erythrocyte chromatins, since 165 bp is close to the minimum DNA content for a two-turn nucleosome structure (Thoma *et al.*, 1979) or chromatosome (Simpson, 1978) with 80 bp per turn, and there can be very little 'linker DNA' as such between adjacent nucleosomes. If short-repeat chromatin were able to form such higher-order structures, constraints would thereby be placed on the way in which the nucleosomes could be arranged in the solenoid. For example, any model that required long lengths of linker DNA between nucleosomes would be specifically excluded.

The first points to be established are whether or not short-repeat chromatin condenses by closing turns of six nucleosomes, and whether folding is continuous or discontinuous. Following our earlier approach (Butler and Thomas, 1980; Bates *et al.*, 1981) we have studied the NaCl-induced condensation of a series of fragments of increasing length, from mononucleosomes up to ~30 nucleosomes. Our results, presented here, identify a single abrupt structural transition in this fragment range. This is a change in behaviour above ionic strength 25 mM for hexanucleosomes, which is consistent with the formation of a unit of higher-order structure. The absence of any further abrupt transitions for larger fragments up to 30 mer is consistent with continuous helical folding into several turns of solenoid with about six nucleosomes per turn.

Results

Characterisation of short-repeat chromatin from cerebral cortex

Fractionation of ox cortical nuclei gave two populations, as previously described for rabbit (Thompson, 1973): N^1, which were largely neuronal nuclei, and N^2 which consisted almost entirely of glial nuclei. The N^1 nuclei were the source of chromatin with short nucleosomal DNA repeat length (Thomas and Thompson, 1977) which was generated by digestion with micrococcal nuclease.

All chromatin samples used for analytical ultracentrifugation or electron microscopy were routinely analysed for histone content in SDS-polyacrylamide gels (not shown) to check that there was no proteolysis. The H1 content of N^1 nuclei is low compared with that of other nuclei (D.L. Bates *et al.*, unpublished data; Greenwood *et al.*, 1981), whereas that of N^2 nuclei is normal, i.e., about one H1 per nucleosome (Bates and Thomas, 1981).

Figure 1a shows the DNA content of typical chromatin fragments from N^2 nuclei. The DNA repeat length of most samples to be analysed in the analytical ultracentrifuge was checked by redigestion with micrococcal nuclease; the redigestion pattern for one such sample at low ionic strength is shown in Figure 1b. The repeat length, determined as described earlier (Thomas and Thompson, 1977) was 162 ± 4 bp. In the case of very short chromatin fragments (<8 nucleosomes) which gave too few bands on redigestion for accurate determination of repeat length, the DNA of the intact fragment was identified by its co-migration in an agarose gel with an identifiable DNA band (e.g., trinucleosome, tetranucleosome) of a complete short-repeat 'ladder' of bands. The same short repeat length was found (data not shown) after redigestion of chromatin that had been frac-

*To whom reprint requests should be sent.

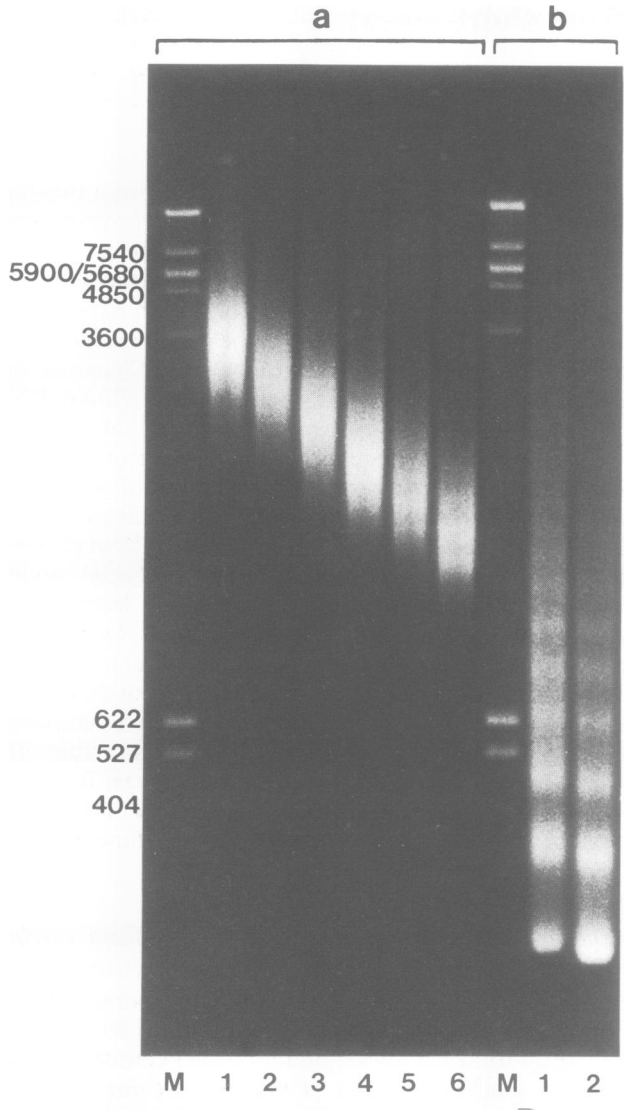

Fig. 1. DNA from N^1 chromatin analysed in a 1.25% (w/v) agarose gel. **(a)** DNA from nucleosome oligomers fractionated in a 5–50% (w/v) sucrose gradient; **tracks 1–6** correspond to adjacent gradient fractions. **(b)** DNA obtained by redigestion of the largest oligomer from **(a)**, for 4 min **(track 1)** or 8 min **(track 2)**. Digestion was carried out in 5 mM triethanolamine HCl, 1 mM Na_2EDTA, 0.25 mM PMSF, pH 7.5, as described in Materials and methods. Marker tracks (M) contain *Eco*RI restriction fragments of λ DNA and *Hpa*II fragments of pBR322. DNA sizes are in base pairs.

tionated in a sucrose gradient containing 60 mM NaCl but otherwise as described (see Materials and methods). This shows that ultracentrifugation at moderate ionic strength does not perturb the short repeat length of the samples, at least up to ~65 mM. [Moreover, the continuity of behaviour in the analytical ultracentrifuge (see below) shows that no novel structural change occurs above this ionic strength.] In contrast with the short repeat length obtained on redigestion of neuronal chromatin from N^1 nuclei, chromatin from glial (N^2) nuclei gave the expected DNA repeat length of ~200 bp (Thomas and Thompson, 1977) on redigestion (data not shown).

Only fragments up to ~30 nucleosomes long have been used in this study. Somewhat surprisingly, we found that larger fragments did not have the expected short repeat length on redigestion; these findings will be reported in greater detail elsewhere.

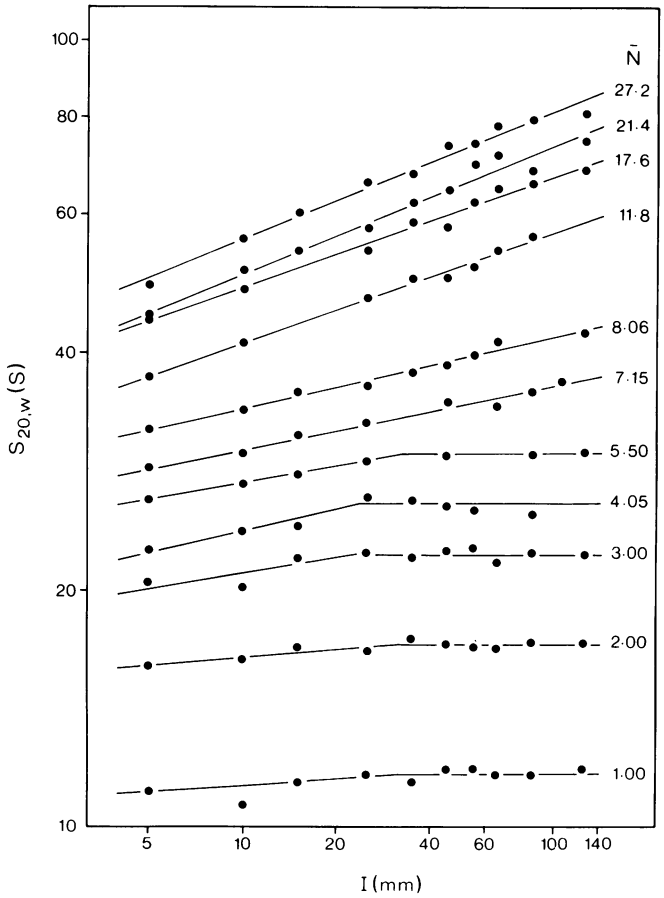

Fig. 2. Ionic strength dependence of the sedimentation coefficients of short-repeat nucleosome oligomers. $\bar{N}$ represents the weight-average size, expressed as the number of nucleosomes, of the oligomer preparation used for each sedimentation analysis.

Sedimentation measurements as a function of ionic strength

Figure 2 shows the ionic strength dependence of the sedimentation coefficients for a range of N^1 chromatin fragments of different weight average sizes, expressed as numbers of nucleosomes ($\bar{N}$). The linearity of the double logarithmic plots for oligomers >7 nucleosomes indicates a simple power-law relationship of the form $S \propto I^n$, as observed previously for chromatin from rat liver (Butler and Thomas, 1980; Thomas and Butler, 1980) and chicken erythrocytes (Bates *et al.*, 1981). Moreover, the gradients of the lines and the absolute sedimentation coefficients are very similar to those of oligomers of corresponding size prepared in an identical manner from rat liver nuclei (Butler and Thomas, 1980). (Thus the lower mass per nucleosome in short-repeat chromatin than in rat liver chromatin may be counteracted by closer juxtaposition of adjacent nucleosomes, leaving the sedimentation coefficient virtually unaltered.) There is a change in behaviour around $\bar{N} = 6$. For smaller chromatin oligomers the plots show a discontinuity at an ionic strength of ~25 mM: below this ionic strength the relationship $S \propto I^n$ holds true, while above this ionic strength there is no dependence of S upon I.

The transition in behaviour at six nucleosomes is also clearly demonstrated in double logarithmic plots of sedimentation coefficient *versus* nucleosome oligomer size (S *versus* $\bar{N}$) at two ionic strengths, one just below that at which the discontinuity occurs (25 mM) and the other above it (65 mM)

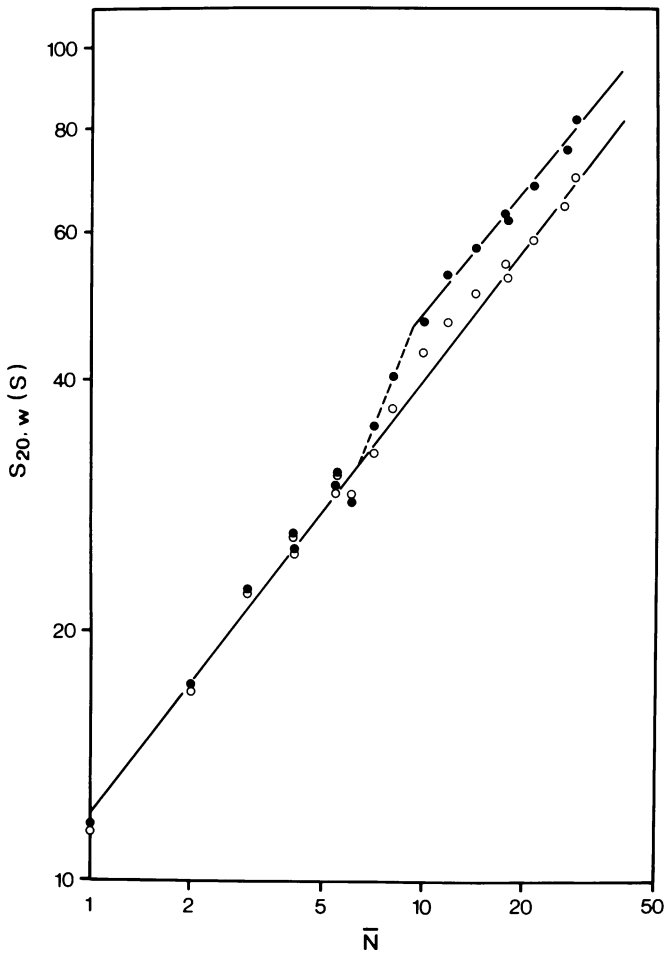

Fig. 3. Dependence of sedimentation coefficient upon size of short-repeat nucleosome oligomer. Sedimentation coefficient interpolated to ionic strength 25 mM (○) and 65 mM (•).

(Figure 3). For this short-repeat material the gradient of the 25 mM plot and the gradient of the 65 mM plot on either side of the discontinuity are very closely described by the relation $S \propto \bar{N}^{0.5}$, which is similar to that found for rat liver (Thomas and Butler, 1980).

The discontinuity for chromatin shorter than $\bar{N} \sim 6$ and its disappearance for chromatin larger than ~6 nucleosomes has been observed previously for both rat liver and chicken erythrocyte chromatins (Butler and Thomas, 1980; Bates *et al.*, 1981). It was taken to represent the formation of a stable unit of higher-order structure which could only be achieved by oligomers at least six nucleosomes long. The unit of higher order structure was taken to be one closed turn of a solenoid (Butler and Thomas, 1980). Our present results for neuronal chromatin therefore suggest that the number of nucleosomes required to form one turn of a solenoid is the same for neuronal chromatin of repeat length 162 bp as for rat liver chromatin and chicken erythrocyte chromatin, of repeat lengths 200 and 215 bp, respectively. In other words the size of a turn of solenoid appears to be independent of the length of linker DNA.

Electron microscopy

Chromatin samples at the various ionic strengths used for measurement of sedimentation coefficients were also fixed and examined by electron microscopy. Figure 4 shows chromatin of $\bar{N} = 22$ at ionic strengths 5 mM and 75 mM. At 5 mM the chromatin exists as a beaded nucleosome filament; the short lengths of DNA occasionally visible between nucleosomes must have been pulled off the nucleosome cores during sample preparation for microscopy, since short-repeat chromatin has little or no free linker DNA.

At 75 mM ionic strength the chromatin is clearly more condensed, although it is not immediately apparent whether it forms solenoids. The range of solenoid sizes that would be expected for this sample ($\bar{N} = 22$; range 14–42 nucleosomes) would be 2–7 turns, with corresponding solenoid dimensions 20–70 nm long and 25–30 nm wide (cf., Finch and Klug, 1976; Thoma *et al.*, 1979). Many of the structures do indeed appear in the micrographs as circular objects 30 nm in diameter, or as square objects or rectangular objects up to 70 nm long. We interpret these, respectively, as short 30 nm diameter solenoids standing on their ends or lying on their sides. Several such images are shown in Figure 5, the side-on views (Figure 5b) being mainly of the longer, more easily recognised, solenoids. The appearance of the electron micrographs thus supports the conclusion drawn from the sedimentation measurements that salt-induced condensation of short-repeat chromatin results in structures similar to those formed by fragments of comparable size from rat liver or chicken erythrocyte chromatins, with repeat lengths of 200 and 215 bp, respectively. Long fragments of these chromatins have been characterised as forming solenoids at high ionic strength (Finch and Klug, 1976; Thoma *et al.*, 1979; Suau *et al.*, 1979; Butler and Thomas, 1980; Thomas and Butler, 1980; McGhee *et al.*, 1980; Bates, *et al.*, 1981; Lee *et al.*, 1981).

Discussion

Two lines of evidence suggest that neuronal chromatin with a short nucleosome repeat length, which lacks any linker DNA in the usual sense, condenses *in vitro* in the same way as chromatins from rat liver and chicken erythrocytes which have linkers of 34 (i.e., 200–166) bp and 49 bp, respectively, between chromatosomes. First, the response of sedimentation coefficient to ionic strength for a range of fragment sizes up to 30-mers is virtually identical, both qualitatively and quantitatively, with that for fragments of similar size from rat liver chromatin. In particular, the discontinuity in behaviour around $\bar{N} = 6$ suggests that despite the much shorter repeat length in neuronal chromatin, the same number of nucleosomes is required to start the formation of stable higher-order structures. Secondly, the appearance of the condensed structures in electron micrographs is entirely consistent with that expected for short solenoids either standing on their ends or lying on their sides, given the inevitable heterogeneity in the fragment size in any sample.

The appearance of the fragments up to $\bar{N} = 30$ is very similar to that of similarly sized fragments from rat liver or chicken erythrocyte chromatin (not shown). In those cases more extensive microscopy has been carried out on much longer fragments (Thoma *et al.*, 1979; Bates *et al.*, 1981); in the case of neuronal chromatin this has not yet been possible because of the difficulties encountered in obtaining long lengths of short repeat chromatin.

In summary, we deduce that despite its short repeat length, neuronal chromatin, at least up to a weight-average size of ~30 nucleosomes, forms structures *in vitro* similar in general architecture to those formed by rat liver and chicken erythrocyte chromatins (although of course differing in the absence

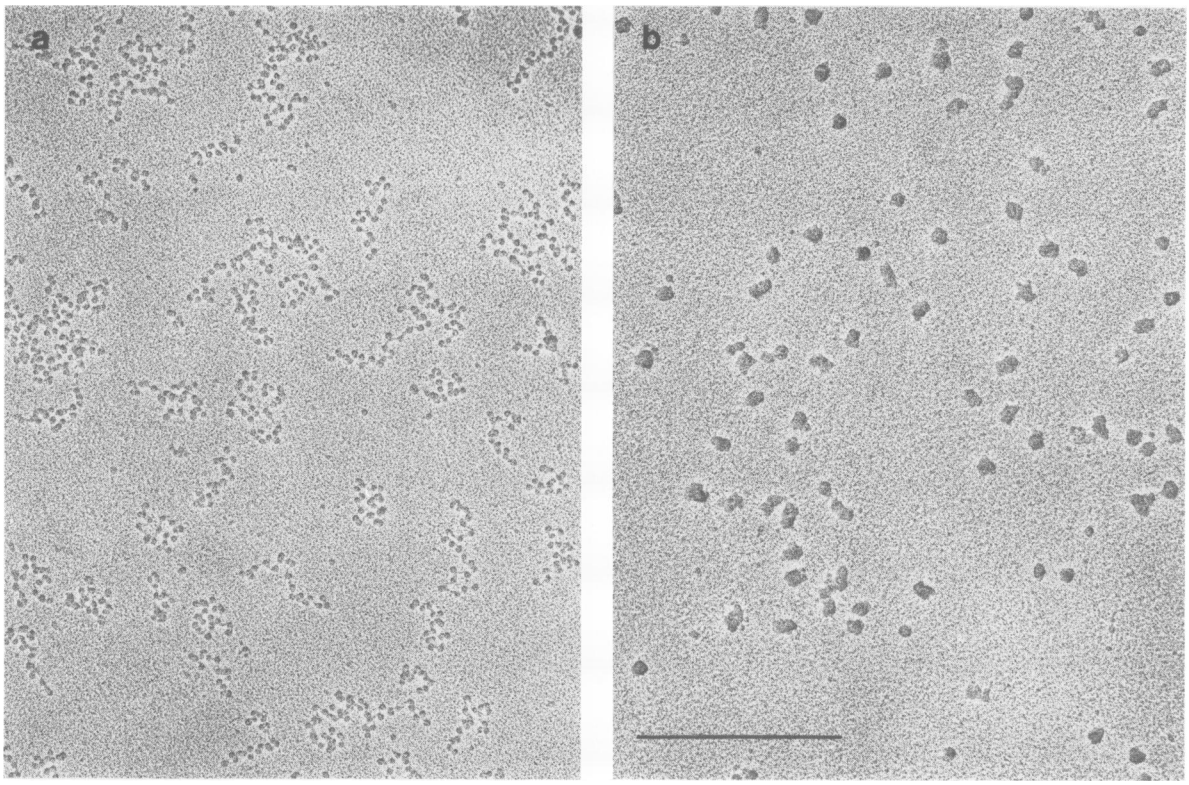

Fig. 4. Electron micrographs of short-repeat chromatin (N = 22; range 14–42 nucleosomes). The samples were fixed at ionic strengths of **(a)** 5 mM, and **(b)** 75 mM. The DNA of this sample is shown in Figure 1a (track 1). Scale bar = 500 nm.

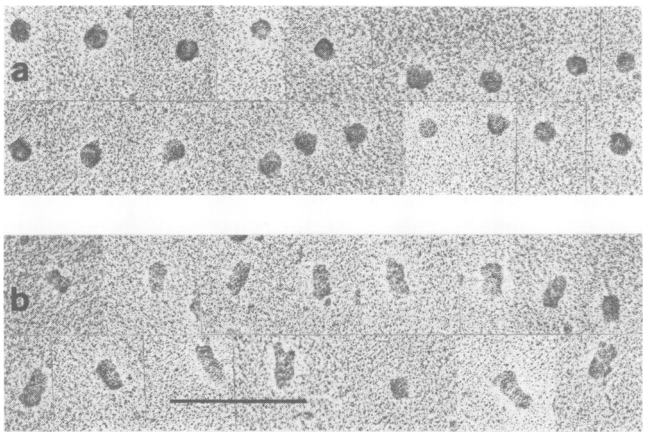

Fig. 5. Electron micrographs of condensed short-repeat chromatin (N = 22; range 14–42 nucleosomes) fixed at ionic strength 75 mm. Structures were selected to show **(a)** circular objects, taken to be short 30 nm diameter solenoids standing on their ends; and **(b)** square or rectangular objects, taken to be short 30 nm diameter solenoids lying on their sides. Scale bar = 250 nm.

of linker DNA). This is despite the low H1 content of nuclei from cortical neurons. Indeed, perhaps formation of stable higher-order structures with a low H1 content is possible only in the absence of linker DNA. It will be of particular interest to establish whether 30 nm condensed fibres exist in cortical neurons *in vivo*. In contrast to inert, heterochromatic chicken erythrocyte nuclei, where closely packed 30 nm fibres are abundant (Davies *et al.*, 1974), nuclei from cortical neurons are transcriptionally active, and the diffuse chromatin is dispersed throughout the nucleus (e.g., Austoker *et al.*, 1972; Thomas and Thompson, 1977).

The formation of (albeit short) solenoids *in vitro*, despite the absence of linker DNA, limits the possible ways in which the nucleosomes can be arranged in the solenoid, if the nucleosomal DNA in short-repeat chromatin is coiled in two turns of 80 bp per turn around the histone core (Finch *et al.*, 1977; Simpson, 1978). While elucidation of the detailed arrangement of the nucleosomes will require further work, some points are apparent. A solenoid with the nucleosomes in edge-to-edge contact, and with their faces radial and nearly parallel or slightly inclined to the solenoid axis (Thoma *et al.*, 1979; Lee *et al.*, 1981; McGhee *et al.*, 1980; Suau *et al.*, 1979) could well tolerate a short DNA path between nucleosomes. Even in this structure, the DNA would probably have to link the nucleosomes at their inner edges (i.e., in the central hole of the solenoid), in order to minimise the length of linker required, and would follow a continuous superhelical path. There is no possibility of alternating directions for the nucleosomes (McGhee *et al.*, 1980); this would require too great a linker length. Other arrangements, such as those with the faces of the nucleosomes facing outwards in the solenoid (rather than radially arranged), or alternatively with the faces perpendicular to the solenoid axis (Suau *et al.*, 1979), would seem far less likely. If the linker DNA is not essential for the packing of nucleosomes into the solenoid, its path must be relatively unconstrained and in a typical solenoid it might well occupy the central hole, bound to H1.

Materials and methods

Preparation of nuclei from ox cerebral cortex

Nuclei were prepared by a method based on that of Thompson (1973). Ox brains, fresh from the slaughterhouse, were collected in ice cold 0.32 M sucrose, 1 mM $MgCl_2$, 0.25 mM phenylmethylsulphonyl fluoride (PMSF), 0.5% (w/v) Nonidet P40 (NP-40), and all subsequent operations carried out

on ice or at 4°C. Brains were freed of meninges and the cerebral cortices removed and rinsed in 0.32 M sucrose, 1 mM $MgCl_2$, 0.25 mM PMSF, 0.5% (w/v) NP-40. The weighed cortices were then homogenised (~50% w/v) in a total volume of 300–320 ml in the same buffer with five strokes of a motor-driven Aldridge type homogeniser. The homogenate was filtered through one layer of muslin, and centrifuged at 10 000 r.p.m for 15 min in the SS-34 rotor of a Sorvall RC-5B centrifuge. The crude nuclear pellets were resuspended in 2.0 M sucrose, 1 mM $MgCl_2$, 0.25 mM PMSF and centrifuged at 22 000 r.p.m. for 30 min in the Beckman SW27 rotor. The resulting pellets were resuspended in ~15 ml of 2.4 M sucrose, 1 mM $MgCl_2$, 0.25 mM PMSF and the suspension distributed evenly between six tubes for the Beckman SW50.1 rotor. The suspension (~2.5 ml) was overlayered with a roughly equal volume of 2.0 M sucrose, 1 mM $MgCl_2$, 0.25 mM PMSF and centrifuged at 32 000 r.p.m. for 30 min. This resulted in two populations of nuclei (Thompson, 1973), those at the interface of the 2.4 and 2.0 M sucrose (N^1) which were enriched in neuronal nuclei, and those that pelleted below the 2.4 M sucrose (N^2) which consisted almost entirely of glial nuclei. The nuclei were washed twice in the 0.34 M sucrose-buffer A of Hewish and Burgoyne (1973) containing 0.25 mM PMSF, and resuspended in the same buffer at A_{260} = 50, as measured in 1 M NaOH. Neuronal and glial nuclei were identified under phase contrast optics on the basis of standard morphological criteria (Thompson, 1973; Austoker *et al.*, 1972 and references cited therein) and counted in a haemacytometer (Weber Scientific International Ltd.). A typical preparation starting with ~150 g of ox cerebral cortex yielded 3.5 x 10^8 nuclei in the N^1 fraction (of which ~65% were of neuronal and ~35% of glial origin) and 1.2–1.3 x 10^9 nuclei in the N^2 fraction, of which 95–99% were of glial oigin. A higher A_{260} per nucleus for the N^1 than for the N^2 population presumably reflects the higher RNA content in neuronal than in glial nuclei (e.g., Austoker *et al.*, 1972; Thompson, 1973) and possibly differences in ploidy (Bregnard *et al.*, 1979).

Preparation of chromatin

Chromatin was prepared from nuclei by digestion with micrococcal nuclease (Noll *et al.*, 1975) essentially as described previously (Butler and Thomas, 1980). For nuclei at A_{260} = 50 (measured in 1 M NaOH), an enzyme (Boehringer) concentration of 15 U/ml was used for 5–12 min depending on the range of fragment sizes required. 2.5 ml of soluble chromatin at A_{260} = 12–24 in 0.2 mM Na_2EDTA, 0.25 mM PMSF at pH 7.0 was loaded on to a linear sucrose gradient (5–50% w/v, 36 ml) containing 5 mM triethanolamine HCl, 1 mM Na_2EDTA, 0.25 mM PMSF, pH 7.5 and centrifuged in a Beckman SW28 rotor at 28 000 r.p.m. for 20 h, or at 21 500 r.p.m. for 17 h depending on the fragment sizes. Gradients were fractionated and monitored as described earlier (Bates *et al.*, 1981). Fractions were dialysed where necessary against 5 mM triethanolamine HCl, 1 mM Na_2EDTA, 0.25 mM PMSF, pH 7.5.

Determination of DNA sizes and nucleosome oligomer lengths

DNA was extracted from chromatin and analysed in horizontal agarose gels as described previously (Bates *et al.*, 1981) except that ethidium bromide (0.5 μg/ml) was included in both the gel and the running buffer. Gels were calibrated with *Eco*RI restriction fragments of bacteriophage λ DNA, and *Hpa*II fragments of pBR322 DNA. Weight-average nucleosome oligomer sizes were estimated from the DNA lengths (Butler and Thomas, 1980; Thomas and Butler, 1980) assuming a nucleosome repeat length of 162 bp for ox neuronal chromatin, obtained by digestion of nuclei as described by Thomas and Thompson (1977) for rabbit nuclei (data not shown).

Redigestion of chromatin fragments

In order to determine the repeat lengths of chromatin fragments fractionated in sucrose gradients, the chromatin in 5 mM triethanolamine HCl, 1 mM Na_2EDTA, 0.25 mM PMSF, pH 7.5 was diluted with the same buffer to A_{260} = 1.0, and digested in the presence of 2 mM $CaCl_2$ at 27°C with 20 U/ml micrococcal nuclease for 2–32 min. The reaction was stopped by the addition, on ice, of Na_2EDTA (pH 7.0) to 30 mM, and the DNA extracted and analysed in agarose gels as above. Repeat lengths were determined from the slopes of graphs of DNA size plotted against oligonucleosome band number (Thomas and Furber, 1976; Thomas and Thompson, 1977).

Analysis of the histone content of chromatin

The histone contents of all chromatin samples were routinely checked by electrophoresis in SDS/18% polyacrylamide slab gels (Thomas and Kornberg, 1978).

Analytical ultracentrifugation

Measurement of sedimentation coefficients for various chromatin fragment sizes at a series of different ionic strengths in the range 5–125 mM was carried out exactly as described previously (Butler and Thomas, 1980), except that most of the measurements were performed in a Beckman L8 ultracentrifuge equipped with a Prep UV scanner linked to an Apple /// microcomputer. Data from the scans were stored digitally, and weight-average positions of the boundaries were determined by numerical integration. The ionic strength was adjusted immediately before centrifugation by addition of 1 M NaCl to the sample in 5 mM triethanolamine HCl, 1 mM Na_2EDTA, 0.25 mM PMSF, pH 7.5, at A_{260} ~0.25.

Electron microscopy

Chromatin samples were prepared for electron microscopy using a procedure based on that of Thoma *et al.* (1979) and developed from that described previously (Bates *et al.*, 1981). Chromatin at A_{260} ≤0.2 in 5 mM triethanolamine HCl, 1 mM Na_2EDTA, 0.25 mM PMSF, pH 7.5, was dialysed overnight against the same buffer containing 0–70 mM NaCl. Glutaraldehyde [final concentration 0.1% (v/v), from a 25% (v/v) stock (EMscope)] was then added to the dialysis buffer and fixation was allowed to proceed for at least 16 h at 4°C. The chromatin samples were then diluted to A_{260} ~0.04 in the same buffer containing benzyldimethylalkylammonium chloride (Bayer) added from a stock aqueous solution (0.1% w/v) to a final concentration of 5 x 10^{-4} %. After 30 min at room temperature, 5 μl samples were placed on 400 mesh collodion-coated copper grids and the chromatin was allowed to adsorb for 5 min. The grids were then washed by floating them on drops of distilled water for 10 min and blotted dry without dehydration, in order to avoid shrinkage of the nucleosome filament (E.C. Pearson, unpublished observations; see also Woodcock *et al.*, 1976). Rotary shadowing with platinum and subsequent electron microscopy were as described previously (Bates *et al.*, 1981).

Acknowledgements

We thank Louise Fairall and Catherine Wilson for expert technical assistance and Dr A. Klug for helpful comments on the manuscript. This work was supported partly by grants from the Cancer Research Campaign and the Science and Engineering Research Council to J.O.T.

References

Austoker,J., Cox,D. and Mathias,A.P. (1972) *Biochem. J.*, **129**, 1139-1155.
Bates,D.L., Butler,P.J.G., Pearson,E.C. and Thomas,J.O. (1981) *Eur. J. Biochem.*, **119**, 469-476.
Bates,D.L. and Thomas,J.O. (1981) *Nucleic Acids Res.*, **9**, 5883-5894.
Bregnard,A., Ruch,F., Lutz,H. and Kuenzle,C.C. (1979) *Histochemistry*, **61**, 271-279.
Brown,I.R. (1978) *Biochem. Biophys. Res. Commun.*, **84**, 285-292.
Brown,I.R. (1982) in Guiffrida Stella,A.M., Gombos,G., Benzi,G. and Bachelard,H.S. (eds.), *Basic and Clinical Aspects of Molecular Neurobiology*, Fondazione Internazionale Menarini, Madrid, pp. 98-110.
Butler,P.J.G. and Thomas,J.O. (1980) *J. Mol. Biol.*, **140**, 505-529.
Davies,H.G., Murray,A.B. and Walmsley,M.E. (1974) *J. Cell Sci.*, **16**, 261-299.
Ermini,M. and Kuenzle,C.C. (1978) *FEBS Lett.*, **90**, 167-172.
Finch,J.T. and Klug,A. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 1897-1901.
Finch,J.T., Lutter,L.C., Rhodes,D., Brown,R.S., Rushton,B., Levitt,M. and Klug,A. (1977) *Nature*, **269**, 29-36.
Greenwood,P.D., Silver,J.C. and Brown,I.R. (1981) *J. Neurochem.*, **37**, 498-505.
Hewish,D.R. and Burgoyne,L.A. (1973) *Biochem. Biophys. Res. Commun.*, **52**, 504-510.
Jaeger,A.W. and Kuenzle,C.C. (1982) *EMBO J.*, **1**, 811-816.
Lee,K.S., Mandelkern,M. and Crothers,D.M. (1981) *Biochemistry (Wash.)*, **20**, 1438-1445.
McGhee,J.D., Rau,D.C., Charney,E. and Felsenfeld,G. (1980) *Cell*, **22**, 87-96.
Noll,M., Thomas,J.O. and Kornberg,R.D. (1975) *Science (Wash.)*, **187**, 1203-1206.
Renz,M., Nehls,P. and Hozier,J. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 1879-1883.
Simpson,R.T. (1978) *Biochemistry (Wash.)*, **17**, 5524-5531.
Strätling,W., Müller,U. and Zentgraf,H. (1978) *Exp. Cell Res.*, **117**, 301-311.
Suau,P., Bradbury,E.M. and Baldwin,J.P. (1979) *Eur. J. Biochem.*, **97**, 593-602.
Thoma,F., Koller,Th. and Klug,A. (1979) *J. Cell Biol.*, **83**, 403-427.
Thomas,J.O. and Butler,P.J.G. (1980) *J. Mol. Biol.*, **144**, 89-93.
Thomas,J.O. and Furber,V. (1976) *FEBS Lett.*, **66**, 274-280.
Thomas,J.O. and Kornberg,R.D. (1978) *Methods Cell Biol.*, **18**, 429-440.
Thomas,J.O. and Thompson,R.J. (1977) *Cell*, **10**, 633-640.
Thompson,R.J. (1973) *J. Neurochem.*, **21**, 19-40.
Whatley,S.A., Hall,C. and Lim,L. (1981) *Biochem. J.*, **196**, 115-119.

Woodcock,C.L.F., Safer,J.P. and Stanchfield,J.E. (1976) *Exp. Cell Res.*, **97**, 101-110.
Worcel,A. and Benyajati,C. (1977) *Cell*, **12**, 83-100.
Yabuki,H., Dattagupta,N. and Crothers,D.M. (1982) *Biochemistry (Wash.)*, **21**, 5015-5020.