a repressor can be calculated from the free energy of superhelix formation[4]. The values are tabulated in the last column of Table 1. On the binding of a *lac* repressor, the operator unwinds by about 40°, or approximately one tenth of a helical turn. This small unwinding cannot be reconciled with the prediction of the Gierer model for the operator structure shown in Fig. 1*b*, unless the operator exists as the hairpinned structure in the absence as well as in the presence of repressor. The melting temperature of the operator fragment[2] indicates, however, that it is highly unlikely that the operator assumes the hairpinned structure in the absence of repressor.

We also tried to measure the unwinding of the *lac* operator by the method of Saucier and Wang[14]. No definitive conclusion was obtained by this technique, which is not unexpected since the estimated value for θ is approximately the limit of detection of this technique.

Maniatis and Ptashne[5] have shown, by measuring the amounts of λ repressor bound at equilibrium to linear and superhelical λ DNAs, that virtually no unwinding of the λ operator occurs on binding of the λ repressor. The observed difference in affinity for the two DNAs was less than a factor of two. For superhelical λ DNA isolated from infected cells, which was used in their measurements, σ is expected to be in the range of 0.026 to 0.031 (ref. 15). If λ repressor unwinds the λ operator to the same extent as in the case of the *lac* system, the difference in affinities for the superhelical and linear λ DNA samples used would be approximately a factor of 4 (Table 1). It therefore

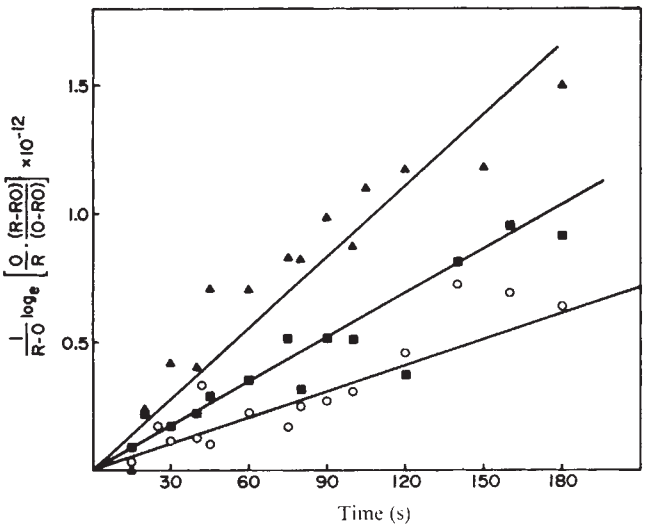

Fig. 3 Rates of association of the repressor to operator DNAs with various degrees of superhelicity. The conditions of the binding assay are the same as described in the legend of Fig. 2 except that the concentrations of the operator DNA varied from 0.4 to 1 × 10^{-12} M and that only a twofold excess of repressor was used. The total operator and repressor concentrations were determined precisely and the concentration of the repressor operator complex (RO) was calculated using a conversion factor estimated by filtering a known amount of RO complex under the same experimental conditions[7]. The reaction was initiated by mixing the repressor and the operator DNA and the association was stopped at appropriate times by transferring 1.5 ml samples into 100 μl of a mixture containing 4 μg of unlabelled λΦ80 d*lac* DNA and 10^{-2} M *o*-nitrophenyl-β-D-fucoside. The presence of about 100-fold excess of unlabelled operator DNA prevents the formation of new labelled RO complexes while the anti-inducer, *o*-nitrophenyl-β-D-fucoside, stabilises the existing labelled RO complexes[11]. Three 0.5-ml samples of that mixture were filtered, washed and counted and the triplicate values were averaged, as described earlier. The background value of the amount of radioactivity retained on the filters in the presence of 10^{-3}M isoprophyl-β-D-thiogalactoside was subtracted from each time point. The data were plotted according to the rate equation for a bimolecular reaction[7], with R and O denoting the total repressor and operator concentrations respectively. The rate constant of association, k_a, was obtained from the slope of the least-square line. ○, σ = −0.001, average of four experiments, k_a = 3.4 × 10^9 mol^{-1} s^{-1}; ■, σ = −0.017, average of two experiments, $k_a = 5.7 \times 10^9$ mol^{-1} s^{-1}; ▲, σ = −0.035, average of three experiments, k_a = 9.2 × 10^9 mol^{-1} s^{-1}.

appears that λ repressor does not unwind the λ operator as much as the *lac* repressor unwinds its operator, if at all.

In the calculation of θ from the free energy of superhelix formation, it has been implicitly assumed that the intercalative dye ethidium unwinds the DNA helix by 12°. Recent results of one of us (J.C.W.) indicate that the unwinding angle for ethidium is likely to be significantly larger than 12° with 26° being the best estimate. This would lead to a proportionately larger value for θ of about 90°. Since the size of the *lac* operator is about 30 base pairs, an unwinding of 40° to 90° suggests a significant change of the operator structure on the binding of the *lac* repressor.

Thus the conclusions of our unwinding measurements are twofold. On one hand, our results suggest that the recognition of the operator by the *lac* repressor is primarily through interactions with groups of the bases in the groves. Recognition either by the Gierer model or by the disruption of a segment of the base pairs, thus exposing a sufficient number of bases, is inconsistent with our results. On the other hand, the specific interaction between the operator and the repressor involves a structural change of the operator, as evidenced by the unwinding measured. This structural change is likely to be important in recognition.

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Subunit structure of chromatin

IN the past, the structure of chromatin has remained obscure despite the efforts of many laboratories and seemed far from a simple solution[1]. It has been known only that chromatin contains a repeating substructure[2,3]. Recently, however, Kornberg proposed[4] that chromatin structure is based on a repeating subunit of 200 base pairs of DNA and two of each of the histones (with the exception of F1

which occurs only once per subunit). Furthermore, he suggested that in chromatin these subunits form a flexibly jointed chain.

Here I present evidence in favour of this model. It is shown that 85% of chromatin can be converted to a subunit by digestion with micrococcal nuclease. This subunit contains about 205 base pairs of DNA and occurs as a discrete complex in solution. As well as demonstrating the existence of a chromatin subunit, the methods described here provide a means of isolating the subunit on a preparative scale and represent a test for the native state of chromatin.

Previous work has shown that a considerable amount of the DNA in rat liver nuclei is cleaved to integral multiples of a unit length upon digestion by an endogenous Ca^{2+} and Mg^{2+}-dependent endonuclease[3]. I have confirmed this result using the enzyme purified from rat liver nuclei, and demonstrated that the same DNA fragments are obtained after digestion with micrococcal nuclease (Fig. 1). A similar

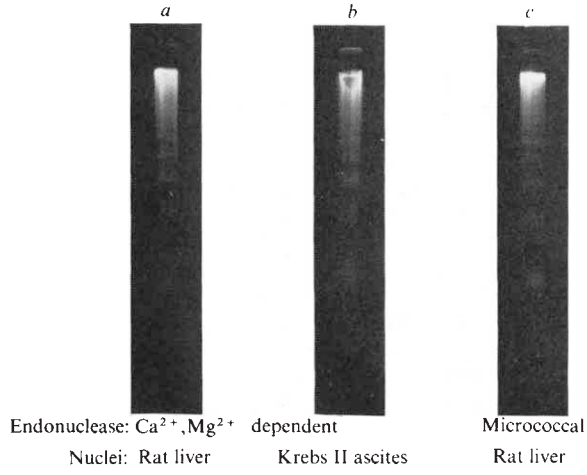

Fig. 1 Polyacrylamide gel analysis of DNA digested *in situ* with micrococcal nuclease or Ca^{2+}, Mg^{2+} dependent endonuclease. *a*, Rat liver nuclei containing the Ca^{2+}, Mg^{2+} dependent endonuclease were incubated at 1.5×10^8 nuclei ml^{-1} as described[3] in the presence of 10 mM $MgCl_2$ and 1 mM $CaCl_2$ for 60 min at 37° C. *b*, Krebs II ascites nuclei which lack the Ca^{2+}, Mg^{2+} dependent endonuclease[6] were incubated as in (*a*) in the presence of 0.5 units ml^{-1} of purified Ca^{2+}, Mg^{2+} dependent endonuclease (M. N., to be published). *c*, Incubation of rat liver nuclei with 300 units ml^{-1} of micrococcal nuclease (Worthington Biochemical Co.) in 1 mM $CaCl_2$ for 15 s at 37° C. After incubation the DNA was extracted, precipitated and analysed on 2.5% acrylamide gels essentially as described[3,6].

result is obtained with DNase I although the pattern is obscured by a very high background. Whereas both the rat liver enzyme and micrococcal nuclease require Ca^{2+}, DNase I requires only Mg^{2+}. Thus, the occurrence of regularly spaced cleavage sites is not dependent on the presence of a specific divalent cation.

Having established that readily available nucleases produce the same digestion pattern as the Ca^{2+}, Mg^{2+} dependent endonuclease, it was possible to answer many questions concerning the hypothetical chromatin subunit. For example, the proportion of chromatin based on this subunit structure could be determined. For this purpose it was necessary to determine the proportion of DNA that can be broken down into monomers (DNA of unit length associated with the hypothetical chromatin subunit of which the sizes of the larger DNA fragments [dimers, trimers, and so on] are integral multiples) or resolved multiples thereof if it is digested *in situ* with saturating amounts of the micrococcal enzyme. It is evident from Fig. 2 that nearly all DNA is converted to the monomer size. In

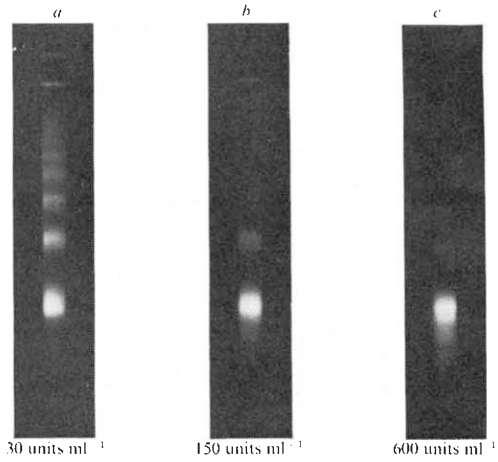

Fig. 2 Titration of chromatin digestion with micrococcal nuclease. Rat liver nuclei were incubated for 6 min at 37° C in a standard reaction mixture as described for Fig. 1*c* with increasing concentrations of micrococcal nuclease: *a*, 30 units ml^{-1}; *b*, 150 units ml^{-1}; and *c*, 600 units ml^{-1}. Incubation mixtures were processed and analysed as described in the legend of Fig. 1.

addition to the analysis of the DNA fragments on gels, the amount of TCA soluble DNA had to be taken into account in order to obtain a reliable value for the proportion of chromatin consisting of the repeating subunit. Under conditions converting all the DNA on the gel to monomers and resolved multiples of it (Fig. 2*a*), 13% of the DNA was acid soluble. This shows that at least 87% of the chromatin consists of the subunit, since the background of DNA between the bands is extremely low (as judged by its evaluation between the well separated first three bands). This estimate represents only a lower limit since some of the TCA soluble DNA is probably due to partial degradation of DNA in the substructure as indicated by the finding that further digestion produced more acid soluble DNA (28% in Fig. 2*c*).

It is evident from Fig. 2 that increasing digestion with micrococcal nuclease is accompanied by the appearance of fragments smaller than the monomer which might arise by cleavage at a few sites located within the subunit. After relatively mild digestion, on the other hand, the monomer can be observed to consist of a doublet (Fig. 3). Increasing digestion shifts the relative intensities of the two bands in favour of the lower band which suggests that it is produced from the upper band. Thus it is conceivable that the upper band is the true monomer while the lower band arises by cleavage at a preferred internal site of the monomer.

To clarify the relationship between the monomer doublet and the higher DNA bands an accurate determination of their size and breadth is required. This was achieved by calibration with a series of sequenced DNA fragments generously supplied by Dr J. W. Sedat. Thus the DNA of a micrococcal nuclease digest, the Hin fragments and a set of sequenced single-stranded Endo IV products of ΦX174 were run in parallel on a 99% formamide slab gel (not shown). From the sharpness of the Hin fragment bands it was apparent that no partial renaturation occurred on the gel. A logarithmic plot of the molecular weight of the Hin (P. G. N. Jeppesen, personal communication) and Endo IV (ref. 5 and unpublished results of J. W. Sedat and F. Sanger) products (the lengths of the Hin fragments had been determined by EM and did not deviate from their true value by more than 10% as judged by comparison with the sequenced Endo IV products) as a function of the square root of their mobility, resulted in a smooth calibration curve which was linear in the range of 200–400 bases. From comparison with this calibration curve the following values were obtained for the monomer doublet, dimer and

trimer: monomer doublet = 205 ± 15 base pairs and 170 ± 10 base pairs; dimer = 405 ± 35 base pairs; trimer = 605 ± 40 base pairs. (The breadth of the bands reflects true heterogeneity in size rather than diffusion in the gel—see also ref. 6.) The sizes of the monomer bands are consistent with an estimate of the average molecular weight of monomer DNA from sedimentation analysis on sucrose gradients[6].

The measurements clearly show that no multiples of the lower monomer band are found and strongly suggest that both dimer and trimer correspond to twice and three times the length of the upper monomer band rather than a linear combination of both monomer bands. The data, therefore, favour a model in which the monomer doublet consists of the real monomer (205 base pairs) and a degradation product of it (170 base pairs) produced by cleavage at a site located approximately 20% within the monomer. No 'monomers', however, are observed arising from cleavage at this site in the dimer, that is, the monomer has never been observed as a triplet (with a third band of 240 base pairs). A possible explanation would be that the internal cleavage site only becomes accessible in the free subunit.

The results presented so far concern primarily the composition of the chromatin subunit. Evidence that the subunit occurs as a discrete complex in solution comes from sucrose gradient analysis of chromatin previously digested with micrococcal nuclease. As demonstrated in Fig. 4 a regular series of seven resolved peaks is observed which have been shown to contain DNA of the size of the monomer (first peak), dimer (second peak), trimer (third peak) and so on. Thus histone–histone interaction between adjacent subunits is not sufficient to hold them together. Since the sucrose gradient is isokinetic (for a particle density of 1.5 g cm^{-3}) the S-values are proportional to the distance migrated in the gradient[7]. By calibration with 30S ribosomal subunits of *Escherichia coli*[8] it was shown that the chromatin subunits sediment at 11.2 ± 0.4S and the chromatin dimers at 15.9 ± 0.5S. These values have been confirmed by sedimentation velocity analysis in an analytical ultracentrifuge. The first two peak fractions when fixed with formaldehyde band both as a homogenous peak with a buoyant density of 1.45 g cm^{-3} in a CsCl gradient, exhibit a protein to DNA ratio of approximately 1.3 and contain in addition to all five major histones some non-histone proteins. Van Holde and coworkers have reported a 12S product of micrococcal nuclease digested chromatin[9]. The 12S material differs from the 11S subunit reported here in size of DNA (about 100 rather than 205 base pairs) and protein to DNA ratio (1.5 rather than 1.3). The 12S material may arise from the 11S subunit through over-digestion (ref. 4, footnote 8).

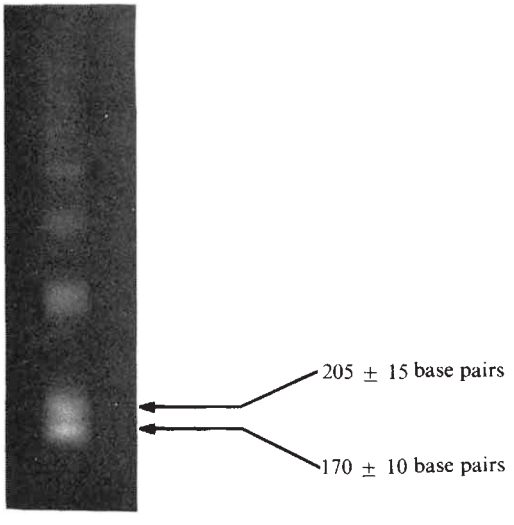

Fig. 3 Monomer doublet after 'mild' micrococcal nuclease digestion. After incubation of rat liver nuclei with micrococcal nuclease (15 s at 37° C, 150 units ml^{-1}) the nuclei were pelleted at low speed, resuspended in 0.2 mM EDTA, and homogenised with a Waring blender. The homogenate was centrifuged for 30 min at 12,000*g* and the supernatant containing the digested chromatin was processed and analysed as described for Fig. 1.

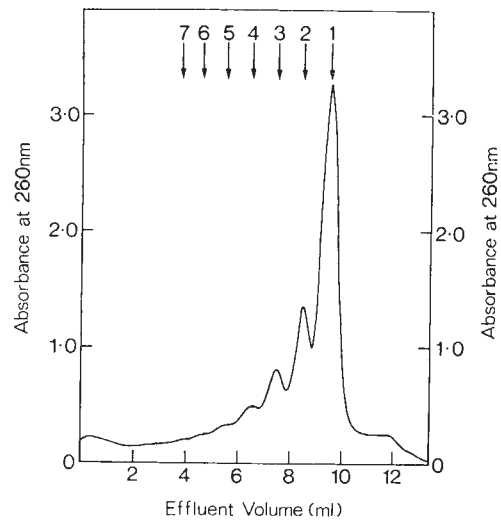

Fig. 4 Sucrose gradient analysis of digested chromatin. Digested chromatin was prepared from rat liver nuclei as described for Fig. 3 except that incubation was for 2 min rather than 15 s. The chromatin supernatant (5.5 A_{260} units) was layered on a 11.5-ml isokinetic sucrose gradient (D. Calhoun, M. N. and H. Noll, in preparation) with $c_1 = 5\%$, $c_2 = 28.8\%$, and $V_m = 55.5$ ml in 0.2 mM EDTA, *p*H 7 and centrifuged for 12.75 h at 28,000 r.p.m. and 4° C in a Beckman SW40 rotor. The gradient was monitored for absorbancy by passing the effluent from the bottom of the tube through a turbulence-free flow cell (Molecular Instruments Co., PO Box 1652, Evanston, Illinois 60201, USA).

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