Volume 1 number 11 November 1974 Nucleic Acids Research

Internal structure of the chromatin subunit

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Received 19 September 1974

ABSTRACT

The digestion of chromatin in situ with DNase I reveals, after denaturation, a regular series of single stranded DNA fragments the lengths of which represent multiples of 10 bases. These experiments are compatible with the DNA being on the outside of the chromatin subunit and suggest that the subunit structure itself contains repetitive structural elements. Possible models are discussed.

INTRODUCTION

In a previous communication[1] experiments have been described which are strongly in favour of the subunit structure of chromatin as proposed by Kornberg[2]. These experiments demonstrated that (i) the digestion of chromatin with micrococcal nuclease produces DNA fragments which are multiples of a unit length as reported earlier for the digestion with an endogenous Ca^{2+}, Mg^{2+} dependent endonuclease[3]; (ii) at least 85% of the chromatin is based on this subunit; (iii) each subunit contains 205 $\pm$ 15 base pairs of DNA; and (iv) the chromatin subunit consisting of all five major histones and 200 base pairs of DNA may be obtained as a discrete entity in solution after nuclease digestion of chromatin. In addition, it was shown that prolonged digestion with micrococcal nuclease produces cuts within the chromatin subunit.

Here I present evidence for a repetitive internal structure of the chromatin subunit and describe experiments suggesting that the DNA is on the outside of the subunit[2]. As in the case of the subunit structure of chromatin the evidence[1,3] is derived from experiments in which chromatin has been digested with an endonuclease, here DNase I. In contrast to micrococcal nuclease[1] and the Ca^{2+}, Mg^{2+} dependent endonuclease[3] which selectively cut between the subunits, DNase I exhibits a preference for a limited number of defined cleavage sites within the subunit.

MATERIALS AND METHODS

Sequenced Endo IV fragments of ϕX174 DNA and pure $tRNA^{Phe}$ from yeast were kindly supplied by E. Blackburn, J. Sedat, E. Ziff and P. Piper. A partial T_1-digest of yeast $tRNA^{Phe}$ was obtained by incubation of 2 A_{260} units of $tRNA^{Phe}$ with 10 μg T_1 in 0.015 ml 3.3 mM Tris-HCl, pH 7.5, 6.7 mM $MgCl_2$, 1.3 mM EDTA for 30 min. at 37°C. A T_1 fragment of 12 bases of a complete T_1-digest of yeast $tRNA^{Phe}$ was isolated from a fingerprint by standard methods[4].

Preparation of DNase I fragments. Nuclei were prepared from rat liver according to Hewish and Burgoyne[3] and incubated at a concentration of 1.5×10^8 nuclei per ml in 0.34 M sucrose, buffer A (ref. 3), 10 mM Na-bisulphite, 10 mM $MgCl_2$ with 300 units/ml of DNase I (Worthington Biochemical Co.) for 30 s at 37°C. The reaction was stopped by adding 20 mM EDTA, 1% SDS, 1 M NaCl, and the DNA was extracted twice with an equal volume of chloroform/isoamylalcohol (24:1). The DNA was either precipitated twice with 70% ethanol at - 20°C or dialyzed extensively against H_2O at 2°C, lyophilized, and resuspended in a small aliquot of H_2O. It was stored frozen at - 20°C until use. The recovered DNA represents 95% of the total DNA present in nuclei.

Analysis on 0.15 cm x 14.5 cm x 15.5 cm 99% formamide slab gels was carried out according to Staynov et al.[5] (gels containing 6% acrylamide) as modified by Maniatis using 20 mM sodium phosphate, pH 7, as supporting electrolyte (T. Maniatis, pers. comm.). The samples were dried, redissolved in 0.010 ml 99% formamide, 20% sucrose, 20 mM Na-phosphate, pH 7, 0.005% bromophenol blue, heated for 30 s at 100°C, and quickly chilled on ice before application to the gel. The gel was run at 70 V for 14 hr at room temperature. It was stained for 10 min. in 1 mM EDTA, pH 7, 20 μg/ml ethidium bromide and photographed immediately.

Analysis on 0.15 cm x 14.5 cm x 15.5 cm 7.5% acrylamide gels was as described by Loening[6]. The gel was run at 30 mA for 6.5 hr at room temperature and stained for 30 min. with ethidium bromide as described above.

RESULTS AND DISCUSSION

The first use of nucleases to study the structure of chromatin was in the work of Clark and Felsenfeld[7]. It has since been reported that the digestion of chromatin with the Ca^{2+}, Mg^{2+} dependent endonuclease[3] and other endonucleases, such as micrococcal nuclease and DNase I, results in the fragmentation of the DNA into

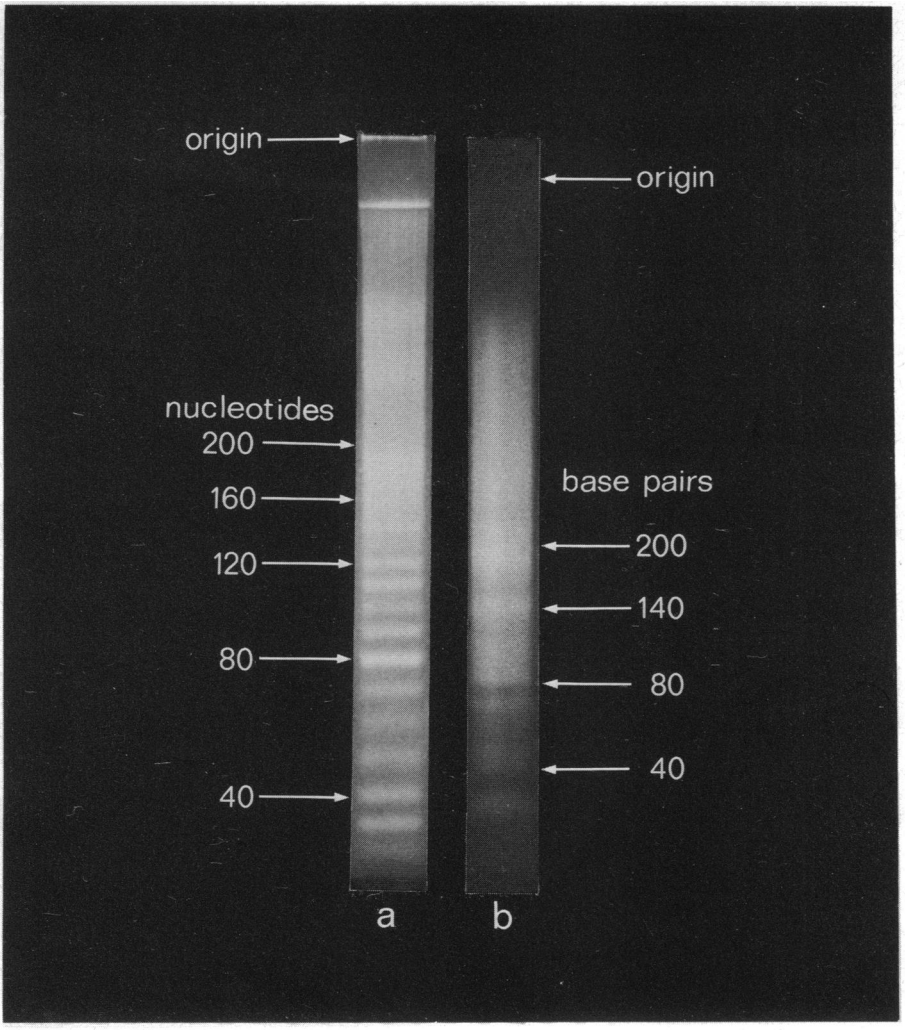

Figure 1

DNA fragments from chromatin digested with DNase I. Chromatin of rat liver nuclei was digested with DNase I in situ, the DNA extracted, concentrated, and analyzed on a 99% formamide gel (a) and a nondenaturing 7.5% acrylamide gel (b) as described in Materials and Methods except that in (b) 5 times as much enzyme was used. The input was 25 μg of DNA in (a) and 18 μg in (b).

multiples of 200 base pairs[1]. While virtually no background is detected between the DNA bands of a micrococcal digest, fragmentation by DNase I produces a rather high "background" between the bands representing multiples of 200 base pairs if analysis is carried out under nondenaturing conditions[1]. However, the analysis of the DNA products obtained by digestion of chromatin with DNase I on a denaturing gel reveals a more detailed and quite striking picture (Fig. 1a). The single stranded fragments form a regular pattern of bands which can be resolved up to a chain length of about 200 bases corresponding to the length of DNA associated with the chromatin subunit. Under conditions which convert all the DNA into the bands of a chain length shorter than 200 nucleotides only 15% of the DNA is TCA soluble. Therefore a major fraction of the chromatin - which has been shown previously to consist of a repeating subunit[1] - exhibits this repetitive internal structure. A series of bands in the region below 200 base pairs may also be obtained if the digested DNA is analyzed under nondenaturing conditions (Fig. 1b). While these bands are less distinct than those in Fig. 1a, the pattern reveals characteristic gaps in which no background seems to appear. The patterns in Fig. 1 reflect the specific interaction of the DNA with the histones within the chromatin subunit since it cannot be obtained by digestion of Krebs II ascites DNA (MW > 2×10^6) free of proteins. Even at 10 times lower enzyme to substrate ratios but otherwise identical conditions of digestion the naked DNA proved to be degraded to fragments shorter than 10 bases. This demonstrates that the specificity is lost when the histones are removed.

In order to calibrate the length of the single stranded DNase I fragments (Fig. 1a) they were run alongside a number of sequenced markers. Thus, sequenced Endo IV fragments of ØX174 DNA of 35, 48, 63, 65, 194, and 260 bases were run as well as a T_1 fragment of yeast $tRNA^{Phe}$ of a chain length of 12 bases, yeast $tRNA^{Phe}$ (76 bases), and a partial T_1 digest of yeast $tRNA^{Phe}$ which produces the main fragments of 61, 42, and 25 bases. Caution is indicated if DNA and RNA fragments are compared. As Fig. 2 proves both the DNA and tRNA fragments exhibit the same mobility. However, larger RNA fragments (e.g. 5.8S RNA from yeast) seem to exhibit a higher mobility by as much as 10% compared with DNA of the same chain length (M. Noll, unpublished observation).

A semilogarithmic plot of the chain length of these markers as a function of

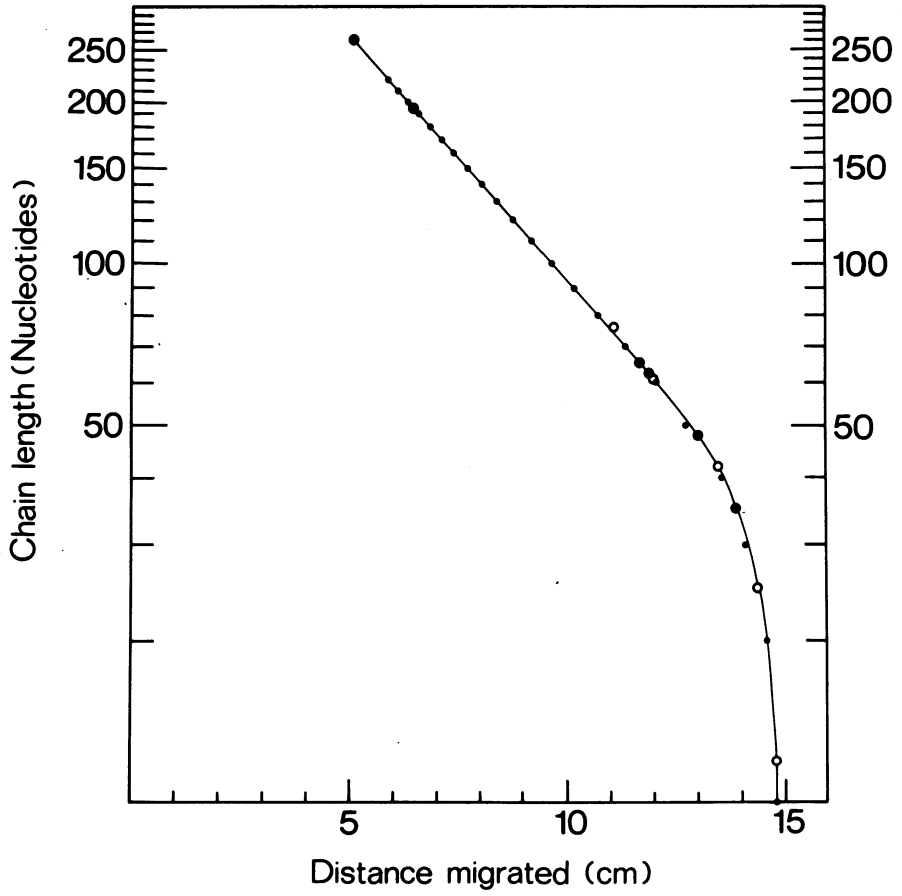

Figure 2: Calibration of single stranded DNA fragments. Sequenced DNA (●) and tRNA (O) fragments were used to determine the chain lengths of the single stranded DNA fragments (•) obtained by digestion of chromatin with DNase I. For detailed description see text and Materials and Methods.

their mobilities exhibits a linear relationship in the range of 60 to 260 bases (Fig. 2). The interpolated values for the length of the DNase I fragments show a striking result. The chain lengths form an arithmetic series of 10, 20, 30, etc. bases which can be resolved up to 220 bases on the best gels. Thus, digestion of chromatin in situ reveals not only a repeat in structure corresponding to the chromatin subunit but also a much shorter repeat reflecting a repetitive structural element within the subunit. In addition, it shows that all subunits are alike with respect to this repetitive feature of their internal structure.

A preliminary estimate of the lengths of the DNA in the bands on the nondenaturing gel suggests that they are also multiples of 10 base pairs. The picture presented in Fig. 1b does not show sharp bands but does show sharp gaps. Thus, the background is very low between 160 and 140, 80 and 70 as well as between 40 and 30 base pairs. The same gaps are observed when extensive digests of chromatin with micrococcal nuclease are analyzed on denaturing or nondenaturing gels. However, the characteristic banding pattern of Fig. 1a cannot be obtained even after prolonged digestion with micrococcal nuclease (M. Noll, unpublished work).

Even though single stranded fragments with lengths of each multiple of 10 bases are obtained by DNase I digestion of chromatin this does not prove that the cleavage sites on each DNA strand occur every 10 bases. Such an interpretation may gain support from the finding that the higher bands get converted to the lower bands with increasing digestion (not shown). Further, it is likely that the actual length of the DNA associated with the chromatin subunit is also a multiple of 10 base pairs since even after very limited digestions ($> 50\%$ of the fragments > 200 bases) no products are detected between the bands. The bands exhibit a characteristic intensity pattern - with a predominant band of 80 bases and relatively weak bands at 60, 100 and 130 bases - which only changes during extensive digestion when the larger bands are degraded to the lower bands. This suggests that not all cleavage sites within the subunit are equivalent.

The high accessibility of the DNA in chromatin clearly favours a model in which the DNA is on the outside of the chromatin subunit[2]. This is supported by electron microscope observations (J. Finch, unpublished work). The regular repeat of the DNase I cleavage sites within the subunit might then be visualized basically by two different models. (i) The DNA is wound around a histone complex[2, 8] in a superhelical form without kinks. Only those sites of the sugar-phosphate backbone which are most exposed to the surroundings are attacked by the DNase I molecules. These sites are expected to occur every 10 bases on each strand reflecting the pitch of the DNA double helix. (ii) The DNA is not bent but kinked -which has been suggested to me by F.H.C. Crick - around the histone core with straight segments alternating with kink regions occurring every 10 base pairs. The fragments are produced by cuts either at the kink positions or in the straight regions depending on which sites are protected by the histone complex. While in the first model it is expected that the two strands are cut with a stagger of 5 bases in the

second model no stagger occurs if the strands are cut at the kinks. A mixed model exhibiting both features has also to be considered.

ACKNOWLEDGEMENTS

I am grateful to Drs. E. Blackburn, J.W. Sedat, E.B. Ziff and P.W. Piper for the sequenced Endo IV fragments of øX174 DNA and a preparation of pure yeast $tRNA^{Phe}$ and to Ms. D. Rhodes for her help in the isolation of the T_1 fragment. I thank Drs. F.H.C. Crick and R.D. Kornberg for many stimulating discussions. This work has been supported by an EMBO Long-Term Fellowship.

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