YEAST CHROMATIN STRUCTURE

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1. Introduction

It has recently become clear that the chromatin of higher eukaryotes has a repeating structure resembling a string of beads [1–3] (or 'nucleosomes' [4]), probably closely packed [1,5]. Each bead comprises 200 base pairs of DNA [6,7,8] and a histone octamer [9] whose composition [10] is taken to be $(H3)_2(H4)_2(H2A)_2(H2B)_2$. The DNA is wound around the histone octamer [1,11,12] and a molecule (see [1]) of the fifth histone, H1, is probably associated with the region linking one repeat unit to the next [8,13].

The question arises whether the chromatin of primitive eukaryotes such as yeast is constructed in the same way. Histones H2A, H2B, and H4 have been found in yeast but histone H3 has been reported absent [14]. Lohr and Van Holde [15] obtained evidence for a DNA repeat of 135 base pairs by digestion of yeast chromatin with micrococcal nuclease and suggested that the repeat size (smaller than the 200 base pairs of higher eukaryotes) might be related to the absence of H3, thereby raising the possibility of a substantially different type of repeat unit in this primitive eukaryote.

The results reported here suggest that this is unlikely to be so. Yeast nuclei contain all four of the main histones H2A, H2B, H3 and H4 in roughly equal amounts, and the DNA repeat is about 165 base pairs. Structural similarities between the nucleosomes of yeast and higher eukaryotes are apparent from the prominent intermediate in digestion containing 140 base pairs of DNA which they both give with micrococcal nuclease, and from the cleavage of the DNA at multiples of 10 bases along each strand by DNase I. The major difference, whose significance remains to be determined, is the shorter length of DNA contained in the nucleosome, a difference which recent results [16,17] suggest may be common to the fungi.

2. Materials and methods

2.1. *Nuclei*

Rat liver nuclei were prepared as described by Hewish and Burgoyne [18]. Nuclei were prepared from commercial ('NG & SF' brand, British Fermentation Products, Felixstowe, UK), fresh, pressed, baker's yeast (*Saccharomyces cerevisiae*) essentially as described by Wintersberger et al. [19]. The formation of spheroplasts using β-glucuronidase (Sigma) (0.4 ml/g cells) was generally complete in about 2 h at 30°C.

Method A: Spheroplasts were lysed by suspension in medium containing 18% ficoll [19]. The step gradient [20] used for subsequent purification of nuclei [19] was not entirely reproducible, and all fractions were carefully monitored by microscopy for the presence of nuclei. All buffers used from the spheroplast lysis step onwards contained 10 mM sodium bisulphite and 0.5 mM phenylmethylsulphonyl fluoride (PMSF) (added from a 50 mM stock solution in isopropanol) to minimise proteolysis.

Method B: In some preparations 0.5% Nonidet P-40 was included to facilitate lysis of the spheroplasts, and to increase the yield of nuclei, in a procedure otherwise identical with that in method A except that bisulphite was omitted and 1 mM PMSF was used, but the nuclei were more difficult to purify and did not give clean nuclease digestion patterns.

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2.2. *Nuclease digestions*

Rat liver nuclei were digested with micrococcal nuclease [6] and DNase I [11] as described. Yeast nuclei were digested at A_{260} = 10 with 30 U/ml micrococcal nuclease, or with 150 U/ml of DNase I at 37°C under conditions otherwise identical to those used for rat liver nuclei.

2.3. *DNA extraction*

Digested nuclei were extracted directly in 1 M NaCl, 1% SDS with isoamyl alcohol–chloroform (1:24) [21]. (In some cases the nuclei were first treated with RNAse A (5 μg/ml) followed by proteinase K (15 μg/ml) for 30 min each to guard against spurious bands when the products were analysed in gels.) The aqueous layer containing DNA was either dialysed exhaustively against double distilled water and freeze-dried, or the DNA was precipitated with 4 vols absolute ethanol at −20°C for 24 h.

2.4. *Gel electrophoresis*

(a) Nuclear proteins were analysed in SDS–18% polyacrylamide slab gels prepared, run and stained as already described [9]. Nuclei were pelleted, redissolved in sample buffer containing 1 mM PMSF at 80–100°C, heated at 100°C for 1 min, and the cooled samples applied directly to the gel.

(b) Double-strand DNA was analysed (after micrococcal nuclease digestion) in 2.5% polyacrylamide slab gels containing 0.5% agarose in the buffer system of Loening [22]. Gels were run at 80 V until the Bromphenol blue tracking dye had migrated 10 cm (about 3.5 h), stained with ethidium bromide (20 mg/l) for 15 min at 4°C, destained for 15 min in 1 mM EDTA at 4°C, and photographed immediately under short wavelength wavelength u.v. light.

(c) DNA was analysed in single-strand form (after DNase I digestion) in 8% polyacrylamide–7 M urea slab gels in Tris-borate-EDTA buffer [23,24]; samples were loaded in 7 M urea–0.1 M KOH–1 mM EDTA [24]. Gels were run at 150 mA until the bromophenol blue tracking dye had migrated 10 cm (about 3.5 h), and then treated as described above.

3. Results

3.1. *The histone content of yeast nuclei*

The electrophoretic mobility in SDS–polyacrylamide gels of each of the four main histones (H2A, H2B, H3 and H4) is virtually independent of the source of the histones for several eukaryotes (e.g. calf thymus, rat liver, chicken erythrocyte, ascites tumour cells, myeloma cells). Fig.1 shows that yeast nuclei give bands with roughly the same electrophoretic mobility as histones H2A, H2B, H3 and H4 from rat liver; the four components of yeast are present in comparable amounts as judged from yeast chromatin (fig.1b) prepared (albeit in low yield [15]) by the nuclease method [25]. Preliminary amino acid analyses of material eluted from preparative SDS–polyacrylamide slab gels show contents of basic amino acid residues comparable with those found for histones of higher eukaryotes. There are also certain other similarities with the histones of higher eukaryotes e.g. H4 has a high glycine content, H2A a high alanine and leucine content.

Yeast nuclei contain material with the same electrophoretic mobility as H1 from rat liver (fig.1a) but other properties of the material argue against its being histone H1. For instance, it appears to be less susceptible than the other histones to the action of endogenous protease whereas the H1 of higher eukaryotes (calf thymus, rat liver) is most rapidly degraded by both endogenous and added proteases; it is present in apparently larger amount than the other four histones; only a small proportion is extracted into 5% perchloric acid, in contrast with other H1 histones; it is present in much lower amounts in nuclei prepared by method B than in those from method A (compare fig.1(a) and 1(b)). Amino acid analysis of the putative H1 eluted from an SDS gel showed a moderately high content of basic amino acids, but a much lower ratio of lysine: arginine than for H1 from higher eukaryotes.

3.2. *A structural repeat in yeast DNA*

Comparison of the products of micrococcal nuclease digestion of rat nuclei and yeast nuclei (fig.2(c) and (d)) shows that in each case the DNA contains a regular array of sites accessible to the nuclease. The result for yeast is in qualitative agreement with that of Lohr and Van Holde [15].

3.3. *The size of the DNA repeat in yeast nuclei*

The size of the smallest DNA fragment from yeast decreases as digestion proceeds (fig.2 (d)–(f)). After

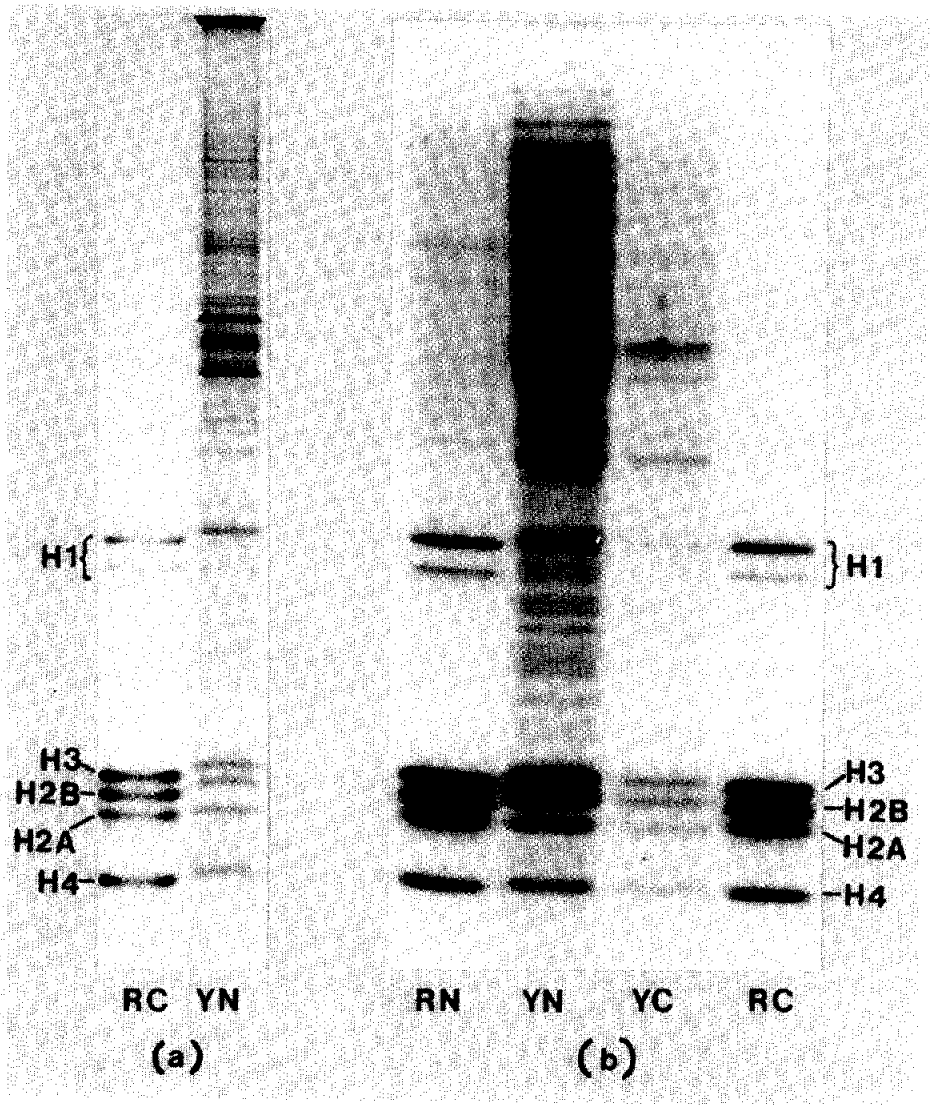
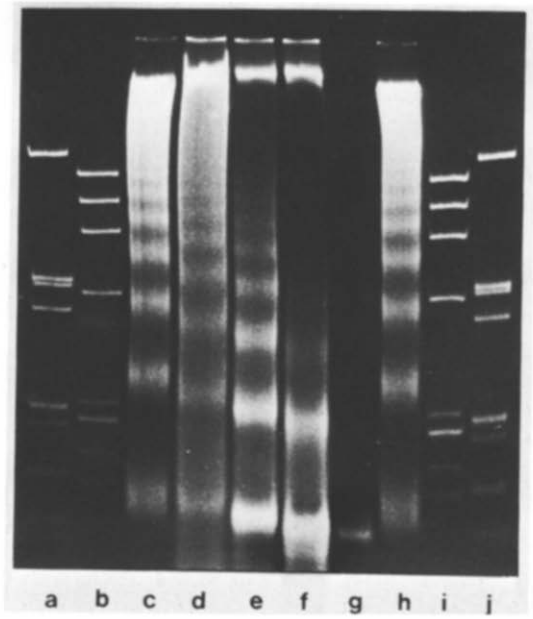


Fig.1. Comparison of the nuclear proteins of yeast with rat histones by electrophoresis in SDS 18%–polyacrylamide gels. (a) Rat liver chromatin (RC), yeast nuclei (YN) (prepared by method A). (b) Rat nuclei (RN); yeast nuclei (YN) (prepared by method B) and chromatin (YC) made from these nuclei by the nuclease method [25]; rat chromatin (RC). Migration was from top to bottom.



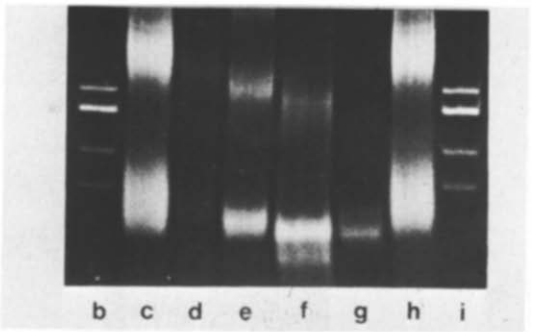


Fig.2. DNA fragments from micrococcal nuclease digestions of rat and yeast nuclei compared in a non-denaturing gel with DNA markers of known size. Top: (a) Hha I digest, and (b) Hae III digest, of ϕX RF DNA; (c) rat liver nuclei; (d) yeast nuclei, 5 sec digestion; (e) yeast nuclei, 1 min digestion; (f) yeast nuclei, 3 min digestion; (g) 140 base pair DNA from extensively digested rat liver nuclei; (h) rat liver nuclei; (i) Hae III digest, and (j) Hha I digest, of ϕX RF DNA. The sizes of the Hae III and Hha I markers are given in table 1. Migration was from top to bottom. (The mobilities for the rat and yeast fragments given in table 1 do not refer to the gel shown here, which is deliberately overloaded for photographic contrast, but are instead taken from an otherwise identical gel with one third the loading.) Bottom: Lower portion of a gel similar to that shown above but with a loading more suitable to show (f).

brief digestion it is about 170 base pairs (fig.2 (d)), roughly the same size as the smallest fragment from rat nuclei (fig.2 (c)). It is possible that the value of about 170 base pairs for yeast is an underestimate of the true DNA content of the repeat unit, since it has been shown for rat liver nuclei that the unit size DNA is shortened rapidly from 200 base pairs to 170 base pairs by further digestion at 37°C [25]. The true size of the repeat was determined for rat nuclei from the difference in size between successive multiples of the unit [8]. When this approach was applied to the products of digestion of yeast nuclei at different times (c.f. fig.2) the results shown in table 1 were obtained. The sizes of the digestion products (in base pairs) were read from a calibration curve constructed with the use of the two sets of restriction fragments of phage ϕX 174 replicative form (RF) DNA. A plot of log (base pairs) versus mobility gave a smooth curve (not shown). The size of the monomer DNA from yeast was estimated as 170, 162 and 155 base pairs after 5 sec, 1 min and 3 min digestion respectively. Like the monomer, all the oligomers (n-mers) were reduced in size as digestion proceeded, but the average difference in size between successive oligomers was 165 ± 6 and 165 ± 4 base pairs after 5 sec and 1 min digestion, as given by the slope of a plot of the number of base pairs in a band containing n-mer DNA versus n, for each digestion time; digestion for 3 min was too extensive to give meaningful results. The true size of the DNA repeat is therefore about 165 base pairs. The difference between this value and that reported [8] for rat liver chromatin (198 ± 6 base pairs) is genuine: the repeat for rat liver chromatin measured here in a gel alongside the yeast samples is estimated as 197 ± 5 base pairs (see table 1), in excellent agreement with the value reported by Noll and Kornberg [8].

3.4. *A relatively stable intermediate from digestion with micrococcal nuclease*

With progressive digestion of yeast nuclei at 37°C the length of the DNA in single nucleosomes liberated by digestion decreases from 170 base pairs (measured to the midpoint of the gel band) to 155 base pairs; reduction in size is accompanied by a sharpening of the leading edge of the band in the gel, indicative of a long-lived intermediate which is found to contain 140 base pairs (fig.2 (e)). This is compared with the DNA

Table 1
Sizes of DNA fragments from mobilities in a polyacrylamide gel (see legend to fig.2 for details)

Markers			Micrococcal nuclease digests				
Restriction enzyme digests of ϕX RF DNA (Gel mobilities used to construct calibration curve)			The size (in base pairs) of the *n*-mer DNA (from *n*-nucleosomes) read off from the calibration curve using mobility taken to midpoint of band				
Band No.	Size[a] (in base pairs) Hae III	Hha I	*n*-mer[b]	Rat	Yeast		
				30 sec	5 sec	1 min	3 min
1	1300	1630	5-mer	960	840	820	
2	1100	640	4-mer	760	670	660	
3	870	615	3-mer	570	510	490	450
4	610	540	2-mer	368	335	328	303
5	320	310 315	1-mer	175	170	162	155
6	285 300	285				(140)[c]	(140)[c]
7	230	200					
8	190	135					
9	115	123					

[a] As given in [32].
[b] In order of increasing mobility.
[c] Sharp front.

extracted from monomer nucleosomes produced from rat liver nuclei by extensive digestion with micrococcal nuclease (fig.2 (g)), subsequently purified on a sucrose gradient [6] and shown [8] to contain 140 base pairs of DNA. One more extensive digestion the 140 base pair fragment from yeast begins to break down (fig.2 (f)). These results indicate that, as in nuclei from higher eukaryotes, the first product of digestion is susceptible to further nuclease attack, and comparative stability is achieved in the 'core particle' [26] which contains 140 base pairs of DNA [8,13,27].

3.5. *Digestion of yeast nuclei with DNase I*

DNase I has been shown to make single strand cuts at multiples of 10 bases in rat liver chromatin

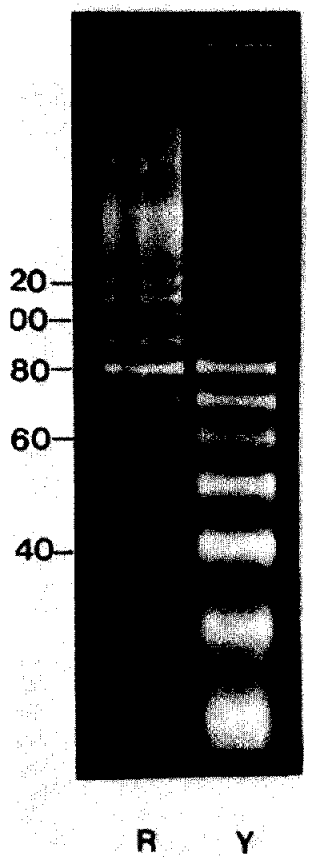


Fig.3. DNA fragments from DNase I digestion of rat (R) and yeast (Y) nuclei analysed in a denaturing gel containing 7 M urea. Samples were treated with RNase A and proteinase K before electrophoresis. The sizes for the rat liver bands have been established [11]. Migration was from top to bottom.

[11], and this high accessibility of the DNA to nuclease is taken as evidence that the DNA is on the outside of the nucleosome. Yeast nuclei give a similar digestion pattern on a denaturing gel (fig.3); slight differences in the relative intensities of the bands in the two cases may reflect different details of DNA–histone interaction within the nucleosome.

4. Discussion

The results described here suggest strongly that yeast contains H2A, H2B, H3 and H4. Earlier reports of Franco et al. [14] had shown H2A, H2B and H4 in chromatin extracted from whole cells; Wintersberger et al. [19] had claimed the presence of H3 in chromatin prepared from purified yeast nuclei, but since this claim was based on fractionations in acid–urea gels, in which resolution of H3 from H2A and H2B was not achieved, it left the issue in doubt. H3 in other nuclei (e.g. rat liver) is the most susceptible of the four main histones to proteolysis [25] and it is possible, especially in view of the abundance of proteases in yeast [28], that this could account for the apparent absence of H3 [14].

The presence of H1 in yeast is still equivocal. One possibility is that the H1 of yeast is substantially different from that of calf thymus and rat liver. For example, if yeast H1 had a lower content of basic amino acids than the H1 from higher eukaryotes it might be less sensitive to proteolysis (e.g. by a trypsin-like enzyme); if it had a lower lysine content than the H1 from other species it might be less soluble in 5% perchloric acid.

A full complement of the four main histones in yeast, taken together with the DNase I digestion pattern and a regular pattern of digestion of the DNA by micrococcal nuclease, suggests strongly that the structure of yeast chromatin is very similar to that of other eukaryotes. In these the arginine-rich histones H3 and H4 are presumed to play a critical structural role [29]; the presence of oligomers of the histones in yeast may be investigated by the methods already used for other sources [9,10,29].

Despite the similarity in structure between the chromatin of yeast and higher eukaryotes, the DNA repeat is smaller in yeast and recent studies on *Aspergillus nidulans* (154 base pair repeat [16]) and *Neurospora crassa* (170 base pair repeat [17]) suggest that this result may hold for the fungi in general. The smaller repeat could be related to the absence of H1 or to the presence of an H1 rather different in composition from that of higher eukaryotes. Since both *Aspergillus* [30] and *Neurospora* [31] contain an H1-like histone, a connection between a small repeat size and the total absence of H1 is ruled out, but a correlation with altered composition of H1 cannot be excluded.

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References

[1] Kornberg, R. D. (1974) Science 184, 868–871.
[2] Olins, A. L. and Olins, D. E. (1974) Science 183, 330–332.
[3] Woodcock, C. L. F. (1973) J. Cell Biol. 59, 368 (a); Woodcock, C. L. F., Safer, J. P. and Stanchfield, J. E. (1976) Exp. Cell Res. 97, 101–110.
[4] Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Cell 4, 281–300.
[5] Finch, J. T., Noll, M. and Kornberg, R. D. (1975) Proc. Nat. Acad. Sci. USA 72, 3320–3322.
[6] Noll, M. (1974) Nature 251, 249–251.
[7] Burgoyne, L. A., Hewish, D. R. and Mobbs, J. (1974) Biochem. J. 143, 67–72.
[8] Noll, M. and Kornberg, R. D., submitted to Proc. Nat. Acad. Sci. USA.
[9] Thomas, J. O. and Kornberg, R. D. (1975) Proc. Nat. Acad. Sci. USA 72, 2626–2630.
[10] Thomas, J. O. and Kornberg, R. D. (1975) FEBS Lett. 58, 353–358.
[11] Noll, M. (1974) Nucleic Acids Res. 1, 1573–1578.
[12] Pardon, J. F., Worcester, D. L., Wooley, J. C., Tatchell, K., Van Holde, K. E. and Richards, B. M. (1975) Nucleic Acids Res. 2, 2163–2176.
[13] Shaw, B. R., Herman, T. M., Kovacic, R. T., Beaudreau, G. S. and Van Holde, K. E. (1976) Proc. Nat. Acad. Sci. USA 73, 505–509.

[14] Franco, L., Johns, E. W. and Naviet, J. (1974) Eur. J. Biochem. 45, 83–89.
[15] Lohr, D. and Van Holde, K. E. (1975) Science 188, 165–166.
[16] Morris, N. R. (1976) Cell, in the press.
[17] Noll, M. (1976) Cell, in the press.
[18] Hewish, D. R. and Burgoyne, L. A. (1973) Biochem. Biophys. Res. Comm. 52, 504–510.
[19] Wintersberger, U., Smith, P. and Letnansky, K. (1973) Eur. J. Biochem. 33, 123–130.
[20] Bhargava, M. M. and Halvorson, H. O. (1971) J. Cell Biol. 49, 423–429.
[21] Marmur, J. (1961) J. Mol. Biol. 3, 208–218.
[22] Loening, U. E. (1976) Biochem. J. 102, 251–257.
[23] Maniatis, T., Jeffrey, A. and van de Sande, H. (1975) Biochemistry 14, 3787–3794.
[24] Maxam, A. and Gilbert, W., personal communication.
[25] Noll, M., Thomas, J. O. and Kornberg, R. D. (1975) Science 187, 1203–1206.
[26] Sahasrabuddhe, C. G. and Van Holde, K. E. (1974) J. Biol. Chem. 249, 152–156.
[27] Sollner-Webb, B. and Felsenfield, G. (1975) Biochemistry 14, 2915–2920.
[28] Juni, E. and Heym, G. A. (1968) Arch. Biochem. Biophys. 127, 79–88.
[29] Kornberg, R. D. and Thomas, J. O. (1974) Science 184, 865–868.
[30] Felden, R. A., Sanders, M. M. and Morris, N. R. (1976) J. Cell Biol. 68, 430–439.
[31] Goff, C. (1976) J. Biol. Chem., in the press.
[32] Jeppeson, P. G. N., Sanders, L. and Slocombe, P. (1976) Nucleic Acids Res. 3, 1323–1339.