Biochem. J. (1979) **177**, 917–923
Printed in Great Britain

Analysis of Histones from the Yeast *Saccharomyces carlsbergensis*

By ALBERT PASTINK, THEO A. BERKHOUT, WILLEM H. MAGER and RUDI J. PLANTA
Biochemisch Laboratorium, Vrije Universiteit, de Boelelaan 1085, *Amsterdam, The Netherlands*

(*Received* 21 *August* 1978)

Basic chromosomal proteins were isolated from the chromatin of the yeast *Saccharomyces carlsbergensis* by extraction with H_2SO_4 and were purified by ion-exchange chromatography. Electrophoresis of the purified fraction on acetic acid/urea gels revealed the presence of four main components. These four proteins were identified as histones H2A, H2B, H3 and H4 on the basis of their amino acid composition, molecular weight and solubility properties, all of which are very similar to the corresponding properties of the various histone proteins from other eukaryotic organisms. A fifth basic protein could be isolated from yeast chromatin by extraction with $HClO_4$. The available evidence indicates this protein to be an H1-type histone. Yeast thus appears to contain a complete set of histone proteins which are strongly homologous to the histones occurring in higher eukaryotes.

The chromatin of all higher eukaryotic cells contains a group of very basic low-molecular-weight proteins, the histones. In animals as well as in higher plants these proteins are very well characterized. They appear to have been highly conserved during evolution. In all cases these basic proteins can be subdivided into five main types: H1, H2A, H2B, H3 and H4 (Elgin & Weintraub, 1975).

Much less is known about histones in lower eukaryotes. This lack of information is due to the difficulties in purifying the histones from chromatin, which in lower eukaryotes is present in rather small amounts as compared with higher cells. Histone proteins have been detected in algae (Iwai, 1964; Bradley *et al.*, 1974; Jardine & Leaver, 1978), slime moulds (Mohberg & Rush, 1969; Charlesworth & Parish, 1975), ciliated protozoa (Hamana & Iwai, 1971; Lipps & Hantke, 1974; Caplan, 1975; Felden *et al.*, 1976; Johmann & Gorovsky, 1976) and fungi (Indik *et al.*, 1975; Goff, 1976). The occurrence of histones in yeast has been studied in three strains. The presence of histones H2A, H3 and H4 in the yeast *Saccharomyces cerevisiae* has been demonstrated (Franco *et al.*, 1974; Brandt & von Holt, 1976). Histones H2B and H1 have so far not been positively identified in yeast.

In this paper we demonstrate the presence of histones H2A, H2B, H3 and H4 in the yeast *Saccharomyces carlsbergensis*. In addition evidence is presented for the occurrence of an H1-type histone in this yeast strain.

Materials and Methods

Isolation of histones

Saccharomyces carlsbergensis (strain S74) was grown in a medium containing 1% (w/v) glucose, 0.5% (w/v) bacteriological peptone, 0.3% (w/v) yeast extract and 0.3% (w/v) malt extract. Cells were harvested when the culture had reached the stationary phase. The cells were resuspended in 0.05M-sodium phosphate buffer (pH 6.5), containing 1mM-$MgSO_4$, 1mM-phenylmethanesulphonyl fluoride, 40mM-$NaHSO_3$ and 3mM-2-mercaptoethanol, and disrupted with glass beads in a Braun homogenizer (Braun A.G., Melsungen, Germany). Cell debris was removed by low-speed centrifugation and chromatin was isolated in essentially the same way as described by Tonino & Rozijn (1966). The chromatin was washed once with 0.05M-sodium phosphate buffer (pH 6.5), containing 1mM-$MgSO_4$, 1mM-phenylmethanesulphonyl fluoride, 40mM-$NaHSO_3$, 3mM-2-mercaptoethanol, 0.5% (w/v) Nonidet P-40 and then twice with a buffer containing 0.05M-Tris/HCl (pH 8.0), 0.15M-NaCl, 1mM-$MgSO_4$, 1mM-phenylmethanesulphonyl fluoride, 40mM-$NaHSO_3$, 3mM-2-mercaptoethanol and 0.5% (w/v) Nonidet P-40. The chromatin isolated in this way had an RNA/DNA ratio of about 4 (w/w) and a protein/DNA ratio of about 6 (w/w). These values are quite similar to those published by Wintersberger *et al.* (1973) for *Saccharomyces cerevisiae*.

The chromatin was extracted with 0.2M-H_2SO_4 and histones were precipitated from the 30000$g_{av.}$ supernatant by addition of 4vol. of ethanol. Extraction of the chromatin with 0.74M-$HClO_4$ was performed as described by Johns (1964).

Purification of yeast histones

Histones were purified by ion-exchange chromatography on a Bio-Rex 70 column. Bio-Rex 70 resin (Bio-Rad Laboratories, Richmond, CA, U.S.A.; 200–400 mesh) was washed and equilibrated with 8% (w/v) guanidine hydrochloride in 0.1M-sodium

phosphate buffer (pH 6.8) as described by Luck *et al.* (1958). The resin was packed in a column of 60cm × 2.5cm. Proteins, dissolved in 8% (w/v) guanidine hydrochloride in 0.1M-sodium phosphate buffer (pH 6.8), were applied to the column and chromatographed at a flow rate of 30ml/h. After elution of the flow-through fraction, the concentration of guanidine hydrochloride was raised to 40% (w/v) to elute the bound proteins. The protein content in the effluent was assayed by turbidity measurements (Bonner *et al.*, 1968). The protein fractions were dialysed against 2% (v/v) acetic acid for 48h and freeze-dried.

Electrophoresis

Proteins were analysed on disc gels (10 or 18cm) containing 15% (w/v) polyacrylamide, 2.5M-urea and 0.9M-acetic acid as described by Panyim & Chalkley (1969). Gels were stained for 30min with 0.5% (w/v) Amido Black 10B in ethanol/acetic acid/water (20:7:73, by vol.) and destained electrophoretically in 7% (v/v) acetic acid. In some cases the gels were further destained in 0.1M-$FeCl_3$ as described by Spiker & Key (1976).

For determination of molecular weights, proteins were electrophoresed on disc gels containing 15% (w/v) polyacrylamide and 0.1% (w/v) sodium dodecyl sulphate as described by Panyim & Chalkley (1971).

We also utilized a two-dimensional electrophoresis system, which is a combination of the two systems described above. In the first dimension we used an 18cm acetic acid/urea gel. After electrophoresis the gel was soaked in 0.025M-glycine/NaOH (pH 10) containing 0.1% (w/v) sodium dodecyl sulphate (Panyim & Chalkley, 1971). Subsequently the gel was polymerized on top of a 15% (w/v) polyacrylamide slab gel (20cm × 20cm × 0.2cm) containing 0.1% (w/v) sodium dodecyl sulphate (Panyim & Chalkley, 1971). Electrophoresis was performed at 60V for 16h.

Gels containing sodium dodecyl sulphate were stained for 16h with 0.1% (w/v) Coomassie Brilliant Blue in 25% (v/v) methanol/12% (w/v) trichloroacetic acid/3% (w/v) sulphosalicylic acid and destained by diffusion in a solution containing 7% (v/v) methanol and 7% (v/v) acetic acid.

Fractionation of yeast histones

Yeast histones were fractionated by the method described by Johns (1964), as modified by Oliver *et al.* (1972).

Amino acid analysis

Yeast histones were electrophoresed on 18cm acetic acid/urea gels as described above. After staining, individual bands were cut from the gel and homogenized by syringing. Protein was then eluted by shaking with 70% (v/v) acetic acid for 48h at 4°C (Hardy, 1975). After sedimentation of the gel fragments, the eluates were dialysed against water and freeze-dried. Protein samples were hydrolysed *in vacuo* in 6M-HCl at 110°C for 24h. Analysis of the amino acid composition was performed by using a Biotronik LC-6000 amino acid analyser (Biotronik, Munich, Germany).

Assays for DNA, RNA and protein

DNA assays were performed by the diphenylamine method by using calf thymus DNA as a standard (Burton, 1956). RNA was measured by the orcinol method with yeast rRNA as a standard (Ceriotti, 1955). Protein was assayed as described by Lowry *et al.* (1951), with lysozyme as a standard.

Results

Isolation and purification of histones

When yeast chromatin was treated with H_2SO_4 and the proteins extracted were electrophoresed on acetic acid/urea gels several strong bands migrating at approximately the same positions as calf thymus histones could be seen (Fig. 1, lane b). In addition, a large number of weaker bands representing slower-moving proteins was also visible. Fractionation of the proteins extracted from chromatin on a Bio-Rex 70 column by elution with different concentrations of guanidine hydrochloride (see the Materials and Methods section) resulted in a significant further purification of the putative yeast histones (Fig. 1, lane d). Approx. 50% of the protein applied to the column was not bound by the resin in the presence of 8% (w/v) guanidine hydrochloride. This fraction contains most of the slower-moving components present in the protein mixture (Fig. 1, lane c). The putative histone proteins are almost exclusively contained in the fraction eluted with 40% (w/v) guanidine hydrochloride. They are, however, still slightly contaminated with other material (Fig. 1, lane d). The main components visible in lane (d) of Fig. 1 were designated A, B, C and D in order of increasing mobility. Component D co-migrates with calf thymus histone H4 and, like histone H4, is split into two bands. Component A is also split into two bands. In order to identify and characterize proteins A–D further we determined the molecular weight, amino acid composition and solubility properties of the individual proteins purified by ion-exchange chromatography and polyacrylamide-gel electrophoresis as described above.

Determination of molecular weights

The molecular weights of the individual yeast histones were determined on gels containing sodium dodecyl sulphate. In order to be able to identify the various proteins after electrophoresis under these

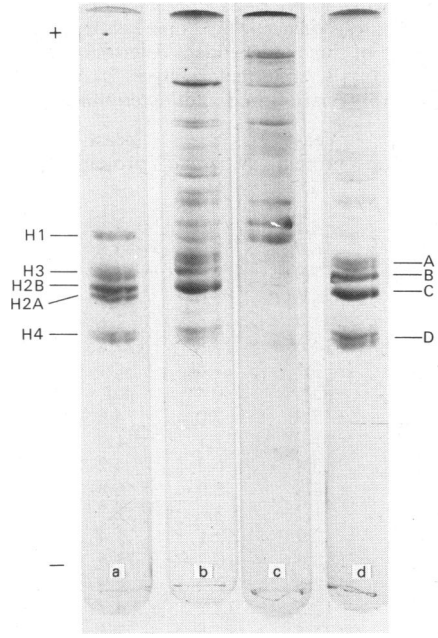

Fig. 1. *Gel electrophoresis of the proteins extracted from yeast chromatin with H_2SO_4*

Lane a, calf thymus histones; lane b, total protein extracted from yeast chromatin; lane c, flow-through fraction after chromatography on a Bio-Rex 70 column; lane d, protein eluted with 40% (w/v) guanidine hydrochloride from the Bio-Rex 70 column. Calf thymus histones were designated according to Panyim & Chalkley (1969). Electrophoresis was performed on 10cm acetic acid/urea gels as described in the Materials and Methods section.

conditions, we used a two-dimensional separation technique, employing an acetic acid/urea gel for the first dimension and a sodium dodecyl sulphate-containing gel for the second dimension (see the Materials and Methods section). The results are shown in Fig. 2(*a*). The order of migration of the yeast proteins in the presence of sodium dodecyl sulphate was the same as on acetic acid/urea gels, except that protein B instead of moving behind protein C now migrated somewhat faster than it. Neither protein A nor protein D showed splitting into multiple bands in the presence of sodium dodecyl sulphate.

Fig. 2(*b*) shows a one-dimensional separation of proteins A–D on a gel containing sodium dodecyl sulphate (lane a). Using calf thymus histones (Fig. 2*b*, lane b) as molecular-weight markers (Panyim & Chalkley, 1971), we calculated the mol.wt. of proteins A and C to be 14000, whereas proteins B and D had mol.wts. of 12500 and 11000 respectively.

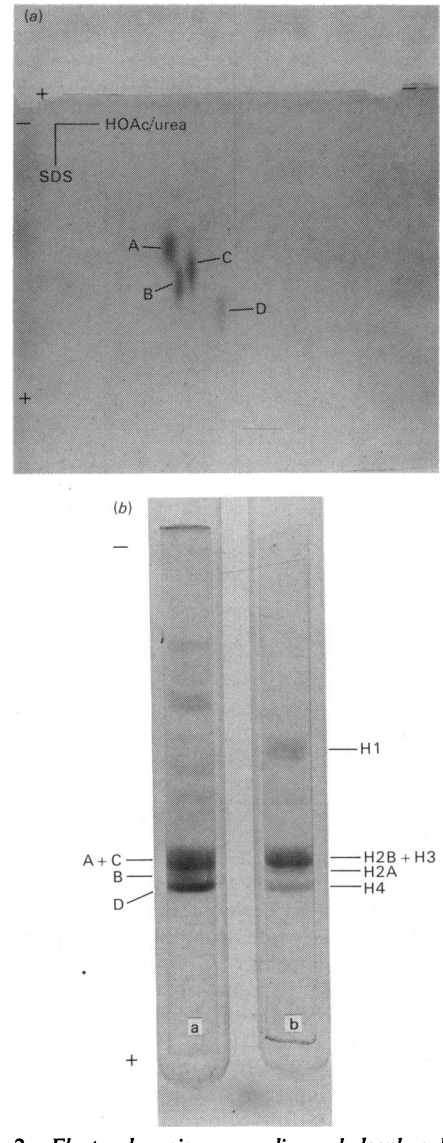

Fig. 2. *Electrophoresis on sodium dodecyl sulphate-containing gels*

(*a*) Electrophoresis of chromatographically purified yeast chromosomal proteins extracted with H_2SO_4 on an 18cm acetic acid (HOAc) urea gel (first dimension) and a sodium dodecyl sulphate (SDS)-containing slab gel (second dimension). Electrophoresis was performed as described in the Materials and Methods section. (*b*) Electrophoresis on 10cm sodium dodecyl sulphate-containing disc gels. Lane a, purified basic chromosomal proteins from yeast; lane b, calf thymus histones.

Amino acid analysis

Table 1 shows the amino acid composition of proteins A–D purified by Bio-Rex 70 column

Table 1. *Amino acid analysis of yeast basic chromosomal proteins compared with Neurospora crassa histones*
Individual yeast basic chromosomal proteins were obtained after electrophoresis on acetic acid/urea gels as described in the Materials and Methods section. Values are expressed as the average of three independent determinations. No corrections have been made for destruction of amino acids or incomplete hydrolysis. Amino acid analyses for *Neurospora crassa* histones were taken from Goff (1976). All values are mol/100mol. Abbreviation: n.d., not determined.

Amino acid residue	Yeast protein A	*N. crassa* H2B	Yeast protein B	*N. crassa* H2A	Yeast protein C	*N. crassa* H3	Yeast protein D	*N. crassa* H4	Yeast protein E	*N. crassa* H1
Asx	6.0	7.0	8.3	8.2	4.4	5.6	4.1	5.0	7.9	7.2
Thr	7.6	7.7	5.0	4.1	7.0	5.6	6.7	7.7	3.8	6.3
Ser	10.8	11.8	7.8	5.8	7.7	8.4	7.9	3.2	7.4	5.7
Glx	10.5	7.6	9.9	9.7	11.3	11.8	8.6	7.0	9.7	8.5
Pro	3.2	4.9	2.2	3.9	2.2	4.0	1.4	1.5	1.6	7.6
Gly	9.0	5.0	10.8	12.1	6.8	7.0	14.6	14.8	17.8	5.8
Ala	13.1	12.7	14.4	12.3	11.5	10.1	8.1	7.4	6.1	21.1
Cys	n.d.	<0.1	n.d.	<0.1	n.d.	0.5	n.d.	0.4	n.d.	0.4
Val	4.9	5.1	4.5	5.6	4.9	5.0	5.3	7.0	4.8	4.9
Met	0.6	0.8	0.2	0.1	0.4	0.4	—	1.1	1.9	1.2
Ile	4.9	5.3	4.3	4.4	4.8	5.0	5.4	6.2	4.2	2.0
Leu	6.6	4.7	10.6	11.2	9.6	8.9	8.8	8.0	6.8	4.8
Tyr	2.1	3.7	2.0	2.4	1.4	2.4	1.7	3.6	1.7	1.5
Phe	1.9	2.1	1.3	1.3	4.0	3.2	2.9	2.1	3.4	1.8
Lys	10.8	15.8	8.8	9.2	10.6	9.5	10.0	10.2	14.5	16.8
His	2.2	1.7	2.0	2.8	1.7	1.9	2.0	2.2	2.9	1.5
Arg	5.7	4.4	7.8	6.9	11.9	11.1	12.5	12.2	4.7	3.4
Percentage of basic amino acids*	18.7	21.9	18.6	18.9	24.2	22.5	24.5	24.6	22.1	21.7
Lys/Arg	1.9	3.6	1.1	1.3	0.9	0.9	0.8	0.8	3.1	4.9

* Sum of histidine, lysine and arginine.

chromatography followed by electrophoresis on an acetic acid/urea gel. The two components of both protein A and protein D were taken together for this analysis. Obviously proteins A and B are lysine-rich proteins, whereas proteins C and D are arginine-rich proteins. Comparison of these amino acid compositions with those of the individual histones from *Neurospora crassa* (Goff, 1976) revealed that protein A closely resembles histone H2B, that protein B is very similar to histone H2A and that the amino acid compositions of proteins C and D closely match those of histones H3 and H4 respectively.

Solubility properties

The solubility properties of proteins A–D were determined by applying the differential extraction procedure of Johns (1964) as modified by Oliver *et al.* (1972) to the protein fraction eluted from the Bio-Rex 70 column by 40% guanidine hydrochloride. Treatment with 5% (w/v) $HClO_4$ predominantly leaves protein A in solution. Traces of proteins B and C can also be detected in the extract (Fig. 3, lane a). Proteins C and D, and to a lesser extent protein B, are soluble in ethanol/1.25M-HCl (4:1, v/v), whereas protein A is completely insoluble under these conditions (Fig. 3, lanes b and c). Finally dialysis of the ethanol/1.25M-HCl (4:1, v/v) extract against ethanol causes preferential precipitation of protein C (Fig. 3, lanes d and e). These results again indicate a close resemblance between protein A and histone H2B, between protein B and histone H2A and finally between proteins C and D and histones H3 and H4 respectively.

Destaining in $FeCl_3$

The identification of protein A as an H2B-type histone is further supported by the fact that protein A loses nearly all detectable dye on destaining of an acetic acid/urea gel in $FeCl_3$, whereas the other three proteins remain clearly visible (Fig. 4).

Extraction of chromatin with $HClO_4$

Bio-Rex 70 chromatography of the H_2SO_4 extract of yeast chromatin did not reveal the presence of an H1-type histone. When chromatin was extracted with 0.74M-$HClO_4$, however, one main component was present in the extract which migrated at almost the same position as calf thymus histone H1 (Fig. 5). We designated this protein as protein E. The molecular weight of protein E as determined on gels containing sodium dodecyl sulphate is 17000 (results not shown). Determination of the amino

acid composition of this protein, after electrophoresis on acetic acid/urea gels, revealed a high lysine/arginine ratio (Table 1). Its content of alanine and proline, however, is rather low, as compared with histone H1 from *Neurospora crassa*, whereas its content of glycine is quite high.

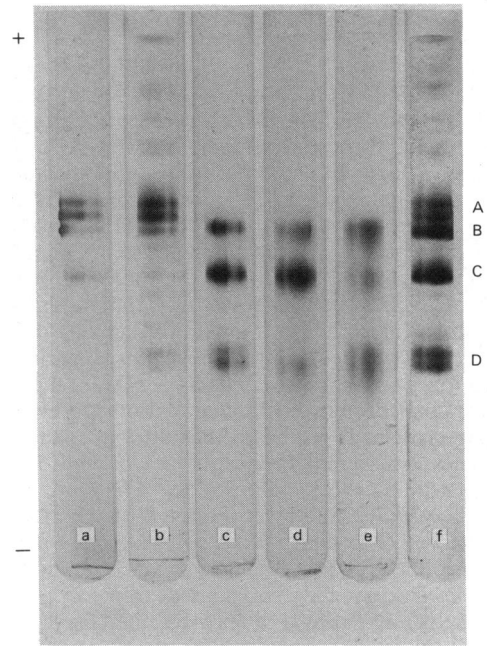

Fig. 3. *Electrophoretic analysis of acid-extractable chromosomal proteins from yeast after chemical fractionation*
Proteins were fractionated by the method of Johns (1964) as modified by Oliver *et al.* (1972). Electrophoresis was performed on 18cm acetic acid/urea gels as described in the Materials and Methods section. Lane a, $HClO_4$ extract; lane b, ethanol/1.25M-HCl (4:1, v/v) precipitate; lane c, ethanol/1.25M-HCl (4:1, v/v)-soluble fraction; lane d, protein fraction precipitated during dialysis against ethanol; lane e, protein fraction remaining in solution during dialysis against ethanol; lane f, total protein before chemical fractionation.

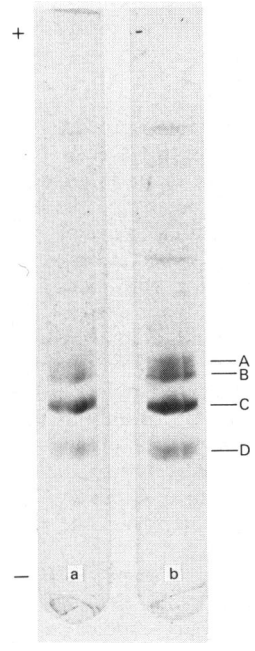

Fig. 4. *Sensitivity of protein A to $FeCl_3$ destaining*
Chromatographically purified basic chromosomal proteins from yeast were electrophoresed on 10cm acetic acid/urea gels as described in the Materials and Methods section. After electrophoretic destaining, further destaining followed in 0.1M-$FeCl_3$ as described by Spiker & Key (1976). Lane a, protein pattern after $FeCl_3$ destaining; lane b, protein pattern before destaining in $FeCl_3$.

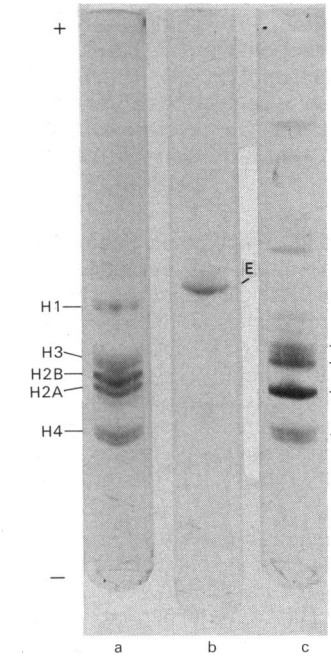

Fig. 5. *Gel electrophoresis of proteins extracted with $HClO_4$*
Extraction of yeast chromatin with 0.74M-$HClO_4$ was performed as described by Johns (1964). Electrophoresis was performed on 10cm acetic acid/urea gels as described in the Materials and Methods section. Lane a, calf thymus histones; lane b, $HClO_4$ acid extract from yeast chromatin; lane c, H_2SO_4-extractable proteins after Bio-Rex 70 chromatography.

Discussion

Protein A

The amino acid composition of protein A is very similar to that of histone H2B from *Neurospora crassa* (Table 1). The main differences are the somewhat lower lysine content and the higher content of glutamine and glutamic acid of protein A. Like histone H2B from higher eukaryotes, protein A is sensitive to destaining with $FeCl_3$ (Spiker & Key, 1976). Similarly to plant histone H2B (Spiker & Key, 1976), but unlike animal histone H2B (Johns, 1964), protein A is partially soluble in $HClO_4$. In addition protein A resembles histone H2B from higher eukaryotes in its insolubility in ethanol/1.25M-HCl (4:1, v/v) (Fig. 3). Finally the molecular weight of protein A is identical with that of calf thymus histone H2B (Panyim & Chalkley, 1971). Therefore we conclude that protein A is the H2B-type histone in yeast.

Unlike vertebrate histone H2B, yeast H2B is resolved into two bands on acetic acid/urea gels (Fig. 1). This probably indicates that this yeast protein species can be modified (e.g. by acetylation), as can histone H2B from *Tetrahymena pyriformis* (Johmann & Gorovsky, 1976).

Protein B

The amino acid composition of protein B closely resembles that of histone H2A from *Neurospora crassa* (Table 1). It is rich in lysine, alanine and leucine, and both its ratio of lysine to arginine and its content of basic amino acids are very similar to the corresponding values reported for *Neurospora crassa* H2A (Goff, 1976). Furthermore, like histone H2A from higher eukaryotes (Johns, 1964), protein B is soluble in ethanol (Fig. 3). Its molecular weight is the same as that of calf thymus histone H2A (Panyim & Chalkley, 1971). We therefore consider protein B to be the H2A-type histone in yeast.

Protein C

In amino acid composition, this protein shows a very close resemblance to histone H3 from *Neurospora crassa* (Table 1). Moreover, like histone H3 from higher eukaryotes (Johns, 1964), protein C is soluble in ethanol/1.25M-HCl (4:1, v/v) and is precipitated in ethanol (Fig. 3). The molecular weight of protein C is the same as that of calf thymus histone H3 (Panyim & Chalkley, 1971). So protein C is very likely to be the H3-type histone in yeast.

Protein D

The amino acid composition of protein D is closely similar to that of histone H4 from *Neurospora crassa*. Protein D shares its solubility in ethanol (Fig. 3) with histone H4 from higher eukaryotes (Johns, 1964), and its molecular weight is the same as reported for calf thymus histone H4 (Panyim & Chalkley, 1971). Furthermore, like histone H4 from higher eukaryotes, protein D is resolved into two bands on electrophoresis in acetic acid/urea gels (Fig. 1), which presumably reflects the presence of acetylated residues in a portion of the protein molecules. We therefore conclude that protein D is the H4-type histone in yeast.

Protein E

Protein E has some important features in common with histone H1 from *Neurospora crassa*. Its solubility in $HClO_4$ is characteristic for H1-type histones (Johns, 1964). Its high lysine/arginine ratio also indicates that it is an H1-type histone. However, unlike *Neurospora crassa* histone H1, protein E shows a low alanine and proline content and a high glycine content. In addition, the molecular weight of protein E is somewhat lower than that reported for calf thymus histone H1 (Panyim & Chalkley, 1971).

Though we used proteinase inhibitors during the isolation procedure, we cannot exclude the possibility that protein E is a degradation product of a larger protein. It is known that during isolation of histones *N*-terminal fragments can be split off by proteolytic enzymes (Bartley & Chalkley, 1970; Brandt *et al.*, 1975). Moreover, the *N*-terminal region of histone H1 in higher eukaryotes is rich in alanine and proline (Elgin & Weintraub, 1975). Therefore protein E may have originated from the actual histone H1 by removal of an *N*-terminal fragment.

It is unlikely that protein E is a yeast equivalent of the so-called high-mobility-group proteins isolated from the chromatin of higher eukaryotic cells by extraction with $HClO_4$ since its content of acidic amino acids (Table 1) is distinctly different from that characteristic for these high-mobility-group proteins (Johns, 1964; Franco *et al.*, 1977). Protein E also is not a ribosomal protein, because treatment of isolated yeast ribosomes with $HClO_4$ did not result in the extraction of an E-type protein.

In conclusion, the results presented in this paper demonstrate that yeast chromatin contains the normal complement of histone proteins as far as histones H2A, H2B, H3 and H4 are concerned. The similarity of yeast histones H2A, H2B, H3 and H4 to their respective counterparts from higher eukaryotes once more emphasizes the high evolutionary conservation of the moderately lysine-rich and arginine-rich histones. The identification of histone H1 in yeast is less certain. Protein E seems to be a good candidate for the yeast H1-type histone, but it is not certain whether protein E might be a degradation product of the actual histone H1, which in other organisms is known to be highly susceptible to proteolytic degradation.

Note Added in Proof (Received 6 October 1978)

After submission of this paper, both Suchiliene & Gineitis (1978) and Sommer (1978) reported the presence of an H1-type histone in the yeast *Saccharomyces cerevisiae*. Their results are in good agreement with the data presented in this paper.

This work was supported in part by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for Pure Research (Z.W.O.). The authors are indebted to Miss M. Samson for skilful technical assistance, to Miss A. Huysmans for performing the amino acid analysis and to Dr. H. A. Raué for critically reading the manuscript.

References

Bartley, J. & Chalkley, R. (1970) *J. Biol. Chem.* **245**, 4286–4292

Bonner, J., Chalkley, G. R., Dahmus, M., Fambrough, D., Fujimura, F., Huang, R. C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivera, B. & Widholm, J. (1968) *Methods Enzymol.* **12B**, 3–65

Bradley, D. M., Goldin, H. H. & Claybrook, J. R. (1974) *FEBS Lett.* **41**, 219–222

Brandt, W. F. & von Holt, C. (1976) *FEBS Lett.* **65**, 386–390

Brandt, W. F., Böhm, L. & von Holt, C. (1975) *FEBS Lett.* **51**, 88–93

Burton, K. (1956) *Biochem. J.* **62**, 315–319

Caplan, E. B. (1975) *Biochim. Biophys. Acta* **407**, 109–112

Ceriotti, G. (1955) *J. Biol. Chem.* **214**, 59–70

Charlesworth, M. C. & Parish, R. W. (1975) *Eur. J. Biochem.* **54**, 307–315

Elgin, S. C. R. & Weintraub, H. (1975) *Annu. Rev. Biochem.* **44**, 725–774

Felden, R. A., Sanders, M. M. & Morris, N. R. (1976) *J. Cell Biol.* **68**, 430–439

Franco, L., Johns, E. W. & Navlet, J. M. (1974) *Eur. J. Biochem.* **45**, 83–89

Franco, L., Montero, F. & Rodriguez-Molina, J. J. (1977) *FEBS Lett.* **78**, 317–320

Goff, C. G. (1976) *J. Biol. Chem.* **251**, 4131–4138

Hamana, K. & Iwai, K. (1971) *J. Biochem. (Tokyo)* **69**, 1097–1111

Hardy, S. J. S. (1975) *Mol. Gen. Genet.* **140**, 253–274

Indik, Z. K., Keller, B. J. & Marks, D. B. (1975) *Arch. Biochem. Biophys.* **170**, 315–325

Iwai, K. (1964) in *The Nucleohistones* (Bonner, J. & T'so, P. O. P., eds.), pp. 59–71, Holden-Day, San Francisco

Jardine, N. J. & Leaver, J. R. (1978) *Biochem. J.* **169**, 103–110

Johmann, C. A. & Gorovsky, M. A. (1976) *Biochemistry* **15**, 1249–1256

Johns, E. W. (1964) *Biochem. J.* **92**, 55–59

Lipps, H. J. & Hantke, K. G. (1974) *Chromosoma (Berlin)* **49**, 309–320

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Luck, J. M., Rasmussen, P. S., Satake, K. & Tsvetikov, A. N. (1958) *J. Biol. Chem.* **233**, 1407–1414

Mohberg, J. & Rush, H. P. (1969) *Arch. Biochem. Biophys.* **134**, 577–589

Oliver, D., Sommer, K. R., Panyim, S., Spiker, S. & Chalkley, R. (1972) *Biochem. J.* **129**, 349–353

Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130**, 337–346

Panyim, S. & Chalkley, R. (1971) *J. Biol. Chem.* **246**, 7557–7560

Sommer, A. (1978) *Mol. Gen. Genet.* **161**, 323–331

Spiker, S. & Key, J. L. (1976) *Arch. Biochem. Biophys.* **176**, 510–518

Suchiliene, S. P. & Gineitis, A. A. (1978) *Exp. Cell Res.* **114**, 454–458

Tonino, G. J. & Rozijn, T. H. (1966) *Biochim. Biophys. Acta* **124**, 427–429

Wintersberger, U., Smith, P. & Letnansky, K. (1973) *Eur. J. Biochem.* **33**, 123–130