PRELIMINARY NOTES

BBA 21144

On the occurrence of histones in yeast

In recent studies on the role of histones (reviewed by BUSCH[1]) attention is focussed on the possibility that histones function as gene suppressors in the process of tissue differentiation. Experimental evidence is accumulating, however, which indicates that histones, and the very lysine-rich histone in particular, also function in the structural organization of chromatin and chromosomes (*e.g.* refs. 1, 2).

In view of these supposed functions we may expect histones to be absent in unicellular organisms, particularly in those that do not show condensed metaphase chromosomes. Good evidence for the presence of histones in bacteria is lacking[3], while a very lysine-rich type of histone is found in unicellular organisms in which condensed chromosomes do occur, such as Chlorella[4] and Tetrahymena[5].

As yeast is an organism that does not form condensed chromosomes during cell division[6], but, in contrast to bacteria, contains a well defined nucleus[6], we thought that an investigation on the presence of histones in yeast could be very useful in the study of the function of histones.

The present communication reports the extraction of proteins resembling histones from yeast chromatin.

From pressed baker's yeast ("Koningsgist", Delft) chromatin can be obtained by the following procedure. 20 g of yeast, suspended in 35 ml 0.05 M phosphate buffer (pH 6.5), containing 1 mM $MgSO_4$ (Medium A), is disintegrated by shaking with 45-ml glass beads (diameter 0.25 mm) for 5 min in the apparatus of MERKENSCHLAGER[7]. The glass beads are allowed to settle and the supernatant is then decanted and centrifuged for 40 min at 8000 $\times$ *g*. A sediment is obtained which consists of 2 layers. The brown loosely packed upper layer is resuspended by shaking with fresh Medium A and next centrifuged for 20 min at 8000 $\times$ *g*. The resulting pellet is washed twice with Medium A and once with Medium B (0.05 M Tris buffer (pH 8.0) containing 1 mM $MgSO_4$). The washed pellet is resuspended in Medium B to a concentration of 0.5 mg DNA/ml and homogenized in a Potter–Elvehjem type homogenizer with a very close-fitting Teflon pestle. Of the homogenate, 10 ml is layered on a sucrose gradient consisting of 10 ml 2 M sucrose and 10 ml 1.5 M sucrose, both in Medium B, and centrifuged for 1–2 h in a SW-25 swinging-bucket head at 25000 rev./min. The purified chromatin is then obtained as a pellet. All manipulations are performed in the cold (0–5°). The isolated chromatin contains DNA, RNA and protein in a ratio of 1:0.3:4.3.

A weakly basic protein, further called histone, can be extracted from the chromatin with cold 0.25 M HCl. The amino acid composition of the extracted protein is given in Table I (first column).

Fractionation of this histone was achieved by chromatography on CM-cellulose[8]. Two fractions could be eluted with 0.17 M acetic acid–0.05 M NaOH–0.42 M NaCl

(pH 4.2) and with 0.02 M HCl. Each of these fractions comprised about half of the protein applied to the column. In contrast to thymus histones[8], yeast histones do not contain a fraction that can be eluted with 0.01 M HCl.

Both fractions are completely soluble in the reagent of MIRSKY AND POLLISTER[9], as are all histones. Electrophoresis of the isolated fractions on polyacrylamide gel at pH 4.2 showed that they differed greatly in electrophoretic behaviour and that

TABLE I

AMINO ACID COMPOSITION OF YEAST HISTONES

Values are expressed as moles %. A refers to acidic amino acids; B to basic amino acids.

Amino acids		*Whole yeast histone*	*Fraction 1*	*Fraction 2*	*Trichloroacetic acid-extractable*
Asp	A	7.4	7.8	6.7	11.0
Glu	A	10.3	10.7	10.8	12.3
Gly		7.4	6.5	7.5	17.1
Ala		10.1	11.3	9.3	8.0
Val		5.7	5.3	6.0	3.7
Leu		8.5	8.2	9.0	4.4
Ile		5.8	5.7	5.8	2.7
Phe		2.6	2.1	3.2	1.7
Tyr		2.7	2.7	2.2	1.7
Trp*		0.5	0.4	0.4	—
Ser		8.4	9.1	7.4	13.2
Thr		5.9	6.1	6.1	5.2
Pro		4.1	4.3	3.6	5.9
Met		0.6	0.7	0.5	0.5
Cys		0	0	0	0
Arg	B	7.4	5.5	9.2	2.8
His	B	2.1	2.0	2.1	2.1
Lys	B	10.8	11.6	10.4	7.8
NH_3**		7.0	8.5	7.9	—
B/A		1.1	1.0	1.2	0.5
Lys/Arg		1.5	2.1	1.1	2.8

* Tryptophan determined according to BEAVEN AND HOLIDAY[11].
** These values were consistent with those for amide ammonia estimated directly.

both were still rather heterogeneous. The amino acid composition of the 2 fractions (Table I) shows that a very lysine-rich type of histone is absent in yeast. Both fractions, however, show some resemblance with the other fractions of thymus histones, although they are less basic in terms of the ratio of basic to acidic amino acids (B/A, Table I). Electrophoresis at pH 9 in starch gel containing 4 M urea showed that both fractions moved to the cathode. Since ammonia was found in the amino acid analysis (Table I), the basicity in these electrophoresis experiments must be ascribed mainly to the presence of acidic amino acids in the amide form. To exclude the possibility that Fraction 1 does contain a very lysine-rich histone contaminated with an acidic protein, we extracted whole yeast histone with 5 % trichloroacetic acid[10]. Only 5 % could thus be extracted and amino acid analysis (Table I) revealed that the extracted protein had no resemblance at all to the very lysine-rich histone found in other organisms.

That the acid-extractable proteins were present in the chromatin associated

with the DNA, could be shown in further experiments. We found that extraction of the purified chromatin with water yielded an extract in which all of the material behaved as a single nucleoprotein complex during sucrose-gradient centrifugation, and this complex contained essentially all of the acid-extractable proteins found in the chromatin.

From the foregoing experiments we can conclude that the acid-extractable proteins in yeast chromatin are histones, as they are basic proteins associated with the DNA. These proteins, however, although having some resemblance with the histones of higher organisms, differ from them in several respects. Particularly striking is the absence of a very lysine-rich type of histone, which is found in all higher organisms and which is probably the predominant type of histone in unicellular organisms like Chlorella[4] and Tetrahymena[5]. This would support the view advanced above that the very lysine-rich type of histone appears to be present only in those organisms that show condensed chromosomes. We are therefore convinced that the very lysine-rich histone plays an essential role in the structural organisation of metaphase chromosomes.

The very interesting question whether the yeast histones with their different composition are also able to inhibit the priming function of DNA in the RNA-polymerase reaction is now being investigated.

The authors wish to thank Dr. C. H. MONFOORT for the amino acid analysis. This work was supported in part by U.S. Public Health Service Research Grant TW-50-03 and by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

Laboratory for Physiological Chemistry,
The State University,
Utrecht (The Netherlands)

G. J. M. TONINO
TH. H. ROZIJN

1 H. BUSCH, *Histones and other nuclear Proteins*, Academic Press, New York, 1965, p. 91.
2 V. C. LITTAU, C. J. BURDICK, V. G. ALLFREY AND A. E. MIRSKY, *J. Cell. Biol.*, 27 (1965) 124A.
3 J. S. ROTH, *Nature*, 207 (1965) 599.
4 K. IWAI, in J. BONNER AND P. TS'O, *The Nucleohistones*, Holden-Day, San Francisco, 1964, p. 59.
5 K. IWAI, H. SHIOMI, T. ANDO AND T. MITA, *J. Biochem. Tokyo*, 58 (1965) 312.
6 TH. H. ROZIJN AND G. J. M. TONINO, *Biochim. Biophys. Acta*, 91 (1964) 105.
7 M. MERKENSCHLAGER, *Biochem. Z.*, 329 (1957) 332.
8 E. W. JOHNS, D. M. P. PHILLIPS, P. SIMSON AND J. A. V. BUTLER, *Biochem. J.*, 77 (1960) 631.
9 A. E. MIRSKY AND A. W. POLLISTER, *J. Gen. Physiol.*, 30 (1946) 117.
10 E. H. DE NOOY AND H. G. K. WESTENBRINK, *Biochim. Biophys. Acta*, 62 (1962) 608.
11 G. H. BEAVEN AND E. R. HOLIDAY, *Advan. Protein Chem.*, 7 (1952) 369.

Received April 25th, 1966