The Fractionation of Histones Isolated from *Euglena gracilis*

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1. The histones of *Euglena gracilis* were separated by gel filtration into five fractions.
2. Each fraction was characterized in terms of its electrophoretic, solubility and compositional properties.
3. *Euglena gracilis* clearly contains histones corresponding to vertebrate H1, H2B, H3 and H4 fractions, although they all differ in containing more lysine.
4. The remaining *Euglena* histone is considered to be homologous to vertebrate histone H2A, but it differs in having a much higher ratio of lysine to arginine.
5. The *Euglena* histone H1 appears to be lacking in aspartic acid.
6. Electrophoresis in the presence of sodium dodecyl sulphate indicates that the molecular weights of the *Euglena* histones are close to those of the homologous vertebrate histones.

The histones of higher organisms have been extensively characterized in recent years, and their role in the structure and function of chromatin is being elucidated (Elgin & Weintraub, 1975). However, much less is known about the histones of lower organisms. They appear to be absent from prokaryotes (Leaver & Cruft, 1966; Raaf & Bonner, 1968; Makino & Tsuzuki, 1971), except that a protein resembling histone H2B[†] has been found in *Thermoplasma acidophilum* (Searcy, 1975). Histones are present in slime moulds (Mohberg & Rusch, 1969), the fungi *Aspergillus nidulans* (Felden *et al.*, 1976) and *Neurospora crassa* (Goff, 1976), baker's yeast (Franco *et al.*, 1974; Brandt & von Holt, 1976), the flagellate *Euglena gracilis* (Jardine & Leaver, 1975) and the ciliated protozoa *Tetrahymena pyriformis* (Hamana & Iwai, 1971; Johmann & Gorovsky, 1976), *Stylonychia mytilus* (Lipps & Hantke, 1974) and *Oxytricha* spp. (Caplan, 1975). The histones of the ciliate *Paramecium aurelia* appear to be only weakly basic (Isaacks & Santos, 1973), in contrast with those of the other ciliates studied. However, the occurrence of histones in lower eukaryotes is apparently not universal, since they have not been found in fission yeast (Duffus, 1971) or in dino-flagellates (Rizzo & Noodén, 1974*a,b*).

A comparison of the histones of lower organisms may yield information about the evolution of these proteins and perhaps of the organisms themselves. Furthermore, comparison of protozoal histones with those of higher organisms might provide insight into structural and functional relationships of the individual histones. However, only for *Tetrahymena* and *Neurospora* have the histones of lower eukaryotes

been fully characterized (Hamana & Iwai, 1971; Johmann & Gorovsky, 1976; Goff, 1976), and partial fractionations have been achieved for the histones of baker's yeast (Franco *et al.*, 1974; Brandt & von Holt, 1976), the slime mould *Physarum* (Mohberg & Rusch, 1969) and *Aspergillus* (Felden *et al.*, 1976).

Euglenoid organisms are a taxonomically isolated group, exhibiting a number of primitive characteristics that include chromosomes condensed throughout the cell cycle and an unusual mitosis (Leedale, 1967, 1968). The latter is characterized by the retention of the nucleolus and nuclear membrane, together with the absence of centromeres and a conventional spindle apparatus. Similar features are found in dinoflagellates (Kubai & Ris, 1969; Ris & Kubai, 1970; Haapala & Soyer, 1973), but we now show that, in contrast with these organisms, *Euglena* contains a full complement of five histones, which closely resemble those of higher organisms.

Materials and Methods

Culture of cells and isolation of nuclei

Euglena gracilis Klebs strain Z (Culture Collection of Algae and Protozoa, Botany School, University of Cambridge, Cambridge, U.K.) was grown in 9 litres of an acidic heterotrophic medium (Hutner *et al.*, 1966) contained in 10-litre bottles. The cultures were continuously illuminated by four 60 cm 20 W fluorescent tubes (Philips Reflectalite 35) and mixed by pumping in air sterilized by miniature line filter (model LF32; Microflow, Fleet, Hants., U.K.). The cells were harvested in late exponential phase by using the flow-through rotor of a Sharples Super centrifuge (model 1A; Pennsalt, Camberley, Surrey, U.K.) operated at approx. 20000 rev./min.

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† Ciba nomenclature for histones (Bradbury, 1975).

The cells were mixed with 2 vol. of 0.44 M-sucrose/10 mM-MgCl₂/2% (v/v) acetic acid/2% (v/v) Triton N101/0.1% (w/v) spermidine. The pH of this medium is 3.3. All the above constituents in the medium are necessary for providing adequate stabilization of nuclei during the isolation procedure while acetic acid and the detergent Triton N101 solubilize unwanted cellular material, notably chloroplasts (Jardine & Leaver, 1977). The suspension was frozen and thawed three times by using a -60°C deep-freeze cabinet, and was then further diluted with 2 vol. of solution as above but without Triton N101. All succeeding operations were performed at 0-4°C.

The cells were broken by passage through a pre-cooled French pressure cell (Aminco, Silver Springs, MD, U.S.A.) at 33 MPa piston pressure applied with a manual hydraulic ram. The suspension of broken cells, which has a pH of approx. 3.6, was left at 0-4°C for 5 min to allow paramylon granules (carbohydrate-storage particles) to aggregate, and these were then sedimented by centrifugation at 50g (*r*_{av.} 11 cm). The supernatant was carefully removed by pipette, and any nuclei trapped in the paramylon residue were recovered by its resuspension in 0.44 M-sucrose/10 mM-MgCl₂ (adjusted to pH 3.6 with acetic acid) and repetition of the aggregation and centrifugation. The supernatants were combined and nuclei sedimented by centrifugation at 1300g (*r*_{av.} 11 cm) for 15 min. The nuclei were further purified by resuspension of the pellet in 0.44 M-sucrose/10 mM-MgCl₂/0.2% Triton N101/0.03% spermidine (adjusted to pH 3.6 with acetic acid) and centrifugation at 600g (*r*_{av.} 11 cm). This purification procedure was repeated twice.

Preparation of histone

Chromatin was prepared from the nuclei by a method based on that of Spelsberg & Hnilica (1971). The nuclear pellet was homogenized successively with (a) 0.35 M-NaCl (Johns & Forrester, 1969), (b) 0.08 M-NaCl/0.02 M-EDTA adjusted to pH 6.3 with NaOH, (c) 0.35 M-NaCl and (d) twice with 1.5 mM-NaCl/0.15 mM-sodium citrate (adjusted to pH 7.0 with HCl). On each occasion the homogenate was centrifuged at 20000g (*r*_{av.} 7 cm), the final product being a chromatin gel.

Histone was isolated by a modification of the technique of Mohberg & Rusch (1969). To the chromatin gel was added an equal volume of water, followed by 2 vol. of 2 M-CaCl₂ with rapid mixing. The viscous solution was stirred at 600 rev./min for 2 h and centrifuged at 25000g (*r*_{av.} 7 cm) for 15 min. The pellet was resuspended in 2 vol. of 1 M-CaCl₂, stirred overnight and then centrifuged as before. A 100% (w/v) solution of trichloroacetic acid was added to the supernatants to give a final concentration of 25% (w/v) and the precipitates were collected by centri-

fugation at 15000g (*r*_{av.} 7 cm). The combined precipitates were extracted three times with 0.25 M-HCl over a period of 24 h, each time with thorough homogenization followed later by centrifugation at 10000g (*r*_{av.} 7 cm) to remove the insoluble material. Histone was precipitated from the combined HCl extracts by the addition of 7 vol. of acetone. The histone was dried by washing it several times with acetone followed by diethyl ether, and finally evaporation of the residual ether. Histone prepared by this method was identical electrophoretically with histone prepared by direct acid extraction of *Euglena* nuclei or chromatin, except that no faint low-mobility bands were present, indicating freedom from contaminating non-histone proteins.

Gel exclusion chromatography

Euglena histone was separated into different components by gel filtration in Bio-Gel P-100 with 0.01 M-HCl/0.02% (w/v) Na₂SO₄ as eluent (Sommer & Chalkley, 1974). Histone (15 mg) was dissolved in eluent containing 1% 2-mercaptoethanol and applied to a previously equilibrated column (95 cm long × 2.5 cm diam.). The flow rate was maintained at 6.5 ml/h and 3 ml fractions were collected. The eluate was monitored for A₂₃₀ by using a 30 μl flow cell in a Cecil (CE 272) spectrophotometer. Appropriate fractions were pooled, concentrated by dialysing against acetone (adjusted to 0.1 M with respect to HCl by addition of conc. HCl) and precipitated with 7 vol. of acetone. The precipitates were dried as described for whole histone.

Polyacrylamide-gel electrophoresis

Histone was analysed electrophoretically in the presence of 2.5 M-urea by the method of Panyim & Chalkley (1969) by using the Shandon disc electrophoresis system. Rod gels (0.6 cm × 9 cm) containing 15% (w/v) acrylamide were pre-run at 50 V before application of the sample in 8 M-urea/1 M-acetic acid. A potential of 90-100 V was applied for 4-6 h, after which the gels were stained for 1-2 h with 0.5% (w/v) Amido Black 10B in methanol/water/acetic acid (5:5:1, by vol.). Excess stain was removed by washing in the stain solvent at 55°C.

Histone was also electrophoresed in 15% polyacrylamide rod gels in the presence of sodium dodecyl sulphate at pH 10 (Panyim & Chalkley, 1971). The proteins were applied in 4 M-urea/0.1% (w/v) sodium dodecyl sulphate/0.01 M-glycine, pH 10, together with 10 μl of 0.01% (w/v) Bromophenol Blue to provide a visible marker of the progress of electrophoresis. The gels were stained as above.

Rod gels were scanned at 600 nm by using a Gilford densitometer modified for use with the Unicam SP.500 optical system.

To compare the electrophoretic patterns of two samples, a 'split gel' technique was used. Protein was applied to either side of a rod gel after division of the space in the glass tube above the gel into two compartments with celluloid. After initial electrophoresis to allow the protein to enter the gel, the celluloid was removed and the remaining procedure was as before.

Preparative gel electrophoresis

The electrophoretic system used was that of Panyim & Chalkley (1969) described above except that the gel contained 6.25M-urea. A short column (2–3 cm) of gel was cast in a Buchler Poly-Prep 100 apparatus. A 2–3 mg sample was dissolved and layered on the surface of the gel, and a potential of 150V applied. The flow rate across the lower gel surface was about 20 ml/h, 1.5 ml fractions being collected. Protein was estimated turbidimetrically in each fraction by the addition of $\frac{1}{3}$ vol. of a 100% (w/v) solution of trichloroacetic acid and measurement of the A_{400} (Luck *et al.*, 1958). Appropriate fractions were pooled, the protein was centrifuged down (2000g for 15 min, r_{av} . 11 cm), redissolved in 0.25M-HCl, precipitated with acetone and dried as for whole histone.

Amino acid analyses

Histone fractions were hydrolysed in 6M-HCl at 105°C for 24 h in sealed evacuated tubes. The hydrolysates were analysed on either a Locarte or a Beckman 120C amino acid analyser. In computation of the results, no corrections were made for hydrolytic losses.

Determination of the solubility of *Euglena* histone fractions in (a) 0.6M-HClO₄ and (b) ethanol/1.25M-HCl (4:1, v/v)

(a) About 2 mg of dried protein was dissolved in 1 mM-HCl, and an equal volume of 1.2M-HClO₄ was added at 0°C (Johns, 1964). After standing overnight, any precipitate was collected by centrifugation at 2000g for 15 min (r_{av} . 11 cm). The supernatants were adjusted to a concentration of 0.2M with respect to H₂SO₄, and any protein therein was precipitated with 7 vol. of acetone. A little of each precipitate was dried and analysed by electrophoresis to identify the components.

(b) The precipitates from the above procedure were then extracted at 0°C with 1 ml of ethanol/1.25M-HCl (4:1, v/v) (Johns, 1964). After centrifugation at 2000g for 15 min (r_{av} . 11 cm), any insoluble material was redissolved in 0.25M-HCl, precipitated with acetone and re-extracted with ethanol/HCl as before. Protein was precipitated from the extracts with acetone, and the precipitates and residues were dried and analysed electrophoretically.

Results

Polyacrylamide-gel electrophoresis of *Euglena* whole histone

Electrophoresis of *Euglena* histone in the presence of sodium dodecyl sulphate yields a pattern of stained bands resembling that obtained with rat liver whole histone (Fig. 1a). Thus there is an indication of similarity in both complexity and molecular size between *Euglena* and vertebrate histones. However, the patterns and band mobilities of *Euglena* and rat liver histones after electrophoresis in acidic urea gels are rather different (Fig. 1b). In particular the *Euglena* histone lacks a slow band corresponding in mobility to the rat H1 fraction.

Gel filtration of *Euglena* histone and electrophoretic properties of resultant fractions

To isolate and characterize the individual histones, *Euglena* whole histone was subjected to gel filtration. The histone was resolved into five main fractions on elution from Bio-Gel P-100 (Fig. 2), with the final absorbance peak being much larger than the others since, besides protein, it contained mercaptoethanol

Fig. 1. Electrophoretic comparison of *Euglena* and rat liver histones

Split polyacrylamide-gel electrophoresis was performed as described in the Materials and Methods section. Samples of *Euglena* histone (E) and rat liver histone (RL) were applied adjacently on the same gel and were electrophoresed in the presence either of (a) 0.1% sodium dodecyl sulphate or (b) 2.5M-urea. The designation of the rat liver histone fractions is according to Panyim & Chalkley (1969, 1971).

Fig. 2. Chromatography of *Euglena* whole histone on Bio-Gel P-100

Column chromatography was performed as described in the Materials and Methods section. Absorbance peaks corresponding to histones are labelled A-E and histone fractions recovered from the eluate are designated in the same way. The peak E absorbance is large, since besides histone fraction E it also contains mercaptoethanol used in the dissolution of the protein before chromatography. The small peaks labelled x and y represent non-histone proteins and, in x, also nucleotide contaminants.

from the solution used to dissolve the histone. The small peaks x and y (the former corresponding to the exclusion volume of the column) represent non-histone contaminants, and were variable in size, sometimes not appearing at all.

Densitometer traces of the patterns given by *Euglena* histone fractions after electrophoresis in sodium dodecyl sulphate and urea/polyacrylamide gels are shown in Fig. 3. The assignment of the bands given by the fractions to bands in the whole histone pattern was confirmed by split-gel electrophoresis (Jardine, 1975). When an individual fraction yielded two or more close-running bands in either of the electrophoretic systems, this is indicated in Fig. 3, although such separations were not always resolved by the gel scanner. Thus in sodium dodecyl sulphate/polyacrylamide gels double bands were given by fractions A and B, whereas in gels containing urea fractions B and E gave double bands and fraction C gave a triple band.

Fig. 3 also indicates that by using the two electrophoretic systems together an unambiguous differentiation of the fractions is achieved. For example, the components of fractions B and D, which have the same mobility in the sodium dodecyl sulphate system, are well separated in urea gels. This differentiation achieved by the systems indicates that the complexity

shown by some fractions is not due to cross-contamination.

Solubility studies on *Euglena* histone fractions

When HClO_4 was added to individual solutions of the Bio-Gel fractions from *Euglena* to give a concentration of 0.6M, fractions A and B remained in solution. Fraction C required a number of hours before complete precipitation occurred, but fractions D and E were precipitated immediately. No protein could be recovered from the supernatants obtained after the precipitation of fractions C, D and E. With mammalian histones, 0.6M- HClO_4 precipitates all fractions except H1 (Johns & Butler, 1962; Oliver *et al.*, 1972).

Despite the fact that fraction B, as well as fraction A, remained in solution after the addition of HClO_4 , the extraction of *Euglena* nuclei or chromatin with 0.6M- HClO_4 released only fraction A (Jardine, 1975).

The *Euglena* fractions B and D dissolved readily in ethanol/1.25M-HCl (4:1, v/v), whereas fraction C was insoluble and fraction E was only sparingly soluble. In the last-mentioned case there was no evidence that fractionation was occurring in the partition, since the electrophoretic pattern of the dissolved protein was identical with that of the undissolved, i.e. a clearly defined double band on urea gels. Ethanol/1.25M-HCl (4:1, v/v) selectively dissolves histones H2A, H3 and H4 from mammalian chromatin (Johns, 1964) or whole histone (Oliver *et al.*, 1972).

Electrophoresis of the fractions recovered after these solubility trials confirmed that no further fractionation had occurred, thus indicating that no more than five main types of histone are present in *Euglena*. However, the procedures were found to be useful in removing cross-contamination between the Bio-Gel fractions when this occurred.

Amino acid analyses of *Euglena* histone fractions

Before analysis each Bio-Gel fraction was further purified as necessary by the selective extraction/precipitation procedures described above. In addition, minor contaminants were removed from fraction E by preparative polyacrylamide-gel electrophoresis. The amino acid analyses are shown in Table 1, along with analyses for calf thymus and *Tetrahymena* histones for comparison.

Discussion

The above fractionation studies show that five types of histone occur in the nucleus of *Euglena*. Examination of the properties of the individual fractions indicates that they fall into the same five main categories as the histones of higher organisms,

Fig. 3. Densitometer traces of polyacrylamide gels after electrophoresis of *Euglena* histone fractions and whole histone in the presence of (a) 0.1% sodium dodecyl sulphate and (b) 2.5M-urea

The identities of bands given by fractions to bands in the patterns for whole histone (confirmed by split-gel electrophoresis; Jardine, 1975) are indicated by the vertical dashed lines. Where a fraction has been separated into close-running double or triple bands (not always resolved by the densitometer) this has been indicated by vertical arrows.

and hence each fraction can be designated according to the Ciba nomenclature (Bradbury, 1975).

Fraction A

The amino acid composition, particularly the high content of lysine and alanine, indicates that this is an H1 histone. This conclusion is supported by its similarity in molecular weight to mammalian histone H1,

shown by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, and by its solubility in 0.6M-HClO₄. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis gives two bands, which suggests that it may be heterogeneous, like vertebrate histone H1 (Kinkade & Cole, 1966).

Euglena histone H1 has some unusual features, notably the apparent absence of aspartic acid, and an unusually high content of lysine. The lack of aspartic

Table 1. *Amino acid analysis of Euglena histone fractions compared with calf thymus and Tetrahymena histones*

Euglena histone fractions were prepared by gel filtration on Bio-Gel P-100 and further purified by selective extraction and precipitation procedures as described in the text. In addition, fraction E was purified by preparative gel electrophoresis. Amino acid analyses for calf thymus and *Tetrahymena* histones were taken from Hnilica *et al.* (1971) and Johann & Gorovsky (1976) respectively. All values are mol/100mol. N.D., Not determined.

Amino acid residue	<i>Euglena</i> fraction A	Calf thymus H1	<i>Tetra-hymena</i> H1	<i>Euglena</i> fraction B	Calf thymus H2A	<i>Tetra-hymena</i> X	<i>Euglena</i> fraction C	Calf thymus H2B	<i>Tetra-hymena</i> H2B	<i>Euglena</i> fraction D	Calf thymus H3	<i>Tetra-hymena</i> H3	<i>Euglena</i> fraction E	Calf thymus H4	<i>Tetra-hymena</i> H4
Lysine	35.0	27.9	28.7	16.7	12.9	13.0	18.2	16.6	15.2	12.9	10.1	11.7	12.3	9.8	10.8
Histidine	0.9	0.0	2.0	0.7	2.7	2.7	1.9	2.2	1.3	1.9	2.5	2.7	2.1	2.0	1.9
Arginine	2.3	1.7	2.3	6.1	9.4	5.7	6.1	6.2	5.4	12.3	13.8	9.5	11.7	14.1	14.9
Aspartate	0.1	2.0	8.1	5.8	5.6	9.4	5.7	4.9	8.2	4.3	4.5	6.8	4.3	5.0	5.5
Threonine	3.4	5.5	8.8	5.4	5.0	5.9	5.3	6.1	5.5	6.0	6.4	5.7	6.4	6.6	5.6
Serine	4.5	6.9	7.5	5.9	5.0	7.8	7.4	10.7	9.9	3.5	3.8	6.7	3.1	2.5	3.5
Glutamate	3.2	3.4	7.4	7.1	9.0	10.1	7.2	7.6	9.4	11.8	10.3	12.1	9.0	6.2	8.4
Proline	10.1	10.2	6.8	6.1	4.0	4.5	3.2	4.8	4.5	4.4	4.3	3.8	1.9	1.3	1.4
Glycine	3.8	6.9	3.4	11.3	9.1	8.7	8.4	5.5	7.3	5.9	5.9	6.1	14.6	16.0	16.4
Alanine	22.6	25.3	14.8	12.5	13.2	9.3	12.0	10.1	9.5	12.1	13.8	10.0	7.0	7.4	7.5
Half-Cystine	0.0	0.0	N.D.	0.0	0.0	N.D.	0.0	0.0	N.D.	0.6	0.4	1.1	0.0	0.0	N.D.
Valine	5.5	4.3	3.8	5.6	6.0	5.2	5.4	7.0	5.3	5.2	4.6	4.0	7.2	8.0	6.5
Methionine	0.6	0.0	0.7	0.4	0.2	1.0	1.9	1.5	1.6	0.9	1.5	1.3	0.2	1.1	1.5
Isoleucine	1.1	0.8	3.0	2.4	4.3	4.8	5.7	4.9	4.6	4.7	4.8	4.9	5.9	5.5	4.9
Leucine	4.3	4.3	1.9	10.1	10.2	9.2	6.6	4.9	7.5	8.6	9.1	8.4	8.5	8.0	7.9
Tyrosine	1.6	0.5	0.0	1.9	2.3	1.1	1.5	3.8	1.5	1.3	2.0	1.3	3.0	3.5	2.8
Phenylalanine	1.2	0.5	0.6	1.8	1.0	2.1	3.5	1.6	3.3	3.7	2.0	4.0	3.0	2.2	2.5
Lys/Arg ratio	15.2	16.4	12.5	2.7	1.4	2.3	3.0	2.7	2.8	1.0	0.7	1.2	1.1	0.7	0.7
Basic/acidic ratio	11.6	5.4	2.1	1.8	1.7	1.1	1.8	2.0	1.2	1.7	1.8	1.3	2.0	2.3	2.0

acid is noteworthy, since published sequences (Croft, 1973, 1974) do not include any such proteins apart from basic sperm-specific nuclear proteins such as the protamines. The ratio of basic to acidic residues (11.6) is significantly higher than for any other H1 histone except that of *Oxytricha* (Caplan, 1975). Compared with mammalian H1 histones, *Euglena* histone H1 has a high mobility on acidic urea/polyacrylamide gels. This feature is shared by other protozoal H1 histones, namely those of *Tetrahymena* (Gorovsky *et al.*, 1974) and *Oxytricha* (Caplan, 1975).

Fraction D

The presence of cysteine in this fraction suggests that it belongs to the H3 class, and the formation of a single oxidation product (Jardine, 1975) implies that it contains only one cysteine residue per molecule. The apparent molecular weight, as indicated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, and overall composition are close to those for vertebrate histone H3. However, unlike the so-called arginine-rich vertebrate histone H3, it contains slightly more lysine than arginine, although the total content of basic residues is the same. *Tetrahymena* histone H3 is also slightly lysine-rich, but has a lower proportion of basic residues and a slightly smaller molecular weight (Johmann & Gorovsky, 1976).

Fraction E

The composition of this fraction closely resembles that of vertebrate histone H4, but again this fraction is lysine-rich rather than arginine-rich (see fraction D above). However, in this instance the *Tetrahymena* H4 histone is arginine-rich (Johmann & Gorovsky, 1976). The molecular weights of *Euglena* and vertebrate H4 histones appear (by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis) to be closely comparable. *Euglena* histone H4 (like the vertebrate fraction) can be resolved into two bands on urea gels, presumably indicating the presence of acetylated residues. However, this *Euglena* fraction contrasts with vertebrate and plant H4 histones in not being very soluble in ethanol/1.25M-HCl (4:1, v/v).

Fraction C

The composition of this histone places it in the H2B class, although it contains slightly more lysine than the vertebrate fraction. Like vertebrate histone H2B, it is insoluble in ethanol/1.25M-HCl (4:1, v/v) and in 0.6M-HClO₄. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis suggests that *Euglena* histone H2B might have a slightly lower molecular weight than vertebrate histone H2B.

The *Euglena* histone H2B is resolved into three bands in urea/polyacrylamide gels, unlike vertebrate histone H2B, which gives a single band (Panyim *et al.*, 1971). This probably indicates that the *Euglena* fraction undergoes modification (e.g. acetylation), as does the *Tetrahymena* fraction (Johmann & Gorovsky, 1976).

Fraction B

This histone resembles mammalian histone H2B in lysine and arginine content, but with respect to the remaining amino acids it is more like the mammalian histone H2A. It also resembles histone H2A in its solubility in ethanol/1.25M-HCl (4:1, v/v) and in its elution from Bio-Gel P-100. Moreover, it behaves like histone H2A in being solubilized, along with *Euglena* histone H4, when chromatin is extracted with 2% (w/v) NaCl in 80% (v/v) ethanol (Jardine, 1975), a technique devised by Johns (1967) for the selective extraction of histones H2A and H4. We therefore consider it reasonable to designate this fraction as *Euglena* histone H2A, particularly as the other possible classification, histone H2B, has already been filled by *Euglena* fraction C.

Euglena histone H2A resembles the histone X of *Tetrahymena*, which, although anomalous, also appears to be the counterpart of histone H2A (Johmann & Gorovsky, 1976). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis indicates this *Euglena* histone H2A consists of two closely related species, another property that it shares with *Tetrahymena* histone X.

General discussion

The above demonstrates that *Euglena* histones may be classified into the same five types as are found in higher animals. Although each *Euglena* fraction (with the possible exception of histone H2A) has a broadly similar composition to the homologous mammalian histone, a consistent difference, reflected in the amino acid composition of *Euglena* whole histone (Jardine & Leaver, 1977), is that all the *Euglena* fractions have a significantly higher content of lysine. However, apart from the H1 fraction, both the total content of basic amino acids and the ratio of basic to acidic amino acids are close to those of the homologous mammalian fraction.

Separation of *Euglena* histones by gel filtration on Bio-Gel P-100 is clearly effective. It is worth noting that the elution order of the *Euglena* histones (as classified above) is the same as for calf thymus (Sommer & Chalkley, 1974) or *Tetrahymena* histones (Johmann & Gorovsky, 1976), namely H1, H2A, H2B, H3 and lastly H4.

Histones H3 and H4 have been remarkably conserved in higher organisms (DeLange *et al.*, 1969,

1973; Hooper *et al.*, 1973; Brandt *et al.*, 1974a,b). These histones are clearly present in *Euglena*, *Tetrahymena* (Johmann & Gorovsky, 1976), *Neurospora* (Goff, 1976) and yeast (Brandt & von Holt, 1976), although they differ in certain respects from those of higher organisms. For example, *Euglena* histones H3 and H4 and *Tetrahymena* histone H3 are not arginine-rich, and *Tetrahymena* histone H3 and *Neurospora* histone H3 have a much lower ratio of basic to acidic residues. Protozoal H4 histones, however, show less variation than the H3 histones and are remarkably similar to the H4 histones of higher organisms.

The moderately lysine-rich histones H2A and H2B are commonly regarded as being less highly conserved with respect to amino acid sequence than are histones H3 or H4 (Elgin & Weintraub, 1975). In plants these histones appear to be replaced by others that exhibit different electrophoretic properties (Nadeau *et al.*, 1974; Spiker, 1975; Brandt & von Holt, 1975). It is therefore noteworthy that typical H2B histones are found in *Euglena*, *Tetrahymena* (Johmann & Gorovsky, 1976) and *Neurospora* (Goff, 1976) and that H2A histones, although showing points of contrast with vertebrate H2A histones, are also present.

The primitive nature of the mitosis and nuclear morphology exhibited by *Euglena* are not reflected in the characteristics of the histones. However, Haapala & Soyer (1975) have shown that *Euglena* chromosomes display essentially the same chromomere organization as higher eukaryotes, and contrasting with that found in dinoflagellates (Haapala & Soyer, 1973). The latter, although like *Euglena* in having chromosomes condensed throughout the cell cycle, do not have histones (Rizzo & Noodén, 1974a,b).

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