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Histones of the Unicellular Alga *Olisthodiscus luteus*†

Peter J. Rizzo,*,‡ William Bradley,§ and Rodney L. Morris‡

Biology Department, Texas A&M University, College Station, Texas 77843, and Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

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ABSTRACT: The four major histones of the marine unicellular alga *Olisthodiscus luteus* were prepared from isolated nuclei and fractionated by molecular exclusion chromatography and preparative gel electrophoresis. The isolated histones were characterized by amino acid analysis and peptide mapping and compared to calf thymus histones. Using the above and various other criteria, we have identified these four histones in terms of their vertebrate counterparts. We conclude that *O. luteus* chromatin contains an H1-like histone, in addition to the highly conserved histones H3 and H4. A new histone (HOl) is described that serves the role of H2A and H2B in the nucleosome core. It is possible that H2A and H2B of higher organisms arose from an archetypal protein similar to HOl, through the usual gene duplication process.

Although the histones of higher plants and animals are now well characterized, little is known about histones of the lower plants that are collectively known as the algae. Histones have been reported in *Chlorella* (Iwai, 1964), *Volvox* (Bradley et al., 1974), *Euglena* (Jardine & Leaver, 1978; Bre et al., 1980), *Olisthodiscus luteus* (Rizzo, 1980), the endosymbiont nucleus of *Peridinium balticum* (Rizzo, 1982), and the red alga *Porphyridium* (Barnes et al., 1982). Only for *Euglena* (Jardine & Leaver, 1978) have these histones been characterized in terms of their vertebrate counterparts.

O. luteus is a wall-less unicellular marine alga, belonging to the Chrysophyceae (Gibbs et al., 1980). It can be grown to high cell densities in a chemically defined medium (McIntosh & Cattolico, 1978) and has been used successfully for chloroplast studies (Cattolico, 1978; Ersland et al., 1981; Aldrich et al., 1982). Since this alga is naturally wall-less, nuclei can be isolated in high yields (Rizzo & Burghardt,

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‡Texas A&M University.
§Baylor College of Medicine.

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1983). The nuclear DNA is typically eukaryotic in that it is composed of 45% repeat sequences, interspersed with single copy sequences (Ersland & Cattolico, 1981). This DNA is organized into nucleosomes that are morphologically similar to vertebrate nucleosomes (Rizzo & Burghardt, 1980), although electrophoretic analysis of the histones revealed only four major components (Rizzo, 1980). Digestion of *O. luteus* chromatin with micrococcal nuclease revealed a DNA repeat length of about 220 base pairs, which is slightly longer than that of higher eukaryotes, due to longer linker regions (Shupe et al., 1980). Digestion with DNase I showed the same ladder of DNA fragments, in multiples of 10 base pairs, as seen in higher eukaryotes (Shupe & Rizzo, 1983). Thus, the chromatin of *O. luteus* appears to be typically eukaryotic with the exception of a longer average DNA repeat length and the presence of four rather than the usual five major histone components.

We have isolated these four histones and characterized them in terms of their vertebrate counterparts, using amino acid composition and peptide mapping as the major criteria. We conclude from these studies that *O. luteus* contains an H1-like histone in addition to the highly conserved histones H3 and H4. No major histone components were found to correspond to H2A or H2B, but a new histone that we call HOl is described.

Materials and Methods

Cell Culture and Nuclear Isolation. O. luteus (Carter) was cultured in a chemically defined medium (McIntosh & Cattolico, 1978) at 22 °C on a 12-h-light/12-h-dark cycle. Nuclei were isolated during the logarithmic growth phase by method C as described elsewhere (Rizzo & Burghardt, 1983). Briefly, cells were lysed by homogenization in a buffer containing 1.0 M hexylene glycol, 1.0 mM PIPES[1] (pH 7.5), 1.0 mM $CaCl_2$, 25% (v/v) glycerol, and 0.5% (v/v) Nonidet P-40. The lysing buffer was made 1 mM in phenylmethanesulfonyl fluoride just before cell disruption by the addition of an appropriate amount from a 0.1 M stock in 2-propanol. Nuclei were purified by differential centrifugation through 1 M sucrose in the above buffer. Details of the culture conditions and nuclear isolation procedure are described elsewhere (Rizzo & Burghardt, 1983). Nuclei were either used immediately or frozen at −20 °C. No difference in the histone gel pattern was observed between frozen and fresh nuclei.

Isolation of Histones. In early experiments histones were prepared by extraction of isolated nuclei with 0.4 N H_2SO_4 (Rizzo, 1980). Later, the $CaCl_2$ extraction procedure of Mohberg & Rusch (1969) was adopted to obtain a more quantitative extraction of histones. This histone extraction procedure was used for nearly all of the experiments described in the present paper. However, in the final stages of the present study an even better method was employed for extracting histones (Urban & Zweidler, 1983). This method involves displacement of histones with protamine and was performed essentially as described except that a brief sonication was used instead of a micrococcal nuclease digestion.

Gel Electrophoresis. Histones were analyzed by electrophoresis using the $NaDodSO_4$ system described by Laemmli (1970) and modified for histones by Thomas & Kornberg (1975). Gels of 0.8 mm × 9.2 cm × 6.4 cm were formed in a microslab apparatus (Idea Scientific, Corvallis, OR) similar to that described by Matsudaira & Burgess (1978). Electrophoresis was carried out at room temperature at 175 V for 75–90 min. The gels were stained with Coomassie Blue for 30 min and destained by diffusion as described (Thomas & Kornberg, 1975). These gels were also used for molecular weight determination, using calf thymus histones as standards.

Column Chromatography. O. luteus histones were extracted from pooled, frozen nuclei. The 0.4 N H_2SO_4 extracts were precipitated with 8 volumes of cold acetone at −20 °C overnight. The precipitate was collected by centrifugation at 30000*g* for 30 min, washed twice with acetone, and dried under vacuum. The 1 M $CaCl_2$ extracts were dialyzed overnight against 200 volumes of 0.4 N H_2SO_4 with three changes, during which time a precipitate formed that was collected by centrifugation at 30000*g*. The histones in the supernatant were recovered by acetone precipitation as described above. Because some histones were also present in the 0.4 N H_2SO_4 dialysis precipitate, this pellet was extracted with 0.4 N H_2SO_4 and the histones in the extract were acetone precipitated as described above. The two histone pellets were combined and dissolved in 8 M urea–1% (v/v) mercaptoethanol to give a concentration of 8–10 mg/mL. The sample was stored at 4 °C overnight and then subjected to chromatography on a Bio-Gel P-60 column according to Bohm et al. (1973) at room temperature. Column dimensions were 120 × 1.5 cm with a pressure head of 70 cm and a flow rate of 3–4 mL/h. The column was previously equilibrated and eluted with 0.05 M NaCl–0.02 N HCl–0.02% (w/v) NaN_3, and 2-mL fractions were collected (assayed by absorption at 230 nm). Appropriate fractions were pooled, dialyzed against 0.01 N HCl, and lyophilized.

Chromatography on Sephadex G-100 was at room temperature on a 0.9 × 120 cm column with a pressure head of 10 cm and a flow rate of 10 mL/h. The column was previously equilibrated and eluted with 50 mM sodium acetate–5 mM sodium bisulfite (pH 5.1). This also served as the sample buffer. Pooled fractions (2 mL) were dialyzed against 0.01 N HCl and lyophilized.

Preparative Gel Electrophoresis. Preparative $NaDodSO_4$ gel electrophoreses were performed as described above except that a Bio-Rad Model 220 slab gel system was used. The gel dimensions were 10 cm × 14 cm and either 1.5 or 3 mm thick. Samples were run at room temperature overnight at 50 V for a total of 800 V·h. Bands were visualized by staining with cold 0.25 M KCl as described by Hager & Burgess (1980) and excized with a clean razor blade. The gel was supported on a glass plate that was kept cold by a bed of crushed ice. This prevented the bands from fading during the cutting procedure. Gloves were worn during the entire procedure to avoid contact with fingers. The gel slices were then soaked in a minimum volume of 1 mM dithiothreitol in cold distilled water with two changes of 10 min each and transferred to $NaDodSO_4$ electrode buffer. Protein was eluted from diced gel slices by using an Isco electroelution chamber fitted onto a Gelman horizontal electrophoresis chamber. Elution was at room temperature for 4 h, and the eluted histone was precipitated by adding 1 volume of 50% (w/v) trichloroacetic acid.

Amino Acid Analysis. Samples for amino acid analysis were hydrolyzed for 22 h in 5.7 N HCl at 110 °C in a Beckman Model 119 amino acid analyzer. The histones were fractionated by P-60 column chromatography first and then by preparative gel electrophoresis. In some cases the P-60 column was omitted.

Peptide Mapping. One-dimensional peptide mapping in the presence of $NaDodSO_4$ was carried out by the method of

[1] Abbreviations: PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); Tris, tris(hydroxymethyl)aminomethane; EDTA, (ethylenedinitrilo)-tetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate.

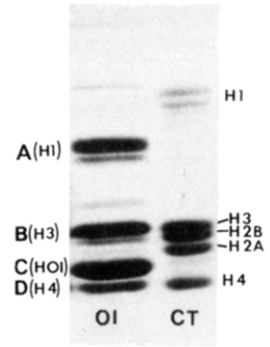

FIGURE 1: Electrophoretic pattern of *O. luteus* and calf thymus histones in 18% $NaDodSO_4$ gels. Migration was from top to bottom and proteins were stained with Coomassie Brilliant Blue. Ol = *O. luteus*; CT = calf thymus histone. *O. luteus* histones were prepared by protamine extraction of nuclei. The four major *O.* Luteus bands are labeled A–D, with their suggested vertebrate counterparts given in parentheses.

Cleveland et al. (1977). All experiments involved limited proteolysis with *Staphylococcus aureus* V8 protease (Miles Laboratories). About 10 μg/well whole histone was electrophoresed in a microslab apparatus as described above except that the thickness of this first slab gel was 0.5 mm instead of 0.8 mm. Following electrophoresis the gel was stained for 10 min with Coomassie Blue and rinsed several times with destain solution containing 5% (v/v) methanol plus 10% (v/v) acetic acid, to allow easy visualization of the bands against the background. The bands were cut out with a clean razor blade and either frozen at −20 °C or used immediately for the second slab gel. No difference was observed in the peptide patterns produced from frozen or fresh gel slices.

The gel slices were equilibrated with buffer containing 0.1% (w/v) $NaDodSO_4$–1 mM Tris (pH 6.8) by soaking for a total of 15 min with three changes and occasional swirling. The gel slices were then placed vertically in the wells of an 0.8-mm gel with a pair of forceps and covered with the protease digestion buffer containing the equilibration buffer above plus 10% (v/v) glycerol and 200–500 μg/mL *S. aureus* V8 protease. Electrophoresis was then performed at 10 mA at room temperature for 1–15 min until the Coomassie Blue was in the middle of the stacking gel. The power was turned off to allow digestion to proceed for 1 h, after which time electrophoresis was resumed at 20 mA for about 1.5 h, when the ion front reached the bottom of the separating gel. These gels were either stained with Coomassie Blue or silver stained according to the procedure described by Wray et al. (1981).

Relative Proportions of O. luteus Histones. Gels of *O. luteus* histones were scanned with a Quick Scan Jr. densitometer at 525 nm. The relative areas of the histone peaks were determined by outlining with an electronic graphics calculator (Pneumonics 1224).

RESULTS

Molecular Weights and Relative Proportions. An electrophoretic study of *O. luteus* histones has been published (Rizzo, 1980), which showed that four major components were detected by several different gel systems. A profile of *O. luteus* histones is shown in Figure 1 for reference. The designations of the various histones in terms of their vertebrate counterparts were derived primarily from amino acid analyses and peptide mapping studies described in the present paper. The relative proportions of the histones were estimated by determining the areas under peaks obtained from densitometer scans of nuclei subjected to electrophoresis (data not shown). Nuclei were used instead of nuclear extracts for densitometry in order to eliminate variability resulting from differential extraction of the various histones. In particular, we have found that H3 and H4 are difficult to extract quantitatively from nuclei and chromatin using a variety of histone extraction procedures. The stain ratios thus obtained were as follows: H1 = 0.7; H3 = 1.0; HOl = 2.0; H4 = 1.0. Molecular weights of the four major histones were estimated by $NaDodSO_4$ electrophoresis as described under Materials and Methods except that the gels were approximately twice as long, to allow a better separation of the bands. Calf histones were used as molecular weight standards. The molecular weights thus obtained were as follows: H1 = 19 000; H3 = 15 600; HOl = 13 000; H4 = 11 600.

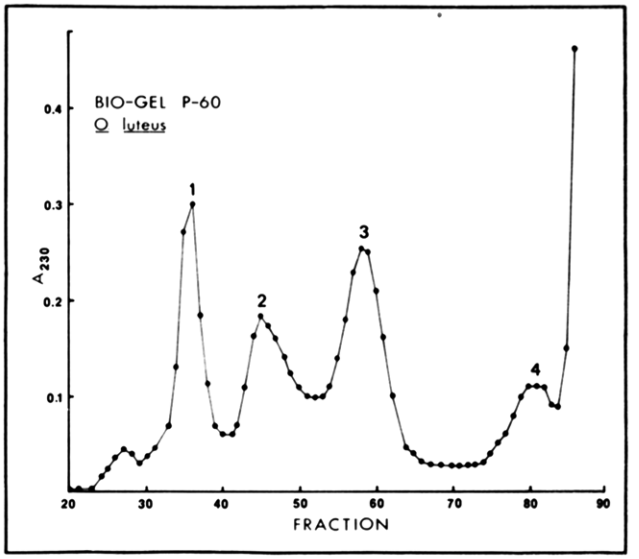

FIGURE 2: Elution profile of *O. luteus* histones chromatographed on a Bio-Gel P-60 column, as described under Materials and Methods. Approximately 10 mg of histone was applied to the column. The small peak beginning at fraction 23 is aggregated histone eluting in the flow through and was therefore not numbered. The high absorbance immediately following peak 4 is the urea from the sample buffer.

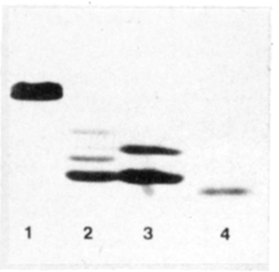

FIGURE 3: Electrophoretic pattern of aliquots taken from the fraction of highest absorption in each of the four peaks shown in Figure 2. The 18% $NaDodSO_4$ gel was stained with Coomassie Brilliant Blue, and migration was from top to bottom. Numbers correspond to the peaks in Figure 2, in their order of elution.

Column Chromatography. O. luteus histones were partially fractionated by chromatography on Bio-Gel P-60 columns. Aside from a small peak of aggregated whole histone that eluted in the void volume (fractions 25–30), four peaks were observed (Figure 2). However, as shown in Figure 3, the peaks are not all homogeneous. While peaks 1 and 4 contain *O. luteus* histones H1 and H4, respectively, peaks 2 and 3 each contain more than one histone component. Thus, in our hands, chromatography on Bio-Gel P-60 is adequate only for the separation of H1 and H4, due mainly to the poor separation of HOl from H3. A possible interpretation of the data in lanes 2 and 3 of Figure 3 is that the major component of lane 2 and

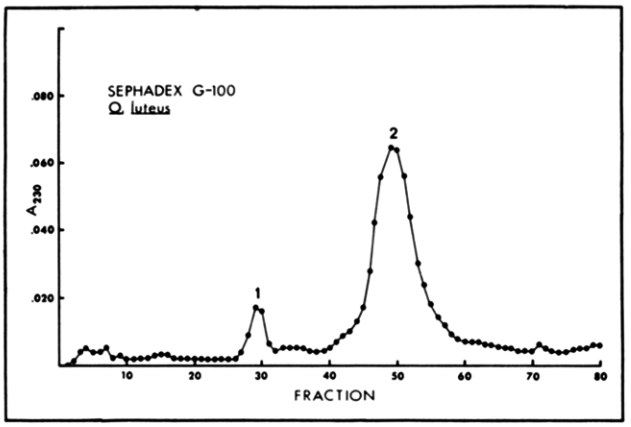

FIGURE 4: Elution profile of peak 3 from Figure 2 rechromatographed on a Sephadex G-100 column, as described under Materials and Methods.

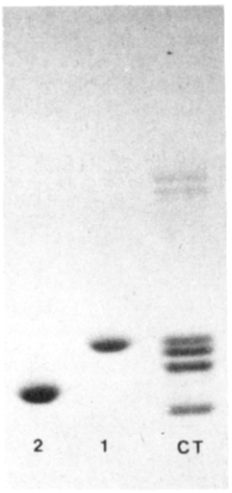

FIGURE 5: Electrophoretic pattern of the pooled fractions of peaks 1 and 2 from Figure 4. Migration was from top to bottom, and the 18% $NaDodSO_4$ gel was stained with Coomassie Brilliant Blue. The numbers correspond to the peaks in Figure 4. Fraction 1 = H3; fraction 2 = HOl.

the fast component of lane 3 represent analogues of H2A and H2B, rather than just the presence of HOl in both peaks. However, since their peptide maps are identical (data not shown), this possibility appears unlikely.

Since peak 3 contained only two components, pooled fractions were lyophilized and chromatographed on Sephadex G-100. For the P-60 column of Figure 2, fractions 55–62 were dialyzed against distilled water, lyophilized, and chromatographed as described under Materials and Methods. Figure 4 shows the column profile obtained, and Figure 5 shows the components of the two peaks. Although a clean separation of HOl and H3 was obtained with this second fractionation, the recovery of H3 was much lower than that of HOl. The reason for this selective loss of H3 is not understood but may be related to the low amounts of protein involved (see A_{230} absorption in Figure 4). Thus, a preferential loss of H3 would not have to be very large in actual amounts of protein, in order to cause a substantial change in the relative proportions of HOl and H3 going from the P-60 column to the G-100 column. This could be tested by loading much more total histone on the P-60 column or by pooling peak 3 material from several columns.

Amino Acid Analysis. O. luteus histones were analyzed for amino acid composition to help identify these histones in terms of their vertebrate counterparts by using calf thymus histones for comparison. Three of the *O. luteus* histones were thus matched with calf thymus histones, and these are shown in Table I. These data suggest that *O. luteus* contains a lysine-rich H1-like histone, in addition to the highly conserved arginine-rich histones H3 and H4. For the *O. luteus* H1-like histone, only the major component of the doublet was used. The comparisons of *O. luteus* H3 with calf H3 and *O. luteus* H4 with calf H4 are shown graphically in parts A and B Figure 6, respectively, which shows the similarity much more dramatically. The amino acid composition of H4 is slightly closer to that of its vertebrate counterpart than is H3. The amino acid composition of the lysine-rich histone HOl was compared to those of H2A and H2B histones from various organisms, including yeast, *Euglena*, *Tetrahymena pyriformis*, *Neurospora crassa*, *Physarum polycephalum*, and many others. The histones that showed the closest resemblance to HOl are given in Table II, along with calf thymus H2A and H2B. Of these, HOl was most similar to histone H2A from *P. polycephalum*.

Table I: Comparison of the Amino Acid Composition of Three *Olisthodiscus* Histones with H1, H3, and H4 from Calf Thymus

amino acid	band A	calf H1[a]	band B	calf H3[a]	band D	calf H4[a]
Lys	19.5	26.8	10.4	10.0	9.8	11.4
His	1.7	0	1.3	1.7	2.2	2.2
Arg	1.8	1.8	10.0	13.0	12.6	12.8
Asx	6.1	2.5	7.0	4.2	6.4	5.2
Thr	5.0	5.6	6.2	6.8	5.7	6.3
Ser	6.4	5.6	4.8	3.6	3.5	2.2
Glx	7.8	3.7	11.2	11.6	9.0	6.9
Pro	5.4	9.2	4.5	4.6	3.0	1.5
Gly	12.4	7.2	8.3	5.4	13.8	14.9
Ala	16.3	24.3	11.9	13.3	7.6	7.7
$^1/_2$-Cys	0	0	tr[b]	1.0	tr	0
Val	4.4	5.4	5.6	4.4	7.3	8.2
Met	tr	0	0.9	1.1	0.9	1.0
Ile	3.3	1.5	4.0	5.3	5.2	5.7
Leu	6.7	4.5	9.3	9.1	7.3	8.2
Tyr	tr	0.9	2.1	2.2	3.6	3.8
Phe	3.3	0.9	2.6	3.1	2.6	2.1
basics	23.0	28.6	21.7	24.7	24.6	26.4
Asx + Glx	13.9	6.2	18.2	15.7	15.4	12.1
Lys/Arg	10.8	14.9	1.0	0.8	0.8	0.9
basic/acidic	1.7	4.6	1.2	1.6	1.6	2.2

[a] Mayes & Johns (1982). [b] Trace.

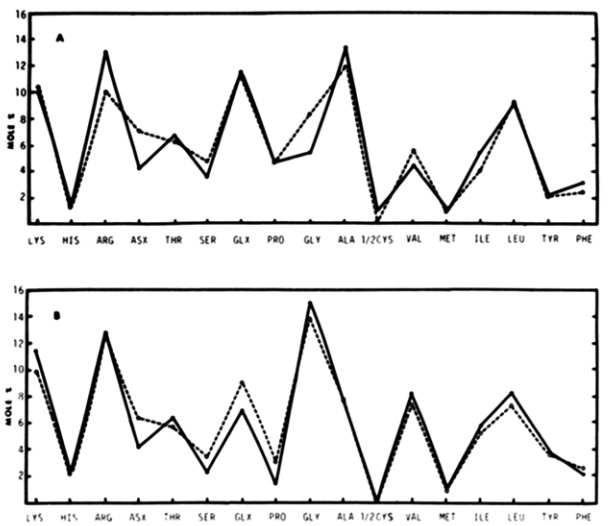

FIGURE 6: Graphic representation of amino acid analysis data for H3 and H4. Panel A = H3; panel B = H4; solid line = calf thymus; broken line = *O. luteus*.

Table II: Amino Acid Composition of HOl and Selected Histones[a]

	O.l. HOl	*D.m.* D2	*E.g.* H2A	*E.g.* H2B	*P.p.* H2A	*P.p.* H2B	calf H2A	calf H2B
Lys	9.9	9.5	12.9	18.2	10.7	16.2	10.9	16.0
His	1.4	3.6	2.7	1.9	2.4	4.0	3.1	2.4
Arg	6.8	6.8	9.4	6.1	7.8	5.3	9.3	6.4
Asx	7.1	5.7	5.6	5.7	7.0	4.7	6.2	4.8
Thr	6.9	5.0	5.0	5.3	4.3	6.9	3.9	6.4
Ser	6.8	6.6	5.0	7.4	7.8	9.3	3.1	11.2
Glx	9.9	10.0	9.0	7.2	10.5	9.3	9.3	8.0
Pro	4.0	2.7	4.0	3.2	4.5	3.9	3.9	4.8
Gly	10.6	11.1	9.1	8.4	11.1	7.4	10.9	5.6
Ala	9.7	13.9	13.2	12.0	10.3	10.4	13.2	10.4
$^1/_2$-Cys	0.4		0	0	0.2	0.3	0	0
Val	4.4	5.9	6.0	5.4	5.4	7.0	6.2	7.2
Met	1.9	0	0.2	1.9	0.3	1.5	0	1.6
Ile	7.4	6.8	4.3	5.7	5.3	3.6	4.7	4.8
Leu	7.4	10.0	10.2	6.6	8.5	5.0	12.4	4.8
Tyr	3.0	1.6	2.3	1.5	3.0	4.4	2.3	4.0
Phe	2.3	0.8	1.0	3.5	1.4	2.0	0.8	1.6
basics	18.1	19.8	25.0	26.2	20.9	25.5	22.4	24.8
Asx + Glx	17.0	15.7	14.6	12.9	17.5	14.0	15.5	12.8
Lys/Arg	1.5	1.4	1.4	3.0	1.4	3.1	1.2	2.5
basic/acidic	1.1	1.3	1.7	2.0	1.2	1.8	1.5	1.9

[a] *O.l.* = *O. luteus*; *D.m.* = *D. melanogaster* (Palmer et al., 1980); *E.g.* = *Euglena gracilis* (Jardine & Leaver, 1978); *P.p.* = *P. polycephalum* (Mende et al., 1983); calf = calf thymus (Mayes & Johns, 1982).

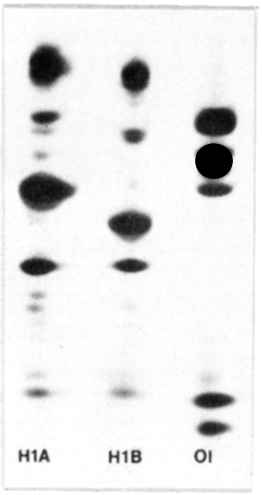

FIGURE 7: Peptide mapping of *O. luteus* H1 (band A, major component) and calf thymus H1. Migration was from top to bottom, and the 18% NaDodSO$_4$ gel was silver stained.

Peptide Mapping. *O. luteus* histones were also compared with their vertebrate counterparts by limited proteolysis with *S. aureus* V8 protease. Figure 7 shows the results of a peptide mapping experiment in which the H1-like histone from *O. luteus* was compared with H1A and H1B from calf. For *O. luteus*, only the major component of the doublet was used. The patterns obtained are complex but it is clear that while *O. luteus* band A is an H1-like histone, the peptide map differs markedly from that of calf thymus H1A and H1B. The component of highest molecular weight in Figure 7 is undigested histone in all three samples.

A comparison of the amino acid composition of HOl with that of the five calf histones showed that HOl most closely resembles H2A and H2B (Table II). It was therefore of interest to compare the peptide maps of these three histones (Figure 8A). In each case the left lane of the pair shows the pattern obtained when the protease was diluted 10-fold. The spot of highest molecular weight is undigested histone for all six samples. Although there may be some peptides in common between HOl and the calf histones, the overall patterns are clearly different, which is in agreement with the amino acid analysis data.

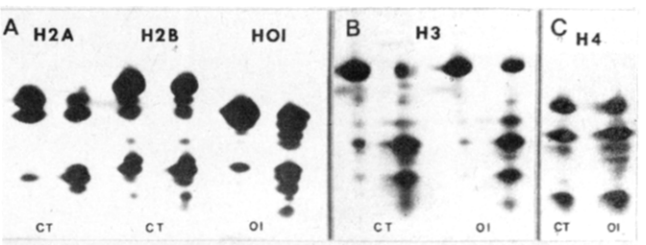

FIGURE 8: Peptide mapping of *O. luteus* HOl, H3, and H4 along with calf thymus H2A, H2B, H3, and H4. Migration was from top to bottom, and the 18% NaDodSO$_4$ gel was silver stained. Panel A = comparison of HOl with calf thymus H2A and H2B; panel B = comparison of *O. luteus* H3 with calf thymus H3; panel C = comparison of *O. luteus* H4 with calf thymus H4.

Figure 8B shows the results of peptide mapping experiments comparing *O. luteus* H3 with calf H3. The left lane of each pair shows the pattern obtained when the protease was diluted 10-fold. The spot of highest molecular weight is undigested histone for all four samples. In contrast to the H1-like histone and HOl, the H3 peptide maps are strikingly similar. The peptide maps are also similar for *O. luteus* and calf H4 (Figure 8C), although only two major fragments are generated. No digestion was observed when the protease was diluted 10-fold (data not shown). Again, the spot of highest molecular weight is undigested histone. The data shown in Figure 8 thus support the amino acid analysis results, indicating close homologies between H3 and H4 from *O. luteus* and calf.

Discussion

O. luteus chromatin is composed of nucleosomes that are morphologically indistinguishable from vertebrate nucleosomes (Rizzo & Burghardt, 1980). However, an electrophoretic study of *O. luteus* nuclear histones showed that four rather than five major components were present (Rizzo, 1980). This previous study included two-dimensional analyses (urea followed by NaDodSO$_4$ and urea followed by urea plus Triton), which failed to detect additional histone components. It was therefore of interest to identify these four histones in terms of their vertebrate counterparts. We believe that *O. luteus* chromatin contains an H1-like histone, in addition to the highly conserved histones H3 and H4. No histones could be identified as H2A or H2B, but instead, a new histone (HOl) is present,

which may substitute for H2A and H2B.

H1. The 19-kilodalton protein is an H1 or at least an H1-like histone in its amino acid composition (Table I), its molecular weight, and its solubility in 5% perchloric acid (Figure 1). Furthermore, it stains purple rather than blue with Coomassie Brilliant Blue R-250, which is characteristic of H1 (Duhamed et al., 1980). This histone is also H1-like in that it is located in the linker DNA as evidenced by micrococcal nuclease digestion experiments (Shupe & Rizzo, 1983) and in that it has the lowest affinity for Triton binding in Triton–urea gels (Rizzo, 1980). Although this histone always appears as a doublet in $NaDodSO_4$ gels, one component of the doublet is much more abundant (Figure 1). Whether the two components are subtypes or a result of proteolytic degradation is not presently known.

H3 and H4. The highly conserved histones H3 and H4 are both represented in *O. luteus*. It is interesting that while *O. luteus* H3 and H4 are both very similar in amino acid composition to their vertebrate counterparts, they have slightly higher apparent molecular weights and are slightly less basic. The amino acid analysis data are supported by peptide mapping data, which shows that definite homologies exist. *O. luteus* H3 and H4 are located in the nucleosome core rather than in the linker DNA (Shupe & Rizzo, 1983), in accord with the location of vertebrate H3 and H4.

HOl. Of the five calf histones, the most likely counterpart for HOl would be either H2A or H2B, based mainly on amino acid composition. However, we believe that the overall properties of this histone differ from those of calf H2A and H2B significantly enough to conclude that HOl is not analogous to either. After comparing the amino acid composition of HOl with a large number of known histone amino acid compositions, we found that it most closely resembled *Physarum* H2A and protein D2 from *Drosophila melanogaster*. It would thus be interesting to compare the peptide maps of these three histones and also to see if HOl antibodies will react with the other two histones or with any of the histones listed in Table II. We are in the process of making antibodies to HOl. An intriguing possibility is that HOl may represent a histone ancestral to H2A and H2B. Since the relative proportion of HOl is twice that of H3 and H4, it probably serves the role of H2A and H2B in *O. luteus* nucleosomes. Previous studies with micrococcal nuclease have shown that HOl is located in the nucleosome rather than the linker DNA (Shupe & Rizzo, 1983). It should be mentioned that although there are minor bands that comigrate with calf thymus H2A and H2B (see Figure 1), they are present in insufficient quantities to play an adequate role in nucleosome formation. Concerning the question of whether HOl constitutes one or more than one protein, we conclude that the present data suggest one major protein. However, since HOl appeared in approximately equal amounts in peaks 2 and 3 of the P-60 column, it is possible that HOl is composed of two proteins and we have been unable to separate them other than by P-60 chromatography.

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